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PERFUSION SCANNING USING ^{99m}Tc -HMPAO DETECTS EARLY CEREBROVASCULAR CHANGES IN THE DIABETIC RAT

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Background: ^{99m}Tc -HMPAO is a well-established isotope useful in the detection of regional cerebral blood flow. Diabetes gives rise to arterial atherosclerotic changes that can lead to significant end organ dysfunction, prominently affecting perfusion to the heart, kidneys, eyes and brain. In the current study, we investigated the role of ^{99m}Tc -HMPAO cerebral perfusion scans in detecting early vascular changes in the diabetic brain.

Methods: Cerebral perfusion studies were performed on both control and streptozotocin-(STZ) induced diabetic male Wistar rats. Rat brain imaging using a gamma camera was performed for each group 0.5, 2, 4, and 24 hours post ^{99m}Tc -HMPAO injection. Data processing for each cerebral perfusion scan was performed by drawing a region of interest (ROI) circumferentially around the brain (B). Background (BKG) due to signal from the soft tissue of each rat was subtracted. Brain ^{99m}Tc -HMPAO uptake minus background counts (net brain counts; NBC) were then compared between the two groups.

Results: The NBC (mean \pm SD) for the STZ group were statistically significantly higher ($p = 0.0004$) than those of the control group at each of the time points studied.

Conclusion: ^{99m}Tc -HMPAO brain scan may be useful in the detection of early atherosclerotic changes in the diabetic rat brain.

Background

Diabetes leads to early atherosclerotic changes through a number of mechanisms, including the formation of advanced glycosylation end products and its influence on serum lipid composition. The vascular complications of diabetes are significant, and can lead to a severe arteriopathy prominently affecting the heart, kidneys and eyes. The brain can also be affected, causing impairments in memory and learning.

Nuclear medicine studies have previously been utilized for the functional investigation of diabetic pathophysiology. For example, in the podiatric literature, nuclear medicine imaging has been shown to be helpful in the management of the diabetic foot [1]. The role of cerebral nuclear medicine imaging techniques in the detection of CNS manifestations of diabetes has been studied previously using ^{18}F fluorodeoxyglucose (^{18}F -FDG) positron emission tomography (PET), to assess brain glucose metabolism [2, 3] in diabetic patients. Four studies have examined cerebral perfusion in diabetes using SPECT [4-7], three in type 1 diabetics and one in type 2 diabetics.

Technetium 99m -hexamethylpropylene amine oxime (^{99m}Tc -HMPAO) cerebral perfusion scanning is a well-known nuclear

medicine test used to detect variations in regional brain blood flow. ^{99m}Tc -HMPAO is the most common radiotracer used for SPECT and planar brain imaging; it is a lipophilic radiotracer that crosses the blood brain barrier (BBB). ^{99m}Tc -HMPAO is thought to accumulate in the brain through its intracellular conversion from a lipophilic to a hydrophilic form within the brain parenchyma [8]. Under most conditions, blood flow is coupled to increases in cerebral metabolism; hence ^{99m}Tc -HMPAO images can be used to represent the functional status of the brain.

To our knowledge, no prior studies have investigated the use of ^{99m}Tc -HMPAO uptake in the detection of vascular signal changes in early diabetic rats. Therefore, in the present study we investigated the potential role of ^{99m}Tc -HMPAO cerebral perfusion scanning in the detection of early vascular changes in new-onset diabetes.

Methods

Materials

A HMPAO (Exametazime) kit was purchased from Amersham (UK). ^{99m}Tc was eluted from a fresh ^{99}Mo - ^{99m}Tc generator (Amersham, UK). All other reagents used in this study were supplied by Sigma-Aldrich (UK).

Animals

Adult male Wistar rats (n = 12) of ~200 g weight were raised and handled in accordance with ethical standards, approved by the institutional ethics committee as recommended by the Helsinki Declaration.

Preparation of ^{99m}Tc -HMPAO

Fresh elutes of technetium (^{99m}Tc) were used each time to prepare the ^{99m}Tc -HMPAO following the manufacturer's instructions and recommendations. In brief, 1110–2960 MBq of $^{99m}\text{TcO}_4$ in 5 ml of saline were added to a freeze-dried Exametazime kit to produce ^{99m}Tc -HMPAO.

Induction of experimental type 1 diabetes

Experimental type 1 diabetes was induced in rats by intra-peritoneal (i.p.) injection of 55 mg/kg streptozotocin (STZ) dissolved in citrate buffer. Control rats were injected with buffer only.

Blood glucose determination

Blood samples were collected from the tail vein. Basal glucose levels were determined prior to STZ injection, using an automated blood glucose analyzer (Glucometer Elite XL). Sample collections were then made 48 h after STZ injection and blood glucose concentrations were determined and compared between groups. Rats with blood glucose concentrations above 300 mg/dl were declared diabetic and were used in the experimental group. One week after the induction of experimental diabetes, imaging was performed.

Experimental protocol

In order to control pain, an intravenous line was placed in the dorsal tail vein of each rat 10–15 minutes before the time of the radiopharmaceutical injection. Each rat was subsequently anaesthetized by intraperitoneal (i.p.) injection with 0.5 ml of 0.5 g intraval sodium 10 minutes before ^{99m}Tc -HMPAO injection. The level of anesthesia achieved with this regimen lasted for ~4 hours. Another i.p. injection of 0.5 g intraval sodium was administered before imaging at the 24 hour point. This anesthetic agent is believed to have a negligible effect on both blood pressure and

the biodistribution of the radiopharmaceutical. 129.5 MBq of ^{99m}Tc -HMPAO was injected within 30 minutes of ^{99m}Tc -HMPAO preparation and followed by as a line push administered via the fixed intravenous line. Each rat underwent a brain scan 30 minutes after ^{99m}Tc -HMPAO injection.

Gamma camera imaging

Each scan was performed using a single-head gamma (γ) camera (Philips camera; Odyssey LX) equipped with a high-resolution parallel hole collimator connected to a Dell computer. The matrix was 128 X 128 pixels and the photo peak was focused at 140 keV with a symmetric 10% window. Planar (posterior static view) images were obtained using a 5 minute acquisition at each time point. Each anaesthetized rat was fixed with plastic tape on a fixing board against the table of the γ camera during acquisition. The rat's head was localized in the center of the field of view and a zoom factor of 4 was applied during each acquisition time.

Image processing

Data processing for brain scans was done by drawing a region of interest (ROI) circumferentially around the brain (B) and around the soft tissues of the neck of each rat as a background measurement (BKG). Brain ^{99m}Tc -HMPAO uptake minus background (net brain counts; NBC) in control rats were determined first and expressed as the mean \pm standard deviation (mean \pm SD). NBC in diabetic-STZ rats were then determined. The ROIs were drawn with a fixed pixel size in order to maintain comparability between groups.

Data presentation and statistical analysis

All data, unless otherwise stated, were expressed as the mean \pm standard deviation (mean \pm SD). A Kruskal-Wallis non-parametric analysis of variance (ANOVA) test was used to evaluate differences between the STZ diabetic group and the control group. In addition, differences between the two groups at each time point were assessed.

Statistical analysis was performed using SPSS version 13.0 software (Chicago, USA).

Results

Induction of diabetes by STZ resulted in a significant increase in blood glucose concentration (mean \pm SD), from 180 ± 6 mg/dl (pre-STZ) to 350 ± 8 mg/dl (post-STZ) after one week of induction.

The net ^{99m}Tc -HMPAO brain counts (NBC) in the control group (mean \pm SD) at 0.5, 2, 4 and 24 h were $67, 766 \pm 10, 405; 49, 439 \pm 6, 960; 37, 080 \pm 5, 459$; and $2, 017 \pm 302$ counts per second (cps) respectively. NBC for the STZ group at 0.5, 2, 4 and 24 h were $241, 296 \pm 3, 548; 148, 215 \pm 2, 316; 111, 254 \pm 1, 682$; and $6, 817 \pm 76$ cps respectively. There was a highly significant difference between the control and STZ groups ($p = 0.004$) and there was no significant difference between each time point at both control and STZ groups ($p = 0.416$) using the non-parametric test equivalent to the analysis of variance (ANOVA), the Kruskal-Wallis test. Figure 1 shows ^{99m}Tc -HMPAO net brain counts (NBC) for both control and STZ-treated rats.

Discussion

Our results indicate that there are highly significant changes in the cerebral vasculature that occur early in the brains of diabetic rats, as demonstrated by an increase in ^{99m}Tc -HMPAO NBC. We found evidence of significantly increased ^{99m}Tc -HMPAO brain uptake one week after the induction of diabetes in the diabetic-STZ group compared to the control group at all time points from 0.5 h to 24 h. This may be due to early pathophysiological changes, such as vasodilatation in the early stages of diabetes associated with increased glucose metabolism. In agreement with our results, several prior studies have reported a significant increase in overall basal cerebral blood flow (CBF) rates in STZ-induced diabetic rats [9-11] as well as in diabetic patients using ^{99m}Tc -HMPAO SPECT [4, 5]. In contrast, one clinical study reported a 25–30% decrease in CBF in type 2 diabetic patients compared to control subjects

[7]. These apparent inconsistencies may actually reflect time-dependent differences in the pathophysiologic course of diabetes; however, more definitive longitudinal studies are needed to evaluate this hypothesis.

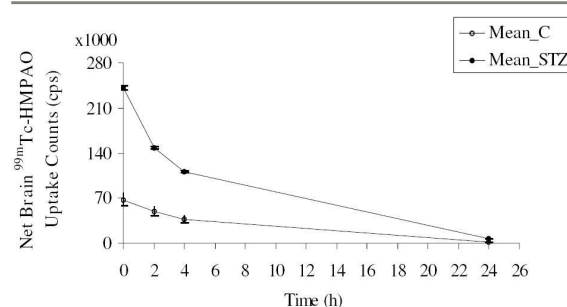


Figure 1. ^{99m}Tc -HMPAO net brain counts (NBC; in cps) versus imaging time points: 0.5, 2, 4 and 24 h for control (mean \pm SD): - - Mean_C, open circles and diabetic-STZ (mean \pm SD) group: - - Mean_STZ, black circles.

Typically, blood flow parallels the rate of glucose metabolism in the cerebrum. The increased ^{99m}Tc -HMPAO brain uptake observed in this study may be related to increased demand for, and/or accumulation of glucose uptake one week after the induction of diabetes.

^{99m}Tc -HMPAO uptake was reported to accumulate significantly and to relate to an enhanced rate of glucose metabolism in malignant cancer cells such as in human breast tumor cell lines (MCF-7) *in vitro* [12]. This illustrates one manner in which CBF and glucose metabolism may be related. However, not all studies are consistent: in a group of patients with newly diagnosed type 1 diabetes, no changes in cerebral glucose metabolism were detected [13]. Furthermore, a group of patients with long-standing diabetes exhibited a 15–20% reduction in cerebral glucose metabolism, again possibly reflecting a time-dependent process [13]. Another potential confounding factor is the comparison of studies performed with different imaging modalities and radiopharmaceuticals, as the latter study was performed using [^{18}F]-2-

deoxy-2-fluoro-D-glu-cose (^{18}F -FDG) PET [13].

Conclusion

$^{99\text{m}}\text{Tc}$ -HMPAO brain scan may be useful in the early diagnosis of cerebral vasculopathic changes in early diabetic rats. Future studies should be performed to confirm our findings and to further delineate the relationship between diabetes and CBF characterized by $^{99\text{m}}\text{Tc}$ -HMPAO.

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THE USE OF YELLOW FLUORESCENT HYBRIDS TO INDICATE MATING IN *TRYPANOSOMA BRUCEI*

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Background: *Trypanosoma brucei* undergoes genetic exchange in its insect vector, the tsetse fly, by an unknown mechanism. The difficulties of working with this experimental system of genetic exchange have hampered investigation, particularly because the trypanosome life cycle stages involved cannot be cultured in vitro and therefore must be examined in the insect. Searching for small numbers of hybrid trypanosomes directly in the fly has become possible through the incorporation of fluorescent reporter genes, and we have previously carried out a successful cross using a reporter-repressor strategy. However, we could not be certain that all fluorescent trypanosomes observed in that cross were hybrids, due to mutations of the repressor leading to spontaneous fluorescence, and we have therefore developed an alternative strategy.

Results: To visualize the production of hybrids in the fly, parental trypanosome clones were transfected with a gene encoding Green Fluorescent Protein (GFP) or Red Fluorescent Protein (RFP). Co-infection of flies with red and green fluorescent parental trypanosomes produced yellow fluorescent hybrids, which were easily visualized in the fly salivary glands. Yellow trypanosomes were not seen in mid gut or proventricular samples and first appeared in the glands as epimastigotes as early as 13 days after fly infection. Cloned progeny originating from individual salivary glands had yellow, red, green or no fluorescence and were confirmed as hybrids by microsatellite, molecular karyotype and kinetoplast (mitochondrial) DNA analyses. Hybrid clones showed biparental inheritance of both nuclear and kinetoplast genomes. While segregation and reassortment of the reporter genes and microsatellite alleles were consistent with Mendelian inheritance, flow cytometry measurement of DNA content revealed both diploid and polyploid trypanosomes among the hybrid progeny clones.

Conclusion: The strategy of using production of yellow hybrids to indicate mating in trypanosomes provides a robust and unequivocal system for analysis of genetic exchange. Mating occurred with high frequency in these experimental crosses, limited only by the ability of both parental trypanosomes to invade the salivary glands. Yellow hybrids appeared as soon as trypanosomes invaded the salivary glands, implicating the short, unattached epimastigote as the sexual stage. The recovery of diploid, triploid and tetraploid hybrids in these crosses was surprising as genetic markers appeared to have been inherited according to Mendelian rules. As the polyploid hybrids could have been produced from fusion of unreduced gametes, there is no fundamental conflict with a model of genetic exchange involving meiosis.

Background

Trypanosomes (Euglenozoa: Kinetoplastea [1]) are wide-spread and ubiquitous parasites of vertebrates, but the best known species are those that cause disease in humans and domestic livestock. So far genetic exchange has been demonstrated experimentally in two species, *Trypanosoma brucei* [2] and *T. cruzi* [3]. However, details of the mechanism remain elusive and the frequency of genetic exchange in nature is controversial [4, 5]. Determining how trypanosomes achieve genetic exchange is not only important for understanding gene flow in these pathogens, but also has relevance to the study of the early evolution of eukaryotes, as trypanosomes arise from a deep branch of the eukaryote tree [6].

Genetic exchange is not an obligatory part of the trypanosome life cycle and, for example, occurs only in a proportion of experimental flies co-infected with two different *T. brucei* strains [2, 7]. *T. brucei* undergoes a complex life cycle involving both mammalian and bloodsucking insect (tsetse fly) hosts [8]. Bloodstream form trypanosomes, taken up by the fly as it feeds, first differentiate into pro-cyclic forms and multiply within the midgut, before moving forward to invade the salivary glands via the foregut and mouthparts [9]. Genetic exchange most likely occurs in the fly salivary glands, because hybrids were found only in trypanosome populations derived from the salivary glands, not midguts, in analysis of crosses using selectable drug resistance markers [10, 11]. However, this approach did

not identify the life cycle stage involved, since detection relied on outgrowth of double-drug resistant hybrids and therefore only procyclics and metacyclics (via blood-stream forms) were actually examined. These results also leave open the possibility that mating occurs not in the salivary glands but en route, among the migratory forms (asymmetric dividers, long and short epimastigotes [9, 12]) that travel from the proventriculus at the anterior end of the midgut, through the foregut and thence to the salivary glands.

What happens during trypanosome mating remains a mystery; no-one has observed it directly and our current knowledge relies on genotypic comparisons of parents and progeny. Mendelian inheritance of genetic markers in hybrid progeny points to the occurrence of a meiotic division during genetic exchange in *T. brucei* [5, 13-18], although a naturally occurring haploid stage has not been observed [19, 20]. Most hybrid progeny are diploid like the parental trypanosomes, but triploid hybrids also occur [10, 11, 13, 21-23]. The observation that kinetoplast (mitochondrial) DNA is inherited from both parents in hybrid progeny [24-26] supports the hypothesis that fusion of the parental mitochondria, and hence cells, occurs during genetic exchange. Thus, genetic exchange in *T. brucei* involves both meiosis and fusion, but the order of these events is uncertain [5, 27]. The genomes of both *T. brucei* and *T. cruzi* contain meiosis-specific genes [28], but for *T. cruzi*, although fewer crosses have been done, the evidence points to fusion of the parental trypanosomes without meiosis, as hybrids inherited both parental alleles at a number of loci [3].

To find out more about the mechanism of genetic exchange in *T. brucei*, we need to examine intermediate stages in the process. The first step is to pinpoint the developmental stage and the region of the fly where genetic exchange takes place and for this we have developed approaches based on detection of fluorescent hybrids. Initial attempts based on segregation of reporter and repressor genes in hybrid progeny were successful

but problematic, as spontaneous mutations of the repressor system leading to reporter gene expression could not be ruled out [29], and thus we could not be certain that all fluorescent trypanosomes were hybrid. A simpler system based on the co-expression of two reporter genes, green and red fluorescent proteins (GFP and RFP), allows hybrid progeny to be detected by dual fluorescence, i.e. appear yellow by fluorescence microscopy in contrast to the green or red parental trypanosomes. This system has proved to be successful for the production and easy visualization of hybrids, as briefly reported in [30], and here we present a detailed analysis of four independent crosses.

Results and Discussion

Experimental cross between red and green trypanosomes

To visualize the production of hybrids in the fly, parental trypanosome clones were transfected with a gene encoding Green Fluorescent Protein (GFP) or Red Fluorescent Protein (RFP). The correct integration of the *GFP* and *mRFP* constructs into the rRNA locus was confirmed by PCR across border regions (Fig 1). We previously established that the *GFP* reporter gene was expressed throughout the trypanosome developmental cycle in the tsetse fly [29], and confirmed that this was also the case for *mRFP* by experimental fly transmission of the red trypanosome line. A series of crosses was set up by mixing approximately equal numbers of bloodstream forms of the red and green trypanosome lines with the first blood meal fed to groups of recently emerged (24–48 hours) tsetse flies. Flies were dissected 3–63 days later and examined for trypanosome infection; a detailed description of the infection results is presented in our companion paper [31], which examined the dynamics of co-infection with the red and green trypanosome clones, and therefore only details relevant to the experimental cross are included here. While almost all midgut infections examined consisted of a mixture of red and green trypanosomes, the composition of the salivary gland trypanosome populations was highly

variable, often with disparity between the two glands of the pair (Table 1). We assume that this reflects characteristics of the colonization process, whereby each salivary gland is invaded separately by a small number of migrating trypanosomes which serves as a

founder population. Only 22 of 60 flies (36.7%) had a mixture of red and green trypanosomes in one or both salivary glands, a far lower proportion than expected considering the high rate of mixed midgut infections.

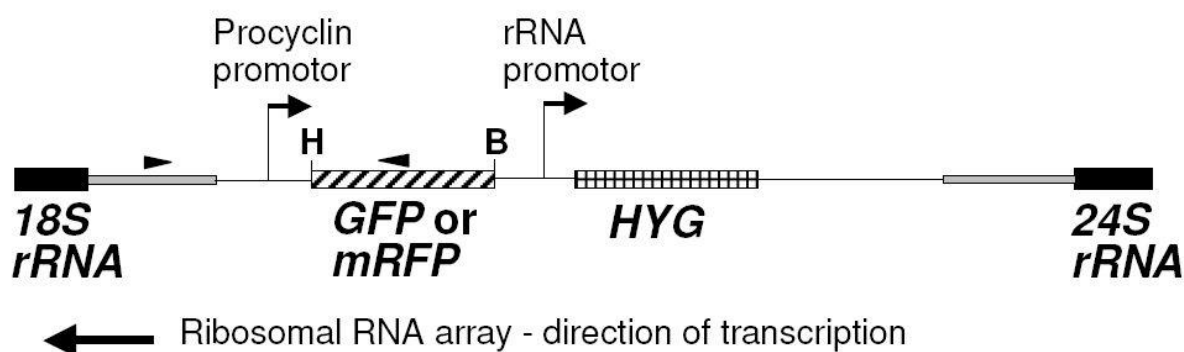


Figure 1. Diagram of reporter constructs. Diagram of reporter construct integrated into the non-transcribed spacer of the ribosomal DNA locus of *Trypanosoma brucei*. The *GFP* and *mRFP* genes were exchanged as a *Hind*III-*Bam*HI cassette. The boxes represent the genes indicated and the grey boxes represent the rRNA intergenic region, part of which was used for targeting the construct to this location. Thin line represents plasmid construct. The arrowheads show positions of primers used to check integration site. H = *Hind*III, B = *Bam*HI.

Yellow hybrid trypanosomes were found either inside or spilling out of the salivary glands of 17 flies, all of which had a mixture of red and green trypanosomes in one or both glands (Table 1; Fig. 2). Yellow trypanosomes were not observed in salivary glands containing only red or only green trypanosomes, even when the other gland of the pair contained a mixed infection of both parents (8/60: 3 flies with 1 mixed + 1 red gland, 5 flies with 1 mixed+ 1 green gland), or when both parents were present in the pair of glands, but not each individual gland, i.e. one gland with green trypanosomes only and one with red trypanosomes only (4/60) (Table 1; [31]). This demonstrates that mating takes place among trypanosomes that have reached the salivary ducts or glands and not among those able to mix en route in the foregut or mouthparts. The fact that yellow hybrids were found in 17 of the 22 flies in which one or both salivary glands had a mixed infection, suggests that all glands con-

taining a mixture of parental trypanosomes would eventually produce hybrids; in this experiment, mixed glands without yellow trypanosomes had either been dissected at early timepoints (14 – 17 days), or had very low numbers of one of the parents. The earliest timepoint at which yellow trypanosomes were observed in the salivary glands was 13 days after infection; in previous crosses, hybrids have generally appeared at least 28 days after infection [5, 7]. These yellow trypanosomes were epimastigotes, as shown by the morphology and close proximity of the kinetoplast to the nucleus (Fig 3). Also, during live imaging, yellow trypanosomes were seen that were clearly attached inside the intact salivary gland (see additional file 1: Movie1), confirming that hybrid formation had occurred by the attached epimastigote stage. On no occasion were yellow trypanosomes observed among midgut trypanosomes: 411 midguts with a mixture of red and green trypanosomes were examined [31].

Nor were yellow trypanosomes found among the foregut migratory forms on their way from the proventriculus to the salivary glands; these developmental stages (proventricular trypomastigotes, asymmetric dividers, short and long unattached epimastigotes) were examined in the salivary exudate from 58 individual flies (see next section).

Table 1. Salivary gland infections

| Salivary gland trypanosome infection | No. of flies | No. of flies with yellow trypanosomes |
|--------------------------------------|--------------|---------------------------------------|
| Both glands mixed | 13 | 10 |
| 1 mixed, 1 green | 5 | 4 |
| 1 mixed, 1 red | 3 | 2 |
| 1 mixed, 1 uninfected | 1 | 1 |
| 1 green, 1 red | 4 | 0 |
| Both green | 11 | 0 |
| Both red | 2 | 0 |
| 1 green, 1 uninfected | 11 | 0 |
| 1 red, 1 uninfected | 10 | 0 |
| Total | 60 | 17 |

From all the above evidence, we conclude that mating occurs in situ in the salivary glands and not among migratory forms en route to the salivary glands. The most likely candidate for the sexual stage is the short epimastigote soon after this stage invades the glands, as yellow epimastigotes were observed at the very early timepoint of 13 days.

Intermediate stages

We searched for intermediate stages at early timepoints during salivary gland invasion and colonization. Individual flies were monitored for production of trypanosomes in samples of salivary exudate (a mixture of saliva and regurgitated foregut contents) from day 8 to 28 after infection by inducing them to probe onto a warm microscope slide [32], and finally examined for trypanosome infection of the salivary glands by dissection 13–28 days after infection, as previously reported [31]. Of 58 flies which extruded trypanosomes in the salivary exudate on one or more occasions, 34 (59%) produced both red and green trypanosomes simultaneously, and

one of these flies also produced yellow trypanosomes in a sample obtained on day 14 after infection, comprising one unattached epimastigote with 2 kinetoplasts, presumably in cell division, and a multinucleate trypanosome (Fig 3B). Two multinucleate cells were also found in a mixed gland containing yellow hybrids dissected on day 15 after infection (not shown). However, these were the only cells with unusual morphology noticed and all other trypanosomes examined in salivary exudate or dissected glands corresponded to the expected morphological stages, namely migratory forms (proventricular trypomastigotes, asymmetric dividers, short and long unattached epimastigotes) and salivary gland epimastigotes and metacyclics.

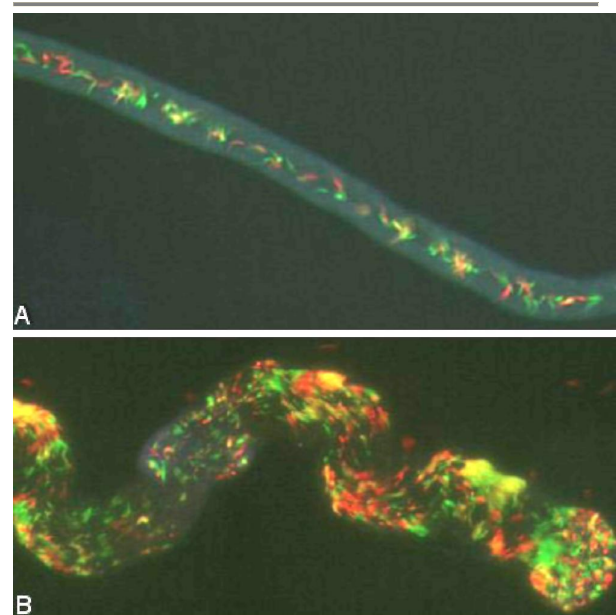


Figure 2. Yellow hybrids in salivary glands. Dissected salivary glands with mixed infection of red, green and yellow trypanosomes. A. Salivary duct from 27 day infection. B. Portion of salivary gland showing blind end from 20 day infection. Reproduced from [30] with permission. Trypanosomes are 20–30 μ m in length.

As reported previously [31], only 15 (26%) of the 58 flies that extruded trypanosomes in the salivary exudate were found to have established a salivary gland infection;

of these, six contained a mixed infection and five also had yellow trypanosomes; the other fly was dissected at a nearly stage of salivary gland colonization. In the mixed glands red and green trypanosomes were occasionally seen in close proximity (e.g. Fig 4), but otherwise appeared to be typical epimastigotes. Thus, other than occasional multinucleate trypanosomes, we observed no candidate intermediate stages in genetic exchange. The comparative rarity of putative intermediate stages in flies at early stages of salivary gland colonization contrasts with the ready observation of hybrid trypanosomes in older flies and suggests that trypanosome mating may be a transient and rapid event. Detailed investigation of epimastigotes attached inside the salivary glands at this early stage of establishment was not attempted due to their small number, inaccessibility to reagents and obscuration by the much larger fly epithelial cells.

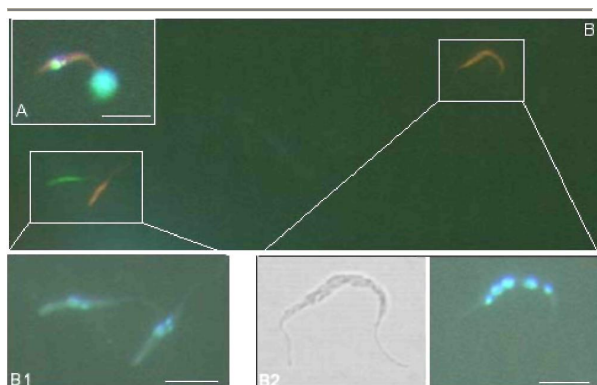


Figure 3. Yellow fluorescent trypanosomes recovered from saliva or glands. A. Yellow epimastigote from fly salivary gland dissected at 13 days stained with Hoechst 33342. B. Trypanosomes in a salivary exudate sample obtained from an infected fly at day 14; main picture shows the unfixed trypanosomes by fluorescence microscopy and insets show Hoechst 33342 and brightfield images. B1 are unattached epimastigotes and the yellow epimastigote has two kinetoplasts; B2 appears to be a multinucleate cell. Scale bar = 10 μ m.

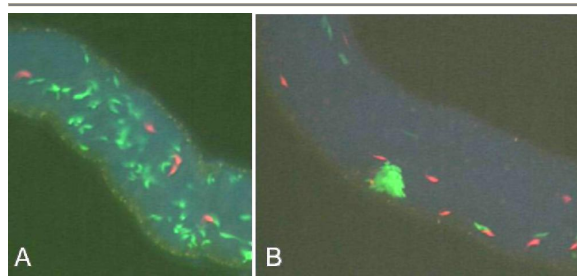


Figure 4. Early establishment in salivary glands. Close proximity of red and green trypanosomes in salivary glands at early establishment. Flies dissected at A. 20 days and B. 15 days after infection. Trypanosomes are 20–30 μ m in length.

Analysis of progeny clones

Trypanosome populations from four flies with mixed salivary gland infections (three flies dissected at 4 weeks, one at 8 weeks) were analysed in detail. Each population should represent the progeny of one independent genetic cross at minimum. Red, green, yellow and/or non-fluorescent clones were recovered from each of the 4 trypanosome populations, giving a total of 63 clones. All clones were checked by PCR for presence of the *mRFP* and/or *GFP* genes, and for correct integration of these genes by PCR analysis of flanking regions (not shown). All yellow clones contained both *mRFP* and *GFP* genes as expected, while the presence of neither gene could be demonstrated in the non-fluorescent clones. The reporter genes were confirmed to be in the ribosomal locus by PCR using a flanking primer paired with a primer in the reporter gene (Fig 1; results not shown).

Molecular karyotypes of parental and progeny clones were compared by pulsed field gel electrophoresis (PFG). All progeny clones showed a non-parental karyotype, whatever their colour (e.g. Figs 5A and 6A), as a result of reassortment and recombination of parental chromosomes. It was evident that some progeny clones from the same fly had identical karyotypes (e.g. Fly 18 clones 1 and 5, and Fly 22 clones 1, 13 and 14, Fig 5A; Fly 1 clones 20, 26 and 27, Fly 22 clones 2 and 6, and clones 15 and 16, Fig 6A); these

observations were borne out by hybridization with a probe for 18S rDNA, which is carried on several different chromosomes (arrays on chromosomes II, III and VII[33], e.g. Fig 5B and 6B). *GFP* and *mRFP* genes were localized to different chromosomes carrying rDNA arrays by hybridization, with *mRFP* on chromosome III and *GFP* on chromosome VII (Figs 5 and 6). Individual chromosomes were identified by localization of genes for β -tubulin(chromosome I), *PFR1* (chromosome III), *CROT1* (chromosomes IV and VIII), *KRET1* (chromosome VII), 5SrRNA (chromosome VIII). The presence of the *GFP* or *mRFP* gene served to distinguish the two homologues of the pair, and it was obvious that *GFP* had switched from its original position on the smaller chromosome VII homologue (VIIa) in parent 1738 to the larger homologue(VIIb) in some hybrid clones (e.g. Fly 22 clones 1, 13 and 14, Fig 5C, 5G); of 5 green or yellow hybrid genotypes examined, 1 had *GFP* on VIIa and 4 had *GFP* on VIIb. There was also some evidence of size variation in the chromosome III homologue carrying the *mRFP* gene (e.g. Fig 6E), but the small differences in size between chromosome III homologues, coupled with run perturbations of individual gels, made this difficult to establish unequivocally. Extra chromosomal bands are clearly visible in the hybridization results for Fly 18 clone 3 (Fig 5 panels D, E and F); this clone was found to have a 4N DNA content (see below).

Microsatellite analysis confirmed that all progeny clones were hybrid, since each had inherited one allele from each parent at the II-PLC, III-2 or XI-53 loci (Fig 7); these microsatellite markers are on chromosomes II, III and XI respectively. In all but one of the hybrid progeny clones,

the inheritance of these markers was consistent with Mendelian reassortment of unlinked genes in diploid F1 progeny, ie. each clone had one allele from each parent at each locus. The exception was a red clone, Fly 3 clone 8, for which the microsatellite

profile was identical to that of parent J10 for 2 loci (genotype XIX, Table 2). The karyotype of this clone was similar but not identical to that of J10 (Fig 6A) and chromosomal differences were evident from hybridization with rDNA probes (Figs 6B, 6C). This clone also had kinetoplast DNA (kDNA) from the 1738 parent (see below) and therefore without doubt is a genetic hybrid. The simplest explanation for the anomalous microsatellite genotype of this clone is that it resulted from more than a single round of mating.

The 63 progeny clones resolved into 29 different genotypes by combining the karyotype and microsatellite results (Table 2). Analysis of the allele frequencies for the 29 genotypes revealed no deviation from Hardy-Weinberg expectations (data not shown). As the III-2 microsatellite locus is on the same chromosome as the *mRFP* gene, one III-2 allele should co-segregate with the reporter gene. Allele *a* occurs in 3 genotypes with *mRFP* and 11 without, while allele *b* occurs in 9 genotypes with *mRFP* and 6 without (Table 2). This suggests that there has been frequent recombination between the III-2 and rRNA loci on chromosome III. On the map of chromosome III [34] these 2 loci are separated by approximately 600 kb.

Kinetoplast DNA inheritance

Inheritance of kDNA mini- and maxicircles was examined by PCR analysis and restriction digestion (Fig 8). The parental trypanosomes differed in a polymorphic *HinfI* restriction site in the maxicircle gene for cytochrome oxidase (*COXI*), allowing the maxicircle type of individual progeny clones to be determined. While most clones had only a single parental maxicircle type, a few had both parental maxicircles present (Fig 8A). We have not previously detected mixed maxicircle networks in hybrid clones, although this was reported by others [25]; this probably results from the greater sensitivity of PCR as a detection method compared to direct analysis of purified kDNA as done previously [24, 26].

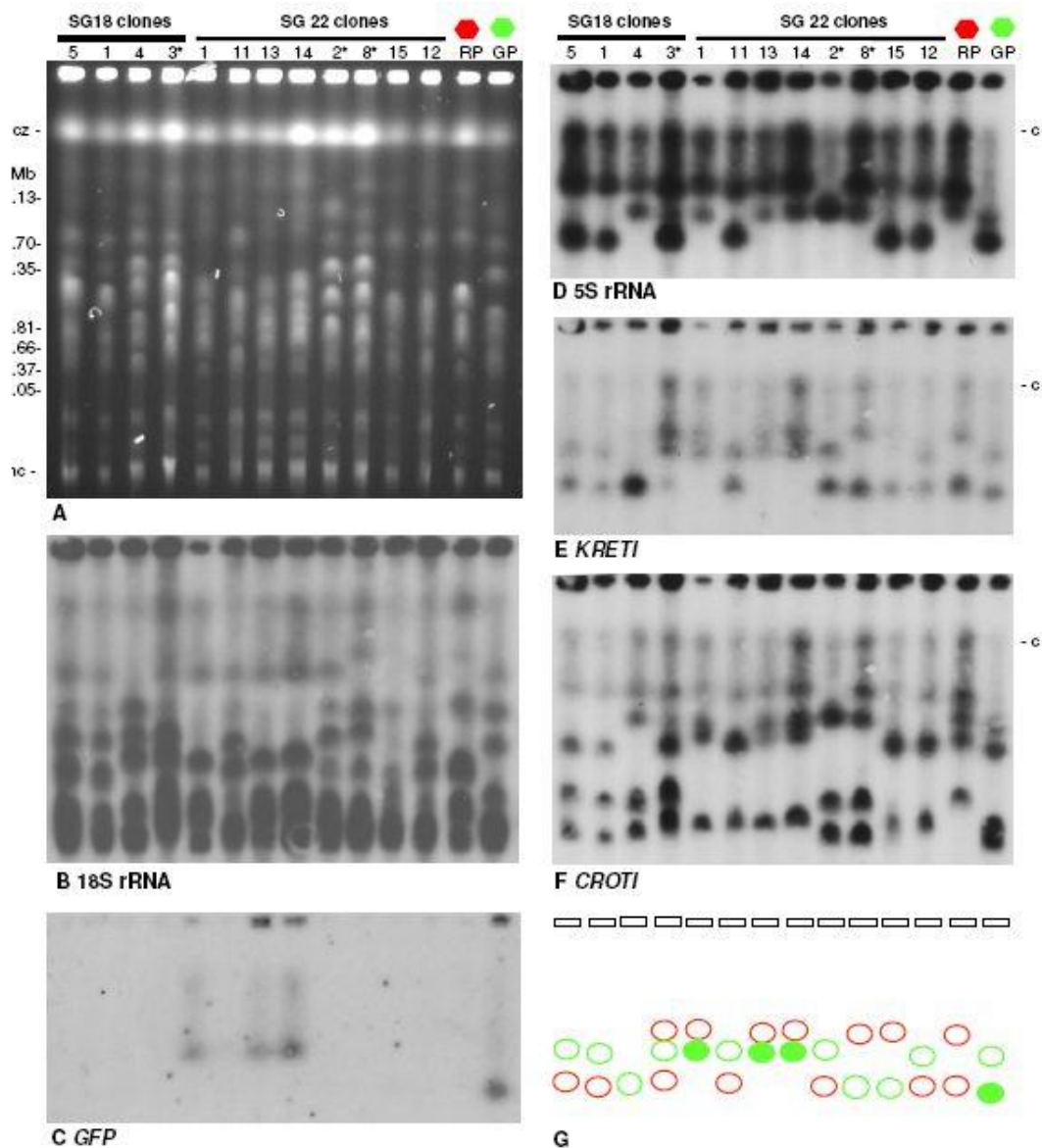


Figure 5. Comparison of green parental and progeny clones by PFG electrophoresis. A. Ethidium bromide stained gel comparing the molecular karyotypes of parental and progeny clones, including red parent J10 (RP) and green parent 1738 (GP). The other lanes show the karyotypes of various hybrid progeny clones from 2 different flies; asterisks denote clones with a 4NDNA content; all other hybrid clones and the parents had 2N DNA contents. Size marker: chromosomal DNAs from *Hansenula wingei*. Cz = compression zone, a region of the gel where several large chromosomal bands are trapped. Mc = mini-chromosomes of approx. 100 kb in size. B – F. Autoradiographs showing results following hybridization with the probes indicated. All blots were washed to 0.1 M SSC at 65°C. G. Diagram indicating the *KRET1* chromosomal band (filled) that also hybridized with the *GFP* probe in parent 1738 and hybrid progeny, Fly 22 clones 1, 13 and 14. The origins of each chromosomal band have been arbitrarily assigned to either the red parent J10 (red circles) or the green parent 1738 (green circles) according to size; each hybrid clone can be deemed to have inherited one chromosome from each parent, except hybrid clone Fly 18–3, which has an extra chromosomal band that may have originated from either parent. This clone also has extra chromosomal bands in hybridizations for chromosomes IV (*CROTI*) and VIII (5S rRNA; *CROTI*).

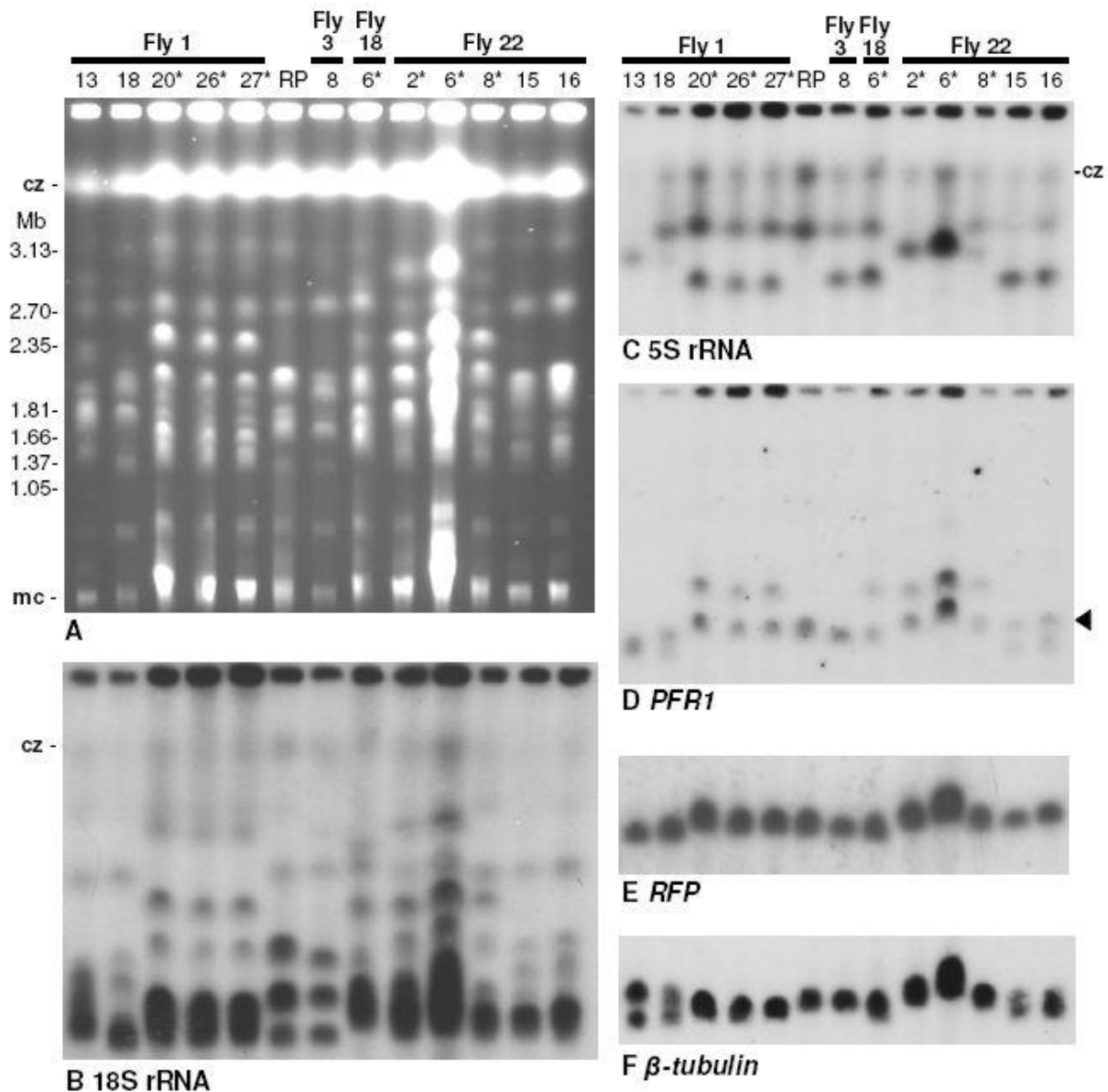


Figure 6. Comparison of red parental and progeny clones by PFG electrophoresis. A. Ethidium bromide stained gel comparing the molecular karyotypes of red fluorescent clones, including red parent J10 (RP). The other lanes show the karyotypes of various hybrid progeny clones from 4 different flies. Size marker: chromosomal DNAs from *Hansenula wingei*. Cz = compression zone, a region of the gel where several large chromosomal bands are trapped. Mc = minichromosomes of approx. 100 kb in size. B – F. Autoradiographs showing results following hybridization with the probes indicated. All blots were washed to 0.1 \times SSC at 65°C. The arrow (panel D) indicates the *PFR1* chromosomal band that also hybridizes with the *RFP* probe (panel E). The asterisked clones had 4N DNA contents; all other clones were diploid like the parents except for Fly 1 clone 18, with DNA content 3N. These hybridization results give no hint that any of these clones, whatever its DNA content, has extra chromosome bands.

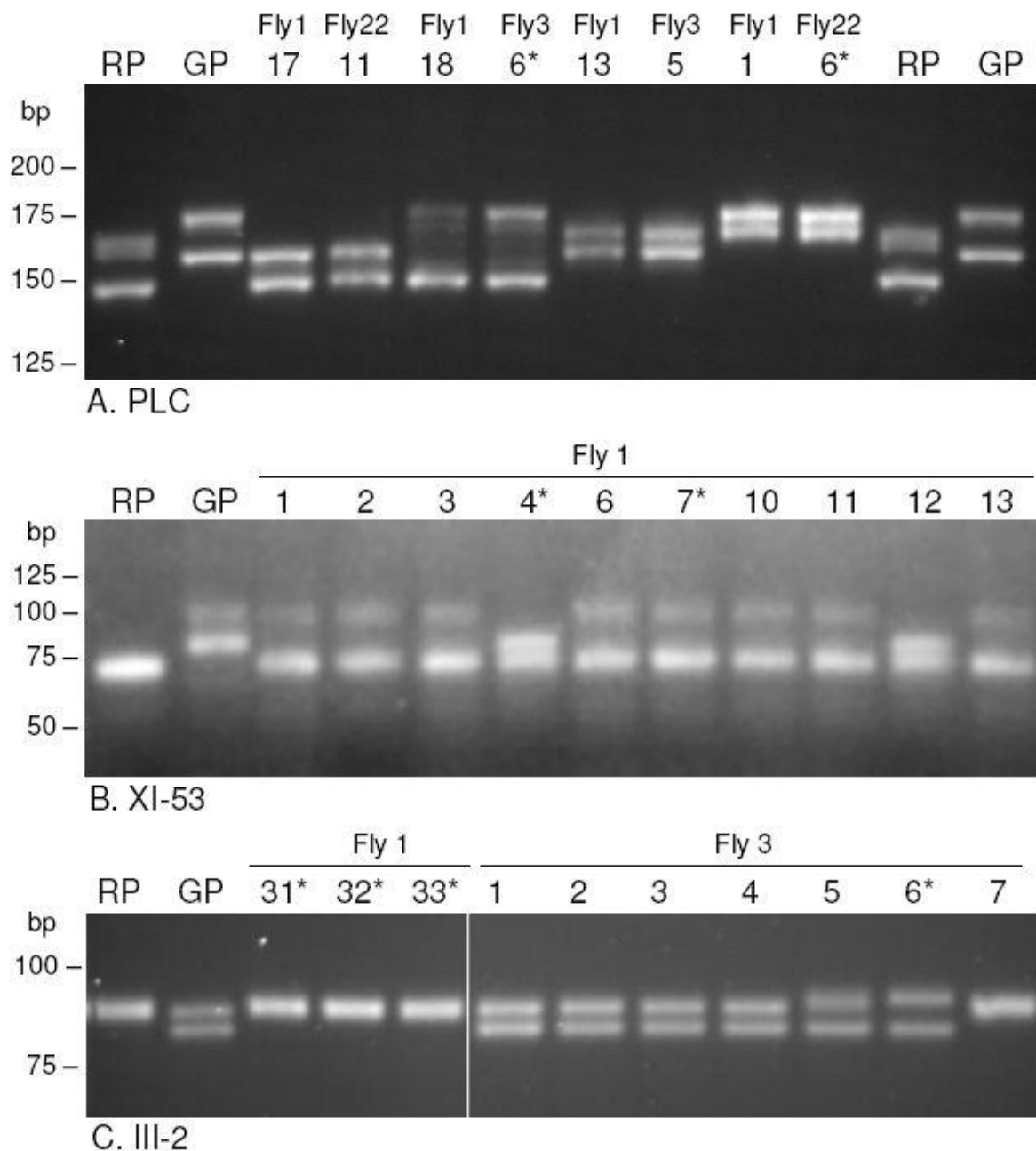


Figure 7. PCR amplification of microsatellite alleles. Results of PCR amplification of 3 microsatellite loci. RP = red parent J10; GP = green parent 1738; other lanes show alleles amplified from individual hybrid progeny clones from 3 flies. Both parental trypanosomes are heterozygotic at the II-PLC and III-2 loci, but only GP is heterozygotic at the XI-53 locus; for II-PLC the larger allele of RP appears as 2 closely spaced bands, but is inherited as a single band in progeny clones. The parental origin of each allele can be unequivocally identified by size for II-PLC, but for XI-53 RP is homozygous and for III-2, alleles a, b and d are closely similar in size and required long gel runs and application of samples in adjacent lanes to be distinguished. Each progeny clone has 2 strongly amplified bands and appears to have inherited one allele from each parent. Asterisked clones had 4N DNA contents; all other clones and parents were diploid, except for Fly 1 clone 18, which had 3N DNA content.

Individual minicircles from the parental trypanosomes were cloned and sequenced in order to design primers for PCR detection of specific minicircles. Minicircles from both parents could be seen in all progeny clones (Fig 8B), showing that minicircle networks are hybrid whatever the maxicircle type. The presence of kDNA elements attributable to both parents in progeny clones is evidence that mitochondria and therefore whole cells fuse during mating. Bi- and uniparental maxicircle inheritance fits with the hypothesis that the kDNA networks fuse during mating, generating an initial network containing both maxicircle types, which subsequently resolves to a single maxicircle type by progressive vegetative divisions [24, 25]; the minicircle network remains mixed due to the large number and variety of minicircles present. This idea is supported by the observation that some clones with an identical genotype by karyotype and microsatellite analyses (above) had maxicircles of different parental type, e.g. Fly 18 clones 1, 5 and 7. A recent result showing that maxicircles are held in a bridge structure between separating kDNA networks during division [35], points to the possibility that a more sophisticated mechanism may operate during resolution of hybrid networks.

There did not appear to be any bias for inheritance of one parental maxicircle type: 11 hybrid genotypes inherited J10, 18 inherited 1738, and 5 inherited both parental types (total > 29, as some hybrid genotypes differed in maxicircle inheritance, as mentioned above). It is note-worthy that the hybrid clone that was closely similar to the J10 parent, Fly 3 clone 8, had maxicircles of the other parental type and a hybrid minicircle network.

DNA contents

DNA contents of progeny and parental clones were measured by flow cytometry of fixed and permeabilized procyclic cells stained with propidium iodide. The 488 nm laser of the flow cytometer excites propidium

iodide but not mRFP, and we confirmed that unstained parental red or green fluorescent trypanosomes gave identical back-ground levels of fluorescence to wildtype. On an initial flow cytometer run, the DNA contents of both parents and a representative clone from each of the 29 hybrid genotypes was measured; on subsequent runs, measurements were repeated on regrown samples of the parents and selected hybrid clones, either the clone used on the first run or another clone of the same genotype, to check that results were reproducible.

Most progeny clones had approximately the same DNA content as the parents, consistent with diploidy (Fig 9). However, many polyploid clones were also identified, where the G1 peak was consistent with 3N or 4N (Fig 10). Although 3N hybrids have been reported in several of our previous crosses, e.g. [22], 4N progeny have not been observed except possibly in the first experimental cross reported, where it was thought that fusion of diploids produced an unstable tetraploid [13]. Of the 29 genotypes of hybrid progeny, 19 had DNA contents consistent with 2N (4 green, 4 red, 1 yellow, 10 no fluorescence), 1 with 3N (red) and 9 with 4N (4 red, 2 yellow, 3 no fluorescence); polyploid genotypes (consistent with 3N or 4N) were found among hybrid progeny from all 4 flies examined and thus had been generated independently in a minimum of 4 crosses (Table 2).

The 4N G1 peak was consistently found in repeated runs of the same clone or clones of the same genotype, but histograms sometimes also had a smaller peak equivalent to a 2N G1 peak (e.g. SG1 clone 15, Fig 9), interpreted as a mixture of diploid and tetraploid cells. This may indicate reduction of the tetraploid cell to diploid as reported earlier for *T. brucei* [13] and described for the yeast *Candida albicans* [36]. This requires further investigation of long-term cultures of polyploid clones.

Table 2. Genotypes and phenotypes of hybrid clones and parents

| Genotype (no. of clones) | Fluorescence | Microsatellite alleles | | | Maxicircle type (no. of clones) | DNA content |
|--------------------------|--------------|------------------------|-------|-------|---------------------------------|-------------|
| | | II-PLC | XI-53 | III-2 | | |
| J10 mRFP | Red | ab | aa | ab | J10 | 2N |
| 1738 GFP | Green | cd | bc | cd | 1738 | 2N |
| SG1 | | | | | | |
| I (3) | Green | bd | ac | ac | J10 | 2N |
| II (1) | Green | bd | ac | bc | 1738 | 2N |
| III (1) | Green | bc | ac | bc | 1738 | 2N |
| IV (5) | None | bd | ac | ac | 1738 | 2N |
| V (1) | None | bd | ac | ac | mix | 2N |
| VI (1) | None | bd | ab | ac | 1738 | 2N |
| VII (1) | None | bd | ac | ad | 1738 | 4N + 2N |
| VIII (1) | None | ac | ab | ad | J10 | 2N |
| IX (3) | None | bd | ac | ac | 1738 | 2N |
| X (2) | None | bd | ab | bc | 1738 | 2N |
| XI (1) | Red | bc | ac | ac | 1738 | 2N |
| XII (1) | Red | ad | ac | bd | 1738 | 3N |
| XIII (3) | Red | ad | ac | bc | 1738 | 4N + 2N |
| XIV (1) | Yellow | bd | ab | ad | J10 | 4N + 2N |
| XV (8) | Yellow | ad | ac | bd | 1738 | 4N + 2N |
| SG3 | | | | | | |
| XVI (4) | None | bc | ac | ac | 1738 (3), mix (1) | 2N |
| XVII (1) | None | ad | ab | bc | 1738 | 4N + 2N |
| XVIII (1) | Red | bc | ab | bc | J10 | 2N |
| XIX (1) | Red | ab | aa | ad | 1738 | 2N |
| XX (1) | Yellow | bc | ab | bd | mix | 2N |
| SG18 | | | | | | |
| XXI (3) | None | bc | ab | bc | J10 (2), 1738 (1) | 2N |
| XXII (1) | None | ac | ab | bd | 1738 | 4N |
| XXIII (1) | None | bc | ab | ac | J10 | 2N |
| XXIV (1) | Red | ac | ac | bc | 1738 | 4N |
| SG22 | | | | | | |
| XXV (9) | Green | bc | ac | ad | J10 (6), mix (3) | 2N |
| XXVI (2) | None | ac | ab | ad | J10 | 2N |
| XXVII (2) | Red | bd | ac | bd | J10 | 4N + 2N |
| XXVIII (1) | Red | ac | ab | bc | J10 | 4N + 2N |
| XXIX (2) | Red | bc | ac | bc | mix | 2N |

Considering the results overall, the majority of hybrid genotypes fit with a conventional meiotic division of adiplod cell, with Mendelian patterns of inheritance demonstrated by microsatellite alleles and homologous chromosomes. However, an uncomfortably large number of hybrid genotypes (10 of 29) do not fit this model, because they have DNA contents consistent with polyploidy. Both sorts of hybrid (diploid and

polyploid) were found in all 4 independent crosses examined. Just like the diploid clones, the polyploid hybrid clones uniformly showed 2 amplified bands for the II-PLC and III-2 microsatellite loci, for which assignment of parental alleles was unequivocal (J10 is homozygotic for the XI-53 locus). Simple fusion of the 2 parental genomes, as recorded for *T. cruzi*[3], would have resulted in hybrid progeny with 4 alleles at each microsatellite

locus and evidence of extra chromosomes in PFG analysis. However, extra chromosomal bands were demonstrated in only one 4N genotype, Fly 18clone 3, which had a minimum of 3 copies of chromosomes IV, VII and VIII (Fig 5 panels D, E and F). Of course, the presence of extra chromosomal bands will sometimes be hidden by co-migration of chromosomal bands on the PFG gels, but this has occurred rather too often

here to be a satisfactory explanation. A total of 5 different chromosomes (I, III, IV, VII, VIII) were screened in the PFG analysis with sufficient size variation between parental homologues of chromosomes III, IV, VII and VIII to facilitate detection of aneuploidy. Taking the microsatellite and PFG results together, the inescapable conclusion is that individual parental homologues have been duplicated in these clones.

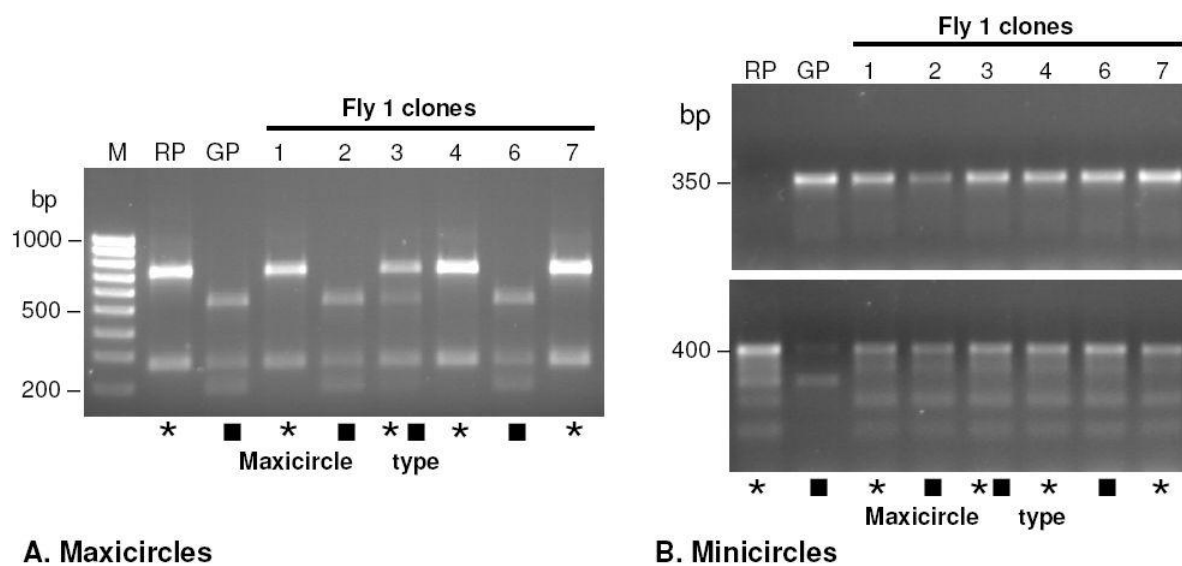


Figure 8. Analysis of kinetoplast DNA inheritance. A. Maxicircle fragments resulting from *HinfI* digestion of PCR amplified *COXI* gene. * J10 (RP) maxicircle type; ■ 1738 (GP) maxicircle type. Inheritance is uniparental except for Fly 1 clone 3, which has maxicircles of both parental types; this result was confirmed in a second reaction. B. PCR products resulting from amplification of genomic DNA using two different sets of minicircle-specific primers. All hybrid progeny clones have the 1738 (GP) minicircle (top) and the J10 (RP) minicircle (bottom) notwithstanding the type of maxicircles inherited from the parents (indicated by the * and ■ symbols as above).

Can the polyploid genotypes have been generated through errors of a conventional sexual cycle or are they the result of a separate parasexual process? We have previously argued that 3N hybrids result from fusion of diploid and haploid nuclei and thus support, rather than conflict with, meiosis as part of the process of genetic exchange in *T. brucei* [10]. One of the 4N genotypes analysed here (Fly18 clone 3) also fits into this scheme as it has the extra chromosomal bands expected from fusion of randomly selected diploid or

haploid cells/nuclei, but the majority of 4N genotypes look more like the products of genome endoreplication. In other organisms, polyploidy quite commonly arises in hybrid taxa and such taxa produce unreduced gametes at high frequency, which is thought to be the result of problems in pairing homologous chromosomes that have diverged in sequence in the separate taxa before hybridization [37]. This suggests another explanation for the large proportion of *T. brucei* polyploids observed here, if *T. brucei* homologues from

different strains are sufficiently divergent to cause failure of meiotic pairing. This might happen on the first round of mating if parental genomes fuse before meiosis, or on subsequent rounds when F1 hybrids attempt to undergo meiosis. Although there is no data on the extent of chromosome divergence between the trypanosome strains used here, divergence in the subtelomeric arrays of VSG genes [33], both in size and sequence, would surely be expected, even on homologues from a single strain. This would also account for the variability in the frequency of polyploids observed in different laboratory crosses, as a variety of trypanosome strains have been used. Thus the formation of all the diploid and polyploid hybrids described here can be explained by a model involving meiosis, and this remains the simplest hypothesis.

Since the genotypes of all the diploid clones from these crosses (except Fly 3 clone 8) conform to expectations for the F1 generation, we have assumed that only a single round of mating has taken place, but there is no a priori reason to believe this; moreover, the genotype of Fly 3 clone 8 is more readily explained as the product of an F1 rather than parental cross. The high frequency of polyploid hybrids might also arise from mating among F1 hybrids, if this leads to problems in meiotic pairing of homologous chromosomes as discussed above. On the other hand, if only the short epimastigotes newly arrived in the salivary glands can mate, then this would favour an initial burst of mating followed by sporadic events if further migratory trypanosomes reach the salivary glands.

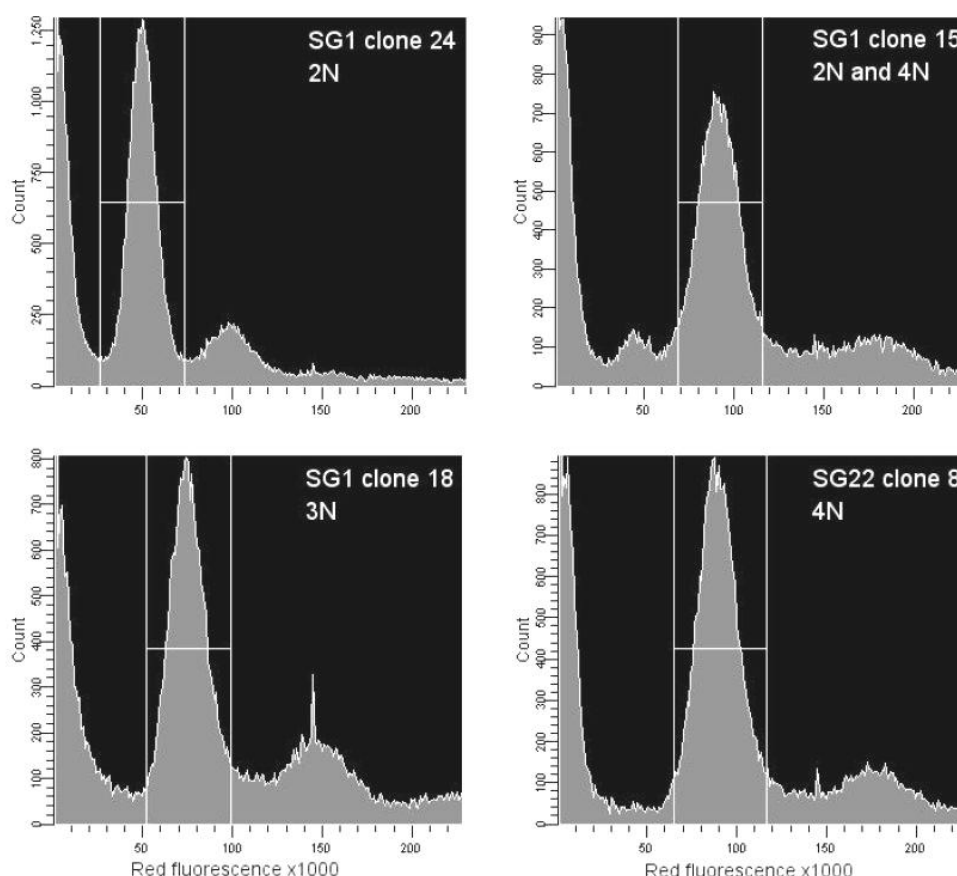


Figure 9. Flow cytometry histograms. Flow cytometry histograms for 4 hybrid trypanosome clones as indicated. Red fluorescence in arbitrary units (x axis) against cell count (y axis). The G1 peak for each histogram is gated; an additional smaller G1 peak is present in SG1 clone 15.

Both 2N and 4N clones were successfully transmitted through tsetse, demonstrating that polyploidy does not compromise completion of the full developmental cycle. The morphology of 4N cells was comparable with that of 2N cells, although the nucleus appeared to be slightly larger in the 4N cells (not shown). However, it is doubtful whether such polyploid trypanosomes would persist if produced in nature, unless polyploidy conferred sufficient advantages to outweigh the cost of replicating twice as much DNA, for example by doubling the available repertoire of *VSG* genes. This could also be achieved by expansion of individual chromosomal *VSG*

arrays though, and the high proportion of pseudogenes in these arrays already points to combinatorial diversity as a key mechanism to increase the antigen repertoire [33]. While the DNA contents of *T. cruzi* strains vary markedly in nature [38, 39], much lower levels of variation have been reported in *T. b. brucei* [40]. On the other hand, comparative analysis of trypanosome genome sequences indicates that a major duplication event involving chromosomes IV and VIII occurred at some stage in the evolutionary history of *T. brucei* that evidently conferred sufficient benefit to persist [41].

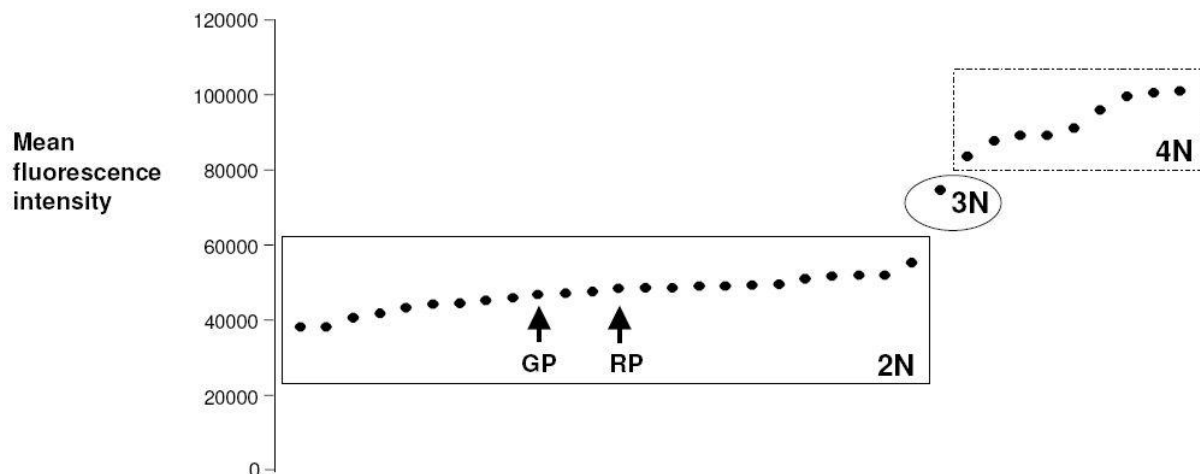


Figure 10. Analysis of DNA contents by flow cytometry. Values for mean red fluorescence intensity of the G1 peak of each histogram, representing the DNA content of the trypanosome clone, are plotted in ascending order. The values for the diploid J10 (RP) and 1738 (GP) parents are indicated. Values for hybrid clones within the solid box are also consistent with diploidy (mean 47042, s.d. 4364). Values for hybrid clones within the hatched box correspond with 4N (mean 94083, s.d. 13091); the remaining clone (circled, Fly 1 clone 18) had a value of 74611, corresponding to a 3N DNA content.

Conclusion

In this experimental *Trypanosoma brucei* cross, genetic exchange readily occurred when the two different strains were together in the same salivary gland, but did not occur in other infected organs despite very high densities of parental trypanosomes. Trypanosomes therefore need to reach the salivary ducts or glands before they can

mate. This, together with the fact that the first hybrid cells observed were epimastigotes, indicates that the life cycle stage that mates is the unattached epimastigote. Relatively few short epimastigotes constitute the founder population that invades and colonizes each salivary gland [31] and the early occurrence (13 days) of hybrid trypanosomes suggests that these trypanosomes mate and

then rapidly populate the salivary glands, leading to the pre-dominance of hybrid over parental genotypes observed here. It seems that the main barrier to genetic exchange is not reluctance of trypanosomes to mate, but rather the low probability of both parental trypanosomes reaching the same salivary gland.

Results from the genetic analysis of hybrid clones were not entirely consistent with a simple model based on meiosis of a diploid cell and Mendelian inheritance of markers, because a high proportion of hybrids were polyploid. The genotypes of these clones were not consistent with simple parental fusion as described in *T. cruzi* [3], and most probably result from a high frequency of formation of unreduced gametes during meiosis. All results from the *T. brucei* crosses described here can therefore be fitted into a model involving meiosis.

Methods

Transfection

The reporter construct pHD67E containing the *GFP* gene was described previously [29]. The *GFP* gene was replaced by a *HindIII-BamHI* cassette containing the *mRFP* gene from a construct kindly supplied by Roger Tsien [42]. Plasmid DNA was purified from bacterial cultures using commercial midiprep kits and trypanosomes were transfected as described previously [29]. Mid-log phase procyclic trypanosomes of isolate J10 (*T. b. brucei* MCRO/ZM/73/J10 CLONE 1; [43]) or 1738 (*T. b. brucei* MOVS/KE/70/1738; [44]), grown in Cunningham's medium [45] supplemented with 10% v/v heat-inactivated foetal calf serum, 5 µg/ml hemin and 10 µg/ml gentamycin (complete medium = CM) at 27°C, were transfected with reporter constructs by electroporation using two pulses of 1.5 kV, 25 µF. Note that the full names of J10 and 1738 are given correctly here. Transfectants were selected 24 hours post-electroporation by the addition of Hygromycin B, 50 µg/ml. The population was checked for fluorescence by microscopy of living cells. Clones were obtained by two rounds of limiting dilution

of procyclics in CM in 96 well plates incubated at 27°C in 5% CO₂.

Trypanosomes were transmitted through male tsetse flies (*Glossina morsitans morsitans*) as described previously [17, 31], supplementing the infective bloodmeal with 60mM N-acetylglucosamine to increase infection rates [46]. Infected flies were maintained on membrane-fed sterile horse blood supplemented with 2.5% w/v bovine serum albumen (Sigma A4503) [47] and 1 mM dATP [48]. Flies were dissected up to 9 weeks following the infective feed. Metacyclics from infected salivary glands were inoculated into mice; bloodstream forms were subsequently transformed back to procyclics to facilitate cloning and preparation of samples by incubation in CM at 27°C.

Microscopy

Living trypanosomes were viewed as wet mounts in CM, blood or phosphate buffered saline (PBS). Whole tsetse midguts or salivary glands were dissected into a drop of PBS and viewed as wet mounts. Dried saliva samples were obtained by allowing flies to probe onto a warm microscope slide before feeding [31, 32]. Cells were fixed in 2% w/v paraformaldehyde at room temperature for 20 minutes and stained with a 1/100 dilution of Hoechst 33342 for 30 minutes to visualize the nucleus and kinetoplast, if required. A DMRB microscope (Leica) equipped with a Colour Coolview camera (Photonic Science) was used for fluorescence and standard microscopy, with ImageProPlus software (Media Cybernetics).

Genotype analysis

Genomic DNA samples from *T. brucei* were prepared from approximately 5 × 10⁷ washed procyclics using a spin column DNA purification kit (Qiagen). Samples for pulsed field gel electrophoresis (PFG) were prepared by lysing and deproteinising trypanosomes in situ in agarose blocks [49]. PCR was performed by standard methods using the following primers: EGFP-R 5' TCAGCTTGCCGTAGGTGG, RFP-R 5' CTCGATCTCGAACTCGTG, SSUsp-G 5'

CAT-GCAACAGTACACTTCAC. Microsatellite alleles were amplified by PCR as described [18] using primers PLC-G5' CAACGACGTTGGAAGAGTGTGAAC, PLC-H3

5'CCACTGACCTTTCATTTGATCGCTTTC, III-2A 5' GGT-GGAATGGAAGATCAGTT, III-2B 5' GTTGAATTGTTGTT-GCTGT, XI-53A 5' CGTGTGTCTTGTATATCTTCT, XI-53B5' TGAATAAACAAAACATGAAACGAC.

These 3 loci were selected on the basis of an initial screen of the parental trypanosomes for allelic variation. Products were resolved by electrophoresis in 1% TAE buffer through 3–5% Metaphor agarose (Cambrex) gels. Chromosomes were separated using a Biorad CHEF-DR III with a 2 phase program (Block 1: switch time 1800 s, voltage 2 V/cm, angle 106°, 15 hours; Block 2: switch time 300–900 s, voltage 3 V/cm, angle 106°, 50 hours) using 0.5% TBE buffer and 0.9% agarose gels. Gels were stained overnight by submersion in electrophoresis buffer containing ethidium bromide (2 µg/ml). Blotting and hybridization were by standard methods [50, 51] using the following PCR-amplified, P³²-labelled DNA fragments as hybridization probes: *GFP* and *mRFP* genes from the plasmid constructs used for transfection; β -*tubulin* from cDNA plasmid clone [52]; 18S rRNA, *PFR1*, *CROT1*, *KRET1*, 5S rRNA genes from *T. brucei* genomic DNA. Kinoplast DNA minicircles were amplified using primers designed to individual sequenced minicircles from parental trypanosome J10: J10F-1 5'GTGCAATGCCTCGTAACTAT, J10F-2 5' CCACCCA-GAAAGCCTTAT; J10G-1 5' AGCAGTGATTGTTACTTGGG, J10G-2 5' TTCCTCCTCTACGCACA. Kinoplast DNA maxicircles were amplified using primers designed to a 982 bp polymorphic region of the cytochrome oxidase subunit I (COI) gene [44] (Accession no. of *T. b. brucei* strain 427 maxicircle = M94286): Max1 5' CCCTA-CAACAGCACCAAGT, Max2 5' TTCACATGGGTTGAT-TATGG.

Measurement of DNA content

Procyclic trypanosomes were harvested from log phase culture, washed 3 times in ice cold Hank's balanced salt solution (HBSS) without Ca²⁺ or Mg²⁺ with 0.5 mM EDTA, fixed with 95% ethanol (final concentration 70% ethanol) and stored at 4°C. Fixed cells were pelleted by centrifugation and resuspended in PBS containing 50 µg ml⁻¹ propidium iodide and 40 µg ml⁻¹ RNase. After incubation at ambient temperature for 30 minutes, cells were recovered by centrifugation and resuspended in PBS. Red fluorescence was measured by flow cytometry using a Beckman FACS DIVA; results were confirmed on a further sample grown from cryopreserved trypanosomes.

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N-HEXANOYL CHITOSAN STABILIZED MAGNETIC NANOPARTICLES: IMPLICATION FOR CELLULAR LABELING AND MAGNETIC RESONANCE IMAGING

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This project involved the synthesis of *N*-hexanoyl chitosan or simply modified chitosan (MC) stabilized iron oxide nanoparticles (MC-IOPs) and the biological evaluation of MC-IOPs. IOPs containing MC were prepared using conventional methods, and the extent of cell uptake was evaluated using mouse macrophages cell line (RAW cells). MC-IOPs were found to rapidly associate with the RAW cells, and saturation was typically reached within the 24 h of incubation at 37°C. Nearly 8.53 ± 0.31 pg iron/cell were bound or internalized at saturation. From these results, we conclude that MC-IOPs effectively deliver into RAW cells *in vitro* and we also hope MC-IOPs can be used for MRI enhancing agents in biomedical fields.

Background

Magnetic particles ranging from the nanometer to micrometer scale are being used in an increasing number of medical applications. The important properties of magnetic particles for medical applications are nontoxicity, biocompatibility, injectability, and high level accumulation in the target tissue or organ; the most important property among those mentioned above is nontoxicity. Magnetic nanoparticles offer attractive and versatile applications in the field of biotechnology, such as DNA and RNA separation, cell separation, drug delivery system (DDS), magnetic resonance imaging (MRI), and hyperthermia [1-6]. For these applications, magnetic iron oxides such as Fe₃O₄ or gamma-Fe₂O₃ are employed as a magnetic phase because they are stable and harmless to the living bodies. To make them bind to a biological

entity, their surfaces are usually modified with an appropriate compound such as polyethyleneglycol (PEG) or streptavidin. Poly-

mers like poly-L-lysine (PLL), poly ethylene imide (PEI) and dextran, and recently chitosan [6] has been used as a stabilizer (coating agent) for iron oxide nanoparticles so as to improve the nanoparticle's biocompatibility and injectability. However, high-level accumulation in the target tissue or organ and cytotoxicity; the most important property of the nanoparticles is remains to be intact.

More or less to improve limitations stated above, several derivatives of chitosan have been proposed based on reactions with the free amino groups. Our research group already investigated the hydrophobic modification of natural chitosan by using three different acyl chlorides (hexanoyl, octanoyl and myristoyl chloride) so as to improve its aqueous solubility and subsequently used them for stabilization of metallic nanoparticles [7-9]. In this paper, we have selected the hexanoyl chloride modified chitosan stabilized iron oxide nanoparticles (Nac-6-IOPs or simply MC-IOPs) as a material of interest and demonstrated its biomed-

cal application like cellular labeling, and MRI using mouse macrophages cell line (RAW cells).

Results and discussion

Synthesis and characterization of MC-IOPs

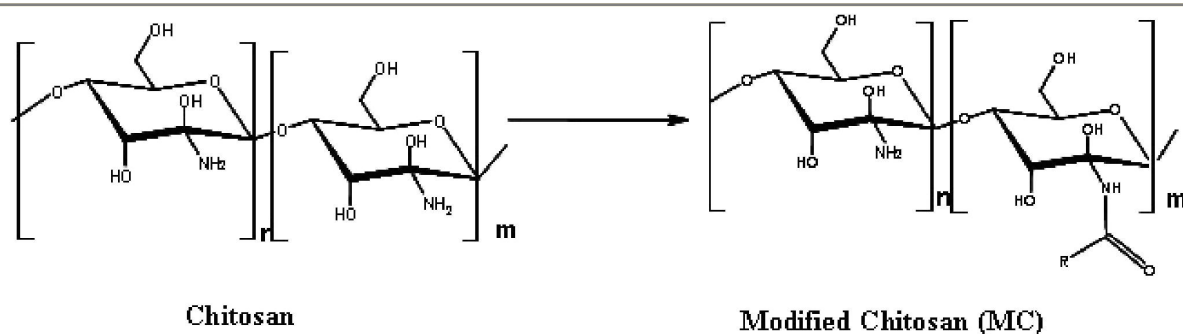
The chemical structure of the native and modified chitosan is shown in Figure 1.

The procedure for synthesis of IOPs, modification process of chitosan, and its detailed characterization was taken from a previously published report [9]. Briefly, Figure 2 shows the fourier transforms infrared (FT-IR) spectra of pure chitosan (a) MC (curve b), IOPs (curve c), and MC-IOPs (curve d). IOPs exhibit strong bands in the low frequency region below 800 cm^{-1} due to the oxide skeleton. The characteristic bands of modified chitosan, amide I, II, and III were shifted 1623, 1510 and 1464 cm^{-1} due to interaction with IOPs. Shifting of such amide bands from higher to lower energies indicates the attachment of IOPs with MC through nitrogen atom [8, 9]. In other regions, the spectra of IOPs have weak bands. The spectrum is consistent with magnetic (Fe_3O_4) and the signals associated to the magnetite appear as broad features at 408.9, 571.5 and 584.5 cm^{-1} [4]. Figure 3 shows the X-ray diffraction (XRD) pattern of IOPs matched with the magnetite (Fe_3O_4) phase as compared to standard XRD patterns reported elsewhere [5]. The sharp peaks which appeared approximately $2\theta = 30^\circ, 35^\circ, 43^\circ, 53^\circ, 57^\circ$ and 62° were due to Fe_3O_4 [1]. Figure 4 shows the transmission electron microscopy (TEM) images of IOPs, and MC-IOPs particles. The morphology of IOPs (Figure 4a) was seen as clustered type, which is the same morphology as reported elsewhere [10]. After modifications, morphology of IOPs was significantly dispersed with an average diameter 10 nm (average over 100 particles) (Figure 4b) in aqueous medium at pH 7.4. The average size of IOPs with and without MC was 10 and 40 nm, respectively (Figure 4a, b and table 1). Figure 4c shows the selected area diffraction (SAD) pattern of MC-IOPs, which is

in exact/or in good agreement with the XRD results. The ring type SAD pattern consists of a cubic inverse spinel structure of magnetite and it indicates good crystallinity of the MC-IOPs.

Figure 4d, high resolution transmission electron microscopy (HRTEM) images further support the interplaner distances of $d = 2.94$ E which is very closed to the plane $d_{220} = 2.97$ E of the magnetite phase in orientation. Taken together, the results of XRD, TEM and HRTEM, show that the synthesized MC-IOPs is highly crystalline as can be found in the pure magnetite phase without phase transformation after conjugation with MC, showing a successful synthesis of magnetic MC-IOPs. The results of dynamic light scattering (DLS) measurements showed a uni-model size distribution of the nanoparticles. The average sizes of the IOPs and MC-IOPs were 60 and 100 nm, respectively, (Table 1, DLS measurement). In contrast to TEM measurement, DLS gave a significantly larger size in the case of both particles. The reason behind this phenomenon is obvious. The particle size measured by DLS technique is larger than that observed by TEM due to the different nature working function of the two instruments.

Moreover, DLS methods differ from TEM in that it measured the hydrodynamic particle size in the dispersion medium. TEM images show the core particle size, without the contribution from the MC; since the MC layer normally collapses onto the IOPs surface when the dispersion medium is evaporated prior to imaging. It is also obvious that the thickness of the stabilizing layer (here MC), when collapsed on the surface of the IOPs, is negligible. Therefore, the difference in diameter measurements obtained by DLS and those obtained by TEM is the size of the stabilizing layer. However, this method is only valid for small particles (diameter < 200 nm), since the size of the stabilizing layer on larger particles is small relative to the experimental error inherent in DLS measurements ($\pm 4\%$).



R = $-(\text{CH}_2)_4\text{CH}_3$ caproyl chitosan

Figure 1. Chemical structure of chitosan and modified chitosan

Table 1. Physiochemical properties of IOPs and MC-IOPs

| Sample | Size (nm) | | Charge (mV) | Size distribution |
|-------------|-----------|-----|-------------|-------------------|
| | TEM | DLS | | |
| IOPs (Pure) | 40 | 60 | -10 | Large |
| MC-IOPs | 10 | 100 | +20.21 | Narrow |

Table 1 shows the ζ -potential of IOPs and MC-IOPs. The polymer of MC being a polycation gives different +ve ζ -potential depending on the pH of the media. The ζ -potential of the MC was decreased at pH 7.4 after incorporation of IOPs. However, the ζ -potential of MC-IOPs (+20.21 mV) at physiological conditions (pH 7.4) is still acceptable for magnetofection of mammalian cells.

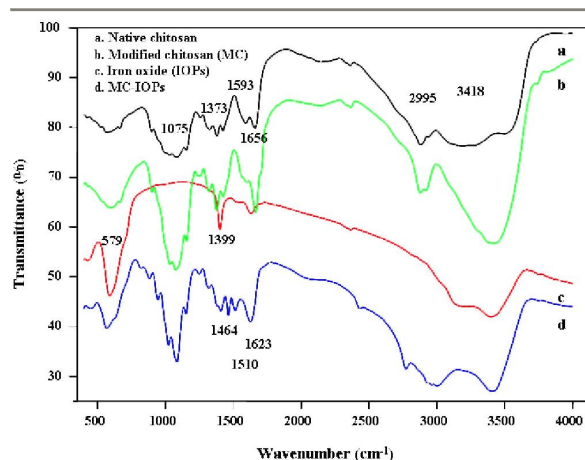


Figure 2. FTIR spectra of (a) pure chitosan, (b) MC, (c) pure IOPs and (d) MC-IOPs.

Figure 5 shows the magnetic properties of magnetic nanoparticles (MC-IOPs). The synthesized MC-IOPs indicate a superparamagnetic behaviour, as evidenced by zero coercivity and remanance on the magnetization loop. A saturation magnetization of ~50 emu/g was determined for the MC-IOPs which is relatively lower than that of the bulk value of Fe_3O_4 (70 emu/g). The higher value of magnetization of MC-IOPs makes them very susceptible to magnetic fields, and easily separates from the solid and liquid phases.

Biocompatibility and cellular labeling of MC-IOPs

MTT assays were performed to evaluate the cytotoxicity corresponding to the biocompatibility of the materials on RAW cell. Figure 6 shows the representative data of cytotoxicities from three different experiments with increasing concentration of the MC-IOPs. The MC-IOPs at low concentration (<10 mg/ml) showed relatively no significant toxicity on the cells. The cell viabilities in the presence of MC-IOPs suspension ranged between 97–120% of the control in all experiments. At a maximum MC-IOPs concentration (>15 mg/ml), the mean cell viabilities of

the cell lines showed about 88–97% viability compared with that of the control. Interestingly, even at high concentrations of MC-IOPs up to 100 mg/ml, which is 9~12-fold higher than the concentration required for high efficiency of intravenous injection, MC-IOPs showed no obvious negative effect on cell viability. This means that the cell viability, after exposure to different concentrations of the MC-IOPs assessed in RAW cells, apparently unaltered in the entire test dosage range from 0.05 to 0.2 mg after the 4 h of exposure, as depicted in Figure 6. The probable reason for high compatibility could be the highly biocompatible natural polymer of chitosan.

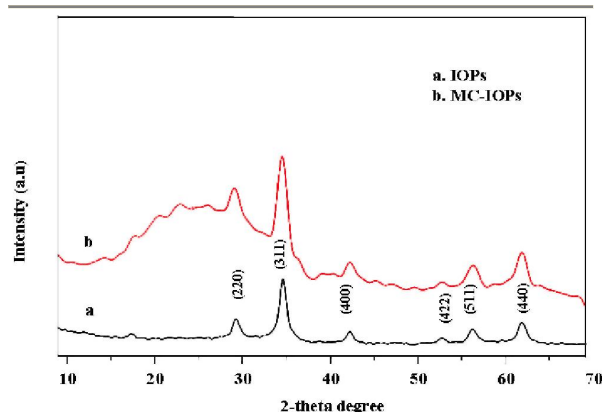


Figure 3. XRD pattern of (a) IOPs, (b) MC-IOPs showing only magnetite reflection.

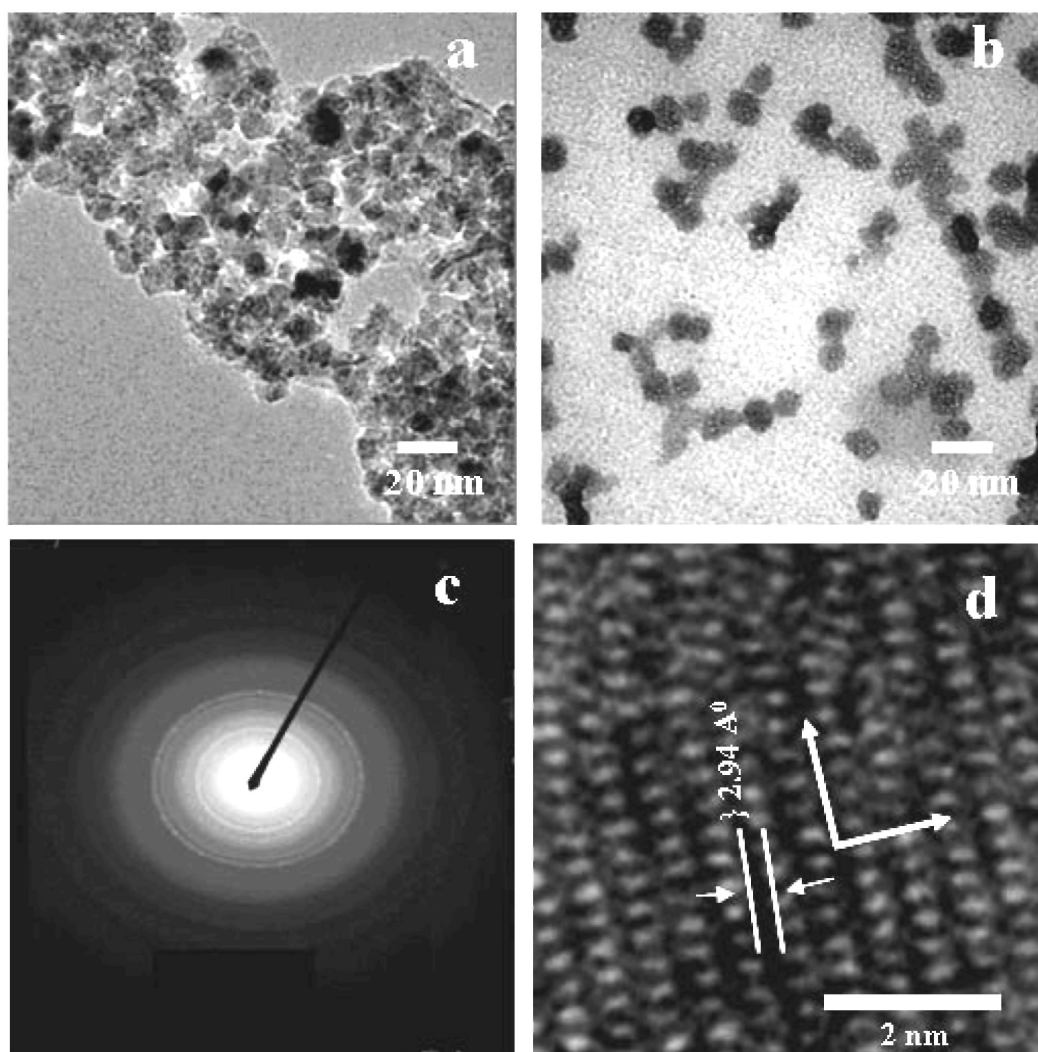


Figure 4. TEM images of (a) pure IOPs, (b) MC-IOPs, (c) SAD pattern of MC-IOPs and (d) HRTEM image of MC-IOPs showing a 10 nm size magnetite nanoparticle with highly polycrystalline nature.

Semiquantitative microscopic analysis showed that the MC-IOPs were incorporated by RAW cells in a concentration and time dependent manner, Figure 7. At the low concentration of the MC-IOPs, only few cells showed intracytoplasmatic Prussian blue positive particles, Figure 7A(b). At a high concentration of the MC-IOPs, virtually all cells contained several Prussian blue-stained, Figure 7A(c), and no cellular loss or damage was observed. Whatever the MC-IOPs concentration, the RAW cells stated internalize the MC-IOPs after 30 min, Figure 7A(b) and 7(c), inset and reached a plateau after 3~4 h, Figure 7A(c). Furthermore, colorimetric quantitative method was used to determine and confirm dose-dependent nanoparticle internalization by observing the RAW cell microscopically (Figure 7B). At our optimal experimental setting based on the morphological observation (10~20 MC-IOPs for 2 h incubation time, Figure 7A(b) and 7(c), the macrophages RAW cells contained an average of 8.53 ± 0.31 pg (iron/cell), Figure 7B. Similarly, Figure 7C demonstrates the side scattering (SSC) distribution of cell shifted with increasing concentration of the MC-IOPs, which means an increase in granularity with increasing MC-IOPs concentration.

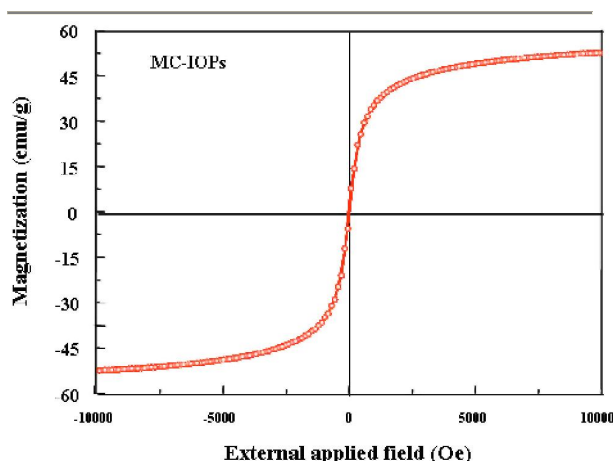


Figure 5. Magnetisation curve of magnetite obtained by VSM at room temperature.

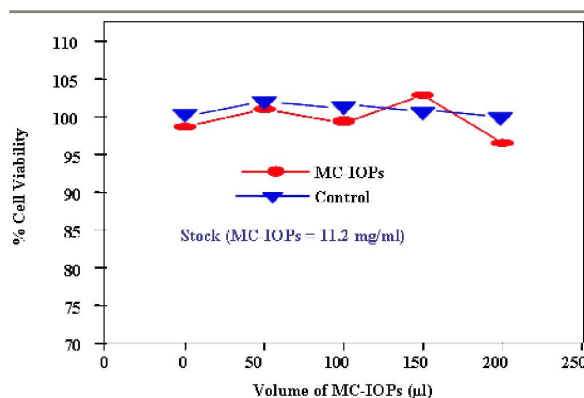


Figure 6. Toxicity evaluation of MC-IOPs on RAW cells by MTT assay. Different volume (50 ~200 μ l) of the nanoparticles was used from the stock MC-IOPs (11.2 mg/ml).

This finding is important because we suspect the phagocytosed MC-IOPs became endosomes and thereby increased the granularity found in flow cytometry. These results further support the semiquantitative microscopic analysis (Prussian blue-stained).

Magnetic resonance (MR) study of MC-IOPs

Figure 8a and 8c, illustrated the signal contrast enhancement performance of the MC-IOPs incubated with RAW cells evaluated in clinical MR imager. This typical array image of the RAW cells, with a concentration gradient of the MC-IOPs in an incubated media solution, is taken by T2 MR sequence. Under T2 weighted pulse sequence evaluation, the signal of each cell pellet was measured as shown in Figure 8a and 8b. The image was further converted into signal intensity by the provided image analysis tool for quantitative measurements. Figure 8c demonstrated the signal difference between cells with and without MC-IOPs incubation. These results clearly indicate that the signal intensity gradually dropped in the iron concentration above 0.1 mg/ml which was in good agreement to the results reported elsewhere [11].

Conclusion

MC-IOPs synthesized by simple precipitation method showed highly crystalline,

superparamagnetic behavior. It also displayed high stability, nontoxicity, enhancement of MR images and the potential endocytose the macrophage cell line. From above preliminary results, we conclude that MC-IOPs could be a better candidate for MR contrast medium.

Experimental methods

Materials

Iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) pure granulated, 99%, iron (II) chlo-

ride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) 99+%, and ammonium hydroxide (14.8 M) were purchased from Fisher Scientific (Pittsburgh, PA). Deionized water purged with nitrogen gas was used in all the steps involved in the synthesis and formulation of iron oxide nanoparticles. Chitosan-100 [viscosity average molecular weight, $M_v = 1.3 \times 10^6$, degree of deacetylation (fraction of free amino group) 78%] was purchased from Wako Pure Chemical Industries, Ltd., Japan.

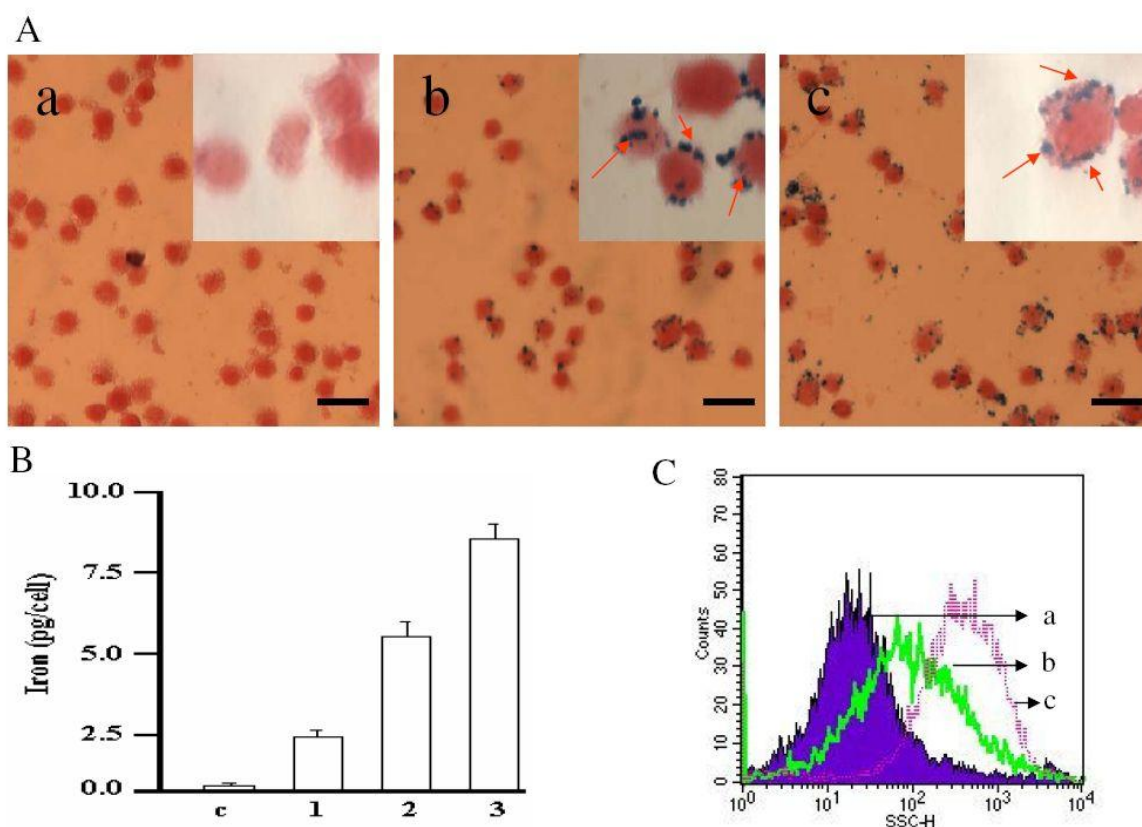


Figure 7. (A) Internalization of MC-IOPs in RAW cells. Cells were cultured with different volume of MC-IOPs (11.2 mg/ml). Cytospin slides were stained with Prussian blue (iron staining) for RAW cells; (a) control cells, (b) and (c) cells incubated with 10 and 20 μl MC-IOPs for 5 h. Inset figure indicate the higher magnification and black arrow denote cell label with particles. Scale bars represent 10 μm . (B) Iron content in RAW cells. Cells were cultured with different concentration of MC-IOPs for 2 h, and incubated for 24 h with fresh medium. C, 1, 2 and 3 represent 0, 5, 10 and 20 μl of MC-IOPs from the stock 11.2 mg/ml, respectively). (C) Flow cytometry of RAW cells incubated with different concentrations of MC-IOPs as described in (A). The SSC signal (SSC-H) is increased with increased concentrations of MC-IOPs. Quantitative iron assessment was performed with a colorimetric method. Values are means of \pm S.D. of iron content per single RAW cells (pg).

Synthesis of iron oxide nanoparticles (IOPs)

Aqueous solutions of 0.1 M Fe(III) (30 mL) and 0.1 M Fe(II) (15 mL) were mixed, and 3 mL of 5 M ammonia solution was added dropwise over 1 min while stirring on a magnetic stir plate. The stirring continued for 20 min under a nitrogen-gas atmosphere. The particles obtained were washed 3 times using ultracentrifugation (25000 \times g for 20 min at 4°C) with nitrogen purged water. The iron oxide nanoparticle yield, determined by weighing of the lyophilized sample of the preparation, was 304 mg.

Modification of chitosan (MC)

The modification process of chitosan was taken from a previously described report [7-9]. Briefly, a mixture of chitosan-100 (0.83 g) and 1.0% aqueous acetic acid (100

ml) was stirred for 24 h to ensure total solubility. The pH was adjusted to 7.0 by slow addition of 0.1 M of NaOH with strong agitation, yielding gel slurry. After addition of 0.02 M of fatty acyl chloride (hexanoyl chloride, FW = 134.61, d = 0.978 g/ml), the resultant solution was diluted 11 times with de-ionized water. After 6 h of continuous stirring, the solution was neutralized (pH 6.8–7.0) by 0.1 M of NaOH and precipitated with acetone. The precipitate, collected by filtration, was washed at 50–60°C with an excess of methanol and decanted. The washing was repeated 4 times to eliminate free fatty acids. Finally, the products were dried under vacuum for 3 days at room temperature. The chemical structure of native and modified chitosan is shown in Figure 1.

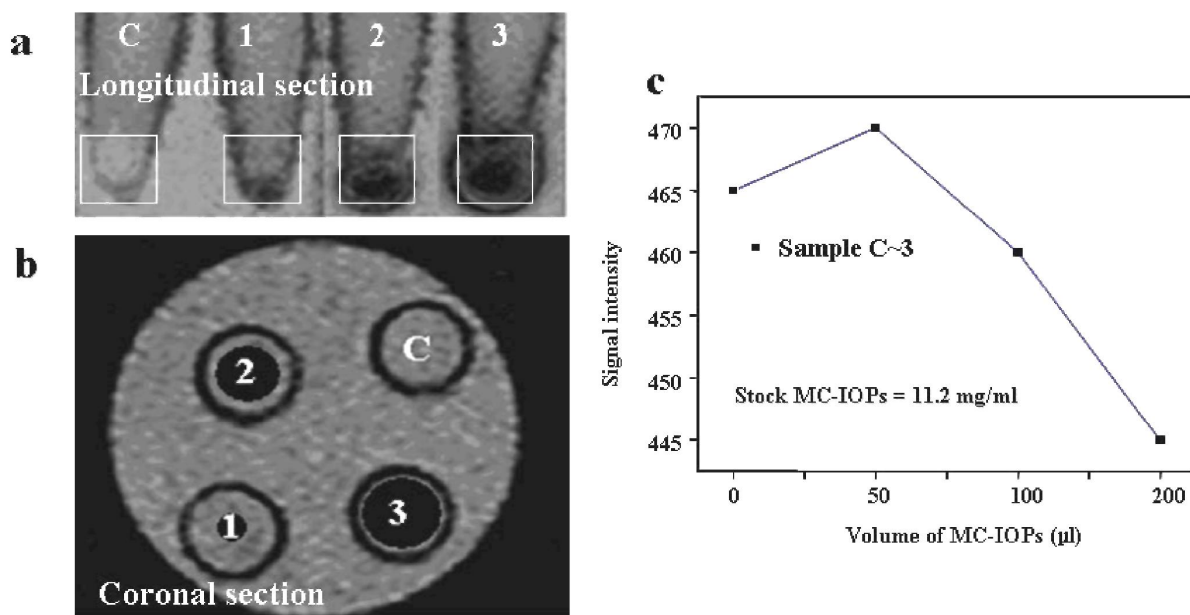


Figure 8. T2 weighted MR images of a representative RAW cells incubated with different volume of MC-IOPs (11.2 mg/ml) for 5 h, (a) longitudinal section, (b) coronal section and (c) signal intensity of sample c to 3 (lane c~3; control, 50, 100 and 200 μ l MC-IOPs, respectively).

Stabilization of iron oxide nanoparticles (MC-IOPs)

Polymer (5.0 ml of 0.33% of *N*-hexanoyl chitosan solutions or MC) was added to the dispersion of the nanoparticles

(100 mg) (the dispersion was cooled to room temperature but not lyophilized) and stirred overnight in a closed container to minimize exposure to atmospheric oxygen to prevent oxidation of the IOPs. These particles were

washed with nitrogen purged water to remove soluble salts and excess polymer. Particles were separated by ultracentrifugation at 30000 rpm (Optima LE-80K, Beckman, Palo Alto, CA) using a fixed angle rotor (50.2 Ti) for 30 min at 10°C. The supernatant was discarded, and the sediment was redispersed in 15 mL of triply distilled water by sonication in a water-bath sonicator (FS- 30, Fisher Scientific) for 10 min. The suspension was centrifuged as above, and the sediment was washed three times with triply distilled water. Nanoparticles were resuspended in triply distilled water by sonication as above for 20 min and centrifuged at 1000 rpm for 20 min at 7–11°C to remove any large aggregates. The supernatant containing MC-IOPs was collected and re-diluted in phosphate buffer at pH 7.4.

Structural characterization of MC-IOPs

FT-IR spectra were recorded at RT using a Perkin-Elmer spectrometer, model 2000. The FT-IR spectrometer was linked to a personal computer loaded with the IRDM (IR Data Manager) program to process the recorded spectra. The specimens were pressed into small discs using a spectroscopically pure KBr matrix. FT-IR measurements were checked by the X-ray diffraction of isolated precipitates. XRD (APD-10, Philips, Netherlands) was performed to identify the structure of the MC-IOPs using Cu K alpha radiation ($\lambda = 1.54056 \text{ \AA}$) between 20° and 90° (2 θ) at 27°C.

Particle size, morphology and ζ -potential analysis of MC-IOPs

The size and morphology of IOPs and MC-IOPs were observed by TEM (JEM-1230, JEOL, Japan) and HRTEM (QUANTA 200F, FEI, USA). The sample for TEM analysis was obtained by placing a drop of IOPs and MC-IOPs suspension diluted by distilled water onto a copper grid without any staining, and drying it in air at room temperature. The average hydrodynamic diameter and the ζ -potential of IOPs and MC-IOPs were determined by DLS and ELS (Zetasizer ZEN 3600, Malvern, UK), respectively. All

DLS measurements were done with an angle detection of 90° at 25°C after diluting the dispersion to an appropriate volume with water. The results were the mean values of two experiments using the same sample.

Magnetic property of MC-IOPs

Magnetic measurement was done using a SQUID magnetometer (MPMSXL-7, Quantum Design, USA). Magnetization curves were recorded for a suspension and solid sample of MC-IOPs at 27°C with an applied magnetic field up to 10,000 Oe.

Evaluation of cytotoxicity

Evaluation of the cytotoxicity was performed by the MTT assay in RAW cells (mouse macrophages cell lines). Briefly, RAW cells suspensions containing 1×10^4 cell/well in DMEM containing 10% FBS were distributed in a 96-well plates, and incubated in a humidified atmosphere containing 5% CO₂ at 37°C for 24 h [12,13]. The cytotoxicity of MC-IOPs was evaluated in comparison with control cells. Cells were incubated for additional 24 h after the addition of defined concentration of MC-IOPs. The mixture was replaced with fresh medium containing 10% FBS. Then, 20 μ l of MTT solution (5 mg/ml in 1 \times PBS) were added to each well. The plate was incubated for an additional 4 h at 37°C. Next, MTT-containing medium was aspirated off and 150 μ l of DMSO were added to dissolve the crystals formed by living cells. Absorbance was measured at 490 nm, using a microplate reader (ELX 800; BIO-TEK Instruments, Inc.). The cell viability (%) was calculated according to the following equation:

$$\text{Cell viability (\%)} = \left[\frac{\text{OD}_{490}(\text{sample})}{\text{OD}_{490}(\text{control})} \right] \times 100$$

Cellular uptake of MC-IOPs

To test cell up take study, RAW cells were prepared and incubated at a concentration of 1×10^6 cells/ml with 5, 10 and 20 μ l MC-IOPs (11.2 mg/ml stock) for 2 h, then incubated with fresh medium overnight. The cells were harvested and measured by flow cytometry using SSC signal.

Similarly harvested RAW cells were further used for Prussian blue staining using

K₄ [Fe(CN)₆] reagents. Iron determination was performed by colorimetric determination method.

Magnetic resonance (MR) study of MC-IOPs

For MR study, MC-IONPs were incubated with RAW cells at different concentration for 24 h. The cells were harvested and washed three times and centrifuged at the cell number 1×10^3 . The cell plates were scanned using 1.5T MR system. Under T2 weighted MR images of MC-IONPs were obtained with 1.5T MR system (Medius Co. Korea, Model Magnum1.5T) by using a spin echo technique. The differences between MR images of cells with and without MC-IONPs incubation were compared.

Abbreviations

MC-Hexanoyl chloride modified chitosan or simply modified chitosan; MRI: Magnetic resonance imaging; IOPs: Iron oxide nanoparticles.

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COMPARISON OF 2D, 3D HIGH DOSE AND 3D LOW DOSE GATED MYOCARDIAL ^{82}Rb PET IMAGING

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Background: We compared 2D, 3D high dose (HD) and 3D low dose (LD) gated myocardial Rb-82 PET imaging in 16 normal human studies. The main goal in the paper is to evaluate whether the images obtained by a 3D LD studies are still of comparable clinical quality to the images obtained with the 2D HD or 3D HD studies.

Methods: All 2D and 3D HD studies were performed with 2220 MBq of Rb-82. The 3D LD were performed with 740 MBq of Rb-82. A GE Advance PET system was used for acquisition. Polar maps were created and used to calculate noise among (NAS) and within (NWS) the segments in the noise analysis. In addition, the contrast between left ventricular (LV) wall and LV cavity was also analysed. For 13 subjects, ejection fraction (EF) on 2D and 3D studies was calculated using QGS program.

Results: For the H20 reconstruction filter, the mean contrast in mid-ventricular short-axis slice was 0.33 ± 0.06 for 2D studies. The same contrast for the 3D HD studies was 0.38 ± 0.07 and for 3D LD, it was 0.34 ± 0.08 . For the 6 volunteers where 3D HD was used, NAS was $3.64 \cdot 10^{-4}$ and NWS was $1.79 \cdot 10^{-2}$ for 2D studies, and NAS was $3.70 \cdot 10^{-4}$ and NWS was $1.85 \cdot 10^{-2}$ for 3D HD studies, respectively. For the other 10 volunteers where 3D LD was used, NAS was $3.85 \cdot 10^{-4}$ and NWS was $1.82 \cdot 10^{-2}$ for the 2D studies, and NAS was $5.58 \cdot 10^{-4}$ and NWS was $1.91 \cdot 10^{-2}$ for the 3D LD studies, respectively. For the sharper H13 filter, the data followed the same pattern, with slightly higher values of contrast and noise. EF values in 2D and 3D were close. The Pearson's correlation coefficient was 0.90. The average difference from 13 subjects was 8.3%.

Conclusion: 2D and 3D HD gating Rb-82 PET cardiac studies have similar contrast, ejection fractions and noise levels. 3D LD gating imaging, gave comparable results in terms of contrast, EF and noise to either 2D or 3D HD gating PET imaging. 3D LD PET gated imaging can make Rb-82 PET cardiac imaging more affordable with significantly less radiation exposure to the patients.

Background

Not long ago [1], we compared 2D with 3D modes in myocardial ^{82}Rb PET imaging at rest. Here, we would like to extend the same comparison to gating myocardial ^{82}Rb PET imaging at rest. The gating imaging provides additional useful information like ejection fraction (EF) and wall thickening. However, it is more demanding due to loss of counts (e.g. bad beats rejection) and dynamic memory limitations. Due to the short half-life of ^{82}Rb (75 s), ^{82}Rb PET cardiac images tend to be count-poor. Additional shifting of counts in different heart cycle phases makes gating ^{82}Rb PET myocardial imaging even more challenging than non-gating imaging. Also, ^{82}Rb biokinetics, i.e., high blood pool activity approximately 2 min after I.V. injection, combined with ^{82}Rb short half-life, requires a careful acquisition protocol in order to obtain images of adequate quality. While 2D and 3D ^{18}F – Fluorodeoxyglucose (FDG) imaging can be optimized based on PET system performance characteristics, mostly described by a noise

equivalent count (NEC) rate [2], the dynamic ^{82}Rb PET cardiac imaging is more complicated. Optimization of ^{82}Rb PET imaging requires taking into account ^{82}Rb biokinetics and ^{82}Rb short half-life, in addition to the PET system performance characteristics.

The main goal in the paper is to evaluate whether the images obtained by a low dose (LD) of 740 MBq (20 mCi) in the 3D myocardial ^{82}Rb perfusion gated PET studies are still of comparable clinical quality to the images obtained with the high dose (HD) of 2220 MBq (60 mCi) in 2D and 3D PET ^{82}Rb perfusion PET gated studies. The reduction in dose by a factor of three has significance in reducing costs associated with ^{82}Rb and the consequent potential of making ^{82}Rb perfusion PET myocardial imaging more affordable. Reducing the patient dose by a factor of three also significantly reduces exposure to the patients.

Methods

All 2D volunteer studies were performed by injecting I.V. 2220 MBq (60mCi) of ^{82}Rb . For six volunteers, 3D studies were

performed with a high dose (HD) of 2220 MBq of ^{82}Rb and for 10 volunteers in the 3D studies, a low dose (LD) of 740 MBq (20 mCi) of ^{82}Rb was used. One volunteer participated twice, i.e., was imaged at two separate occasions, in 2D and 3D HD, and 2D and 3D LD studies, respectively. In all studies, i.e., 2D and 3D LD and 3D HD studies, time per frame was 1 minute and total acquisition time after appropriate delay, was 6 minutes. In the 2D studies there was a delay of 2 minutes, in the 3D LD studies there was a delay of 3 minutes, and in the 3D HD studies there was a delay of 5 minutes. The heart cycle was divided in 8 phases in the 2D gated PET studies. In the 3D gated PET studies, 5 phases were used, due to dynamic memory limitations. These human protocols were approved by the Institutional Review Board of our Medical Center.

The GE ADVANCE (General Electric Medical Systems, Milwaukee, WI) system was used for all acquisitions in both the 2D and 3D modes. The 2D images were reconstructed using a filtered backprojection reconstruction method and Hanning filters with a 0.33 cycles/pixel (H13) and 0.21 cycles/pixel (H20), cutoff frequency, respectively.

The 3D studies were reconstructed using a Kinahan-Rogers [3] algorithm and also using H13 and H20 filters. The matrix size was 128×128 and the pixel size was 4.29 mm. Attenuation correction using an 8-min transmission scan was applied in all studies. In the 2D studies, Bergstrom [4] scatter correction was applied. For the 3D data, scatter correction was performed by fitting the tails of the sonogram to a 2D Gaussian [5]. Transaxial gated slices were transferred to GE Xeleris system for further gated analysis.

A mid-chamber short axis slice was used for analysis (Figs. 1, 2, 3, 4). In addition to end diastoli (ED) and end systoli (ES) images, gated images summed over the cardiac cycles were also used in comparison. The contrast value, which was calculated as a ratio $C = (A-B)/(A+B)$, where A and B are the average activities in the left ventricle (LV)

and LV cavity respectively, was calculated from mid-chamber short-axis summed slice (Figs 5 and 6). The contrast values were used in comparison between 2D vs high dose 3D, 2D vs low dose 3D and high dose 3D vs low dose 3D studies, respectively. We used a paired t-test in the comparison of the contrast values. In our noise analysis, image noise was defined as the coefficient of variation (COV, $100 \times \text{SD}/\text{mean} (\%)$). Summed short axis slices from apex to base were used to create circumferential profiles and polar maps on which we superimposed ROIs (Fig. 7), giving 33 segments. For each segment, the mean value and standard deviation was calculated.

Intrasegmental variance was investigated by calculating variance for each segment, as well as all segmental variances and the average coefficient of variance. F statistics were used to compare 2D and 3D HD studies, between 2D and 3D low dose studies and 3D HD and 3D low dose studies.

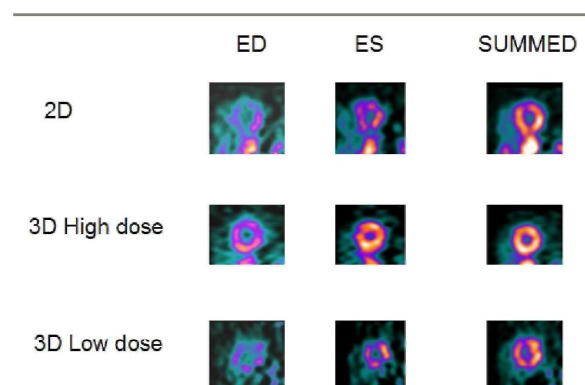


Figure 1. Short-axis slices. Mid-chamber short-axis slices in the 2D, 3D HD and 3D LD volunteer ^{82}Rb gated myocardial PET study. Reconstruction filter was H13, i.e. Hanning filter with 0.21 cycles/pixel cut-off frequency.

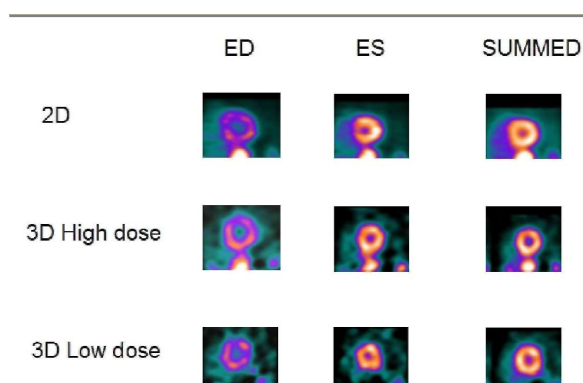


Figure 2. Short-axis slices. The same as on figure 1 but for H20, i.e. Hanning filter with 0.33 cycles/pixel cutoff frequency.

Intersegmental variance was investigated by applying a logarithmic transform on each mean segmental value and performing two-way ANOVA without replication. The effects of different patients, different segmental positions and interaction of different patients and segments (assumed none) on noise were tested. Again, F statistics were used to compare 2D and 3D HD studies, between 2D and 3D low dose studies and 3D HD and 3D low dose studies. EFs were calculated using QGS program. Initially we did not plan to compare EF in 2D and 3D studies due to difference in the number of phases used to cover the heart cycle. As mentioned before, because of the dynamic memory limitations, only 5 phases were used in 3D gated studies. In retrospective comparison between 2D and 3D EFs, 3 sets of data were found to be corrupted and only 13 subject were used in the comparison. Also, our noise and contrast analysis showed that the 3D LD and 3D HD images were very comparable. Therefore, we did not distinguish between HD and LD 3D studies in the comparison between 2D and 3D EFs. A second reason for not splitting between HD and LD 3D studies was the limited number of subjects and we want to keep the same number of studies in 2D and 3D.

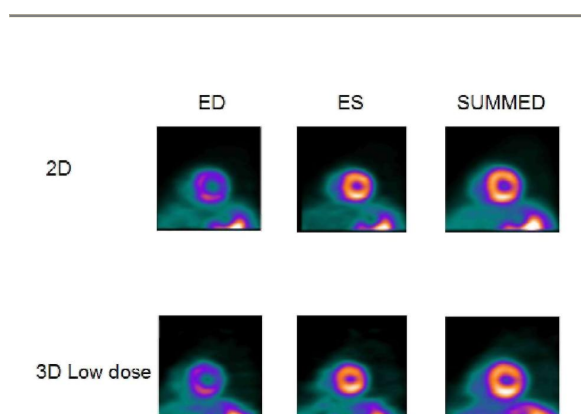


Figure 3. Light volunteer. Results of comparison for light (49 kg, 162 cm) volunteer, for 2D and 3D LD studies.

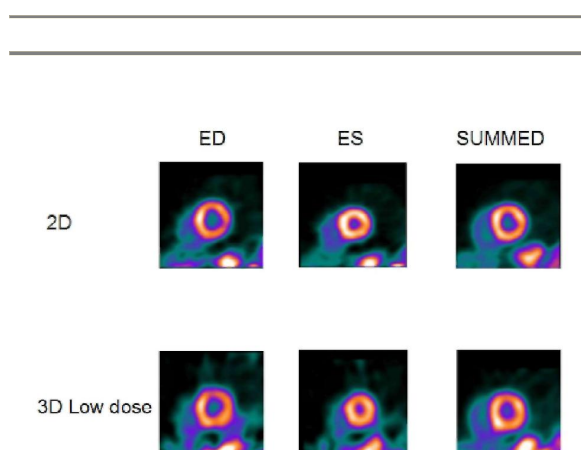


Figure 4. Normal-weight volunteer. Results of comparison for normal-weight (75 kg, 188 cm) volunteer, for 2D and 3D LD studies.

Results

Figures 1 and 2 show the results for a 54-y-old, 183 cm, 90-kg man, who volunteered twice. In the first study, 3D imaging was performed with the high dose of 2220 MBq (60 mCi) of ^{82}Rb . Three months later in a second study, 3D imaging was performed with the low dose of 740 MBq (20 mCi) of ^{82}Rb . In both studies, 2D imaging was performed with the high dose of 2220 MBq (60 mCi) of ^{82}Rb . Figures 1 and 2 show the 2D and 3D high (HD) and low (LD) dose cardiac short-axis slices at end-diastole, end-systole

and summed over all phases, respectively. The figure 1 shows the results for sharper filter H13 with 0.33 cycles/pixel cutoff frequency, and figure 2 the same for the smoother filter H20 with 0.21 cycles/pixel cutoff frequency. The images in fig. 1 are quite noisy and routinely we decided to use smoother filters. For smoother filter H20, Fig. 2, the 3D HD images have slightly better contrast than 3D LD gated images, but both low and high dose 3D images are comparable in quality, that is, in contrast, scatter from adjunct activity and noise to the 2D images. Summed images are even more similar than end-diastole and end-systole images. In our other volunteers studies, 3D HD studies provided slightly better images, i.e., with less amount of noise and slightly better contrast, as summarized in Table 1 and table 2. However, the main goal of our project was to determine whether 3D low dose studies can replace the more expensive high dose 2D or 3D studies, providing images of comparable quality. Therefore, the next two clinical examples are comparing only 2D with 3D low dose studies. Figure 3 compares the 2D and 3D gated end-diastole and end-systole images, and summed over all phases images, in ^{82}Rb PET imaging when the lower dose of ^{82}Rb (740 MBq) was used in the 3D study. The subject is a 49-kg, 162 cm, 20-y-old female.

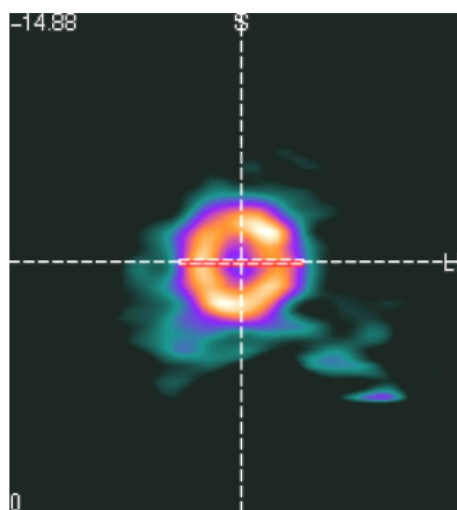


Figure 5. Mid-chamber short-axis slices. Mid-chamber short-axis slices profile ROI.

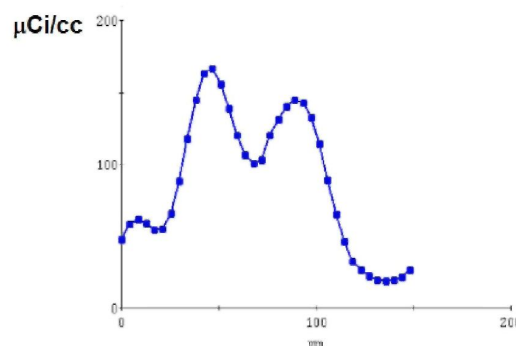


Figure 6. Profile curve. Corresponding profile curve from ROI shown in figure 5.

Table 1. Comparison of contrast values for 2D and 3D high-dose (HD) and 3D low-dose (LD) in ^{82}Rb PET myocardial imaging - smoother filter H20

| | 2D | HD-3D | 2D | LD-3D |
|-----------------|------|---------|------|--------|
| Contrast | 0.33 | 0.38* | 0.33 | 0.34 |
| NAS * 10^{-4} | 3.64 | 3.70 | 3.85 | 5.58 |
| NWS * 10^{-2} | 1.79 | 1.85*** | 1.82 | 1.91** |

*p = 0.04 vs 3D LD, **p = 0.025 vs 2D and 3D HD, ***p = 0.02 vs 2D

NAS = noise among segments

NWS = noise within segments

Here, all corresponding images, i.e., 2D and 3D LD gated and summed are very similar. Only the images for the smoother

H20 filter, which is routinely used, are shown. Figure 4 shows the results for a 75-kg, 188 cm, 32-year-old man. Again, in the

3D study the lower dose was used and only results for routine, H20 filter are given. The 2D and 3D gated and summed images are very alike.

Table 1 and table 2 gives the mean contrast values in mid-ventricular short-axis slice for 2D and 3D low and high dose studies for H20 and H13 reconstruction filters, respectively. The contrast values in 2D and 3D LD studies are very close. The contrast values in 2D and corresponding 3D HD studies are also close, with 3D HD studies having slightly higher values. The p value of 0.04 shows that there was no statistically significant correlation between noise in 3D low and high dose studies. The contrast values are higher for the H13 reconstruction filter than for the smoother H20 (table 1 and table 2). Noise among segments (NAS) and noise within segments (NWS) are also given for 2D and 3D low and high dose studies, for both filters. From table 1 and table 2 one can see that noise among and within the segments

is higher for H13, i.e., for sharper filter than for H20 in 2D and 3D low and high dose studies. Noise among and within the segments were very similar for the 2D and 3D high dose (HD) studies, although there were no statistically significant correlations between noise within segments in 2D and 3D HD studies ($p = 0.02$). In comparison between 2D and 3D low dose (LD) studies, noise among and within the segments was moderately higher for the 3D LD studies. However, again there were no statistically significant correlations between noise among and within segments in 3D LD studies and 2D and 3D HD studies ($p = 0.025$ for H20 filter and $p = 0.001$ for H13 filter). For the sharper filter H13, noise within segments was higher than for the smoother filter H20 and was practically the same in 2D and 3D low and high dose studies. The H13 filter provided relatively high noise images and is not used routinely in clinical practice.

Table 2. Comparison of contrast values for 2D and 3D high-dose (HD) and 3D low-dose (LD) in ^{82}Rb PET myocardial imaging – sharper filter H13

| | 2D | HD-3D | 2D | LD-3D |
|-----------------|------|-------|------|---------|
| Contrast | 0.48 | 0.50 | 0.33 | 0.34 |
| NAS * 10^{-4} | 4.43 | 5.17 | 8.18 | 9.18*** |
| NWS * 10^{-2} | 2.00 | 2.00 | 2.00 | 2.00 |

*** $p < 0.001$ vs HD

NAS = noise among segments

NWS = noise within segments

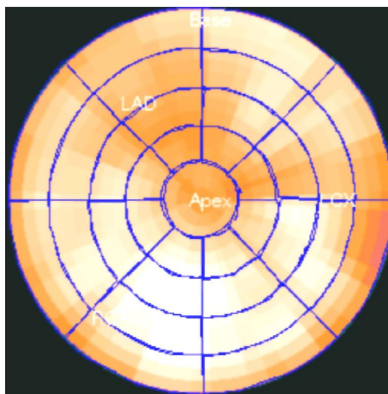


Figure 7. Polar map. Polar map and 33 segmental ROIs used in our comparison.

The comparison between ejection fractions in 2D and 3D gated PET studies are shown in figure 8 and 9. There is a nice linear correlation between ejection fractions. The Pearson's correlation coefficient was 0.90 and there was no significant deviation from linearity ($p > 0.10$). The 2D ejection fractions were generally slightly higher but average difference from 13 subjects was 8.3%.

Discussion

Gated myocardial images are usually count poor. Gated ^{82}Rb PET myocardial imaging is an even greater challenge, because ^{82}Rb has quite a short half-life, making ^{82}Rb

images count poor even without gating. Therefore, 3D gated ^{82}Rb PET myocardial imaging has great appeal due to significantly higher sensitivity than 2D imaging. The first goal of our study was to investigate whether gated 3D high dose (2220 MBq) imaging, i.e., the same dose as in gated 2D images, would achieve better performance due to higher sensitivity. The results of our study indicate that gated 3D high dose images did not provide better images, because of the longer delay in acquisition from the time of injection in 3D HD imaging, as discussed below. The longer delay in 3D HD imaging diminishes the advantages of the higher sensitivity in 3D imaging in comparison with 2D imaging. The second question was to investigate the possibility of replacing high dose gated 2D and gated 3D imaging with gated 3D low dose (740 MBq) imaging. The advantage of 3D LD imaging, due to 3D mode higher sensitivity, is the same count rate as in 2D HD mode, but with a lower injected dose. This could lead to significant cost savings in the purchase of an ^{82}Rb generator and thus could make myocardial ^{82}Rb PET imaging more affordable [1].

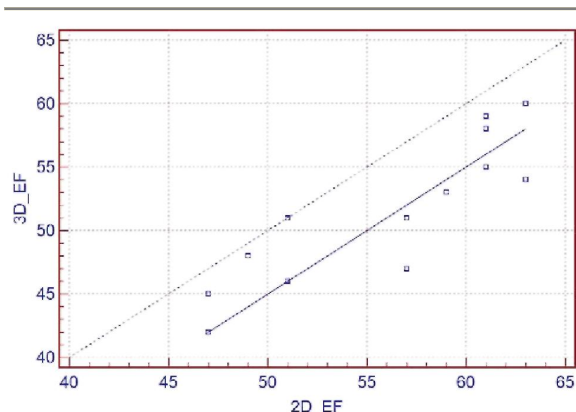


Figure 8. Comparison between 2D and 3D ejection fractions. Passing & Bablok regression scatter diagram with the regression line (solid line), the confidence interval for the regression line (dashed line) and identity line ($x = y$, dotted line), for the 2D and 3D EF. The correlation between 2D and 3D EF was 0.90 and there were no significant deviation from linearity ($p > 0.10$).

Our results show that the contrast values between LV and LV cavity were practically the same in 2D and 3D low and high dose studies. However, the noise in 3D low dose studies has been slightly higher when compared with the 2D and 3D high dose studies. Nevertheless, in spite of the slight increase in noise in the 3D LD studies, the images are very comparable with high dose 2D and 3D images.

Due to the fact that we used 8 phases in the 2D studies and only 5 phases in the 3D studies, the ejection fraction values in 3D studies were slightly underestimated, in average by 8.3%. The same effect was observed in the comparison between 16 and 8 phase gated SPECT studies, where the 8 phase studies show 3.71% lower ejection fractions [6].

Additional improvement in PET detectors [7] and better correction algorithms [8] can make the differences in contrast, ejection fractions and noise even smaller.

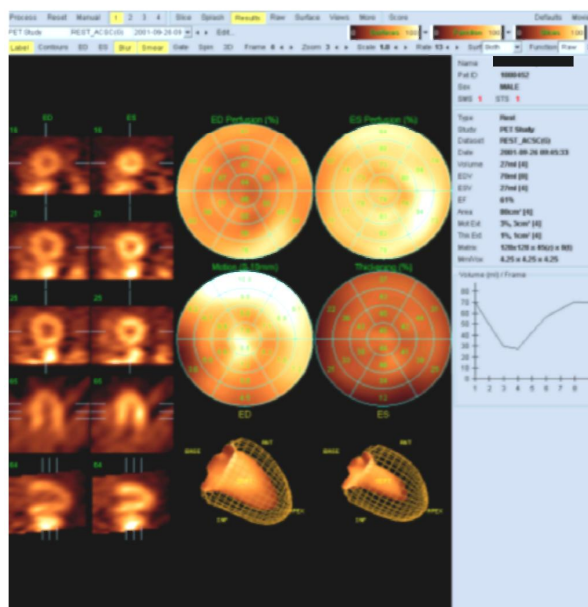
In terms of dosimetry, the effective dose equivalent for 2220 MBq (60mCi) of ^{82}Rb is 2.66 mSv. The kidneys, as the critical organ, receives a dose of 19.98 mGy [9]. For 740 MBq (20 mCi) of ^{82}Rb , the effective dose equivalent is 0.89 mSv with the kidneys receiving only 6.66 mGy, one-third of the dose for 2220 MBq (60mCi).

Conclusion

On our dedicated high counting-rate performance PET system, 3D high dose (2220 MBq) gated PET imaging gives similar contrast and noise level as high dose 2D imaging. However, high dose 3D gated imaging did not achieve a better performance due to a necessary delay in acquisition from the time of injection, and slightly higher randoms and scatter fraction. Low dose (720 mBq) 3D gated imaging, while achieving similar contrast and ejection fractions, resulted in slightly higher noise, compared to either 2D or high dose 3D imaging. In view of these findings, we conclude that 3D low dose acquisition images with optimized filtering can probably give acceptable results with significant cost savings, related to purchasing an

^{82}Rb generator, and considerable decrease in patient exposure.

2D EF



3D EF

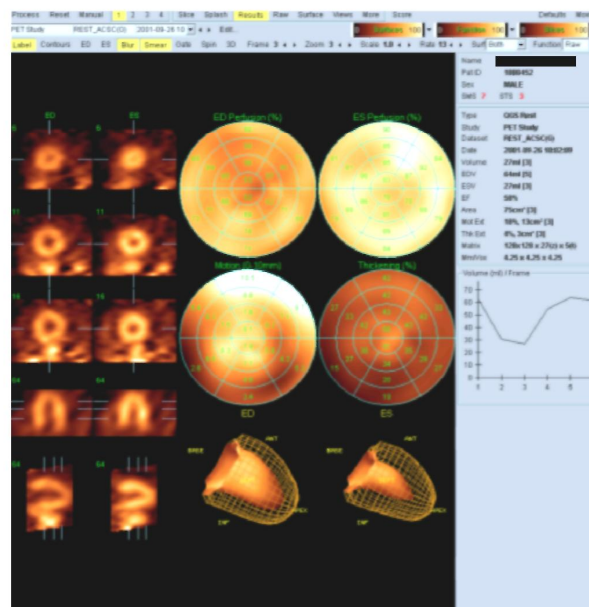


Figure 9. Comparison between 2D and 3D ejection fractions of the same subject. The 2D and 3D EF was 0.61% and 0.58%, respectively.

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NANOBIOTECHNOLOGY TODAY: FOCUS ON NANOPARTICLES

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In the recent years the nanobiotechnology field and the Journal of Nanobiotechnology readership have witnessed an increase in interest towards the nanoparticles and their biological effects and applications. These include bottom-up and molecular self-assembly, biological effects of naked nanoparticles and nano-safety, drug encapsulation and nanotherapeutics, and novel nanoparticles for use in microscopy, imaging and diagnostics. This review highlights recent Journal of Nanobiotechnology publications in some of these areas <http://www.jnanobiotechnology.com>

Bottom-up nanobiotechnology and biology-inspired nanoparticles

Some of the most promising applications of biologically inspired nanoparticles have so far been in nanobiotechnology and in tissue- and cell-specific drug delivery in particular. Unlike liposomes, dendrimers, metal and semiconductor nanoparticles, the nanoparticles made of biopolymers, such as bacterial spores, viruses and alike are naturally uniform in size and offer precise control for the surface-displayed targeting groups and their components. Furthermore, such biological nanoparticles may be produced recombinantly relatively easy and at low cost, and their assembly may be easily followed using a plethora of molecular and cellular approaches and instrumental techniques. The stability of nanoparticles made of biopolymers is one of the limiting factors which will determine the range of their applications. In their most recent paper *Caldeira and Peabody* [1] investigated in vitro assembly and stability of virus-like icosahedral capsid of the RNA bacteriophage PP7 particles. Contrary to expectations, recombinant fusion of the subunits have not stabilized PP7 virus-like particles against thermal denaturation, whilst disulphide bonds between coat protein dimers greatly increased the viral particles' stability.

A loading capacity of nanoparticles made of biopolymers is another important criterion in the use of such nanoparticles as nano-containers for specific targeting applications. A comprehensive study of the assembly and stability of canine parvovirus-like particles (CPV) was conducted by *Gil-*

bert et al [2] who employed a novel strategy, based on Fluorescence Correlation Spectroscopy analysis, to monitor the assembly of a series of truncated monomeric canine parvovirus VP2 structural proteins and their GFP fusions. The truncations ranged from 0 (native protein) to 40 amino acids. Intriguingly, only one truncated variant (-14 amino acids) failed to assemble into a CPV-like particle, which was confirmed independently using more traditional confocal and electron microscopy approaches. The GFP "load" did not prevent nanoparticle assembly.

The ability to manipulate and direct CPV assembly is of critical importance in the field of target-specific drug delivery. Because CPV has naturally high affinity to transferrin receptors (TfRs), which are often over-expressed on tumor cells, CPV might be used for specific targeting of tumour cells directly. *Singh et al* [3] have utilized this ability of CPVs and studied targeting of HeLa, HT-29 and MDA-MB231 cells and the internalization of native and modified ("loaded") CPVs. The assembled CPV-like nanoparticles were found to withstand conjugation with chemotherapeutic drugs, remain intact following their purification and internalise within 2 hours through TfRs receptors.

Biological effects and therapeutic applications

Traditional strategies towards the tissue-specific drug delivery utilise cytotoxic drugs attached to targeting moieties (e.g. towards TfRs receptors mediating cell-specific targeting and internalisation). In their recent report *Mondalek et al* [4] have shown that

nanoparticle internalisation can be enhanced by the use of an external magnetic field and targeted magnetic delivery. To illustrate this, a model similar to the human round window membrane has been developed and superparamagnetic iron oxide (Fe_3O_4) nanoparticles were magnetically transported through three co-cultured layers of cells. Such magnetic gradient-forced transport is minimally invasive, does not compromise epithelial confluence and has the potential to enhance the therapeutic benefits of magnetic nanoparticles-based drugs and reduce their toxicity.

Magnetic nanowires are another example of paramagnetic nanomaterial especially suitable for nanobiotechnology applications due to their size and anisotropy (unlike traditional anisotropic magnetic nanoparticles). *Prina-Mello and co-workers* have shown that Nickel nanowires, grown in alumina membranes, can be introduced into adherent and suspended cells and be used for cell manipulation, identification and separation [5]. The authors have also shown that internalised nanowires can be manipulated (re-oriented) whilst inside the cells without inducing any anisotropy in the population of adherent cells.

In addition to their ever more increasing use in molecular separations and targeting, magnetic nanoparticles were shown to also increase stability, activity and functionality of enzymes immobilised on the surface of the particles [6]. Kinetic studies of free and bound Cholesterol oxidase revealed structural and conformational changes of the immobilised enzyme which resulted in the reduction of activation energy upon binding onto iron oxide (Fe_3O_4) nanoparticles. The binding to nanoparticles further improved the storage stability of the enzyme, increased its tolerance to the variation in reaction pH and its thermal stability (increased twice at 60°C). The above effects were observed with particles ranging between 9.7 and 56.4 nm in size. Protein-nanoparticle interactions and the immobilisation kinetics onto L-aspartic acid-modified iron oxide (Fe_3O_4) nanoparticles and 3-mercaptopropionic acid-modified gold

coated $\text{Fe}_3\text{O}_4/\text{Au}$ nanoparticles has been reported by *Kouassi and Irudayaraj* previously [7].

In an independent study, *Mukherjee et al* reported that the immobilisation of anti-VEGF antibodies on gold nanoparticles increase the ability of these antibodies to induce apoptosis in Chronic Lymphocytic Leukemia B cells [8]. The induction of apoptosis with gold-conjugated anti-VEGF antibodies was significantly higher than the CLL cells exposed to antibodies alone or to unconjugated gold nanoparticles. The authors attribute the effect to the increased concentration of drug and improved intracellular delivery, although improvements in antibody stability, conformational changes and the nanoparticles' cytotoxic effect on the target cells cannot be discounted.

A complex character of the interactions of inorganic nanoparticles with viral particles has been investigated by *Elechiguerra et al* [9]. The authors unequivocally demonstrated size- and site-dependent interaction of silver nanoparticles with gp120 surface glycoproteins of the HIV-virus. The binding and the inhibition of virus binding to host cells is limited to particles ranging between 1 and 10 nm in size.

In contrast to [8, 9], *Williams et al* observed no effect on cell proliferation or any signs of toxicity when *Escherichia coli* were incubated with silica, silica/iron oxide, and gold nanoparticles [10]. Studying the interaction of inorganic nanoparticles with biological targets, whether molecules, viruses, bacteria, cells, tissues or organisms, as well as the nano-safety aspects and the long-term effects of that interaction might present a challenge to the scientific community due to the sheer number of materials, nanoparticle preparation methods and functionalization techniques. There is no universal "nanoparticle" to fit all the cases, and the multitude of "grey goo" scenarios, first hinted at by nanotechnology theorist Dr. K. Eric Drexler in his 1986 book "Engines of Creation", has raised hair on the heads of many safety officers and researchers active in the field, in-

cluding Peter Hoet, Irene Bruske-Hohlfeld and Oleg Salata who's paper on the health risks associated with the nanoparticles remains the most cited paper in the Journal of Nanobiotechnology to date [11].

Concluding remarks

This month the Journal of Nanobiotechnology celebrates 5 years since its creation. On behalf of the Editorial board I would like to thank all the authors for their precious work and excellent manuscripts, the reviewers for their invaluable service to the field and the Editorial Board and many Editors of other BioMed Central publications for their continuous support and encouragement. My special thanks go to the publisher, BioMed Central (London). I would like to invite the wider scientific community to join the fast growing readership of this Open Access Journal and also to consider it for publishing your own work. And finally, my warmest wishes to everybody for the coming Christmas and the New Year (with apologies to the followers of other calendars and religions). This New Year promises to be the warmest ever but we have yet to see a manuscript on the use of *Nano-bio-technology* for solving this truly *Planetary-scale* problem of global warming.

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THE HISTORY OF AFRICAN TRYPANOSOMIASIS

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The prehistory of African trypanosomiasis indicates that the disease may have been an important selective factor in the evolution of hominids. Ancient history and medieval history reveal that African trypanosomiasis affected the lives of people living in sub-Saharan Africa at all times. Modern history of African trypanosomiasis revolves around the identification of the causative agents and the mode of transmission of the infection, and the development of drugs for treatment and methods for control of the disease. From the recent history of sleeping sickness we can learn that the disease can be controlled but probably not be eradicated. Current history of human African trypanosomiasis has shown that the production of anti-sleeping sickness drugs is not always guaranteed, and therefore, new, better and cheaper drugs are urgently required.

Background

African trypanosomiasis is an infectious disease of humans and animals of similar aetiology and epidemiology. The causative agents of the disease are protozoan parasites of the genus *Trypanosoma* that live and multiply extracellularly in blood and tissue fluids of their mammalian hosts and are transmitted by the bite of infected tsetse flies (*Glossina* sp.). The distribution of trypanosomiasis in Africa corresponds to the range of tsetse flies and comprises currently an area of 8 million km² between 14 degrees North and 20 degrees South latitude [1]. Throughout history, African trypanosomiasis has severely repressed the economic and cultural development of Central Africa.

African animal trypanosomiasis or nagana disease is caused by *T. congolense*, *T. vivax* and *T. brucei* spp. In wild animals, these parasites cause relatively mild infections while in domestic animals they cause a severe, often fatal disease. All domestic animals can be affected by nagana and the symptoms are fever, listlessness, emaciation, hair loss, discharge from the eyes, oedema, anaemia, and paralysis. As the illness progresses the animals weaken more and more and eventually become unfit for work, hence the name of the disease "N'gana" which is a Zulu word that means "powerless/useless" [2]. Because of nagana, stock farming is very difficult within the tsetse belt.

Human African trypanosomiasis or sleeping sickness is caused by two subspecies of *T. brucei*, *T. brucei gambiense* and *T.*

brucei rhodesiense, while the third subspecies, *T. brucei brucei*, is only infectious to animals. *T. b. gambiense* is responsible for the chronic form of sleeping sickness in West and Central Africa, whereas *T. b. rhodesiense* gives rise to the acute form of the disease in East and Southern Africa. There are two distinct stages during the course of sleeping sickness. The first or early stage of the disease, also known as the haemolymphatic phase, is defined by the restriction of the trypanosomes to the blood and lymph system [3]. The symptoms of this stage are fever, headaches, joint pains and itching. The second or late stage of the disease, also known as the neurological phase, is characterised by the presence of the parasites in the cerebrospinal fluid [3]. In general, this is when the typical signs of the disease occur: confusion, disturbed sleep pattern, sensory disturbances, extreme lethargy, poor condition and coma. If left untreated, sleeping sickness patients die within months when infected with *T. b. rhodesiense* or within years when infected with *T. b. gambiense*. Wild and domestic animals may play a major role as parasite reservoirs for human infections with trypanosomes [3-5].

Prehistory

Phylogenetic reconstruction based on the genes coding for the small subunit ribosomal RNA suggested that all Salivarian trypanosomes (to which African trypanosomes belong) separated from other trypanosomes approximately 300 million years ago [6]. Probably soon after their emergence, Sali-

varian trypanosomes became gut parasites or commensals of early insects, which evolved around 380 million years ago. With the appearance of tsetse flies some 35 million years ago, trypanosomes have been transmitted to mammals by these bloodsucking insects. The long coexistence of both tsetse flies and game animals may explain why most African wildlife species are tolerant of trypanosomiasis: they become infected by the parasite but show no ill effects [7]. In contrast, domestic animals have yet been unable to develop tolerance or resistance to trypanosome infections within the 13000 years of their breeding.

It is likely that trypanosomiasis has played an important role in early hominid evolution. Probably, the disease had an important role in the selection of trypanosome-resistant early terrestrial hominids. This is evident from the observation that arboreal primates are susceptible to trypanosomiasis while humans, with the exception of *T. b. gambiense* and *T. b. rhodesiense* infections, are resistant [7]. The fact that humans are resistant to all other African trypanosome species indicates that human African trypanosomiasis is a recent event in human development.

Presumably the sustained transmission of trypanosomes between tsetse flies and humans in West Africa has led to the evolution of the less virulent *T. b. gambiense* subspecies [7]. In contrast, the *T. b. rhodesiense* subspecies has remained ill-adapted to humans and is transmitted from game animals to humans [7]. The infectivity of *T. b. rhodesiense* to humans is due to a serum-resistant-associated (SRA) gene [8]. It seems that the SRA gene originated in a single event and then spread through *T. brucei* in East Africa by genetic exchange [9].

Antiquity

It is well established that in ancient times the north coast of the African continent held more lush vegetation than today [10].

Also the flora and fauna of the Nile valley during the Old Kingdom (3000 BC – 2000 BC) was quite different and probably similar to the current region of the Gazelle River (one of the major tributaries of the Nile River) in Sudan [2]. The distribution of tsetse flies should therefore have extended much more northwards and ranged into the Nile delta. Hence, it is reasonable to assume that shepherds and livestock breeders in these regions experienced the problem of trypanosomiasis. This is also evident from the fact that the Egyptians of the Old Kingdom kept their cattle together with game animals [2]. The ancient Egyptians did this not because they were inexperienced in breeding but could only successfully rear trypanotolerant animals. Further evidence for the presence of trypanosomiasis in ancient Egypt comes from the Veterinary Papyrus of the Kahun Papyri dating from 2nd millennium BC [11] in which a cattle disease is described that resembles nagana (Fig. 1). It seems that an ointment made from the fat of particular birds was used as treatment against the bite of flies [12]. During the course of the Middle Kingdom (2000 BC – 1300 BC) the stream course of the Nile River was adjusted and thus the breeding sites of tsetse flies were largely destroyed. At that time the Egyptians gave up raising game animals and discontinued growing pure breeds of the trypanotolerant aurochs (*Bos primigenius*). Instead, they interbred the aurochs with the more efficient Indian zebu cattle (*Bos indicus*) [2]. The gradual eradication of the tsetse fly due to the progressing regulation of the Nile River eventually allowed the ancient Egyptians to raise pure breeds of zebu cattle [2]. In addition, it is told that the horse, which was not introduced until the 16th century BC, was also difficult to breed in ancient Egypt [2]. It is probable that failure in horse breeding was also due to trypanosome infections by tsetse flies.



Figure 1. Last section of the damaged Veterinary Papyrus of the Kahun Papyri about the cattle disease *ushau* dating from 2nd millennium BC. The translation reads as follows: (1) Title: Treatment of the eyes (?) of a bull with *ushau* in winter. (2) If you see a bull with *ushau* (3) in winter, and he is blinded (?), (4) his two eyes are thick; gash thou as (5) above. If you see a bull (6) with *ushau* in winter from cold, (7) since its arrival in (?) the summer, (8) his temples are wrinkled (?), his eyes are running, his stomach groaning (?), (9) he does not walk (?) (10) (11) (12) thou all his body with as is done to one (13) with a bruise (?) [11].

Middle Ages

There are only a few written reports giving evidence for the occurrence of trypanosomiasis in Africa during the Middle Ages. Most of these reports are from the Arabs who kept close trade relations with the West African kingdoms such as Benin, Ghana, Mali and Songhai. One of the first historical records on human trypanosomiasis is by the famous Arabian geographer Abu Abdallah Yaqut (1179–1229). During his journey into Africa he found in the "Country

of Gold" (Wangara [13]) an underground village whose inhabitants and even their dogs were just skin and bones and asleep [2]. This scene is reminiscent of the devastating sleeping sickness epidemic in Uganda at the turn of the 20th century. A first case report of sleeping sickness comes from the Arabian historian Ibn Khaldun (1332–1406). In his historical work he reported that a fellow countryman told him about the death of the Sultan Mari Jata, Emperor of Mali, who died of an illness which, according to the descrip-

tion, corresponds to human trypanosomiasis [14]: "He told me that Jata had been smitten by the sleeping illness, a disease which frequently afflicts the inhabitants of that climate, especially the chieftains who are habitually affected by sleep. Those afflicted are virtually never awake or alert. The sickness harms the patient and continues until he perishes. He said that the illness persisted in Jata's humour for a duration of two years after which he died in the year 775 AM (= 1373/74 in our calendar)." A striking example of the impact of trypanosomiasis on the life and destiny of people is the eastward migration of the Fulbe (Fulani) in the northern parts of West Africa [2]. It is believed that in ancient times the Fulbe people have moved from Egypt or Ethiopia to the area of present-day Senegal. At the beginning of the 13th century when the Sahara was getting increasingly dryer, they were forced to relocate southwesterly into the savannah area. As cattle-breeding was their economic basis, the Fulbe did not risk continuing their migration southward otherwise they would have entered the tsetse belt and lost their herds.

Hence, they moved eastward and settled south of the Sahara but north of the tsetse belt in regions with sufficient grazing land.

Modern Times

Early Modern Times

In early Modern Times, the history of human African trypanosomiasis is closely linked to the slave trade. First accounts of sleeping sickness came from ship doctors and medical officers who worked for slave-trade companies. As sleeping sickness caused increasing losses, ship-owners and slave-traders pressed their ship doctors to investigate this eerie disease. In 1734, the English naval surgeon John Aktins (1685–1757) published the first accurate medical report on African sleeping sickness [15]. However, whereas Aktins described only the neurological symptoms of the late stage of sleeping sickness, the English physician Thomas Winterbottom (1766–1859) published in 1803 a report referring to the characteristic sign of

swollen lymph glands along the back of the neck in the early stage of the disease [15]. He also mentioned that this symptom was known long ago by Arabian slave-traders who refrained from buying slaves with this sign [2]. Although throughout the 19th century, reports on sleeping sickness increased and human African trypanosomiasis became a well-recognised disease, no one had any real idea about the nature of the illness [15].

Discovery of the tsetse fly - trypanosome complex

It was the Scottish missionary and explorer David Livingston (1813–1875) who first suggested that nagana is caused by the bite of tsetse flies. In 1852, he reported the occurrence of a disease in the valleys of the Limpopo and Zambezi rivers as well as at the banks of the lakes Nyasa and Tanganyika from which all the cattle he carried died after they have been bitten by tsetse flies [2]. However, it took another 40–50 years until trypanosomes were identified as the causative agents of nagana and sleeping sickness. In 1895, the Scottish pathologist and microbiologist David Bruce (1855–1931) (Fig. 2) discovered *T. brucei* as the cause of cattle trypanosomiasis (cattle nagana) [16]. The first unequivocal observation of trypanosomes in human blood was made by the British Colonial surgeon Robert Michael Forde (1861–1948) in 1901 when he examined a steamboat captain in The Gambia [17]. He first thought that the organisms he found were worms [15] but the English physician Joseph Everett Dutton (1874–1905) identified them as trypanosomes a few months later and proposed in 1902 the species name *Trypanosoma gambiense* (now *T. b. gambiense*) [18]. In the same year, the Italian physician and pathologist Aldo Castellani (1878–1971) found trypanosomes in the cerebrospinal fluid of sleeping sickness patients and suggested that they cause sleeping sickness [15, 19]. One year later, Bruce provided conclusive evidence that sleeping sickness is transmitted by tsetse flies [15, 20]. At that time, however, he believed that trypanosomes were transmitted mechanically by

tsetse flies [15]. It was the German military surgeon Friedrich Karl Kleine (1869–1951) who showed in 1909 the cyclical transmission of *T. brucei* in tsetse flies [21]. This prompted Bruce to change his original opinion of mechanical transmission of trypanosomes, and instead describe the full developmental cycle of the parasites within their insect host [15]. In the meantime, the two other animal pathogenic trypanosome species *T. congolense* and *T. vivax* were discovered in 1904 and 1905 by the Belgian physician Alphonse Broden (1875–1929) [22] and the German naval doctor Hans Ziemann (1865–1905) [23], respectively. The second human pathogenic trypanosome species, *T. rhodesiense* (now *T. b. rhodesiense*), was eventually recovered in 1910 by the parasitologists John William Watson Stephens (1865–1946) and Harold Benjamin Fantham (1876–1937) [24].

Epidemics and control of the disease

In the 20th century, Africa saw three severe sleeping sickness epidemics. The first one began in 1896 and lasted until 1906, and affected mainly Uganda and Congo [3]. It was a devastating epidemic with 300,000 and 500,000 people estimated to have died in the Congo Basin and the Busoga focus in Uganda and Kenya, respectively [20,25]. The disastrous effects of the epidemic worried the colonial administrations to such an extent that they sent out scientific missions to investigate the disease (see above) and to develop a cure [2, 20]. The French physician Charles Louis Alphonse Laveran (1845–1922) and the French biologist Felix Mesnil (1868–1938) were the first to report in 1902 that sodium arsenite was effective in infected laboratory animals [15]. In 1904, a paper was published by the Canadian doctor Harold Wolferstan Thomas (1875–1931), and the Austrian doctor and zoologist Anton Breinl (1880–1944), informing that the arsenical drug atoxyl could cure experimentally infected animals [2]. It was thought to be better than any other arsenical compound tested so far and relatively atoxic (hence the name) [2]. However, the German physician Robert

Koch (1843–1910), who investigated the trypanocidal activity of atoxyl on sleeping sickness patients on the Ssesse Islands located in the northwest of Lake Victoria, found that the drug was by no means nontoxic; of 1622 atoxyl-treated patients Koch observed 22 cases of atrophy of the optic nerve with complete blindness [2]. In 1907, Koch informed the German scientist Paul Ehrlich (1854–1915) about these complications and advised him to improve the drug atoxyl [2].



Figure 2. Sir David Bruce (1855–1931). The Scottish bacteriologist identified *T. brucei* as the aetiological agent of nagana disease.

Already in 1904 Ehrlich had become interested in the chemotherapy of trypanosomiasis and had developed the dye trypan red, which proved to be both curative and prophylactic for *T. equinum* (a species of trypanosomes that causes Mal de Caderas in horses in Central and South America) in mice but not for *T. brucei* [26]. Eventually, it was Ehrlich's former assistant Wilhelm Roehl (1881–1929) who in 1916, with the help of a

small team of chemists and the German chemical and pharmaceutical company Bayer, developed the first effective drug for treatment of sleeping sickness. The compound, Bayer 205, (later named suramin) is still in use in the therapy of early-stage *T. b. rhodesiense* infections [2]. A year earlier, the American chemist Walter A. Jacobs (1883–1967) and the American immunologist Michael Heidelberger (1888–1991) discovered the organo-arsenical tryparsamide. This was the first drug to treat late-stage sleeping sickness alone, or in combination with suramin, and was also employed in the treatment of animal trypanosomiasis [26]. Both drugs helped to fight the second major sleeping sickness epidemic which began in a number of African countries in 1920 and died down by the late 1940s (Fig. 3) [3,27]. Another important measure towards the control of the 1920s sleeping sickness epidemic was the introduction of mobile teams [20]. This method of systematic case detection and treatment with the aim of elimination of the parasite reservoir was suggested by the French military surgeon Eugene Jamot (1879–1937). In 1926, after long opposition by his superiors in Paris, Jamot was allowed to set up a special service in Cameroon that showed the effectiveness of his approach; within 11 years the prevalence levels of sleeping sickness declined from 60% in 1919 to 0.2–4.1% in 1930 [20]. Subsequently, other colonial powers introduced the method of mobile teams for *T. b. gambiense* sleeping sickness control [20]. Other approaches to the control of African trypanosomiasis were vector control, host reservoir control and game destruction [20]. Vector control was already introduced in 1910 and included the use of differently designed traps and bush clearing. Between 1920 and 1940, reservoir host control and game destruction, which was practised mainly in East Africa on the recommendation of Bruce, resulted in a significant reduction, but never in the extermination, of the tsetse fly population [20]. A third drug for treatment of the early stage of *T. b. gambiense* sleeping sickness, pentamidine, was devel-

oped by the English chemist Arthur James Ewins (1882–1958) of the pharmaceutical company May and Baker in 1937 [28].

With the discovery of its insecticidal properties in 1939, DDT was used by 1949 in the hope of freeing large parts of endemic areas from tsetse flies [2, 20]. Also in 1949, the arsenical melarsoprol, which was developed by the Swiss pathologist, microbiologist and chemist Ernst Friedheim (1899–1989), was introduced for the treatment of late stage human African trypanosomiasis. It was the first and is still the only effective drug for late stage *T. b. rhodesiense* sleeping sickness. Since the 1950s, several drugs have become available for chemotherapy of animal trypanosomiasis. These include the phenanthridine derivatives homidium bromide (Ethidium[®], Novidium[®]) and isometamidium chloride (Samorin[®], Trypamidium[®]), the aminoquinaldine derivative quinapyramine (Anthrycid[®]) and the aromatic diamidine diminazene aceturate (Berenil[®]) [29]. Eventually, the combined employment of chemotherapy, systematic case detection and vector control led to a dramatic reduction in the incidence of sleeping sickness at the beginning of the 1960s (Fig. 3) [27].

Other factors that affected the epidemiology of sleeping sickness in the first half of the last century are the socioeconomic conditions created during the colonisation of Africa. An excellent example of this is the sleeping sickness epidemic in the north-central Uele district of the former Belgian Congo, now known as the Democratic Republic of Congo [30]. Colonisation of this region in the first decade of the 19th century was protracted and brutal. Large numbers of people were displaced and many of them experienced famine. This created an ideal environment for spreading the disease and sleeping sickness became increasingly entrenched and epidemic in this region over the next 15 years. It was not until the mid 1920s that medical services were introduced in the Uele district by the colonial powers. Five years later, the epidemic was under control due, as claimed by the Belgian authorities, to the

medical interventions. However, improvements in nutrition and hygiene are likely to have had as much to do with the decline of sleeping sickness as the medical interventions [30]. By the 1930s, many people in Belgian Congo no longer suffered from in-

tense social and economic disruption and learned how to better cope with the rules and controls of the colonial powers while the Belgians at the same time ceased their social engineering practice of abrupt resiting of whole communities [30].

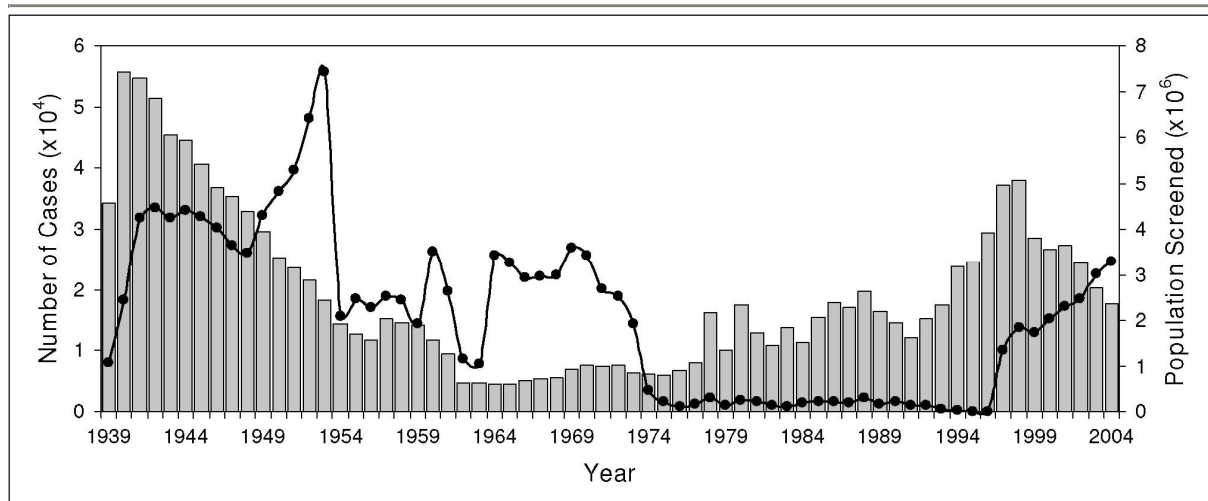


Figure 3. Number of reported cases of sleeping sickness and population screened, 1939–2004. Grey columns, number of reported cases; black circles, population screened. Data derived from [27, 33].

By the mid 1960s, most of the trypanosomiasis-endemic countries became independent and were no longer supported by their former colonial powers. In the aftermath of decolonisation, many African countries experienced political instability and economic ruin with a disastrous effect on the health services. After a decade of low endemicity, the control of trypanosomiasis was no longer a priority. As a consequence, control programmes were stopped and population screening declined to very small numbers of people (Fig. 3) [27]. Concerns about the environmental effect of DDT led to a ban of the insecticide in disease vector control worldwide in the 1970s. The result of all this was that since the mid 1970s there has been a steady increase in the number of reported sleeping sickness cases (Fig. 3) [27]. This was the beginning of the third and most recent sleeping sickness epidemic in the 20th century, mainly affecting Angola, Congo, Southern Sudan and the West Nile

district of Uganda [3,20]. The situation remained unchanged until 1990 when eflornithine (DL- α -difluoromethylornithine, DFMO), a selective inhibitor of ornithine decarboxylase, was introduced for treatment of late stage *T. b. gambiense* sleeping sickness. Eflornithine was initially developed by scientists at the Merrell Research Institute in Strasbourg for treatment of cancer [31], but was then found to be an effective therapeutic agent against *T. b. gambiense*. Although the administration regime is strict and difficult, eflornithine was a welcome alternative to melarsoprol treatment as it is less toxic.

Recent developments and current situation

At the turn of the millennium, the scale of sleeping sickness had almost reached, yet again, the levels of the epidemics seen at the beginning of the century (Fig. 3) [27, 32]. The situation got even worse as the production of eflornithine was ceased and that of melarsoprol was threatened to be discontin-

ued. Fortunately, in 2001 the World Health Organization (WHO) reached an agreement with the pharmaceutical companies Aventis (now Sanofi-Aventis) and Bayer AG to provide sleeping sickness drugs free of charge for endemic countries [3, 32]. The aid organisation *Medicins Sans Frontières* was commissioned with the distribution of the drugs. By 1997, surveillance had been reinforced and since 1998 the number of new cases has dropped steadily (Fig. 3) [33]. At present, the estimated number of infected patients is thought to be between 50,000 and 70,000 [3].

In 2001, the Organisation of African Unity (OAU) launched a new initiative, the Pan African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) to eliminate the tsetse fly from Africa [34]. It was planned to employ an area-wide approach using odour-baited traps, insecticide-treated targets and pour-ons and ultra-low volume aerial spraying of insecticides to reduce the tsetse fly population, and finally the sterile male technique to ensure total elimination of the target *Glossina* species [34].

The sterile male technique was successfully used in the eradication of tsetse flies and thus trypanosomiasis on the island of Zanzibar in 1997 [35]. However, in contrast to the Zanzibar project, which worked because it was on an island (isolated area of 1,651 km²) infested with only one tsetse fly species, the PATTEC initiative has to deal with a vast area of sub-Saharan Africa (~10 million km²) inhabited by at least 7 different *Glossina* species recognised as vectors for transmission of sleeping sickness. Therefore, many scientists are sceptical that the PATTEC project will succeed as similar eradication campaigns failed in the past because the tsetse fly infested areas could not be isolated [36]. The huge costs associated with the eradication project are also a concern as most of the countries involved belong to the most heavily indebted poor countries in the world [36].

The only new drug candidate currently in development for treatment of sleeping

sickness is the diamidine pafuramidine (DB289). In January 2007, pafuramidine had completed enrolment for Phase III clinical trials in the Democratic Republic of Congo and Angola [37,38] which is the final step before the compound can be registered as a drug against human African trypanosomiasis. If successful, pafuramidine would be the first orally available treatment for early stage sleeping sickness. Another approach to improve the treatment of sleeping sickness is the development of a combination therapy. Currently, the anti-Chagas disease drug, nifurtimox, is being tested in combination with melarsoprol or eflornithine in a randomized clinical trial in Uganda [39, 40].

There is also an urgent need for accurate tools for the diagnosis of human African trypanosomiasis. The existing tests for diagnosis are not sensitive and specific enough, due to the characteristically low number of parasites found in the blood of sleeping sickness patients. Therefore, the Foundation for Innovative New Diagnostics (FIND) and the WHO launched, in 2006, a new initiative for the development of new diagnostic tests to support the control of sleeping sickness [41]. It is expected that the new test will allow for early case detection and simplified staging and, thus, will improve disease management and support for the elimination of sleeping sickness as a public health problem.

Conclusion

The history of African trypanosomiasis gives an example of how a disease not only affected the evolution of humans but also the cultural and economic development of people in sub-Saharan regions. From the historical events of the 20th century one can learn that a concerted approach of systematic case detection and treatment is the appropriate method for the control of sleeping sickness and that discontinuation of these control measures will lead to re-emergence and spread of the disease. History has also shown that African trypanosomiasis always prevented the introduction of stock farming in endemic areas. A consequence of this is that much of tropical

Africa is still present today and has not been converted into grassland for cattle breeding.

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THE MAIN CAUSES OF DEVELOPMENT AND CONTEMPORARY TENDENCIES IN CLINICAL COURSE PECULIARITIES OF PELVIC INFLAMMATORY DISEASES IN WOMEN

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The article presents the comparative analysis of peculiarities and tendencies in the clinical course of pelvic inflammatory diseases (PID) for the period of 25 years. 1941 patients with PID were followed-up during the period 1981 to 2006. The tendency to the increase of chronic and destructive forms of PID has been revealed. In the etiology of the disease the prevailing of microbial associations with predomination of opportunistic flora as well as the increase of the role of sexually transmitted infections had been noted.

Pelvic inflammatory diseases (PID) keep the leading position within the structure of gynecological morbidity being the commonest cause of reproductive health disturbances in women [1, 3, 5, 7, 8]. The age of women suffering from PID becomes significantly younger. 70% of patients with salpingitis are under 25, 75% of patients had no labors [7, 8].

The comparative analysis of the onset and the development of PID in patients who had undergone treatment in the curative institutions of Ufa and Kemerovo cities over a period of 25 years (1981-2006) had been performed to estimate the clinical course peculiarities of PID.

Materials and methods

1941 patients with PID aged 16 to 48 were followed-up. In the period 1981 to 1988 there were 470 follow-ups, in the period 1989 to 1996 the follow-ups numbered 703 and in the period 1997 to 2006 they numbered 768. The morbidity pattern of PID is shown in table 1. The study was based on the unique scheme and included studying complaints, anamnesis, general and gynecological status using laboratory and instrumental methods. Microflora of the abnormal discharge from the cervical canal, from the abdominal cavity and the content of abscesses were studied as well. All patients had undergone ultrasound examination of the organs of small pelvis. 136 patients had undergone laparoscopy for the diagnosis and treatment, 125 patients had undergone laparotomy.

Table 1. Structure of PID

| Age | The forms of disease | | | | | | | | |
|--------|-------------------------------|-----------|-----------|---|-----------|-----------|--|-----------|-----------|
| | Salpingo-oophoritis | | | Salpingo-oophoritis in a combination with endomyometritis | | | Salpingo-oophoritis in a combination to a pelvioperitonitis and tubo-ovarian abscess | | |
| | Terms of supervision on years | | | Terms of supervision on years | | | Terms of supervision on years | | |
| | 1981-1988 | 1989-1996 | 1997-2006 | 1981-1988 | 1989-1996 | 1997-2006 | 1981-1988 | 1989-1996 | 1997-2006 |
| < 20 | 32 | 101 | 91 | 21 | 68 | 35 | 15 | 16 | 11 |
| 21-25 | 83 | 99 | 122 | 45 | 49 | 37 | 31 | 50 | 17 |
| 26-30 | 64 | 34 | 70 | 50 | 32 | 31 | 17 | 19 | 58 |
| 31-35 | 26 | 71 | 46 | 29 | 44 | 24 | 17 | 23 | 78 |
| >36 | 12 | 39 | 32 | 12 | 15 | 14 | 16 | 43 | 102 |
| In all | 217 | 344 | 361 | 157 | 208 | 141 | 96 | 151 | 266 |

Results and discussion

The performed comparative analysis proves salpingo-oophoritis to be the prevailing form of PID, the highest morbidity rate for this pathology being for ages 21 to 25. It should be noted that this form of PID mostly occurred in age group under 20. This situation is associated with the change in the beginning of the sexual activity. In 1980s the major part of women began their sexual activity over 19 and the rate of early aged sexual activity was only 5, 4%. But during last 15 years 17% of women began their sexual activity under 17. It is rather notable that in the period 1981 to 1988 the acute forms of salpingo-oophorites prevailed (67%) but in the period 1989 to 1996 their number decreased to 48, 4% and the recurrence of chronic inflammatory process (74, 5%) prevailed in the period 1997 to 2006. In addition, the number of cases with the severe course had increased (34, 6% in 1997-2006 and 20, 4% in 1981-1988). During last 15 years the statistically evident ($p=0,0376$) increase of the destructive forms of PID from 13, 4% in 1989-1996 to 17, 2% in 1997-2006 was marked, they mostly prevailing in women aged over 36 (44, 5% in 1997-2006). In 60, 9% of cases the tubo-ovarian abscess (TOA) prevailed. Diffuse purulent peritonitis developed in 7% of patients with ruptured TOA, 11, 7% of patients showed the development of interintestinal abscesses as a result of microperforation in TOA. The development of destructive PID forms was caused by the prolonged use (over 5 years) of intrauterine device (IUD). The mean duration of IUD use in patients of this group was ($M\pm m$) 6, $1\pm 0,3$ years. During last 10 years the decrease of the destructive forms in patients under 25 was directly associated with declining popularity of this kind of contraception in young women. The development of destructive forms in women of this age group had mainly social cause: drug using, having several sexual partners. The social status of patients with inflammatory diseases of uterine appendages has significantly changed. For instance, in 1980s the patients with PID (59,

1%) engaged in occupational physical activity prevailed and the part of the unemployed was 3, 3% whereas during last 15 years women with mental occupational activity prevailed (39, 6%), the part of the unemployed (up to 34%) having increased significantly. The importance of social factors in the development of PID is supported by the data of other researchers [8, 9]. It should be noted that the significant change of factors contributing to the development of inner genitals inflammatory process had taken place (fig.1). In 1981-1988 these factors most frequently were: pregnancy interruption (23, 7%) and the next menses. In 1997-2006 the development of PID was most frequently caused by intrauterine device use. Very often the onset of the disease was registered (11%) after the beginning of sexual activity. The number of PID increased twice after the surgical and invasive diagnostic procedures, nowadays it being caused by wide scale surgical activity and the increase of using the invasive diagnostic manipulations in obstetrics and gynecology.

The performed study of the etiological structure of PID has also allowed to identify the characteristic tendencies. In 1981-1988 monoinfection played the primary role in the etiology of inflammation and the main causative agents were staphylococcus (66.7%) and colibacillus (14, 8%) [6]. Associations of 2 to 3 causative agents (staphylococcus, streptococcus, proteus) were in 18, 5%. Later staphylococcus had lost its leading significance and the frequency of its revealing in associations of microorganisms had decreased to 11, 2% in the period 1989 to 1996. The combined microflora (2 to 5 causative agents) was revealed most frequently (38, 8%) during this period. In the same period new microbial associations such as *Klebsiella pneumoniae*, *Corynebacterium*, *Gardnerella vaginalis* had appeared. Anaerobic microflora was revealed in 15, 5% of cases with the destructive forms of the disease, in 2, 6% of cases actinomyces were revealed. At the same time the role of sexually transmitted infections (STI) had greatly increased. For in-

stance, *Chlamydia trachomatis* were revealed as the components of microbial association in 12% of cases.

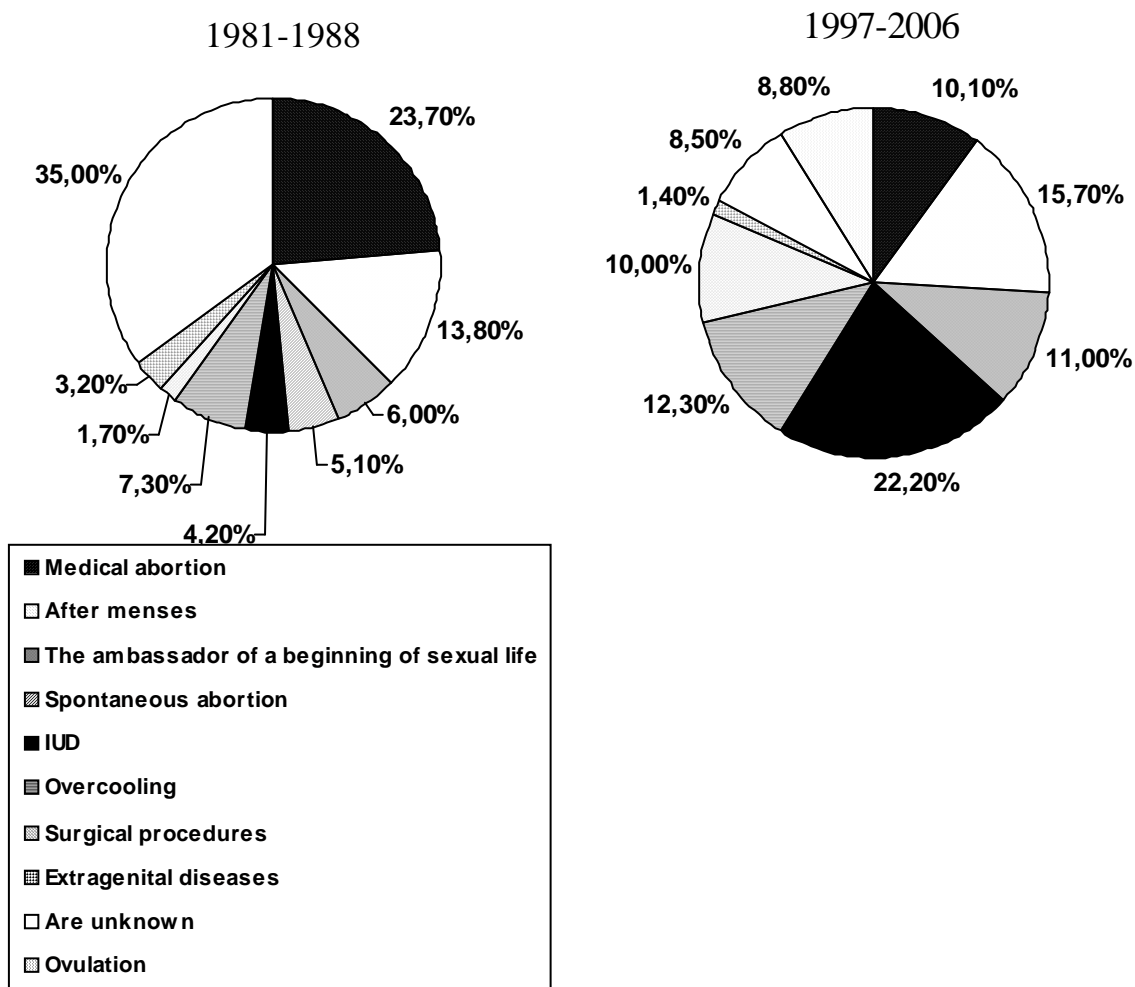


Figure 1. The basic reasons promoting occurrence of disease

The etiological structure of PID in contemporary conditions has got the mixed character [4]. The associations of 3 to 7 microorganisms (52%) are frequently revealed. Among the associations in 18, 7 % of cases enteric bacteria are revealed, in 16, 6% - streptococcus, in 10, 1% - corynebacterium, in 9, 35% - ureaplasma, in 6% - strict anaerobes and in 1, 9% - mycoplasma. The role of chlamydia infection persists to be significant (18, 75%). The identification rate of *Neisseria gonorrhoeae* had decreased to 5, 5% of cases. The rate of disbiotic vaginal conditions had sharply increased, it being 72, 8% nowadays.

The revealed increased role of opportunistic microflora in PID development suggests that today the inflammatory diseases of uterine appendages develop mostly being associated with the disturbances of natural defense factors. In the majority of cases the main role in this process is played by irrational antimicrobial therapy. For instance, 67% of patients with diagnosed vaginal dysbiosis in whom the association of saprogenic microorganisms was revealed had undergone self-medication by wide spectrum antimicrobials before the admission to the hospital.

The practical interest arises the time period from the onset of the disease and the admission to the hospital. On the first day of

the onset of the disease 19, 7 % of women were admitted to the hospital in 1981-1988, during the three days from the onset the rate of the admitted was 31, 1%, the majority (83, 4%) of women consulted a doctor during the period of the initial 7 days. In 1997-2005 only 3, 4% of women consulted a doctor on the first day, 13, 8% - during 3 days, 61% - during 7 days. The duration of the disease before the admission to the hospital was ($M \pm m$) $8, 7 \pm 0, 5$ days that proves the late diagnosis. Statistically the amount of hospitalized patients with PID has evidently decreased ($p=0.0083$) among all hospitalized gynecological patients (22, 9% in 1981-1988 and 15, 0% in 1997-2006), the number of patients with severe purulent inflammatory diseases having significantly increased. The highest increase of this pathology (by 50%) was in age group over 36. The additional difficulties in their treatment were associated with antimicrobial medication during 2 weeks to 1 month before hospitalization. The number of complicated purulent PID requiring emergent surgery on the day of admission has also increased from 2, 6% in 1989-1996 to 9, 6% in 1997-2006, over the half of patients requiring radical surgery (extirpation of uterine with uterine appendages or with uterine tubes). The noted changes are associated with the fact that currently the inpatients have more severe forms of diseases than in 1981-1988, patients with mild forms having been treated in outpatient departments. For instance, in 1981-1988 the majority of patients (67, 4%) were admitted to the hospital in satisfactory condition, 30, 6% patients had the condition of middle severity, 2% of patients were in bad condition. Currently the bad condition is registered on admission to the hospital in 10, 3% of patients, the condition of middle severity – in 44% of patients and satisfactory condition – in 45, 7% of patients. At the same time the amount of latent forms of the diseases keeps high (29, 3%). In 1989-1996 all hospitalized patients with TOA had un-

dergone radical surgery but in 1997-2006 these surgical procedures were performed in 65, 5% of cases only, 18, 75% of surgeries having been organ saving procedures using endoscopic techniques.

The frequency of revealing the symptoms of PID within the analyzed period (fig. 2) should be noted. The predominant complaint of patients with PID was the pain in the low abdominal regions. In 1981-1988 about 90.9% of patients characterized the pain as mild and dull whereas in 1997-2006 this character of the symptom was marked in 44% of patients only, the majority having complained of intensive dull and colicky pain radiating to the sacral bone, rectum and perineal area. Recently the patients with PID complained of disuretic condition (18, 5% in 1981-1988 and 40, 5% in 1997-2006) mostly due to the spread of sexually transmitted infections (STI) among this group of patients. Besides, 21, 5% of patients noted the menses disorder, 31% had gastrointestinal symptoms (nausea and vomiting, bloating, diarrhea) and 90, 5% complained of pathologic discharge from the genitals. Pathologic discharge was mostly noted associated with salpingo-oophoritis and endometritis and with TOA.

During last years most hospitalized patients with PID had the marked temperature reaction. The mean values were ($M \pm \delta$) $37, 7^\circ \pm 0, 7^\circ$. On gynecological examination the thickened, infiltrated and painful uterine appendages were revealed in most patients (44, 8%). During the first examination it was not possible to palpate the uterus and the uterine appendages in 27, 6% of patients due to the tension of frontal abdominal wall and sharp pain on palpation. In 18, 1% of patients a space-occupying lesion in the uterine appendages area was identified, 7, 8% of patients had the adhesion mass of the abdominal cavity organs and 1, 7% of patients experienced tenderness on palpating the uterine appendages area and painful tractions posterior the cervix of the uterus.

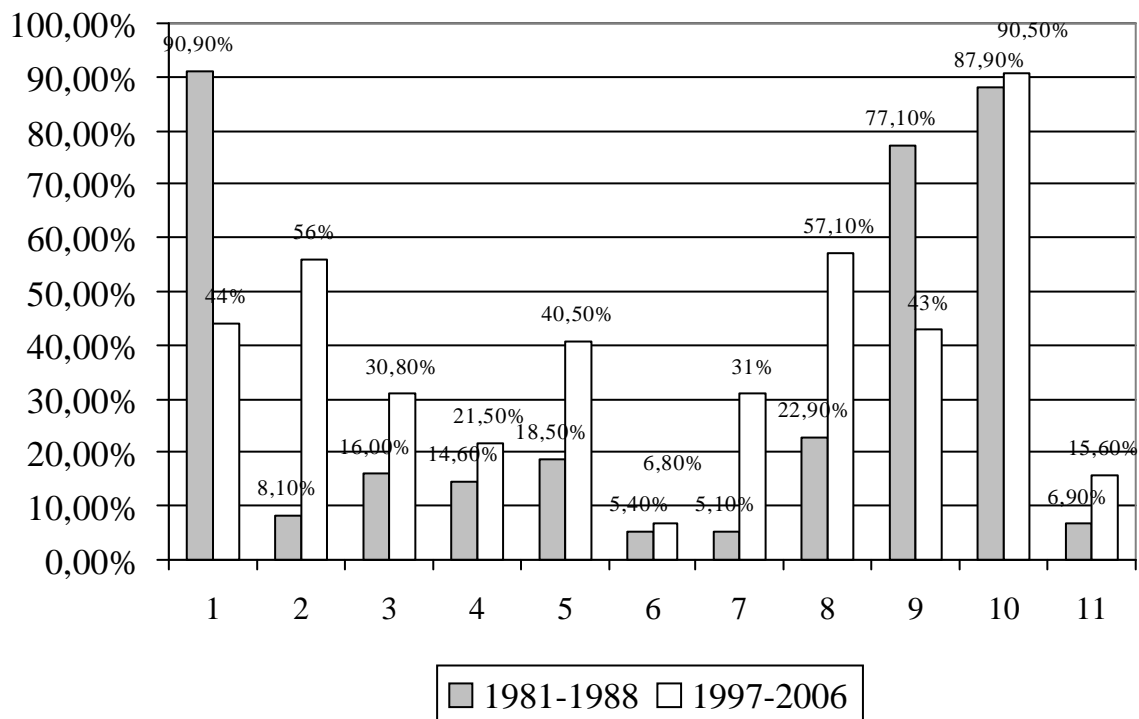


Figure 2. Frequency of revealing of symptoms of PID at an investigated quota of the patients

1 – moderate pain, 2 - sharp pain, 3 – common weakness, fatigue, 4 – infringement of a menstrual cycle as a menometrorrhagia, 5 – disuretic condition, 6 - infringements of a water exchange (thirst, dryness in a mouth, dry language), 7 – nausea and vomiting, 8 – hyperthermia, 9 – subfebrile temperature reaction, 10 – leucorrhoea, 11 – infertility

The peripheral blood samples have a number of peculiarities today. Moderate leukocytosis was revealed in less than a half of patients with acute inflammatory diseases of uterine appendages (21, 1%), the erythrocytes sedimentation rate (ESR) appeared to be increased in less than a half of the examined (43, 1%). The clinical pattern of contemporary acute PID was proved by laboratory findings in complex only, every isolated routine laboratory finding having been informative in less than half of cases. The common laboratory investigations in case on infectious toxic form of acute PID were not always informative. Thus, the expected leukocytosis was proved only in 25, 2% of patients, the band forms shift in the leukocytic formula was observed in 33, 9% and the increased ESR – in 33, 9%. The values of C-reactive protein were from moderate to

highly positive in 30, 9% of patients with exacerbation of chronic PID. At the same time the endogenic intoxication rate was high, the mean values of intoxication leukocytic index being – 4, $1 \pm 0, 5$. One can resume PID to have typical symptoms in 50% of patients. However, in latent forms of the disease the same pathogenetic alterations are observed as in typical forms of PID because slight atypical clinical symptoms are frequently associated with deep involvement of the inner genital organs tissues [8].

Thus the performed analysis had revealed a number of peculiarities in the pattern and clinical course of contemporary PID that consequently requires certain changes in the traditional approach to managing the patients with this pathology. Prevention and treatment of chronic forms of PID is the most difficult problem, the commonest mis-

take being the irrational use of antibacterial agents. In many cases women have been medicating with antibiotics of various groups including the reserve agents that results in cultivation of strains resistant to antibiotics, vegetation of opportunistic flora, allergic reactions, suppression of the immune system functions [2].

Conclusions

1. The significant tendency to the increase of chronic diseases of uterine appendages and mild or latent forms of inflammation has been observed recently. Social, behavioral, genital and extragenital risk factors contribute to the development of inflammatory process in inner genitals and influence the character of its development and the course, the microbial associations of opportunistic microorganisms being predominant in the genesis of PID.

2. High incidence of the destructive PID forms development associated with the use of IUD makes it necessary to use this contraceptive device with more restrictions. Contemporarily it may be recommended to women who had already fulfilled their reproductive function.

3. High incidence of generative function disturbances, as the consequence of PID, requires rational therapy at minimal diagnostic indicators of the inflammatory process as

well as rehabilitative measures after the discharge from the hospital.

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MIDDLE OB AREA LEARNERS' MENTAL CAPACITY AND CNS FUNCTIONAL STATUS FEATURES ACCORDING VARIATIONAL CHRONOREFLEXOMETRY METHOD

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The chronoreflexometrical performance characteristic of the Middle Ob Area residents aged from 7 to 20 years old was carried out on the ground of the simple visual-motor reaction latent period statistical analysis. The approximation of the CNS functional state estimation calculation criteria values and the performance level indexes allowed defining that the CNS functional status of Surgut school children and students had been at the reduced and insignificantly reduced performance level. A diminished attention, deterioration of temporary and precise activity parameters and significant performance lowering as a whole are indicative for such state.

The search for objective criteria of the nervous system development periods' estimation, the formation of its integrated systemic activity is one of important tasks of age-dependent physiological psychology. At that, it is important to know about the CNS age-dependent changes in comparison with other systems and functions of the body with due consideration of alterations in postnatal development sensitive periods (Soroko S.I. with other authors, 2006). A large body of research allows considering mental capacity as children's functional status integral index altering under the influence of endogenic and external cause factors (Grombakh S.M., 1988, Antropova M.V. and other authors, 2006).

Currently, as mostly often used in practice and objective method of the CNS functional state defining in physiological psychology of academic and professional activity the variational chronoreflexometry, at the heart of which a simple sensor-motor reaction latent period statistical analysis lies, is used. A relative simplicity of this method, usability in natural conditions, practical non-effect of training factor give an opportunity to use it as an express-method in applied research on human functional status estimation (Moroz M.P., Chubarov I.V., 2001).

In our research natives of the Middle Ob Area of both sexes aged from 7 to 20 took part, 1 and 2 health groups (1551 learn-

ers of municipal educational institutions of Surgut and the Pedagogical University students took part, among them 768 persons of male sex and 783 persons of female sex). All the examinees were referred to a Slavic nationality group and were born in Surgut by endemic population representatives, who had migrated to the places of new findings development from South-West and Southern areas of Russia and countries of the CIS.

For the purpose of getting information about the CNS functional state and the performance of the Middle Ob Area learners we used the computer program "Express-diagnostics of human functional state and performance" (Moroz M.P., 2003) by "IMATON" production, Saint Petersburg. The examination of school children and students was carried out in a separate sound-insulated laboratory in morning hours.

The Middle Ob Area learners' variational chronoreflexometry showed the following results (Fig. 1-6).

The comparison of average temporal values of a simple visual-motor reaction (SVMR) showed general improvement (reaction time reducing) from junior to senior age groups (Fig. 1). It appeared that the longest latent periods of the SVMR were in children of 7 and 8 years old, and the shortest and steadiest ones in the examined boys and girls aged 19 and 20.

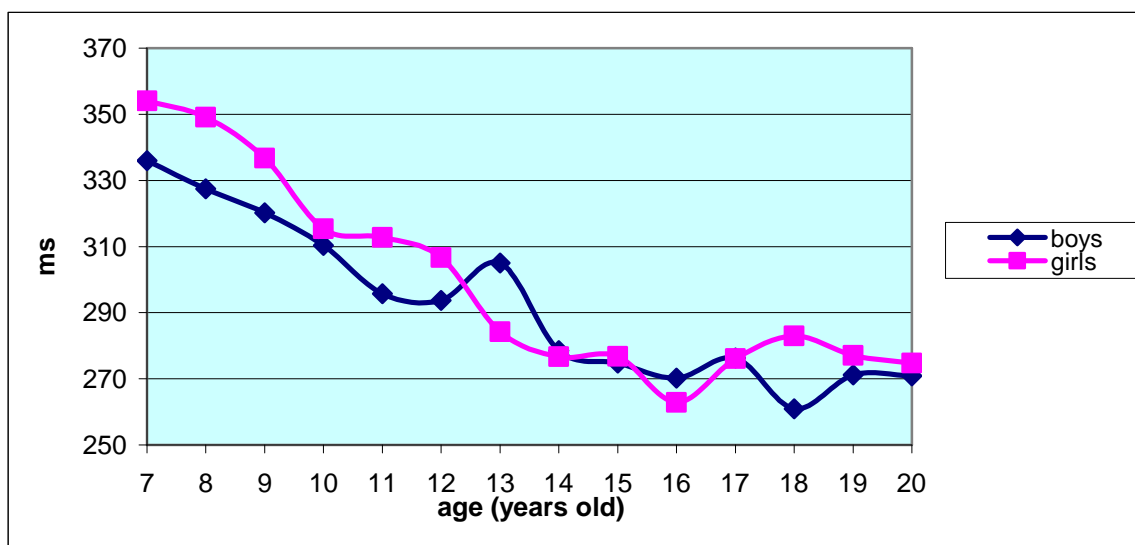


Fig. 1. Time of simple visual-motor reaction in Surgut natives (ms)

As the results of our research testify, an authentic shortening of the SVMR time occurred with the increase of years in Surgut natives of both sexes, that is probably associated with the CNS morphofunctional status and its highest departments' formation.

The reaction time reduction is most noticeable in the period from 7 to 11 years old both in boys and girls. The intensive changes of neurodynamic functions' properties in junior school learners are connected with the accelerated morphological brain maturity, regulation mechanisms improvement and specification of its separate departments, which are responsible for the reception and processing of information (D.A. Farber, N.V. Dubrovinskaya, 1991). At the age of 13 the SVMR speed is authentically higher in girls compared to the data of boys of the same age, that is probably can be associated with the beginning of active pubertal alterations in boys and changes of the functional status of the body as a whole.

During the puberty the pronounced SVMR variations were registered, and only in the ephebic period of ontogenesis the specified factor stabilized.

In 18-20-year-old boys the motor reaction speed is higher, than in girls, the SVMR in 18-year-old boys being authentically lower ($p < 0, 05$) compared to 19-20-year-old boys.

The latent period duration of the sensorimotor reaction to a visual stimulus in non-adults is associated with not only morphofunctional formation of the visual analyzer's peripheral link, but, in a greater degree, of their central structures and, as a whole, the CNS functional state (A.I. Kiyenya, O.V. Kirichenko, 2001).

Boys were more responsive to visual stimulus compared to girls, statistically-valid differences are found out in age groups of 8, 9, 10, 18 years old.

The standard deviation analysis showed that from year to year the spread of VMR values reduced. The task performances improvement with the increase of years can be associated with the anterior cerebral cortex maturation, that manifests itself with the control function intensification, thereby a more selective brain structures' activation is achieved (T.V. Akhutina and other authors, 1999; V.M. Vodlozerov, S.G. Tarasov, 2002; N.Makarenko and other authors, 2005).

Our researches are based on bi-manual testing variant, which allowed investigating the interhemispheric functional asymmetry dynamics as well (Fig. 2, 3). It is known that the interhemispheric asymmetry phenomenon reflects the CNS adaptive activity based on bilateral regulation mechanisms.

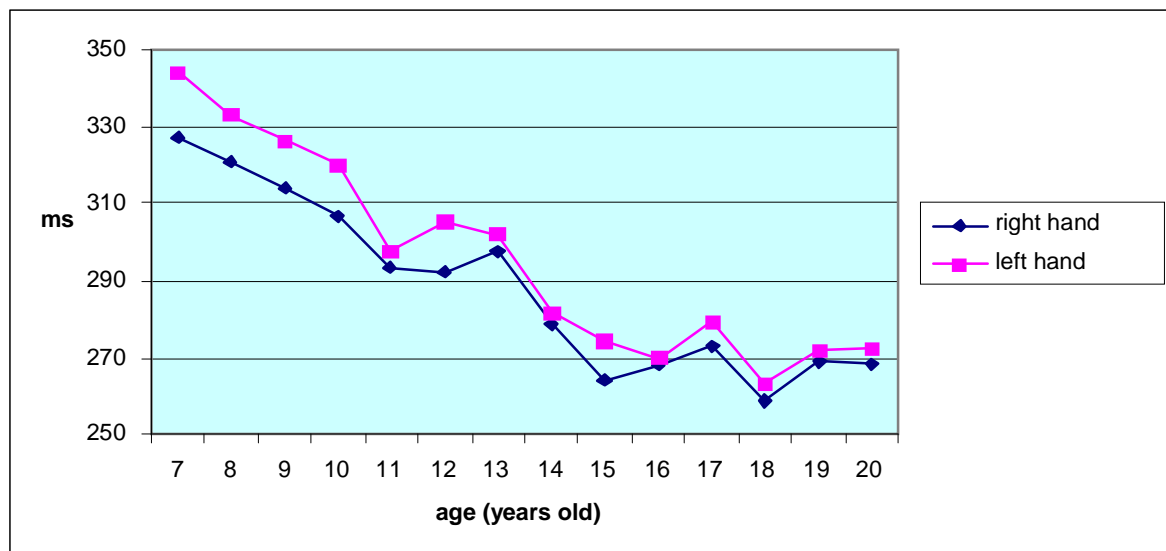


Fig. 2. Simple visual-motor reaction values (mc) in Surgut boys

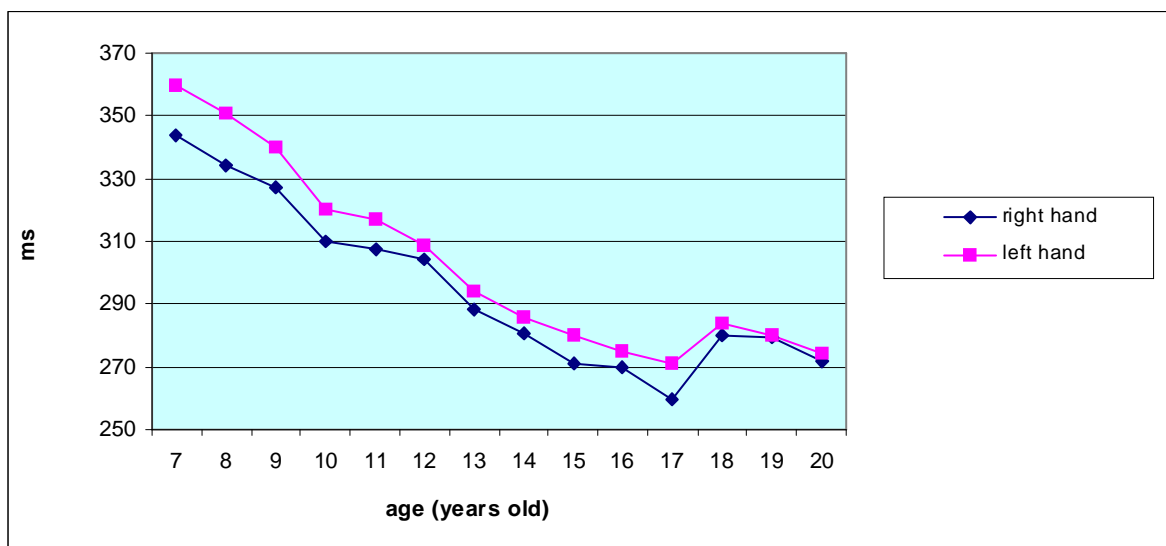


Fig. 3. Simple visual-motor reaction values (mc) in Surgut girls

The information processing by brain in each specific case is defined by the asymmetry of hemispheric activation levels and the dynamic character of interhemispheric relations. The response hand demonstrates a less sensomotor reaction time in the examinees (A.P. Bizyuk, 2005).

In the majority of the examined school children and students the SVMR speed was higher in the right hand.

In the age groups of 11, 14, 16, 19 years old in boys and 12, 19, 20 years old in girls the SVMR values of the right and left

hand were closest to each other or practically coincided. In the age groups of 7, 8, 9, 10, 12 years old in boys and 7, 9, 10 years old in girls maximal differences of the SVMR values of the right and left hand are registered (the differences are authentic, $p < 0, 05$).

A special attention was paid by us to the three quantitative criteria reflecting various sides of the CNS functional state, and also the performance levels: the nervous system functional level; the functionality level of the formed functional system; the nervous

reaction stability (T.D. Loskutova, 1975; M.P. Moroz, 2003).

The first chronoreflexometry criterion, which we analyzed, is the functional level of the system (FLS). Its value is determined mainly by the SVMR absolute indexes, i.e.

by the variation curve position against the absciss.

The given criterion was smoothly growing from 2, 1 to 2, 7 relative units (Fig. 4) over the period of the studied ontogenesis in the examined learners.

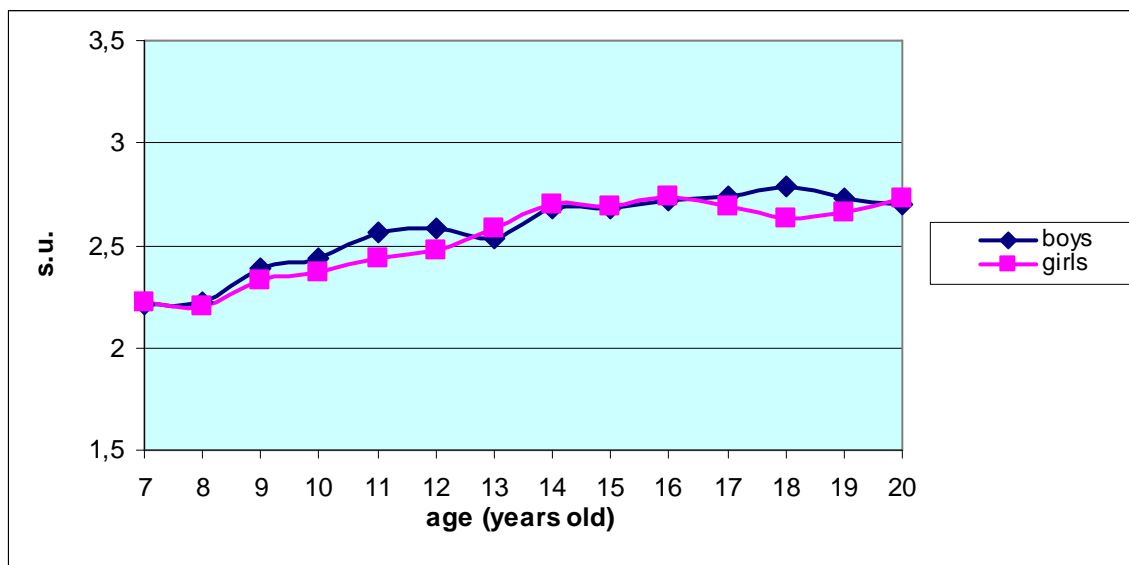


Fig. 4. Values of chronoreflexometry criterion “functional level of nervous system” in Surgut natives (relative units)

In the same age groups in boys and in girls no authentic differences were registered.

However, the fact that the given factor was within the limits of the functional status, which is characterized as “reduced performance”, comes under notice. Thus, according to M.P. Moroz (2003), the chronoreflexometry criterion “functional level of the nervous system” must be from 4, 9 to 5, 9 relative units.

The second criterion – the functionality level of the formed functional system (FL), is the fullest characteristic of the CNS state and allows judging about its ability to form and keep the corresponding functional system long enough.

In school children and students aged from 7 to 20 the FL was within the limits, which corresponded to the state of the char-

acterized as “insignificantly reduced performance” anywhere from 2, 0 to 3, 7 relative units (Fig. 5).

The FL criterion fluctuations were observed in puberty boys. In Surgut natives the FL criterion stabilized only with the beginning of ephebic period of ontogenesis.

The third criterion – the reaction stability (RS), was considered as the CNS states stability criterion. The less the SVMR values variability is, the more this factor value is (T.D. Loskutova, 1975; L.P. Pavlova, 1988).

In our researches the RS criterion in boys was smoothly growing from 7 to 11 years old. In girls the given criterion started growing from 12 only. The reaction stability approached the regulatory values (M.P. Moroz, 2003) only by 19, 20 years old both in boys and in girls (Fig. 6).

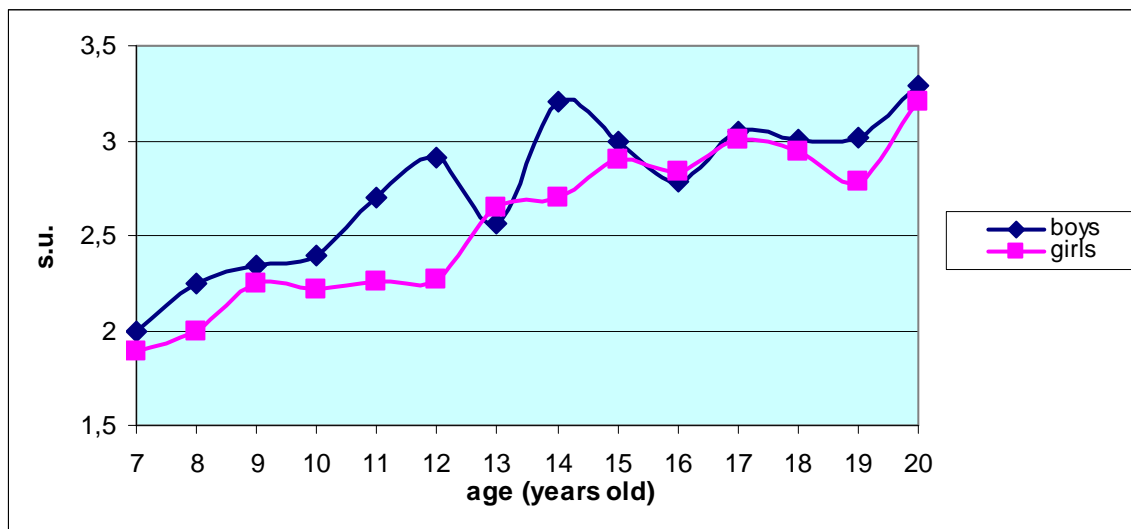


Fig. 5. Values of chronoreflexometry criterion “functionality level of the formed functional system” in Surgut natives (relative units)

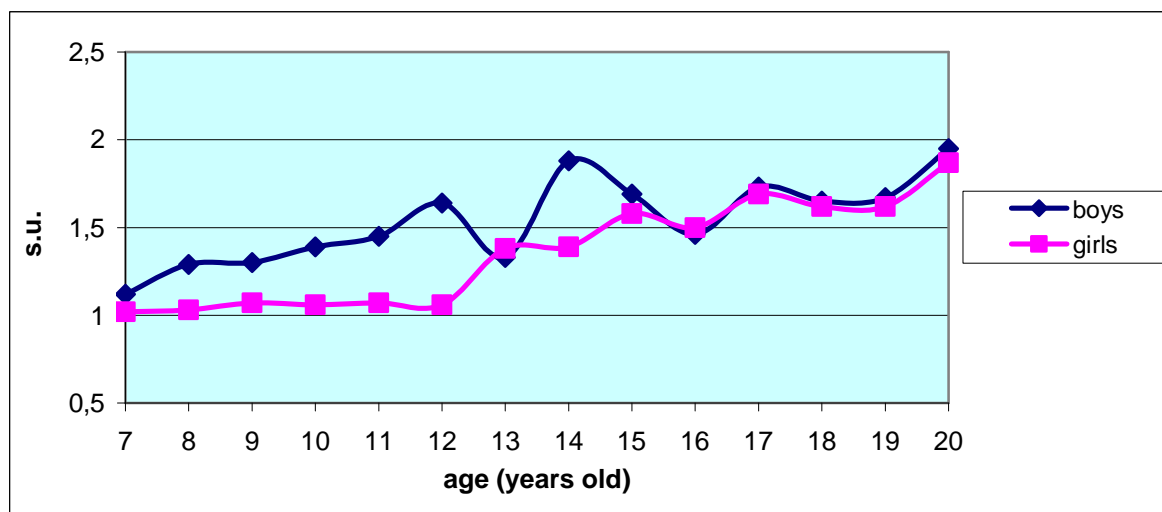


Fig. 6. Value of variational chronoreflexometry criterion “nervous reaction stability” in Surgut natives (relative units)

Thus, the approximation of the CNS functional state estimation calculation criteria values and the performance level indexes allowed defining that the CNS functional state of school children and students had been at the reduced and insignificantly reduced performance level (M.P. Moroz, 2003). For insignificantly reduced performance level the state of diminished attention is indicative, mistakes are assumed, the task performance time increases. The functional state at the level of reduced performance is characterized

by the deterioration of temporary and precise activity parameters and significant performance lowering as a whole.

The findings got by us can be used to control the psychophysiological state of the learners living in specific climate-geographical conditions of the Middle Ob Area. The CNS functional state express diagnostics can be used as an additional method of learners' functional status differential estimation and performance control in various

periods of academic activity with the help of variational chronoreflexometry.

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*Materials of conferences***COMPLEX STUDY OF MECHANISM OF SOME ANAESTHETICS ACTION ON CELLULAR AND ARTIFICIAL MEMBRANES PENETRANCE**

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For the purpose of studying molecular mechanism of some narcotic substances' action we have carried out experiments on bilayer phospholipid membranes with anion-selective channels formed by amphotericin B. It has been found out that $5 \cdot 10^{-4}$ M of cocaine doubled the conductivity of such membranes without affecting that of the unmodified phospholipid bilayers'. It has been demonstrated that anaesthetics are ranged – cocaine, lidocaine, novocaine, - according to their pharmacological action series. It has been supposed that the molecular mechanism of the detected effect is connected with the action of anaesthetics on the lipid bilayer surface charge. The following anaesthetic gases were tried by us on biological and artificial membranes: Halothane, Methoxyflurane, Chloroform and Butanol. It has been established that some compounds, local and general anaesthetics among them, cause the orderliness factor contraction or, in other words, dissolve the membranes. It was shown that the membrane should be in a certain optimal state to function well. After the inhalant addition the membrane resistance began to fall and after 15-20 min achieved a new level. At that the conductivity increased by 1, 6-3 orders more. It was suggested that perhaps the membrane dissolution accelerated their interaction. This assumption was verified while determining the time of two phospholipids membranes' fusion, when the anaesthetic was added into the solution 10mM KSI, wherein the work was carried out. The data obtained show that in the presence of the investigated inhalants the membranes' fusion accelerated several times as much. The membrane resistance reduction, which occurs in the presence of inhalants, doesn't influence their fusion by itself. It was shown on the model membranes that cocaine doubles the penetrance of phospholipid membranes with anion-selective channels, and inhalation anaesthetics of narcotic action accelerate their fusion several times, when dissolving the membranes.

On the *m. Cutaneus pectoris Rana temporaria* nerve-muscle preparation the influence of some local anaesthetics (LA) (norcaine, novocaine, viadril, trimecaine, lidocaine and its analogues QX-314 и QX-572) was studied. All of them possess a postsynaptic action, the miniature endplate potentials' amplitude contraction (MEP) testifying to this fact. Proceeding from the data obtained one can conclude that all the investigated LA promote the emission of Ca^{2+} ions into the

nerve terminal protoplasm. The LA blocking effect, which manifests itself as the MEP amplitude contraction, happens due to the interaction of LA cationic type (i.e. quaternary amine) with the anionic receptors of the electro-excited membranes' sodium channel orifice.

The work was submitted to international scientific conference «Innovation technologies», USA, (New York) December, 19-27, 2007, came to the editorial office 26.10.07.

MECHANISM OF DEVELOPMENT OF GROWTH OF THE OVIDUCT AND BODY OF THE HENS IN POSTNATAL ONTOGENY

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The study of mechanism of the development of a structure reproductive organs of birds remains by a urgent question. The knowledge of features of stages of the development reproductive organs will help to find resources, which will allow increasing efficiency of poultry. Morphology of reproductive organs of birds during development studied many scientists all over the world, however problems of the mechanism of development reproductive organs have study short.

Sharandak V.I. has offers to choose seven periods of growth and development oviduct in postnatal ontogeny: first period – relative rest, which lasts up from hatching to 60-day age; second period – intensive growth and development oviduct, last till 120 days; third period – complete differentiation of the oviduct on departments, last till 150 days; fourth period – beginning oviposition, last about 360 days; sixth period – attenuation oviposition, last about 480 days; seventh period – involution, start since 540 days.

The purpose of our researches was study of mechanism of development of weight of a body and oviduct of the hens "Lohmann Brown" in postnatal ontogeny with the subsequent definition of critical stages its development.

Analysis facts shows, that the intensive gain of alive weight of chickens proceeded with daily up to 150-days age for this interval of time alive weight of chickens has increased by 45, 1 times. As for to weight oviduct, it for the similar period has increased by 5533, 3 times, and the sharp increase of weight oviduct occurred during 120-150 days by 104, 2 times. It is necessary to note, that in the period about daily to 120-days age weight of the oviduct has increased only on 0, 469 g, that is practically did not develop. The age 150 days is to the periods, when alive weight reaches the peak and becomes concerning constant. The change of oviduct length proceeds in the same law, as its weight. So for the period 1-120 days it has

increased by 3, 9 times, and for the period 120-150 days by 6, 6 times. The jump has taken place within 5 months, when the relative gain of length oviduct has made 147, 4 %.

The growth of weight of a body of the hens is active on 1 and 2 months of development, and oviduct on 4 and 5. These parameters are reduced, accordingly on 6, 7 and 6 month.

Thus, having compared the received results on weight of a body, weight and length of oviduct, it is possible to make the conclusion that the increase of alive weight of the hens occurs per the first 5 months and further is stabilized. In development oviduct, opposite, the first 4 months are the period of relative rest. And since 4 months there is an intensive development of an organ. The period 120-150 days, when weight of oviduct is increased by 104 times, and length by 6, 6 times is necessary to consider critical.

The work was submitted to III international scientific conference «Actual problems of science and education», Cuba, March, 19-29, 2008, came to the editorial office 15.01.2008

HOW MUCH HEALTHY IS "APPARENTLY HEALTHY" MEGACITY RESIDENT (IN TERMS OF KRASNOYARSK)

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Nowadays, more than a third of the world's population lives in big cities. Socially meaningful and professionally conditioned stressogenic diseases manifest themselves more acutely in a megalopolis. At the functional body reserves decreasing the priority value is acquired by dyscrasia diagnostics for a complex and effective sanitation of the internal environment. A non-specific resistance cellular link functional-metabolic reserve evaluated by means of chemiluminescent (CL) method on *Tono-Oka et al.* (1983) in the modification of Zemskov V.M. with co-authors (1988) can serve one of the body's adaptative potential criteria. On the basis of the analysis of hemophages' "breathing outburst" kinetics parameters in 1252 persons of various sexes, ages and health states the reserve coefficients RC_S , RC_I and the prooxidant shift evaluation index were calculated. The norm is characterized by the values $RC_S \geq 3, 8$; $RC_I \geq 10$; $EI = 0\%$; a disease - $RC_S \leq 1, 5$; $RC_I \leq 2$; $EI > 33\%$, a pre-existing disease - $1, 5 < RC_S < 3, 8$; $2 < RC_I < 10$; $0\% < EI < 33\%$. The parameters association of "apparently healthy" and absolutely healthy people testified that in 80% of clinically asymptomatic active working age adults of both sexes the non-specific cellular defence functions in conditions of chronic oxidative stress. It is fraught with adaptative mechanisms deterioration. Authentically, in 6% of the selection a phagocytic cells' "func-

tional palsy" has been found out. In children in conditions of constant urbanized environment chemical pollution an extreme adaptative mechanism with a higher prooxidant shift against the imbalance of mineral status and antioxidant components than in adults is realized. In 38% of pregnant women a double rise of cellular immune reactivity reserve capacity "is paid" by three-time intensification of the prooxidant shift and eight-time increase of the endogenous free-radical background. The reserve coefficient RC_S increase adaptative price is the prooxidant shift increase manifested in men 1, 7 times more intensive than in women. The phagocytic functional response is characterized by a reversed quotient (overshoot) of activated and basal production of free radicals and the reserve coefficient RC_I decrease 30 times at inflammatory, 3 times – at noninflammatory diseases and twice – at the pre-existing disease stage. The nonspecific resistance functional disturbances at the pre-existing disease stage are reversible when using the alimentary correction. The therapeutic measures efficiency enhances at their application with due consideration of the cellular immune reactivity original type and reserve corresponding to the body's phase of adaptation to the effects of controlled and uncontrolled factors of the environment.

The work was submitted to the International Scientific Conference "Scientific Research of Higher School on Priority Orientations of Science and Technology", Savona – Hamburg, May, 6-16, 2008. Came to the editor's office on 29.01.2008.

CARBOHYDRATE AND LIPID METABOLISM FACTORS IN GIRLS WITH MENSTRUAL FUNCTION DISORDER DEPENDING ON BODY WEIGHT

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The performances of the last years testify to high frequency of carbohydrate and lipid metabolism deviations from the age norms in girls with menstrual function disorders (MFD). However, these researches were mainly carried out in patients with polycystic ovarian syndrome, anorexia nervosa and Turner's syndrome. At the same time, the most commonly encountered MFD forms among teenagers are oligomenorrhea (OM) and pubertal uterine bleedings (PUB).

The purpose of the present work has become the study of carbohydrate and lipid metabolism features in teenage girls with OM and PUB depending on body-weight index (BWI). For the objective implementation 68 teenage girls were examined, 25 of them having OM and 43 – PUB. In 40 patients the BWI was contained within the confines of age norms (in 13 with

OM and in 27 with PUB). 28 patients had an increased BWI (12 with OM and 16 with PUB). The glucose loading was carried out according to the standard practice, the lipid metabolism state was valued on the content of high-density lipoprotein cholesterol (HDLPC) and triglycerides (TG) in blood. The age deviations frequency of the studied factors was found out. For the evaluation of authenticity of the results the method of Fisher's angular transformation ($P\phi$) was used.

It has been established that the lipid content ratio, the character of which is evaluated as atherogenic one (the HDLPC level decrease and the TG level increase), in patients with normal BWI was registered in 17, 5 % (equally frequent at OM and PUB). At the overweight the specified disorders were revealed 2, 5 times as often (42, 9 %; $P\phi < 0, 001$). The Analysis of glucose tolerance test results has revealed a flat character of the "sugar curve" in 40% of the examinees irrespective of the BWI, that is indicative of carbohydrate metabolism disorders in a significant part of the teenagers with MFD.

The carried out research results testify to a high frequency of lipid and carbohydrate metabolism disorders in girls with MFD not only with an increased, but also with normal BWI, that can be considered as a risk of the metabolic syndrome early formation and it should be born in mind when carrying out a complex of medical and preventive treatment measures.

The work was submitted to V international scientific conference «Present-day problems of experimental and clinical medicine», Thailand, February, 20-28, 2008, came to the editorial office 18.01.2008.

USE OF ULTRASONIC DOPPLEROGRAPHY IN GLAUCOMA PATIENTS

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Topicality

Glaucomatous optic neuropathy (GON) is the core of primary glaucoma. Alongside with ophthalmohypertension and specific changes of visual fields the GON enters into the triad of this disease cardinal symptoms (Yegorov Ye.A., 2002, Alyabyeva Zh.Yu., 2004). The GON pathogenesis includes some factors, one of which is a vascular one - optic nerve head microcirculation disorders (tendency to vasospasms, rheological disorders, venous stasis, perfusion pressure decrease) (Grigoryeva Ye.G., 2002, Galassi F., 2003). However, in spite of native and foreign researchers' works on the ocular blood flow study, the possibilities of modern eye circulatory dynamics and

orbital region research methods have not been studied to the end by the present time.

The purpose is to study the circulatory dynamics in primary open-angle glaucoma (POAG) patients in the ophthalmic artery and central retinal artery by the ultrasonic duplex scanning method with color doppler mapping.

Materials and methods

Patients with obstructive affections in internal and external carotid arteries' regions were excluded from the examination.

We examined 38 patients (38 eyes) with the diagnosis of I-IV stage POAG (the main group). Among them, the I stage was registered in 9 patients, II stage – in 11, III – in 11 and IV – in 7 patients. In all the patients the intraocular tension (IOT) was normalized medicamentally or surgically and averaged $19, 4 \pm 2, 6$ mm hg.

The control group was made up of 20 patients (20 eyes) with mature age-dependent cataract without glaucoma signs. The average age of both groups patients made $68, 3 \pm 2, 4$ years old.

The circulatory dynamics investigation was carried out on the multipurpose ultrasonic high definition imaging system (HDI – High Definition Imaging System) HDI 1500 of the ALT firm, USA, with a broad-band linear transducer of 5-12 mHz. The valuation of hemodynamic parameters was performed in the following vessels: ophthalmic artery and central retinal artery.

The ophthalmic artery was located with the help of the 5MHz frequency transducer, at the depth of 38-42 mm at the vessel's entry into the orbital cavity, transbulbarly through the patient's closed eyelids. The patient's gaze direction was inward and downward. The pathfinder position is upper-external quadrant of the eye-bulb. The location direction – is to the orbital height, $15-30^\circ$ from the median line.

The central retinal artery was defined at scanning plane passing through the optic disk. The blood flow investigation in this artery was performed within the region of 0-10 mm from the place of its entering into the optic nerve trunk thickness up to the eye-bulb, with the following valuation of all parameters.

At the investigation findings analysis the following Doppler frequency shift spectrum parameters were evaluated:

- maximum systolic velocity (V syst., cm/sec);
- end diastolic velocity (V diast., cm/sec);
- average blood velocity (V m., cm/sec);
- resistance index (RI).

Results

It is found out that the maximum systolic velocity is the least variable parameter in the POAG patients. There are no statistically-valid V syst. values differences with those in the control group registered ($p < 0, 05$).

The average blood velocity in the ophthalmic artery in the main group patients decreased against the

control group with the disease progression; the deviation from the control being authentic ($p < 0,01$) at the I stage POAG. Thereafter (II-IV stages) a gradual average blood velocity decrease was registered. It testifies to the importance of this factor in the progression of the process.

At the analysis of the end-diastolic blood velocity changes as the vascular resistance factor, the data were got that a slight V diast. decrease, authentically differing from the norm at the III and IV stages only ($p < 0,05$), takes place at the II-IV stages of the disease. The peripheral resistance index is in the inverse linear dependence on the end-diastolic velocity value (the lower the velocity – the higher the index value, and vice versa), that is why the RI dynamics in the ophthalmic artery according to the glaucoma stages was inversely as the described above changes of the end-diastolic velocity. The changes found out in the CRA look like this: At the initial stages of glaucoma the V syst. in the CRA decreased 1,4 times compared to the control group. The Vm. at the OAG initial stage was 1,5 times as lower. The CRA diastolic blood velocity was decreased beginning with the I stage ($p < 0,05$) and also decreased with the glaucoma progression. The maximal CRA diastolic velocity fall was registered at the IV stage of glaucoma; the diastolic component being missing in one case. It was found out that the peripheral resistance indexes in OAG patients, beginning with the I stage of the disease, is authentically higher than in the group of control.

Conclusion

Ultrasonic methods of diagnostics using Doppler methods allow evaluating quantitative blood flow indexes in the ophthalmic artery and its branches at various stages of open-angle glaucoma, that affords an opportunity to come around to the diagnostics, treatment and prognostication of its course in a more detailed way.

The work was submitted to III international scientific conference «Actual problems of science and education», Cuba, March, 19-29, 2008, came to the editorial office 17.01.2008

ACTIVITY OF SYMPHATO-ADRENAL SYSTEM AT DIFFERENT STAGES OF ONTOGENESIS

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The adaptive process, set as the program of actions by the regulator centres, is accompanied by mobilization of the visceral systems providing the physiological price and result. Research of catecholamines by separate parts discloses local mechanisms of regulation. At the same time functional activity of an organism is achieved owing to integrative processes.

From this point of view studying the general metabolism - adrenaline and noradrenaline is justified.

The symphato-adrenal system excreting catecholamines - noradrenaline and adrenaline - is of great importance for the adaptation of a growing organism to conditions of environment. It carries out its regulator influence on functions of an organism through hormonal mediators which, according to L.A. Orbeli, promote a constancy of the internal environment and its adaptation to varied conditions of life.

At present the significant amount of works has been published which discover the dynamics of excretion of catecholamines and their predecessors in children basically of teenage age, including those with academic and physical loadings.

According to our data, excretion of adrenaline and noradrenaline in urine in children in the age of 6-8 years undergoes the wavy dynamics in both sexual groups, with the greatest expressiveness in boys in the age of 6 and 7 years. Judging from excretion of adrenaline and noradrenaline in urine, the character of synthesis and utilization of catecholamines varies essentially. In this age interval, the chronotropic function of heart is appreciably reconstructed. Physiological preconditions of this age form the adequate mechanisms of urgent adaptation influencing behavioral activity.

Our researches have shown that boys adapt more difficultly for conditions of school: within one academic year the exhaustion dependent on a sex and age of the child develops in children. During educational occupations children in the age of 6-8 years show the changes reflecting a functional condition of an organism. The character and intensity of these changes serve a mark of "physiological cost" of academic load.

The work was submitted to III international scientific conference «Basic Research», Dominican Republic, April, 10-20, 2008, came to the editorial office 19.01.2008.

MORPHO-TOPOGRAPHICAL FEATURES OF LARGE-SIZED OVARIAN CYSTADENOMAS

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Oothecomas occupy one of leading places among all the neoformations occurring in women. Except for mammary tumors the ovarian newgrowths among all the oncological diseases of woman genital sphere are second only to endometrial and endocervical carcinomas on frequency. About 80% of ovarian tumors are of nonmalignant nature and occur mainly in women aged 20-45 years old (Paltsev M.A., Anichkov N.M., 2001), 90% of all the neoformations make

epithelial neoplasms, the serosal ones among them are registered in 70% of the cases (Petrovic O. et al., 1992). In this group 60% are referred to benign tumors (adenocystoma), 15% - to marginal, 25% - to malignant ones. Serosal tumors, which are referred to the newgrowths from the superficial (coelomic) epithelium, afflict women of all ages, but more often – those aged from 41 to 50 years old (Kolosov A.E., 1996).

Clinically similar tumors manifest themselves when achieving certain sizes, most often – 5-10 cm in diameter, and by associated with them complications. We submit our own sectional and surgery observations of large-sized ovarian adenocystomas. In the first case the prior disease – is an ovarian adenocystoma with extremely rare daughter cyst unconnected with the mother one anatomically in another cavity of the body, which was not diagnosed pro vita through twenty years long. The second observation appeared a finding of the operating table. The third one – is an intravital diagnostication of a large-sized oothecoma represented in the form of various tumor growths combination in the juvenile age and a successful operative therapy.

The patient R., 75 years old, had been suffering from various forms of “tumor growth” (abdominal cavity organs, lungs) for the last 20 years. At the autopsy investigation there was a cyst up to 150 mm in diameter, 3000 ml volume, with fancifully crooked bulges located between the intestinal loops, found in the abdominal cavity. In other localizations – in the region of spleen, sigma and appendix – there were independent, anatomically unconnected with each other cysts, each one - up to 100-200 ml volume. Round these formations there was a frank adhesive process registered with the deformation of the close round organs (liver, bile cyst, spleen, gaster). There was no gastrointestinal tract obstruction through. There was sclerosis, hyalinosis and petrification of the thickened up to 10-15 mm large-sized cyst with a keen bulge of the navel ring forwards in the umbilical region.

In the right pleural cavity there was a cyst of 140 mm in diameter with a keen edging of the right lung to the back bone, its compression into a slab to 10 mm thick and mediastinal displacement to the left. Its content represented an odorless opacity grayish fluid. Shtiftic, neutral, cubical and mucus secerning cells were detected there cytologically and pathohistologically. Hialinized connective-tissue papillae emerged in the cyst lumen. The wall itself – is dense, fibroid, with petrification regions. All the cysts represented ovarian adenocystomas with connective-tissue papillae and the epithelium without malignant change phenomena.

The patient G., 72 years old, had been suffering from polycystic ovary for the last 25 years. When, 18 years ago, the greatest one from the cysts had achieved the size of a chicken egg, he was offered an operative therapy, which she refused from.

She was operated on incarcerated umbilical hernia. At the abdominal incision a giant round shape Rockitansky's tumor with dimensions of 190x230x250 mm, weighing 7000 g, was found. The intestinal loops were pushed off to the lateral channels of the abdominal cavity. The cyst wall is thick, fibroid, with large regions of hyalinosis and petrification. Branching and plain, low connective-tissue papillae covered with epithelial cubical, nonsecreting cells typical of ovarian adenocystomas were seen on its internal surface.

In the ectatic umbilical ring and hernial sac a fatty tissue at various stages of necrosis and infiltrative hemorrhage into it was found. There were no clinical data concerning the like formations, though of smaller volume, in other localizations.

The patient K., 23 years old, applied to the gynecological hospital with the complaints for volumetric gain of the stomach, pains irradiating to the small of the back in its lower departments for three weeks, infertility.

A faintly mobile formation with clearly-defined boundaries, soft, elastic, coming up to the navel, was determined at palpation. The stomach was increased up to 18-19 weeks of pregnancy. At the ultrasound investigation of the right ovary a large-sized (d = 170 mm) fluid formation coming from the ovary from the small pelvis to the navel was detected.

The right ovary was resected and the operational material was investigated pathohistologically using standard methods.

The macroscopic investigation testified: the oothecoma is d=160 mm; the wall is fibroid, rather hard, its internal and external surfaces are even, smooth, glossy, well vasculated; the vessels are large, tree-like branching. The cystic content is grayish, transparent, fluid, 3000 ml volume. In one cystic segment there was a cavity of 40 mm in diameter with brown content of fluid consistency, with chondral, osseous and hairy inclusions in the wall, and also a fragment of osseous tissue seemingly resembling a tongue bone. In another segment of the cyst there was a yellow body cyst (d=10mm) immured into the fibroid wall. In the third segment, on its internal surface, there was a papillary excrescence in the form of a thin 10 mm long villus (d=1mm), grayish, with a clean surface located.

The microscopic investigation testified: the ovary capsule is desmogenous, fibroid. In separate departments it is more rich in fibrous structures, in others – vice versa – in cellular fibrocyte- and fibroblast-, tissular lymphocyte- and histiocyte-type elements, with commonly defined, but faintly manifested phenomena of their atypism. In some visual fields on the fibroid capsule there were lipocytes with the coarsened stroma and extensive network of blood channels.

The ovary tissue is rather compact and vasculated, with a greater number of maturing follicles and starting forming white bodies. Fine serosal and

mucic cysts inlaid with one-row cubical and pavement epithelium were noticed. In the cytoplasm of the last cellular formations fine vacuoles, mainly in their apical departments, were seen. Blood vessels are thin-walled, filled with blood unevenly. The overgrown yellow body vaguely divided from the cortical layer of the organ consisted of loosely disposed polygonal cells with foamed and honeycombed cytoplasm rich in glycolipoproteids. The cores – are large, roundish, rich in chromatin. The central region of the yellow body – is ectatic and filled with bloody masses with haemolysis occurrences and without it.

On the internal surface of the serous retention cyst fibroid capsule there was a middlenoded fibromatous formation up to 20 mm in diameter – a dense, hard fibroma rich in fiber structures, with dystrophic changes up to sclerosis and hyalinosis, with a decreased content of cellular elements and vessels. The atypism of the above listed cellular-fiber structures was manifested insignificantly.

In the other segments of the cyst wall there were elements of secerning cilioepithelial cyst (adenocystoma) represented by a papillary excrescence with a soft, faintly fibrous stroma. On its basic membrane there was a one-row cubical epithelium with small apically disposed vacuoles filled with serous fluid. In separate regions the epithelial coating had a two-row structure; the papillary stroma contained a rather developed blood supply. The perivascular fiber structures – are expanded, with ajar venules and arterioles.

In separate high-power fields there were small, so-called “chocolate cysts” of incomplete genesis – organizing and being organized hemorrhages into the serous daughter cyst, and also structural elements of dermo-affinity: osteoid, chondroid tissues and skin derivatives’ anlagen – hair follicles.

Thus, the time protracted ovarian cystadenomas can reach more than considerable sizes and become very rare findings of operating and sectional tables. The epithelial cells of even encapsulated forms of these space-occupying lesions are able to penetrate with the thoracic cavity by hematogenous and lymphogenous ways, implant there and develop to the sizes analogous to the mother formation. The only analogous to the mentioned one case is described by one of us earlier (Razin P.S., Razin A.P., 2000). In the comprehensible literature about the possibility of serous oothecomas spread there were single mentions in the context: “Non-encapsulated variants of serous tumors are able to spread through the peritoneum” (Paltsev M.A., Anichkov N.M., 2001). Clinically, such multilocular ovarian cystadenomas without malignant change occurrence are able to simulate a large variety of diseases, the inclusive of cancerous ones.

The last of the observations represented by us differs by the combination of a large-sized serous cyst and with the regions of adenocystoma and teratoma – a dermoid tumor including various kinds of tissues (osteoid, chondroid and skin derivatives), being a

congenital abnormality. The neoformation is defined by us as a mixed ovarian benign neoplasm, one component of which is represented by a cyst with neoplasm growth from the germinal epithelium (a cystadenoma), another one – by a benign neoplasm of the connective tissue (a fibroma), a third one is of herminogenous genesis (a teratoma in the form of a dermoid cyst containing three tissue types). Taking into account the patient’s syndactylia, which is a congenital malformation as well, there are good reasons to speak about the syndrome of multiple defects, which includes the congenital abnormality of ovaries and bony frame.

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The work is submitted to the Scientific International Conference “Scientific Research of Higher School on Priority Orientations of Science and Technology”, May, 6-16, 2008, Savona – Hamburg, came to the editorial office on 12.02.2008.

ANTITUMOUR EFFECT OF PLANT PEPTIDE EXTRACT PE-PM: PRELIMINARY *IN VIVO* TESTINGS IN MOUSE MODELS OF BREAST CANCER

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Breast cancer (BC) is mostly common among female malignancies. Multistage mechanisms of aetiology and pathogenesis of this disease comprise numerous molecular interactions. Thus, combined therapies are expected more effective against BC. Complementary phytotherapy after radical mastectomy is currently under clinical trial. Moreover, plants are well known resources of new chemotherapeutics. For instance, many currently used drugs were obtained from plants, at least initially (taxol, oncovin *etc.*). Recently discovered anti-cancer properties of plant cyclotides challenged us to search for new potent plant peptides against BC. Therefore, we created data base and analyzed information (including in Russian) about plants with anti-cancer properties used in folk medicine. We isolated a peptide extract – plant mixture (PE-PM) from the mixture of the plants: *Chelidonium majus* L., *Inula helenium* L., *Equisetum arvense* L., and *Inonotus obliquus*. The extraction was performed according to the method described earlier; peptide fractions were characterized by Ion exchange -HPLC, Matrix-Assisted Laser Desorption / Ionization spectrometry (MALDI) and amino acid analysis. No data were available concerning the influence of PE-PM on mammary adenocarcinoma (MAC) growth *in vitro*.

The aim of this study was to reveal local and/or systemic anti-cancer effect of PE-PM *in vivo* using original panel of mouse models that reproduced a number of morphological forms of mammary cancer in human and veterinary patients (especially MAC in cat). Four tests called “point” experiments (5-6 mice of the same sex and similar age *per* group) were performed using transplanted and spontaneous mouse models and local application of the peptide. The mouse models differed in (1) time of drug application in relation to a tumour growth stage (in start model – next day after tumour transplantation or spontaneous tumour detection, and in therapeutic model – application to advanced tumour burden); (2) site of tumour transplantation (sub cutaneous, s.c. or intra peritoneal, i.p. models); (3) an extent of transplanted tumour lethality for the untreated control group (lethal and sublethal models); (4) rate of palpable tumour appearance before their visible manifestation (lag⁺ models with at least two week lasted latent palpable period and lag⁻ models with the absence of a palpable period); and (5)

tumour growth rate after visible tumour manifestation (standard and fast growth). PE-PM (10 mg in 1 ml of physiologic solution) was applied locally (s.c. around a tumour burden in s.c. model and i.p. in i.p. model). Tumours in control mice were injected with physiologic solution alone at the same time and manner. Tumour growth delay and improved survival of treated mice in comparison to the parameters in the control mice showed local and systemic anti-cancer effects, respectively.

A local effect of a single PE-PM injection was firstly probed in a lethal transplanted s.c. start model of slowly appearing lag⁺ MAC of the CBRB females. Earlier this model was shown to reproduce invasive lobular adenocarcinoma in human and veterinary patients. We propose a start mouse model to replicate the situation after radical mastectomy in patient: massive tumour burden removed but residual tumour cells may persist leading to local and/or systemic recurrence later. Need of an additional local treatment is evident as a distinct proportion of BC patients with local relapses require secondary operations. A long lasting palpable tumour growth period in lag⁺ MAC allows revealing even a weak local effect of the testing drug on tumour growth. Moreover, local application of the drug provides detecting both direct and indirect (via immune system) anti-cancer effects. Here, on day 0 CBRB females were s.c. injected to right axillary fat pad with 10⁶ tumour cells obtained from spontaneous slowly growing syngeneic MAC. On day 1 mice were locally treated with 0.1ml PE-PM solution *per* mouse. The local PE-PM effect was monitored for four weeks *post* transplantation (*pt*) by delay in palpable tumour appearance and growth rate in treated mice comparing with those parameters in control mice. As a result, only 67% of treated mice demonstrated tumour palpable nodules at the second week *pt* versus 100% of females in the control group. Significant tumour growth delay was observed on the forth week *pt* as treated animals demonstrated smaller nodules (25% on average) than ones in control mice. Finally, we showed that even a single local PE-PM application to slowly appearing lag⁺ palpable CBRB MAC resulted in prolonged local anti-cancer effect.

As a next step, sublethal i.p. transplanted slowly growing BLRB MAC was used as a model to test anti-cancer effect of a single i.p. PE-PM application. This *in vivo* model is a prototype of *in vitro* test as both drug and tumour cells are interacting at the same location during a long period of time. Moreover, presence of immune components (lymphocytes, macrophages, neutrophils, and mast cells) in peritoneal cavity of tumour bearing host may facilitate indirect drug effect manifestation/s. Furthermore, highly expressed direct and/or indirect anti-cancer effect may cause tumour grafting and growth prevention in treated animals as solid i.p. MAC growth is normally observed in only about half of untreated animals in sublethal model. Here we obtained TC from slowly

growing spontaneous BLRB MAC, transplanted them i.p. to syngenic male recipients using dosage of 10^6 TC *per* mouse. Next day experimental mice received single i.p. injection of PE-PM solution (0.1 ml *per* mouse). Anti-cancer peptide activity was estimated locally by delay of palpable i.p. solid MAC appearance (until the 13th week *pt*) in treated mice and reduced proportion of treated recipients with tumour manifestation (20% versus 57% in control, 16th week *pt*). This implies prevention of tumour grafting and growth in 57-20=37% of treated animals. As a result, survival of treated recipients was improved significantly as they all were alive at the 16th week *pt* when only 72% control recipients survived. Therefore, single local PE-PM treatment caused long term local and systemic anti-tumour effect in transplanted sublethal i.p. slowly growing BLRB mouse model.

As PE-PM exhibited significant anti-cancer effect in slowly appearing and growing transplanted models we further tested it's activity in fast tumour growing model using lethal transplantable s.c. mammary carcinoma in BALB/cJCitMoise (B/c) females and multiple PE-PM local injections. This model characterized by aggressive growth with visible metastatic foci in the lungs. Here syngeneic B/c females received 10^6 TC *per* mouse at day 0. Local PE-PM treatments were provided at days 1, 3, 5, 7, 9 *pt* in a dosage of 0, 1 ml PE-PM solution *per* mouse *per* injection. Multiple treatments resulted in significant s.c. tumour growth delay (starting from the second week *pt*) and survival improvement. All control animals died at the day 44 *pt* whereas 80% of treated recipients survived. Gross morphology examination *post mortem* showed in PE-PM treated animals less visible metastatic foci in the lungs and three fold increased spleen weight comparing with these parameters in control animals. As a result, multiple PE-PM local treatments exhibited significant long term local and systemic anticancer effect in fast growing s.c. BLRB model, probably due to the metastasis spread postponement and immune component involved in the mechanism(s) of action.

As anticancer activity of PE-PM was clearly revealed in both slowly and fast growing transplanted s.c. and i.p. mouse models of human BC we further tested whether PE-PM affect spontaneous MAC growth in females of the BLRB-Rb(8.17)1Iem strain (BLRB, about 90% MAC and/or about 5-10% T-lymphoma/leucosis). It is known to distinguish in this model: (1) lag⁻ and lag⁺ tumours before visible tumour manifestation, latter exhibit distinct palpable latent period during at least during two weeks (morphology of well differentiated MAC, similarly to lobular carcinoma of the human and veterinary patients) and (2) fast and slowly growing tumours after visible tumour manifestation (as a rule, latter are morphologically characterized by the absence of cystic tumour structures, which are common in fast growing mouse MAC, similarly to MAC in the cat). Average tumour

growth rate dynamic of 10-20 spontaneous tumours represents "normal" tumour growth rate curve. Tumour growth above this curve is considered to be fast. Information about tumour morphology, growth rate, and survival of some hundreds females with lag⁻ and lag⁺ (about 1/3 of all MAC) tumours is accumulated in our data base. This information was used as historic control data to analyse tumour growth rate and survival of treated mice. According to earlier described approach, five females aging 12 ± 2 months with spontaneous MAC of various tumour growth steps without associated lymphoma/leucosis were selected to test PE-PM activity. At the therapy start two females exhibited advanced lag⁻ tumours growing fast during the third week after visible tumour manifestation (lag⁻ therapeutic model, tumour diameter 11.9 ± 0.5 mm) and three females had palpable tumours at the first detection (lag⁺ start model, tumour diameter 2.0 ± 0.2 mm). Those five females were locally treated weekly, seven PE-PM applications. Peptide dosages were dependent on tumour size: 0.1 ml of PE-PM solution was locally applied to tumours up to 5mm of diameter, above this size, 0.1 ml of peptide solution *per* each 5mm of tumour diameter was additionally applied each time. Fast initial tumour growth was detected in therapeutic model before PE-PM application. Therefore, tumour growth rate in treated females was compared with average growth dynamic of fast growing historical controls. Multiple PE-PM injections turned initially fast tumour growth down significantly (tumour diameter of 6, 9, 12, 13, 14, 16, 18, 20 mm at weeks 1, 2, 3, 4, 5, 6, 7, 8, respectively) comparing with tumour growth in historical control (tumour diameter 6, 9, 12, 15, 18, 21 mm at weeks 1, 2, 3, 4, 5, 6, respectively). Tumour growth delay in treated females resulted in two fold prolonged survival comparing with survival (6.0 ± 0.2 weeks) of untreated control females bearing similar lag⁻ fast growing MAC. In start lag⁺ model therapy was applied to palpable tumours at the time of the first detection; so, information of initial tumour growth speed was not available. Palpable latent period of tumour growth in PE-PM treated females seemed to be not prolonged. Average tumour growth dynamics of treated tumours after visible tumour manifestation and their average survival time were compared with "normal" tumour growth curve and average survival of historic controls. No differences were detected in nor tumour growth rates and survival. Thus, multiple local PE-PM applications appeared to be less effective in lag⁺ spontaneous start model in comparison to promising results in lag⁻ advanced tumours with fast tumour growth rate.

Data obtained and conclusion made here remind in a way of our wealth experience with local application of immunotherapy by interleukin-2 to treat spontaneous mammary cancer, where therapy benefit was strongly dependent on initial tumour growth rate and time of application in both transplanted and spontaneous mouse models. Together with previously pub-

lished data this may indicate a presence of indirect immune component in a mechanism of PE-PM action. Immunotherapeutic approaches are well known to be tightly dependent on a step of tumour-host interactions and, therefore, to be beneficial for recipients only within distinct period of time. Consequently, immunotherapy would not be worthwhile to apply before or later, as it is appeared here with PE-PM application. In conclusion, both local and systemic effect of multiple PE-PM local applications to advanced mammary tumours by mounting dosages with tumour diameter increasing was detected in fast growing lag⁻ spontaneous BLRB model (morphologically resembling cystic papillary and/or medullary carcinoma of the human and veterinary patients).

“Point” experiment approach proposed here during initial steps of anti-cancer PE-PM efficacy testing procedure *in vivo* revealed mono-directed effect, i.e. tumour growth delay and survival prolongation in all mouse models (CBRB, BLRB and BALB/cJCitMoise). And the amount of used animals is sufficiently decreased.

Finally, our data permit to recommend PE-PM for extensive anti-cancer drug testing and to hope that this kind of therapy would be of benefit for local application after radical mastectomy.

The work was submitted to V international scientific conference «Present-day problems of experimental and clinical medicine», Thailand, February, 20-28, 2008, came to the editorial office 20.12.2007.

METABOLIC DISORDERS IN ELDERLY DIABETES PATIENTS

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The purpose of the work is studying biochemical factors of blood against metabolic syndrome in diabetes patients – elderly men and women.

The object of the investigation was the blood of 20 diabetes patients (the average age of the men was 61, 7±1, 5 years; women - 63, 1±1, 3 years), the disease duration was from 7 to 34 years. As the control the blood of 20 practically healthy donors matching in sex and age was used.

The quantity of leucocytes, the concentration of glucose, total protein, cholesterol (CL), triacylglycerols (TG), low density lipoproteins (LDLP), high density lipoproteins (HDLP), urea, creatinine, also amylase, alanine aminotransferase (ALT), aspartate aminotransferase (AST) were determined in the blood by the unified methods applied in clinical laboratory diagnostics. The concentration of Na⁺, K⁺, Cl⁻, Ca²⁺, Mg²⁺ was determined by the method of flame atomic absorption spectroscopy (Quantum-2A, Russia). The WBC differential was developed; the intoxication leukocytic index (ILI) and allergization index (AI) were calculated.

In diabetes men-patients the glucose concentration in blood made 11, 6±1, 3 mmol/l⁻¹, women-patients - 9, 4±0, 7 mmol/l⁻¹, that is authentically higher than in the control group donors. In the diabetes men-patients' blood the concentration of CL (by 72, 7%), TG (by 43, 1%), LDLP (by 55, 4%) and K⁺ (by 19, 4%) is authentically higher and the concentration of HDLP (by 14, 2%), Na⁺ (by 6, 5%), Cl⁻ (by 5, 5%), Mg²⁺ (by 20, 6%) and Ca²⁺ (by 57, 6%) is lower. In the diabetes women-patients the content of CL (by 70, 1%), TG (by 44, 4%), LDLP (by 63, 0%) and K⁺ (by 8, 7%) is also authentically higher, and that of HDLP (by 17, 4%), Na⁺ (by 5, 9%), Cl⁻ (by 4, 2%), Mg²⁺ (by 17, 6%) and Ca²⁺ (by 68, 9%) is lower.

As a tendency one can consider the amount decrease of amylase, ALT and the increase of creatinine, AST by 16, 0% and 6, 3%; 5, 4 and 15, 7; 6, 3 and 13, 1; 14, 9% and 54, 5% accordingly in men and women, in the blood of diabetes patients.

In the examined healthy people and diabetes men- and women-patients the average ILI values correspond to a light form of endointoxication, the AI values in the diabetes patients are authentically higher and reflect the presence of an allergic process in them.

Thus, hyperglycemia, provoking serious metabolic disorders, retains against the insulinic therapy in elderly men and women suffering from diabetes; a light form of endointoxication and the presence of allergic process have been detected.

The work was submitted to VII international scientific conference «Scientific potential development of higher school», UAE, March, 4-11, 2008, came to the editorial office 14.01.2008.

Short reports

**REVIEW OF "WHAT BUGGED THE
DINOSAURS? INSECTS, DISEASE AND DEATH
IN THE CRETACEOUS" BY POINAR G. JR.
AND POINAR R.**

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Book details

Poinar G Jr, Poinar R: What bugged the dinosaurs? In *Insects, Disease and Death in the Cretaceous* Princeton University Press; 2008. 264 pages. ISBN 978-0691124310

Review

Have you ever wondered whatever happened to the dinosaurs? George and Roberta Poinar have put forward some evidence that maybe it was not just cataclysmic events, such as meteorites falling on the earth. They surmise that perhaps insects transmitted diseases that contributed to the extinction of the dinosaurs. By studying the arthropods trapped in amber during the Cretaceous (65.5 –145.5 million years ago) period, they have revealed some extraordinary micro-organisms concomitant with the ensnared invertebrates.

The period is well described in the opening chapters, showing that fossil evidence and especially amber tells us a great deal about the animal and plant kingdoms during those millions of years. Some chapters start with a speculative scene, painting a picture of life in the Cretaceous, the dinosaurs, the plants they feed from and the insects that breed around them, while others discuss in detail the known scientific facts. Herbivory, both by the dinosaurs and the insects is described in detail and the possibility that insects introduced plant viruses and fungi into the food supply, which may have led to the depletion in resources for the large animals. The dinosaurs did benefit from insects, like the dung beetles that removed the vast waste voided by 55–100 ton dinosaurs, and arthropods were part of the diet of the omnivores.

The authors describe how they believe that arthropods were able to acquire blood meals from the dinosaurs in antiquity. By studying the mouth parts of the insects trapped in amber, they have shown that regardless of the outer skin, whether cold or warm blooded, the micro-predators had found a way to obtain the necessary food for survival. Chapters 12 – 18 describe those blood-sucking arthropods that were extant during the Cretaceous, including, important Nematocera and Tabanids, fleas, lice, ticks and mites. For each group the method of haematophagy is discussed and which organisms could have been transmitted with a few examples of ancient parasites observed in amber. There are separate chapters on the worms, cretaceous diseases, and another on the evolution of pathogens, (erroneously Rickettsia are given as

the cause of human plague). The numerous color plates illustrate the diversity of arthropods in the Cretaceous, while the original line drawings embellish the theory. This is an assiduously written book for entomologists and parasitologists who would like to learn more on the time-encapsulated data from the Cretaceous, and perhaps stimulate the search for more "paleoparasites".

**MUSCULO-ELASTIC COMPLEX IN THE
VALVES OF HUMAN FEMORAL VEIN**

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Condition of the problem

Venous valves are often become an object of researches in normal conditions, in experiments and in pathology [2, 3, 5]. The absence of myocytes in cusps of the venous valves is accepted as correct until now. Therefore they are opening and closing only passively. Solitary muscular elements may occur in thickened subendothelial layer of cusp during the intima proliferation [1]. However, last years the data that valvar cusps contain the smooth myocytes were observed [4].

Material and methods

The work was carried out on both sexes human cadaveres of 20-78 years old. Valves were chosen from the walls of human femoral vein isolately or with the adjacent part of venous wall. Serial histologic sections of 5-7 mkm in thickness in sagittal (from base to tip of the valve) and transverse planes (in plane of the stretched cusp). Sections were stained by picrofuxine, azane, hematoxylin-Fe, orseinum, impregnated by Argentum. The stained by hallocianinum and hematoxylin-Fe total preparations were made from the part of material.

Results

The femoral vein contains 1-5 valves, constant valve situates under the entry of deep femoral vein. Valves constitute the circular folds of inner layers of the venous wall. Internal elastic membrane from the distal segment of vein continues to the axial sector of valvar cusp where it gives branches of different thickness to the parietal sector of cusp. Internal elastic membrane from the proximal segment of vein are loosening in the base of valve, its fragments are determined in the parietal sector of cusp. Transverse muscular bundles and folded bundles of thick collagen fibers prevail here as in the whole cusp. The bulge of the external coat pushes the circular muscular layer of middle coat inside. It conflues with the longitudinal muscular layer of intima. The compact accumulation of myocytes arises in the base of valve – multi-layer circular muscle of valvar cuff. It has the configu-

ration as a parabole, its branches (lateral segments of valvar cuff) separate and grow together by their ends with the same branches of other valvar cuff – comisures of valvar cusps. The longitudinal muscular bundles plating into muscular coat of the proximal (post-valvar) segment of the vein originate from them. Muscular bundles with longitudinal orientation from intima of its distal segment reach the base of valve and divide into branches, which enter usually into one of the cusps, into lateral segments of valvar cusp and into a cusp, usually in axial sector. Circular muscular bundles cross both cuff and cusp, suspending the valve to middle coat of the venous wall. In passage from cuff

to cusp the number and sizes of myocytes as and connective tissue fibers greatly decrease. The continous multilayer muscular stratum transforms to unilayer muscular network which loosens in direction to the free border of cusp with thinning of muscular bundles. They become straight and parallel to the free border of cusp. There are much oblique muscular bundles in the middle part of a cusp passing along lateral segment of valvar cuff and crossing near it's central segment. They form muscular “cupola” above the thin marginal part of cusp, the similar is formed by bundles of collagen and elastic fibres.

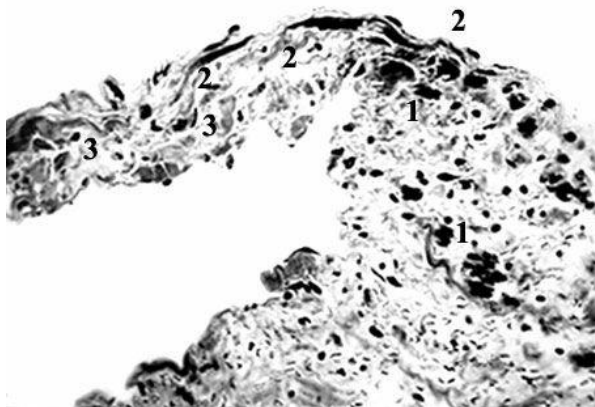


Fig. 1. Valve of the human femoral vein, longitudinal section of the vein: 1 – transverse myocytes in valvar cuff; 2 – longitudinal muscular bundle from valvar cuff enters into the cusp (axial sector); 3 – myocytes in parietal sector of cusp. Picrofuxine. x 350.



Fig. 2. Valve of the human femoral vein, longitudinal section of the vein: 1 – inner elastic membrane from valvar cuff continues into cusp (axial sector); 2 – its fragment in parietal sector of cusp. Orseinum. x 350.

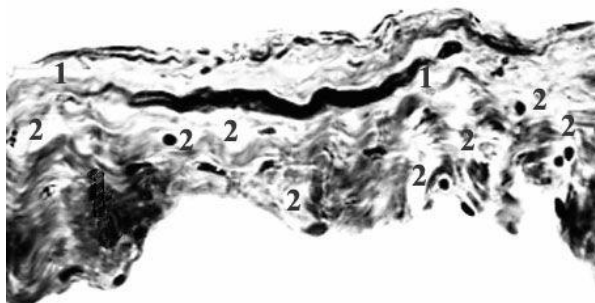


Fig. 3. Cusp of valve of the human femoral vein, longitudinal section: 1 – longitudinal bundle of myocytes in axial sector; 2 – folded bundles of collagen fibres and transverse myocytes in parietal sector. Picrofuxine. x 600.

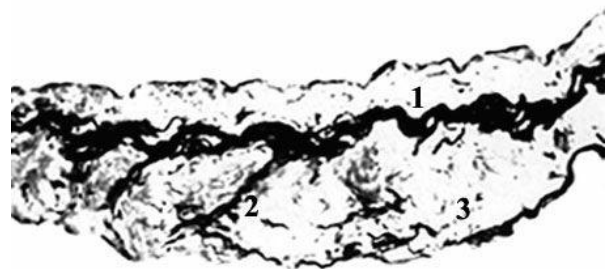


Fig. 4. Cusp of valve of the human femoral vein, longitudinal section: 1 – inner elastic membrane in axial sector; 2 – its branches into thickness of cusp and 3 – fragments in parietal sector.

Conclusion

Valves of femoral vein contain smooth myocytes and elastic fibres. Their fixed parietal part (valvar cuff) counteracts to blood flow by thickening and compacting, concentrating and interlacing of structures. The free luminal part of a valve (valvar cusp) is moveable and can reacts on the blood pressure by displacement and distortion. It is possible to explain the dispersion and reduction of structures, including myocytes, in the direction from base to free border of the valve by the decreasing of the load. Changeable haemodynamic determines the asymmetrical distribution of structures in thickness of a valve: longitudinal shocks of direct blood flow stimulate the development of musculo-elastic complex (amortiser) and longitudinal structures in the axial sector; vortical indirect blood flow extending the valvar sinuses causes the preferred morphogenesis of transverse structures in the parietal sector of cusp including muscular bundles and reserve folds in the bundles of low extensible collagen fibres. The received data allow to suppose that venous valves are able to active counteract to pressure of direct and indirect blood flow due to the complexation of connective tissue fibers with proper muscular

structures of valvar cusps. They increase the durability of valvar cusp and determine its ability for blood flow regulation.

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Materials of conferences

SOLUTION OF THE MAIN MIXED PROBLEM OF THE ELASTICITY THEORY FOR A HALF-PLANE WITH CURVED BOUNDARY

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Let us analyze semi-infinite Region S in Plane Z .

The Function, mapping the lower half-plane $\text{Im}Z < 0$ onto the investigated Region S , is accepted in the form of a polynomial, as it was done in the work [1]:

$$z = \omega(\zeta) = A + B\zeta - \sum_{k=0}^n \frac{c_{2k+1}}{(\zeta + a - bi)^{2k+1}} \quad (1)$$

where $z = x + iy$, $\zeta = \xi + i\eta$, $\eta < 0$, $A, B, c_1, c_3, \dots, c_{2n+1}, a, b > 0$ are real numbers.

Let us assign the boundary values of Displacement Component $g_1(t)$ and $g_2(t)$ at Segment L' of Boundary L of Region S , occupied by an elastic body, and at the rest Segment L'' – boundary values of Stress Component $N(t)$ and $T(t)$. It is necessary to determine the stressed state of Region S .

Along with the given problem we will analyze the problem of stressed state determination, presuming that the main external force vectors (X_k, Y_k) , $k = 1, \dots, n$ are assigned, which are applied to every Segment L_k separately.

We will refer to the above-formulated problems as problems A and B , according to [2]. Thus to obtain a common solution of the given problems let us use the methods developed by N.I. Muskhelishvili [2], as well as in the paper of I.N. Kartsvadze [3]. Let us extend here the approach of the author of paper [3] to the case of a half-plane, subjected to conformal mapping.

As it is known [2], the formulas, expressing the displacement and stress components through Functions $\Phi(\zeta)$ and $\Psi(\zeta)$ of Complex Variable ζ take the form of

$$Y_\eta + X_\xi = 2\{\Phi(\zeta) + \overline{\Phi(\zeta)}\} = 4 \text{Re } \Phi(\zeta), \quad (2)$$

$$Y_\eta - X_\xi + 2iX_\eta = \frac{2}{\omega'(\zeta)} \{\overline{\omega(\zeta)}\Phi'(\zeta) + \omega'(\zeta)\Psi(\zeta)\}, \quad (3)$$

Summing up the left and right parts of these equations, and, then proceeding to conjugate values, we get

$$Y_\eta - iX_\eta = \Phi(\zeta) + \overline{\Phi(\zeta)} + \frac{1}{\omega'(\zeta)} \{\omega(\zeta)\overline{\Phi'(\zeta)} + \overline{\omega'(\zeta)}\overline{\Psi(\zeta)}\} \quad (4)$$

The formula, expressing Displacement Components u and v in Cartesian coordinates, is given by

$$2\mu(u + iv) = \Re \varphi(\zeta) - \omega(\zeta)\overline{\Phi(\zeta)} - \overline{\psi(\zeta)}, \quad (5)$$

where: $\varphi'(\zeta) = \Phi(\zeta)\omega'(\zeta)$, $\psi'(\zeta) = \Psi(\zeta)\omega'(\zeta)$.

If Functions $\varphi(\zeta)$ and $\psi(\zeta)$ are assigned, then Functions $\Phi(\zeta)$ and $\Psi(\zeta)$ are entirely determined. And in case Functions $\Phi(\zeta)$ and $\Psi(\zeta)$ are assigned, Functions $\varphi(\zeta)$ and $\psi(\zeta)$ are determined with accuracy to arbitrary constants. Therefore Eq.(5) may be written in the form of

$$2\mu(u + iv) = \Re \varphi(\zeta) - \omega(\zeta)\overline{\Phi(\zeta)} - \overline{\psi(\zeta)} + \text{const}. \quad (6)$$

Assuming now, that $\omega(\zeta)$ is a rational function, let us apply Function $\Phi(\zeta)$ determination to Region $\text{Im}\zeta > 0$, supposing that

$$\omega'(\zeta)\Phi(\zeta) = -\omega'(\zeta)\overline{\Phi(\zeta)} - \omega(\zeta)\overline{\Phi'(\zeta)} - \overline{\omega'(\zeta)}\overline{\Psi(\zeta)}, \quad (7)$$

where: ξ lies in the upper half-plane.

This operation must be carried out so that the values of Function $\Phi(\zeta)$, determined in the upper half-plane, can be analytically extended to the lower half-plane through the unoccupied segments of boundaries (if there are any).

Taking into account (7), having changed ζ for $\bar{\zeta}$ and proceeded to conjugate values, we obtain

$$\begin{aligned}\omega'(\zeta)\Psi(\zeta) &= -\overline{\omega'(\zeta)}\overline{\Phi(\zeta)} - \overline{\omega(\zeta)}\Phi'(\zeta) - \overline{\omega'(\zeta)}\Phi(\zeta) = \\ &= -\overline{\omega'(\zeta)}\{\Phi(\zeta) + \overline{\Phi(\zeta)}\} - \overline{\omega(\zeta)}\Phi'(\zeta).\end{aligned}\quad (8)$$

Eq. (8) expresses the values of $\Psi(\zeta)$ for the lower half-plane through values of $\Phi(\zeta)$, for the lower half-plane, as well as for the upper half-plane.

The determination of Function $\varphi(\zeta)$ can be extended to the upper half-plane, provided that the relation holds in the upper half-plane

$$\varphi(\zeta) = \int \Phi(\zeta)\omega'(\zeta)d\zeta.$$

Integrating both parts of expression (7) over ξ , and, dropping the arbitrary constant value, we have

$$\varphi(\zeta) = -\overline{\Phi(\zeta)}\omega(\zeta) - \overline{\psi(\zeta)}\quad (9)$$

where: ξ lies in the upper half-plane.

Similarly,

$$\psi(\zeta) = -\Phi(\zeta)\overline{\omega(\zeta)} - \overline{\varphi(\zeta)}\quad (10)$$

where: ξ lies in the lower half-plane.

Thus, Stress and Displacement Components can be expressed through one and the same Function $\Phi(\zeta)$, determined in the lower half-plane, as well as for the upper half-plane.

To fulfill further solution it is necessary to employ Expression (2) and Expression (4), taken in the form of:

$$Y_\eta - iX_\eta = \Phi(\zeta) - \Phi(\bar{\zeta}) + \left\{ \frac{\omega(\zeta)}{\omega'(\zeta)} - \frac{\omega(\bar{\zeta})}{\omega'(\bar{\zeta})} \right\} \overline{\Phi'(\zeta)} + \left\{ \frac{\overline{\omega'(\zeta)}}{\omega'(\zeta)} - \frac{\overline{\omega'(\bar{\zeta})}}{\omega'(\bar{\zeta})} \right\} \overline{\Psi(\zeta)},\quad (11)$$

It is necessary to explain that Expression (11) is obtained after Function $\Phi(\bar{\zeta})$ is subtracted from the right part of (4) and after the same function replaced with its expression obtained from Eq. (8) after having proceeded to conjugate values is added there. Let us notice that the expression determined by Eq. (8) is meant by Function $\Psi(\zeta)$.

For Displacement Components u and v , replacing $\psi(\zeta)$ in Eq. (6) with expression (10), we obtain the following relation

$$2\mu(u + iv) = \aleph \varphi(\zeta) + \varphi(\bar{\zeta}) - \{\omega(\zeta) - \omega(\bar{\zeta})\}\overline{\Phi(\zeta)} + const.$$

From this point on we will also need the expression for $u' + iv'$, where,

$$u' = \frac{\partial u}{\partial \xi}, \quad v' = \frac{\partial v}{\partial \xi}.$$

It can be obtained if we differentiate both parts of Eq. (5) over ξ . Then we have

$$2\mu(u' + iv') = \aleph \omega'(\zeta)\Phi(\zeta) - \omega'(\zeta)\overline{\Phi(\zeta)} - \omega(\zeta)\overline{\Phi'(\zeta)} - \overline{\omega'(\zeta)}\Psi(\zeta).$$

Having added Function $\omega'(\zeta)\Phi(\bar{\zeta})$ to the right part of above relation and subtracted the same Function, replaced with its expression, obtained from Eq. (8) after proceeding to conjugate values, we have

$$2\mu(u' + iv') = \omega'(\zeta)\{\aleph \Phi(\zeta) + \Phi(\bar{\zeta})\} - \omega'(\zeta)\left\{ \frac{\omega(\zeta)}{\omega'(\zeta)} - \frac{\omega(\bar{\zeta})}{\omega'(\bar{\zeta})} \right\} \overline{\Phi'(\zeta)} -$$

$$-\omega'(\zeta)\overline{\omega'(\zeta)}\left\{\frac{1}{\omega'(\zeta)}-\frac{1}{\overline{\omega'(\zeta)}}\right\}\overline{\Psi(\zeta)}.$$
(12)

Let us note that both of Functions $\Phi(\zeta)$ and $\Psi(\zeta)$ are holomorphic in the lower half-plane.

Now it is necessary to ascertain the behaviour of Function $\Phi(\zeta)$, extended by Eq. (7) to the upper half-plane. To do that it is enough to analyze the behaviour of Product $\Phi(\zeta)\omega'(\zeta) = \varphi'(\zeta)$, referring to Eq. (7) which determines Function $\Phi(\zeta)$ when $\text{Im}\zeta > 0$.

Now let us proceed to the analysis of problems A and B.

Let us denote the points of Real Axis $O\xi$ which correspond to the points a_k, b_k of the Cutout L as α_k, β_k , a segment of a real line which corresponds to L' as $O\xi'$, and the rest segment of the line as $O\xi''$.

As we know [1] the solution of the first main problem, it is more convenient to take account of the influence of the forces assigned at Segment L'' separately. Consequently, it can be supposed that Segment L' of Boundary L is free from external stresses.

Then, based on Eqs. (11) and (12) the boundary conditions in both problems are given by

$$\Phi^+(t) - \Phi^-(t) = 0 \quad \text{на } L'',$$
(13)

$$[\omega'(t)\Phi(t)]^+ + \aleph[\omega'(t)\Phi(t)]^- = 2\mu g'(t) \quad \text{на } L'$$
(14)

Eq. (13) proves that Segment $O\xi''$ of the real axis is not saltus function for Function $\Phi(\zeta)$, i.e. that Function $\Phi(\zeta)$ is a holomorphic one on a cut-down plane $O\xi'$ except for finite number of points where it may have poles. The same refers to Function $\omega'(\zeta)\Phi(\zeta)$.

To determine it, let us refer to Eq. (14) representing a boundary condition of the problem well-known in the theory of functions of a complex variable, which is Riemann problem or Gilbert problem. It is completely investigated in the works of N.I. Muskhelishvili [4], F.D. Gakhov [5] and other authors. N.I. Muskhelishvili calls it "boundary value problem of linear conjugation", or "conjugate problem" for short.

Assuming that Function $\omega'(\zeta)\Phi(\zeta)$ may have poles of order not higher than m_1, m_2, \dots, m_l, m in Points $\zeta_1, \zeta_2, \dots, \zeta_l, \infty$ and employing the results of the conjugate problem solution [2, p.397], we obtain for the sought function

$$\omega'(\zeta)\Phi(\zeta) = \frac{\mu X_0(\zeta)}{\pi i} \int_{L'} \frac{g'(t)}{X_0(t)(t-\zeta)} dt + X_0(\zeta)R(\zeta),$$
(15)

where:

$$X_0(\zeta) = \prod_{j=1}^r (\zeta - a_j)^{-\gamma} (\zeta - b_j)^{\gamma-1}, \quad \gamma = \frac{1}{2} - \beta i, \quad \beta = \frac{\ln \aleph}{2\pi}.$$
(16)

with $X_0(\zeta)$ implying such a branch that $\lim_{\zeta \rightarrow \infty} \zeta^n X_0(\zeta) = 1$, and $R(\zeta)$ is a rational function of the form

$$R(\zeta) = \sum_{j=1}^l \left\{ \frac{C_{j1}}{(\zeta - \zeta_j)} + \frac{C_{j2}}{(\zeta - \zeta_j)^2} + \dots + \frac{C_{jm_j}}{(\zeta - \zeta_j)^{m_j}} \right\} + P(\zeta),$$
(17)

where: $P(\zeta)$ is a polynomial of degree not higher than $m + r$.

Coefficients included in Expression (17) are determined based on the additional conditions of Problems A and B.

Let us consider an example of a stamping tool with a footing parallel to axis $O\xi$, provided that the stamping tool moves only vertically, so

$$g'(t) = 0 \quad \text{on} \quad L' \quad (18)$$

Besides, suppose that external forces influencing the stamping tool have a resultant force directed vertically downwards, so

$$X = 0, \quad Y = -p, \quad (19)$$

where: P is a positive constant set in advance.

In this case Eqs. (7) and (8) take the form of

$$\begin{aligned} \omega'(\zeta) \Phi(\zeta) = & - \left(B + \sum_{k=0}^n \frac{(2k+1)c_{2k+1}}{(\zeta + a - bi)^{2k+2}} \right) \overline{\Phi}(\zeta) - \left(A + B\zeta - \sum_{k=0}^n \frac{c_{2k+1}}{(\zeta + a - bi)^{2k+1}} \right) \overline{\Phi}'(\zeta) - \\ & - \left(B + \sum_{k=1}^n \frac{(2k+1)c_{2k+1}}{(\zeta + a + bi)^{2k+2}} \right) \overline{\Psi}(\zeta) \quad \text{npu} \quad \zeta \in \text{Im} \zeta > 0, \end{aligned} \quad (20)$$

$$\begin{aligned} \omega'(\zeta) \Psi(\zeta) = & - \left(B + \sum_{k=0}^n \frac{(2k+1)c_{2k+1}}{(\zeta + a + bi)^{2k+2}} \right) \overline{\Phi}(\zeta) - \left(A + B\zeta - \sum_{k=0}^n \frac{c_{2k+1}}{(\zeta + a + bi)^{2k+1}} \right) \Phi'(\zeta) - \\ & - \left(B + \sum_{k=0}^n \frac{(2k+1)c_{2k+1}}{(\zeta + a + bi)^{2k+2}} \right) \Phi(\zeta) \quad \text{npu} \quad \zeta \in \text{Im} \zeta < 0. \end{aligned} \quad (21),$$

as for rational Function (1) there are poles of order $1, 3, 5, \dots, 2n+1$ in Point $\zeta = -a + bi$ and at infinity, and for Function $\omega'(\zeta)$ there are poles of order $2, 4, 6, \dots, 2n+2$ in the same point $\zeta = -a + bi$. Thus, for Function $\omega'(\zeta) \Phi(\zeta)$ there are poles of order not higher than $2, 4, 6, \dots, 2n+2$ in Point $\zeta = -a + bi$. Assuming that $\Phi(\zeta)$ together with $\omega'(\zeta) \Phi(\zeta)$ disappear at infinity, according to (15) and taking into account (18), we obtain

$$\omega'(\zeta) \Phi(\zeta) = \left(C_0 + \frac{D_1}{(\zeta + a - bi)^2} + \frac{D_2}{(\zeta + a - bi)^4} + \dots + \frac{D_n}{(\zeta + a - bi)^{2n+2}} \right) X_0(\zeta) \quad (22)$$

where: D_1, D_2, \dots, D_n are to be determined.

According to (16)

$$X_0(\zeta) = (\zeta + l)^{-\frac{1}{2} + i\beta} (\zeta - l)^{-\frac{1}{2} - i\beta}$$

For large values of $|\zeta|$ it takes the form of

$$X_0(\zeta) = \frac{1}{\zeta} + \frac{\alpha}{\zeta} + \dots, \quad (23)$$

where: $\alpha = 2\beta li$.

Note that Function $\Psi(\zeta)$ corresponding to Function $\Phi(\zeta)$ that is determined both in the lower and in the upper half planes, should be holomorphic in the lower half plane. But this is not the case, because according to (21) it has a pole in Point $\zeta = -a + bi$.

Constants D_1, D_2, \dots, D_n are determined based on the holomorphy of Function $\Psi(\zeta)$ in Point $\zeta = -a + bi$. Denote them as $D_1^*, D_2^*, \dots, D_n^*$.

Now it is necessary to figure out constant value C_0 . Let us remark here that for large values of $|\zeta|$ it follows from (22) and (23) that

$$\omega'(\zeta) \Phi(\zeta) = \frac{C_0}{\zeta} + o\left(\frac{1}{\zeta}\right).$$

On the other side, Function $\Phi(\zeta)$ for large values of $|\zeta|$ [2, p.339] involves

$$\omega'(\zeta)\Phi(\zeta) = -\frac{X+iY}{2\pi} \cdot \frac{1}{\zeta} + o\left(\frac{1}{\zeta}\right),$$

therefore

$$C_0 = -\frac{X+iY}{2\pi} \quad (24)$$

Thus, Function $\omega'(\zeta)\Phi(\zeta)$ that is set by Eq. (22) is completely determined. Applying (19) and taking into account (24) result in

$$\omega'(\zeta)\Phi(\zeta) = \left(\frac{ip}{2\pi} + \frac{D_1^*}{(\zeta+a-bi)^2} + \frac{D_2^*}{(\zeta+a-bi)^4} + \dots + \frac{D_n^*}{(\zeta+a-bi)^{2n+2}} \right) X_0(\zeta). \quad (25)$$

Formulas for estimating pressure $P(t)$ and tangential stress $T(t)$, influencing the body under the stamping tool, are analogous to those of classical case and take the form of

$$\omega'(t)\{P(t) + iT(t)\} = \frac{\aleph+1}{\aleph} [\omega'(t)\Phi(t)] \quad (26)$$

From this, taking into account Eq. (22), it follows that

$$\begin{aligned} \omega'(t)\{P(t) + iT(t)\} = & \frac{\aleph+1}{\aleph} \left(\frac{ip}{2\pi} + \frac{D_1^*}{(t+a-bi)^2} + \frac{D_2^*}{(t+a-bi)^4} + \dots \right. \\ & \left. \dots + \frac{D_n^*}{(t+a-bi)^{2n+2}} \right) (t+l)^{-\frac{1}{2}+i\beta} (t-l)^{-\frac{1}{2}-i\beta}. \end{aligned} \quad (27)$$

Now, separating the real and the complex parts it is possible to obtain formulas for pressure $P(t)$ and tangential stress $T(t)$.

In conclusion let us emphasize the following circumstance: if we assume for Function (1) that $B=1$, and put to zero all the other coefficients, we obtain a function that allows self-reflecting of the half-plane.

In this case Function (25) becomes

$$P(t) + iT(t) = \frac{ip}{2\pi} \frac{\aleph+1}{\aleph} (t+l)^{-\frac{1}{2}+i\beta} (t-l)^{-\frac{1}{2}-i\beta}. \quad (28)$$

From this it is easy to get formulas well-known in mathematical theory of elasticity, which allow solving a classic problem of a stamping tool with a linear horizontal footing

$$\begin{aligned} P(t) &= \frac{p}{\pi\sqrt{l^2-t^2}} \frac{1+\aleph}{\sqrt{\aleph}} \cos \left[\frac{\ln \aleph}{2\pi} \ln \frac{l+t}{l-t} \right], \\ T(t) &= \frac{p}{\pi\sqrt{l^2-t^2}} \frac{1+\aleph}{\sqrt{\aleph}} \sin \left[\frac{\ln \aleph}{2\pi} \ln \frac{l+t}{l-t} \right], \end{aligned} \quad (29)$$

that was obtained independently by V.M. Abramov [6] who used the method of integral transformations and by N.I. Muskhelishvili [2] who applied methods of the theory of functions of a complex variable.

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The work was submitted to III international scientific conference «Basic Research», Dominican Republic, April, 10-20, 2008, came to the editorial office 12.02.2008.

NOISE AT SAW AND WOODWORKING INDUSTRIES IN RUSSIA: FROM THEORY AND EXPERIMENTS TO PRODUCTION SECTOR WANTS

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The noise deteriorating effect on the efficiency of labour and human health is commonly known. In the past decades the noise quieting problem in developed nations of the world, and our country as well, has become a top-ranking one.

The noise effect on the worker's body goes beyond the influence on the organ of hearing only. Hygienists have found out that in some noisy branches the general morbidity rate rises by (10...15)%. It is proved that even the levels of (40...70) dBA have an effect on the vegetative nervous system irrespective of the subjective noise perception of the person. The habituation of the person to noise is illusive, as noise affects even the sleeping person.

The action of noise is often coupled with the effect of other destructive for human health factors: vibrations, irradiations, dust and gas content, etc. It also accentuates the requirements to restrict noise exposure and promotes the untimely retirement benefits.

It must be emphasized that the USSR was the first in the world to initiate the noise attack by law (The Resolution of the Council of Ministers of the USSR 1960, 1969, 1973).

In 1971 the "Sanitary standards of industrial institutions" CH 245 – 71 and "Hygienic standards of sound pressure and volume tolerance levels at work places" GH 1004 – 73 were developed. The Committee of Measures and Active Measuring Instruments at the Council of Ministers of the USSR established a series of Noise National Standards included into the "System of Labour Safety Standards". Nowadays the sanitary norms CH 2.2.4/2.1.8.562 – 96 work, where the noise norm for working places makes 80 dBA on the sound level.

The requirement toughening for noise from 90 dBA (according to CH 245 – 71) up to 80 dBA forced us to work in 3 directions in this problem solution [1 – 6].

1. The investigation of noise, causes and noise making objective laws of active processing equipment as part of processing lines and departments. The saw and woodworking equipment is, as is known, characterized by a high efficiency, at which one has to appoint process speeds from 40 to 100 (and even faster) m/sec with feed velocities up to 150 m/min.

Let us note that the quantity of working tools (saws, shaft arbors) to provide the efficiency achieves the number of 4-10 and more in one machine. For the moment of our works starting (meeting of 60-70s of the past century) there was no slightest hint in the operating equipment and technological designs to any noise reducing solutions in the constructions, working areas, plant and seliteb (by-plant) territories. On the active processing equipment, at the minimal interference with the construction, we developed the devices with due consideration of rather various methods of noise control: the acoustic suppression in the generation places, sound protection and absorption, acoustic shielding and noise localization methods. A noticeable (5...10 dBA) effect was obtained. In some instances we managed to interfere with the mainstream technology as well, when the equipment location was determined by the technology requirements only. The shop drawings of the devices were sent by us in a great amount at the request of numerous enterprises (not only saw and woodworking industries).

The dominant requirement, which would exclude the rejection of antinoise devices by the workers, was sustained by us in the direction of technological capabilities non-reduction of a machine or aggregate.

2. The results of theoretic and experimental research have served the foundation for the leading engineering materials complex development for production workers, design engineers, original equipment (methods, guidance, instructions, drawing albums) manufacturers. All of them have come through the stages of reconciliation with the State design institutes of the branch, production engineering design consultancies, main engineering department on woodworking processing equipment design, Research and development institutes of the branch and original equipment manufacturers, trade union Central Committee of the branch before the approval of the Ministry of Forest Industry. So that the design engineers "were not afraid" of acoustic designs (and it would be a novelty for them), we carried out a week training. The following years of communication with engineering designers testified that necessary theoretical knowledge on noise, methods of design policy for all possible technological situations, types of shop floors and equipment stock, illustrated with plenty of numerical examples, allow them to lead acoustic designs without any difficulties.

With the appearance of data-flow computers we created the computation algorithms and programs

included into 2 instructions on computer-aided acoustic design systems application.

3. The work with the Machine-tool Industry Ministry, its plants and development laboratories promoted the operating equipment modernization positive results shift to the series-produced one.

Let us note that many technological solutions were performed by us taking into account the world's patent novelty. It, naturally, promoted a more active advance of research ideas into production.

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NON-CONTACT INSPECTION OF ROTATING MACHINE PARTS

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Due to safety and economical reasons the interest to diagnostic and monitoring systems is growing rapidly in industry. Besides safety, there are quality control requirements. The greatest attention at machine-building enterprises is paid to quality control of

working surfaces of commutators and contact rings of electric machines [1]. However, the experience shows that the existing quality control is not efficient enough since it is functioning only in steady-state mode with the help of micrometer heads or industrial indicators and does not take into consideration the whole range of centrifugal, vibratory and temperature loads, influencing the commutator in working electric machine.

The majority of existing diagnostic systems are based on vibration diagnostic and they cannot provide information about cross section of rotating part or its surface quality. In order to fulfill these requirements, a diagnostic system ‘MICROCON’ was designed at Tomsk Polytechnic University. This complex is used for non-contact precision control of cross sections of shafts, commutators and contact rings of machines in static and dynamic modes, measurement of linear micro-movements and vibrations of machine parts.

Designed measuring complex has better technical characteristics compare to other systems due to original design of eddy current sensor, patented method of master correction of measurement results and special mathematical processing methods of measurement data. Eddy current sensor of measuring system has a narrow sensitivity diagram which allows differentiate profile levels of commutator plates with tangential dimension of 1.5 mm or wider.

‘MICROCON’ has high protection from external influences (dust, vapor, oil fog, etc.). It allows determination of object micro-movements, specific electric resistances, as well as surface temperature (if temperature via specific electric resistance is known). Non-contact measurement of specific electric resistance is especially important for thin-film structures supervision.

With the help of the designed diagnostic complex and special mathematical processing methods, the unique experimental data concerning the change of commutator profile of high-speed electric machines during the lifetime period, shape and value of bearing vibrations were collected.

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The work was submitted to XIII international scientific conference on Modern Techniques and Technologies, Tomsk, Russia, 2007, came to the editorial office 16.01.2008.

APPLICATION OF MULTIVERSION PROGRAMMING METHODOLOGY TO CONTINUOUSLY DIFFERENTIABLE FUNCTIONS OF SEVERAL VARIABLES

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In modern nonlinear programming the universal methods allowing solving arbitrary problems have not been elaborated yet. It is conditioned by the fact that real problems of minimization usually differ very much from each other both intrinsically and dimensionally. The basic idea of my research is the elaboration of a system connecting all optimization methods into a single one, so that the advantages of both first- and second-order methods speed and direct search methods universality remained. It is obvious that all the optimization algorithms are of the same specification – they get a function and initial point at the entry, and at the output – return the found optimal point. This property allows us to combine them into one *multiversion system*.

The main idea of the *multiversion programming* is in the introduction of software redundancy due to using several various program modules equivalent on the functional purpose (got the name of *multiversions*), working in time parallel and getting the same data at the entry. The multiversion outputs are conformed by means of a particular multiversion voting algorithm. As a result, all the program module versions operate as an organic whole and return one coherent result irrespective of failures and errors of certain modules. Because of its high efficiency the given method has got a wide spread occurrence and development.

Having applied the multiversion programming ideology to the problem of several variables function optimization, we get the system, in which different optimization methods act as multiversions.

Methods of comparison of multiversions against each other:

- by the function value;
- by the search direction at the last step;

The influence of random search algorithms on the general result:

- the general search speed increases;
- there is no search process circling;

The influence of various voting methods on the efficiency of several variables functions optimization multiversion system:

- the overall majority voting method is invalid;
- the coherent majority voting method shows authentically high results;
- the coherent majority ill-defined voting method shows the best results;
- the weighted voting algorithms often end in results' mismatch when using random search methods;

- the median voting results in the optimization process "circling".

As the carried out experiments showed, the multiversion system of several variables functions optimization doesn't lose its efficiency compared to the best of all methods separately, while in some cases with a complex surface character, functions show even more rapid convergence speed.

It allows using the offered elaboration as a universal method of any several variables continuously differentiable functions optimization while solving academic and practical problems.

The work was submitted to international scientific conference «Innovation technologies», Thailand, February, 20-28, 2008, came to the editorial office 10.01.2008.

CAPACITY ELECTROMECHANICAL SYSTEMS

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Nowadays capacity electromechanical systems are widely used in such applications as microactuators for high-accuracy drives, accelerometers for automotive control and safety, etc. It is due to the development of thin-film technologies. The theory of capacity devices as a whole has also received a development. A new branch of science – film electromechanics – has appeared [1]. The systems under consideration have small sizes – at least some microns in one direction. It is caused by their rather high value of power capacity at given sizes. This fact determines rather perspective branch of systems' application – transducers of micro-transferences.

This paper deals with a mathematical model of the step-type capacity motor with a rolling rotor. The system is represented in figure 1.

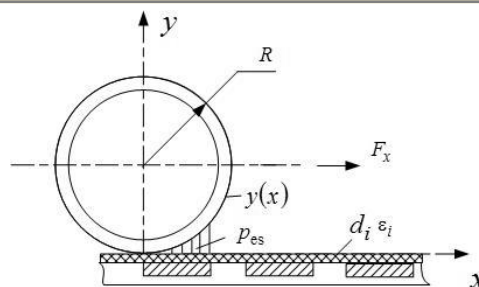


Figure 1. The model of the capacity electro-mechanical system

R – the radius of the rotor, d_i – the thickness of the thin dielectric film on the stator surface, ε_i – permittivity of the dielectric, p_{es} – electrostatic pressure applied to an element of the rotor surface

The stator surface contains electrodes and is covered by dielectric thin film. The rotor represents metal hollow thin-walled cylinder.

Electrostatic pressure is defined as:

$$p_{es} = \frac{\varepsilon_0 E^2}{2} \quad (1)$$

The determinative factor of capacity devices operation is mutual capacitance, i.e. the capacitance between a rotor and a stator. With the purpose of its increase the choice of a design of the motor with a rolling rotor [2] was made. Between the rotor and the stator electrodes the potential differences are applied by turns, and the rotor rolls along the stator's surface under action of the force created by the interaction of electric charges.

The following assumptions were made: 1) the stator and rotor surfaces have no any defects, i.e. the stator surface is a plane and the rotor surface is a cylindrical surface; 2) electric losses (losses in the dielectric) and mechanical losses (losses on friction) are not taken into account; 3) the phenomenon of charge accumulation on the border gas – solid dielectric is not taken into account; 4) the external factors change influence on the system's operation is negligible, i.e. temperature, pressure and humidity practically do not vary.

Thus, it is supposed, that any energy change of the system under consideration is converted into mechanical work.

Let's acquaint the reference frame xoy . Assume, that abscissa x is the generalized coordinate. Hence, the force exerted to the rotor in a direction of x will be directly proportional to the generalized coordinate partial derivative of the system's energy [3], in compliance with the following expression:

$$F_x = - \left. \frac{\partial W}{\partial x} \right|_{U=\text{const}} = - \frac{U^2}{2} \frac{\partial C}{\partial x}, \quad (2)$$

where W – energy of an electric field, C – mutual capacitance.

Thus, the more mutual capacitance change, the more force will be exerted to the rotor at $U = \text{const}$.

In its turn, mutual capacitance is defined by the following expression:

$$C(x) = \frac{\varepsilon_0 S(x)}{y(x) + d_i / \varepsilon_i} \quad (3)$$

and is also the function of x . Here $y(x)$ is the function describing distance between the rotor surface and the stator one.

$$y(x) = R - \sqrt{R^2 - x^2},$$

and the expression in the denominator is the function describing the change of a work gap.

The developed mathematical model allows to estimate one of the basic geometrical parameters of the capacitor electromechanical system of the offered design (the width of the stator electrode) and it will be possible to apply this model to estimate parameters of any other design of the systems under consideration.

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The work was submitted to III international scientific conference «Basic Research», Dominican Republic, April, 10-20, 2008, came to the editorial office 14.02.2008.

*Materials of conferences***PHYTORECLAMATION OF PREBAIKAL REGION'S GREY WOOD SOILS**

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The phytoreclamation, done by means of the introduction of new high-productive plants: *Bunias orientalis*, *Galega orientalis*, *Polygonum divaricatum*, makes a positive effect on basic indices of grey forest soil fertility and productivity in PreBaikal Territory.

A monodirectional, focused on grain production, use of tillable lands in present-day farming systems of Irkutsk Region leads to their advanced degradation. On the tillable lands agrochemical inspection results the humus rich soils areas have reduced by 131, 6 thousand ha for 15 years. For this period of time the humus poor soils areas have increased by 74, 9 thousand ha, and the average humus content soils areas – by 130 thousand ha [1].

Among soils characterized by low and unstable fertility the most common (46, 7% of all tillable lands) in Irkutsk Region are grey wood ones [2].

The state of “out-ploughness” is typical of grey wood soils. This negative phenomenon is associated with the decrease of fresh organic matter content in the soils and soil consistency deprivation. The deterioration of physical-chemical, biological and ecological properties results in their productivity lowering.

The grey wood soils guarantee getting only 8-10 centner of grain from ha.

Various forms of soil modification, among which the replantation (earthing), ruling, sanding, bituminous grouting (soil conditioning), soil chalking, etc., are recommended for the soils enrichment.

The phytomelioration (land reclamation) – is an agroecologically and economically sound method of their fertility rise.

Compared to other methods of land-clearing the use of phytomelioration is 5-20 times as cheaper.

The phytomelioration (vegetative reclamation) is carried out with the help of new and rare plants: *Bunias orientalis*, *Galega orientalis*, *Polygonum divaricatum*, possessing a super high biological productivity and extensive root system. These plants are introduced in Irkutsk Region on the initiative of the author.

A well-developed root system of these plants drains the tith-top soil, improves physical-chemical properties of soils. The roots penetrate with under-plow-layers, extract nourishments out of hardly soluble compounds and carry them into the top-soil. The plants form a super high photosynthetic potential ($PP = 3, 0 - 5, 0 \text{ m}^2/\text{ha}/\text{days}$), produce a great amount of fresh organic matter, which performs various functions: defensive, ecological, physiological and productional.

The manufacturing expenditures on phytomelioration carrying out make 7-10 thousand rubles per 1 ha.

For four years of phytomeliorants cultivating 40-60 ton/ha of dry organic matter, 600 kg/ha of nitrogen are introduced into the soil; the content of water-resistant structural aggregates increasing up to 75%.

After four years of cultivation the soils are used as forerunners for grains for two years. The grains crop yield achieves 30-35 centner/ha (with no application of chemization means), the grains baking quality grows [3].

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The work was submitted to III international scientific conference «Basic Research», Dominican Republic, April, 10-20, 2008, came to the editorial office 23.10.2007.

CATTLE RAISING INNOVATION DEVELOPMENT TRENDS IN NON-BLACK EARTH REGION

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The Russian market of meat and meat food is being integrated into the world's food market, the tendencies and processes of which, in their turn, influence the home meat food market state, to an increasing degree.

In present-day conditions at the high plane of all science branches development the ultimate meat product, as a rule, is a multicomponent one. Therefore, to solve the quality problem of a wide range of products, in the basis of which there is meat crude, it is necessary to think not only of the final meat processing technologies, but also, first of all, of obtaining meat system qualitative components forming the final product. The producer-enterprise is of a great importance as well, so as its technical level, assortment, pricing policy and the reputation in the market. The meat production competitiveness factors can be integrated into four groups: economical, technological, social and ecological ones.

For meat production consumers' market assessment the population survey was held in the region

(288 persons) (Fig. 1). It was found out that 58% of the respondents up to 45% of the total expenditures

for nutrition spend on buying meat and meat products.

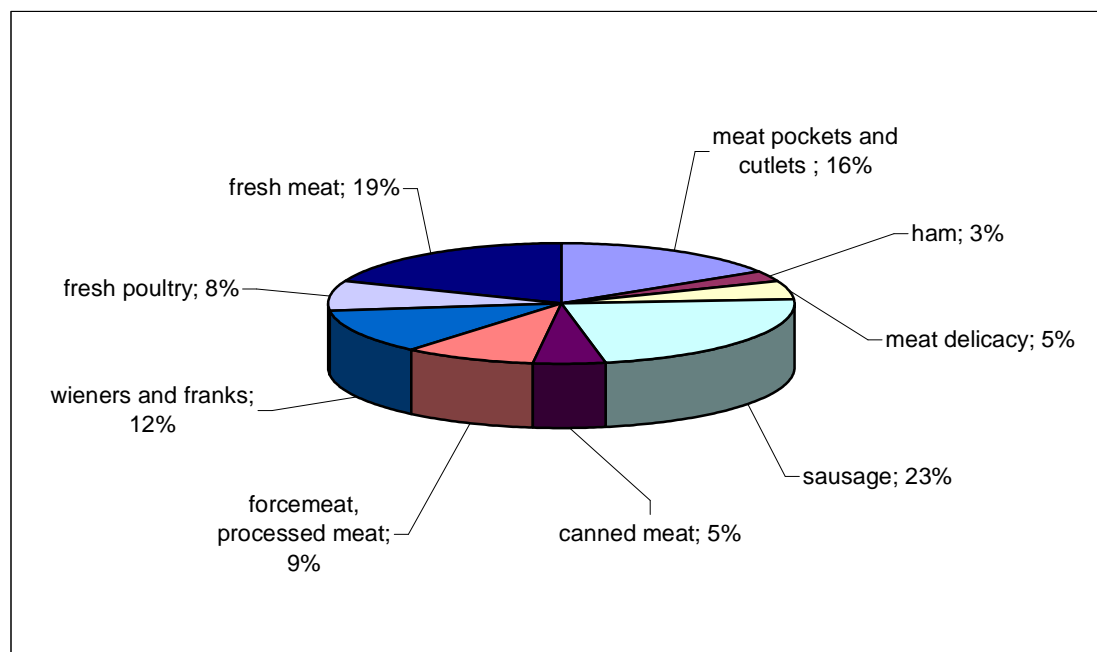


Fig. 1. Structure of consumers' preferences for meat products of respondents of Oryol Region

The respondents' answers study testified that no matter how high the prices on meat and meat products are, it remains one of the most preferable food stuffs ranking second after bread and baked goods in the chain. The study of consumers' preferences for meat products testified that the greatest percentage in the structure of consumption is taken by sausage goods – 35% totally, fresh meat ranks second – 19%, then – semi-finished products – 16%, meat delicacies, canned meat, hams are in the minimal demand.

The Oryol Region meat market competitive environment assessment testified that the leading position is occupied by Moscow commodity producers. It is conditioned both by larger volumes and scales of production and wider range of meat production in price and quality. Under the existing market environment conditions in the competitive struggle the enterprise, conducting competent policy when concluding a contract on raw material supply or increasing its own meat production values, cheapening the production as the result of nonmanufacturing costs reducing, introducing modern resource-conserving technologies, wins.

The economic and financial difficulties, which the Oryol Region APC cattle raising experiences nowadays, are redoubled by the low production level and high costs. In our opinion, such a state of production can have a poisoning effect on the economics as a whole and result in negative tendencies of supplying the population with meat products in the future. Under the circumstances for the meat animals production increase it is necessary to change the fodder base devel-

opment directions, concentrate the efforts and instruments on the fodder production expansion, its quality and ration balance improvement, the fodder payback promotion. The bioclimatic potential of the Oryol Region is favourable to the intensification of fodder production, green and forage production expansion, if it is remembered that in many economies there are large hay acreages and pastures.

The investigations testify that every kilogram of pasture herbage costs to the Oryol Region 2, 4 times, and the digestible protein – 3, 5 times, cheaper than grain production, permanent grasses herbage.

The accelerated development of meat industry requires the solution of a range of problems associated with the coordination of interbranch connections and productions. At the present time in Russia 34 kg of meat on a per caput basis is produced (Oryol Region – 71 kg), while the consumption (thanks to import) makes 55 kg on a per caput basis (Oryol Region – 79 kg).

The low animal production, high labour costs, and also high production goods, fuel and energy costs, the inflation result in the constant production cost development, return rate lowering.

In our opinion, the government's role in the creation of effective market management mechanism levers should be aimed, first of all, at the creation of favourable economic conditions for the enlargement of the output, which is important with a view to national security ensuring.

As long as the problem of meat crude production for the meat processing industry is the most im-

portant and topical one, a livestock breeding development plan was launched in the Oryol Region.

The investments in the Oryol Region cattle raising from various sources in 2008-2009 will exceed 10 billion rubles. In the livestock branch 25 eight-year credit agreements totaling about 6, 8 billion rubles and 52 five-year credit agreements totaling almost 1, 7 billion rubles were contracted. The program is rated for five years. Now seven pedigree factories, six multiplying farms and 76 stud farms, where thoroughbred animals are managed.

The dynamic development of cattle raising in the Oryol Region is the consequence of its close disposition to the capital, one of the principal consumers of the food industry production, and also well-developed fodder base existence.

Together with that, despite the positive dynamics, they fail to reach cardinal changes in cattle raising so far. The livestock and poultry industries development plan, developed by the regional agriculture and food department, is tended to the disadvantages overcoming, animal products output increase and productivity enhancement in the Oryol Region for the period up to 2010.

On the other hand, the embodiment of all the livestock projects declared in the Oryol Region to the

full extent can result in the glut of the whole branch. The fears concerning such a perspective are unlikely groundless. Indeed, the information that the pork production will increase 10 times in the Oryol Region in the coming three years has already been officially announced. As a result, the Oryol Region can enter the trio of leaders (after the Belgorod and Lipetsk Regions) in livestock development in the Black Earth Region.

On the ground of the represented data the Oryol Region competitive space components estimation can be characterized as a "moderate" one: the medium position among the regions of Russia encourages the Region's competitive potential implementation. The decisive role here is played by the close interaction of the chain-work: producer – raw material supplier - production processor – sphere of finished products' realization, its delivering to the customer and consumer response accounting.

The work is submitted to III International Scientific Conference "Problems of International Integration of National Educational Standards", April, 23-27, 2008, Czechia (Prague) – Luxembourg – France (Paris), came to the editorial office on 10.02.2008.

*Materials of conferences***ORGANIZATION CULTURE OF
DISTRIBUTED EDUCATION PROJECT IN
PERSONALITY'S MENTAL SPACE
DEVELOPMENT**

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The surging dynamics of educational processes' globalization and integration, determined by the intense introduction of the latest information and telecommunication technologies, conditions the necessity of working out of innovative pedagogical models and open education concept distribution systems with due consideration of personality development priorities in the period of knowledge society becoming. The present-day cyberspace as a global information field and new universal bio-electronic people's life environment, the most important component of which is the Internet, appears one of the key developments of the existing educational paradigm transformations. In the given context a special topicality is acquired by the problem of working out of the distributed education project's life cycle management strategy methodological foundations as a flexible dynamic heterogeneous open pedagogical system of the interaction organization on the basis of state-of-the-art technologies, the Internet means and services in the development of the personality mental space.

From the system approach viewpoint the distributed education project and the process of its implementation are a complex metasystem, wherein the project itself appears as a controlled system and the project management is a controlling system. The distributed education projects' management - is the methodology of organization, planning, guidance, resources coordination through the project's life cycle for didactic purposes achievement using modern methods and means of Internet-technologies. The development of the projects control methods from the network planning technology to system management of functions and subsets of the project have allowed actualizing the distributed education project's didactic potential in the process of personal- and social-professional becoming of the learner. The projects, wherein the learner should successfully perform various roles and functions, are small or medium in their scale, short-term or medium-term - in their lead time, innovative - in their type, opening new opportunities of the open education quality improvement for the preparation to successful activity in conditions of the formation of a new society characterized as a "networking", "informational", "competent" one - in their goal orientation.

A virtual office of the distributed education project provides a complex use of modern pedagogical, informative, communicative and managerial tech-

nologies for the integration of educational, professional and social media of the project's functioning, that promotes the formation and development of mentality of the personality as a hierarchically higher stage of its becoming [2]. A virtual office of the distributed education project represents a network of individual and collective actors - freely interacting intelligent agents developing the joint project [1]. The core of the project's virtual office is the project's knowledge base management informative-technological system, which includes the following components: a centralized bank of artifacts created at separate stages of the project implementation; records fixing the knowledge and experience accumulated in the course of the project's implementation, which are easy to study and use; an endurance integrated pool with the tuned system of classification and the project's participants' access rights control.

The distributed education project needs to be regarded as a cycle of innovative activity, wherein a special importance is acquired by organization culture values exercising a significant influence on the personality's and the project's team mental space formation and development with an integrated system of mental values, norms and rules of conduct determined by the common purpose availability [4; 5]. The joint-creative activity of the project's participants promotes development of the project-technological type organization culture [3], wherein the values are: the team's mission and the project vision; common purposes and solidarity of the team in their achievement; knowledge and competences of the team members; the floating role of the leader and readiness to obey acknowledging the power of those, whose knowledge and competences are important for the achievement of the project's key marks. As an instrument of cooperation of the distributed education project participants making actual the search and knowledge transfer in the process of its realization, and also a free idea exchange (thread discussions", connected with projects, documents, tasks; "meetings in the net"; coordinated "work floods") within and between the levels of the team's organization hierarchy, synchronous and asynchronous means of the Internet communications, personal and group informative managers of the project's virtual office are used.

In the distributed education project two tasks are solved: objective models of the principal and secondary business processes of a virtual educational institution (school, HEI) are created and the triad of "project - technology - reflection" competences is accustomed [3]; both results of this project are the organization culture values of the personality and the team. The distributed education project life cycle leading management strategy is a purposeful formation and development of the Internet-mentality - steady core foundations of information and world perception,

world outlook and human behavior, which give the personality the properties of uniqueness and the ability of self-actualization in the virtual world by means of mastering and creating the organization culture knowledge society values [6].

During the distributed education project's life cycle the team's organization culture development takes course of several stages [7; 8] according to the achieved maturity level of the project management process:

- the initial level – the organization “power culture” dominates; in conditions of the team formation success depends on individual efforts and peculiarities of the person initiated the project; the unitary mental space of the distributed education project team is lacking;

- the repeated level – the transfer from the “power culture” to the “role culture”; the participants of the project acquire the competence updating experience which is reproduced in new projects; the “mutual discovery” and cognition of individual mentalities’ unique features;

- the definite level – is characterized by the organization “task culture”; the process stops depending on individual merits of separate participants and cannot devolve at a lower level; the mutual enrichment and convergence of the team members’ individual mental spaces begin;

- the controlled level – allows transferring to the organization “personality culture”, taking the team members’ interaction at the cooperation level in the unitary project’s mental space as detailed quantitative indexes are established for the development process and product quality and the process of work on the project, and the project results – are understood and controlled;

- the optimizing level – a self-training and self-developing team continues intense improving of the process on the basis of the current results analysis and innovative ideas and technologies application; a transfer to the organization “personality and task culture” is reached; the total mental space of the project determines the leading orientation of individual mentalities’ transformation in accord with the prior values of organization culture.

Thus, the task-oriented formation of the distributed education project team’s total mental space promoting the development of an individual mental space of a personality is defined by the following factors: the informative interaction of individual and collective actors between each other and with the informative-technological artifacts created during the project’s life cycle; the convergence processes and cross-enrichment of the project participants’ individual mentalities; the innovative intelligent Internet-technologies applied as didactic instruments of the project’s monitoring.

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The work is submitted to III Scientific Conference “The Problems of International Integration of Educational Standards”, Czechia (Prague) – Luxembourg – France (Paris), April, 20-27, 2008. Came to the Editor’s Office on 25.01.2008.

PROJECTIVE CULTURE AS A BASIS OF A FUTURE TEACHER’S READINESS TO PROFESSIONAL ACTIVITY

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Projective culture of a person is rather a new concept. It has entered into scientific use with the developing of technologies of “social engineering”. Meanwhile such property and ability in many respects are initially peculiar to a person because one of the basic characteristics of a “cultural person” is his ability to projective activity, i.e. to productive imagination, creative and free transformation of the reality based on a model of the desirable future. This ability is set by the main point of culture, which is first of all a set of projective ways and results of development and transformation of the world of nature, society and a person himself. The projective culture is a basic characteristic of personality of a teacher and it is formed at the intersection of three components: a system of polycomponental qualities of a person including base and peripheral properties; theoretical and practical readiness of a teacher to projective activity, and also a system of

specialized technologies which a person should master during education.

The theoretical analysis based on the generalization of works in the sphere of social and cultural projection has allowed us to define projective culture as an integrative social and cultural phenomenon, which objectifies a level of development of methodological culture of a teacher. It also has allowed us to offer the following structure of a projective culture: abilities-purposes-values-knowledge-methods.

As an integrated qualitative property of a professional activity, the projective culture is characterized by following features: aspiration to purposeful reorganization of the society; valuable orientability and realizability; refusal from a gnosiscentrism in a projective activity; a criterion character of selection of alternative pedagogical means, their dependence on necessity of realization of specific function, probable character of possible decisions.

Abilities are certain inclinations of a personality. Their development suggests the success of its functioning in different spheres of activity, and with reference to projection – in general, in society, in life. Values are an ethics, philosophy of a profession - its methodology and at the same time - the attitude of the subject to object. Purposes are the desirable results which the teacher anticipates understanding the essence of culture, his profession, experience of today's social and cultural situation, understanding certain problems. Knowledge and methods are instructions about the purposes and ways of activity.

The conditions of mastering the projective culture by a person are the following: motivation of a person to mastering the projective culture and the worked out complex of pedagogical actions for mastering the methodology of projection; the content of education fixed in educational programs; the organization of process of projection as systemforming activity; consideration of education as a process, directed to the expansion of opportunities of a competent choice by a person of his course of life and his self-development; modification of character, type, style of relations in such systems as "teacher - student", "student - teacher"; extension and deepening of life experience of students and of professional skills of teachers; variety of kinds of activity and ways of development of the understanding the reality by the subjects of educational process; socialization of students, increase of their creative potential, motivation of achievements, creation of situation of success, formation of a stable cognitive necessity; self-realization, self-actualization of subjects of the educational process.

The main functions of a teacher's projective culture are based on the specific character of his activity, variety of types of relations and communication, system of value understanding, opportunities of a person's creative self-realization. These functions have the following characteristics: humanistic, individual-

lycreative (aimed at the person's formation, development of his subjectiveness), proactive (prevention of possible negative manifestations in educational process of poly-cultured groups); cultural and creative (determines the abilities of a future teacher to stand to re-formative relation to pedagogical sphere and to his own vital actions); regulatory (suggests a certain teacher's responsibility for ensuring the succession of world, federal and regionally-municipal standards of education, for his choice of means of limits of permissible pedagogical influence); creative (inadmissible teacher's line of conduct which allows to understand, to find the right line of conduct in non-typical situations); cognitive (which represents a complete view of the world, native and regional culture as the essential principles of the culture of a teacher's activity). Taking into consideration the mentioned peculiarities we have singled out the following functions of projective culture: gnoseological, humanistic, communicative, informative, normative, teaching and educative.

The criteria of projective culture were defined by us from the systemic understanding of culture, the distinguishing of its structural and functional components, the interpretation of culture as a process and as a result of the development and creation of pedagogical values, technologies of the professional-creative self-realization of the personality of a teacher.

The experimental research spent on the basis of Stavropol State University, has allowed us to establish, that the basic criteria and indices that show that the projective culture of a teacher is formed are the following: a valuable attitude to pedagogical activity; readiness to the realization of projective activity; creative activity of a teacher; a degree of development of pedagogical thinking as the criterion of projective culture; aspiration to a professional-pedagogical perfection.

The technology of formation of the projective culture is based on the following conceptual ideas: the orientation of didactic process of the higher institutes of education toward the general and professional culture of the teacher; the realizations of strategy of the productive creative education, the joint activity of students and teachers; the formation of educational process in the logic of emphasizing, of amplification of senses of educative-professional work by means of cooperation and co-authorship, enrichment of motives to cognize, development and expansion of relations with professional community. The pedagogical technologies of formation of the projective culture of a future teacher are innovative; social; reflected-creative; dialogue techniques of human mutual relations; creation of the sphere of communication which is adequate for the personal growth; active methods of education; independent work; techniques of a personal reflexion.

The efficiency of the process of formation of the projective culture is defined by the following pedagogical conditions: organization of a complete pedagogical process and its orientation toward the

formation of the projective culture; the creation of the humanitarian sphere of education; the integration of the content of pedagogical education; providing of students with a subjective position in educational process; development of the creativeness of students; development of reflexive positions of the subjects of educational process; orientation to the formation of innovative environment, involving the students in different types of pedagogically-psychological practical work.

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The work was submitted to III international scientific conference «Actual problems of science and education», Cuba, March, 19-29, 2008, came to the editorial office 25.01.2008.

ISSUES OF ADAPTIVE EDUCATIONAL SYSTEM MANAGEMENT

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In traditional knowledge-oriented approach educational system management begins with setting aims which are important for society and with control of their achievement. Educational aim having been stated as a socialization of younger generation concerning educational process becomes a part of external aims category. In turn it needs organization of external educational management. Such external management begins with structuring the contents of education. That means creation of external content standards. Otherwise, the structure of the content, or content standards, in this case become specific external social aim of education. Inasmuch as aims of education are always external in respect to educational process, educational system management comes to content standards manipulation, or to strict regulation of educational process at any level. Such conditions don't allow taking peculiarities and interests of all the participants in educational process – the teacher and the students – into consideration. Therefore, achievement of the aim is of scholastic nature and educational system management is ineffective. Nowadays, educational aim – socialization – is achieved by means of humanization of education. In its turn humanization is often interpreted as child's adaptation to social environment due to the process of education. In pedagogics as a whole

and in education in particular adaptation is interpreted much like in biology, sociology, psychology as external environment adaptation. In general adaptation is a process of adjusting the organism and its functioning to the changed living conditions. The necessary conditions of adaptation are inner resources of the person, in this case biological, his social environment, and their interaction. There are two possibilities of this interaction. First, changes in social environment lead to inner changes. Second, inner changes need some changes in social environment. Implementation of the first variant becomes possible due to *person's adaptability* which is inborn and acquired ability of the person to adapt oneself to diversity of life in any conditions. In terms of pedagogics *adaptation of the student* to social environment is interpreted as socialization, or aim of education, and, as it was already mentioned, is one of the external characteristics of educational process. The results of education, however, are the internal characteristics of the person being taught. So the gap appears, that makes the development of adaptability in educational process impossible. One of the attempts to get rid of such a gap is creation of new concept adaptive educational environment. Under the concept *adaptive educational environment* [1] social and pedagogical system adapting to the conditions of the changing external environment which, on the one hand, aims at the best adaptation to the individual peculiarities of the students, on the other hand, tries to be flexible when meeting the society requirements, is meant.

Adaptive educational environment takes the position in-between the external aims and the internal features of the student, for example, the results of teaching, and has the ability to adapt both sides of educational process. It ensures their interaction due to creation of the system of pedagogical process in the context of some pedagogical theory. The interaction in the context of some pedagogical theory is, in its turn, fulfilled in the course of appropriate educational process, which is *aimed at* achievement of educational purposes by the students and is fulfilled due to its *structure*. Therefore, creation of adaptive educational environment is to guarantee achievement of the educational process aims. That allows us to speak about efficiency of education – a measure of coincidence of the results achieved in reality with educational aims. However, it should be pointed out that it is not always possible to compare the results and the aims because they are on either side of adaptive educational environment – of educational process. Such definition of efficiency of education again causes the problem connected with the elimination of the mentioned gap. Yury Babancky judges the efficiency of the educational process [2] chiefly according to the educational success of the students and also according to their breeding and maturity that is according to their internal characteristics. To his mind, efficiency can be attained only by optimal means those which are more

favorable in present conditions, in other words, in optimal educational process. Optimal educational process is such educational process [3] that is the best for present conditions, real abilities of the student and the teacher according to certain criteria. *The criteria of efficiency* according to Y. Babansky [4] are achievement of that level of progress in studies, breeding and maturity by every student he is really able to achieve during given period of time that shouldn't be under satisfactory level according to existing system of grades; spending established amount of time for class- and homework by teachers and students; and a minimum of necessary efforts on the part of the educational process participants. The first one defines the aims of education, which are connected with knowledge standards according to the scale of grades, that, for sure, refers to the external conditions and is always determined by external social order – the standard. The second criterion of educational process efficiency also refers to the norms of the standard that is time criterion of the standard. The third criterion refers to the inner characteristics which are organized and controlled from the outside, and, therefore, as it is in the first case, efficiency is presented by means of external environment organization and management. There upon educational process structure and course being under control of the external characteristics points out its quality. The extent to which educational process is well-organized and controlled is characterized by such category as intensification of education. *Intensification of education* as a process according to Y. Babansky is an increase in productiveness of educational work of the student and the teacher in every unit of time [5]. In order to control the external conditions the author defines the following *elements of intensification of education*: rise in purposefulness of education; strengthening of learning motivation; making the contents of education more informative; using the active methods and forms of training; increasing the speed of fulfilling the learning activities; development of learning skills; using computers and other new technical devices. All the elements stated above can be defined as external features of educational process. Consequently, we can say that the criteria of educational process efficiency describe the adaptive educational environment as interaction of external and internal elements of pedagogical process according to the first possibility that is changes in social environment lead to inner changes, and educational process intensification category is characteristic of controlling the educational process as adaptive environment. It should be taken into account, however, that educational process organization and control don't allow to get rid of the gap between internal characteristics of the student and external educational environment conditions because the aims of education are external and internal characteristics are uncertain and, therefore, are out of control. Intensification of education in this case is interpreted as average educational work productiveness in-

crease that makes educational process control in order to achieve the aim the purpose of averaged standardized technologically effective educational process. External structure of technological effectiveness of educational process course indirectly becomes the aim of education, and the quality of education is judged by the standardized educational process efficiency as a measure of educational process control for the achievement of external averaged, that is to say uncertain, aims. In this way, the first adaptation possibility implementation by means of control the adaptive educational environment is not enough to achieve the internal aims.

According to the second adaptation possibility inner changes need modification of social environment. To our mind, in terms of pedagogics educational process can become more effective only when there are internal, that is to say personal, aims which will require the changes in the whole of educational process. And intensification of educational process which will lead to the increase in efficiency in the results of education of every student will be interpreted from the point of view of individualization of educational process. The increase of individual educational aims becomes the first element of *individual intensification of education*. For stating and achieving the individual educational aims one should create inner system that is implemented in individual purposeful pedagogical process which should certainly include *inner personal educational aim, individual contents of education, theory-based method of teaching*, that should suit the aim, and *method of control* the results of education. Inasmuch as inner aim of education always depends on a person, pedagogical process construction becomes the object of education, and internal individual pedagogical system becomes its subject. The first stage of constructing the internal individual pedagogical system is defining the internal individual aim of education, which will allow to make the whole process of education individual and also to define other aim-depending characteristics of theoretical system. Defining the aim of education begins with stating the task, which should be based on the inner features of the student. Taking the initial characteristics into account, we are able to create individual contents of education. Thus, in the course of implementation the first stage of pedagogical system construction the aim becomes the theoretical construct of contents of education. The aim of education is still the main element of pedagogical system, is still inside its bounds that is why it is still internal for the system. It can be stated that while defining the aim the unveiling (constructing) of the latent pedagogical object – the person taught – takes place and its replacement for certain construct of the contents which being the personal aim of education and having some material realization also serves as an individual criterion for the level of education. The process of constructing contents of education adaptive to the inner educational aim or constructing

the adaptive contents becomes the second stage of pedagogical system becomes. The process of constructing contents of education adaptive to the aim, or the process of content adaptation, must have two functions: the function of control and the function of subject contents. The function of control the constructing of the adaptive contents means constructing the individual structure of contents of education, which suits the internal aim. In other words, taking inner aims of education as individual construct of education the teacher defines some plan, the path of education, which will allow fulfilling the personal aims to their full and according to which the further educational process of the student will be constructed. Obviously, the individualization of educational aim and its fulfillment due to creation of individual educational path make the educational process individual. The subject function of constructing contents of education adaptive to the aim of education makes us think of the problems of didactics and lies in constructing certain subject contents unveiling educational aims at certain subject level. In other words, concretization takes place that is revealing the aim by means of defining and constructing the subject contents. The process of implementation of the function of control and subject function of constructing the individual (adaptive) contents results in constructing certain internal for the system contents of education unveiling the internal aim of education (adaptive to the aim) or constructing *adaptive educational contents*. Under adaptive educational contents we understand individual educational path definite in subject terms at the level of didactics and implementing internal individual aim of education. Everything stated above makes us come to conclusion that we should speak of the course of education not in the direction of the external unsteady and unstable social norms but in the direction of the internal steady and fairly permanent norms. Under the concept of the internal norms we should understand the contents of education itself. In this case the aim of education is not a process but education itself that is its internal and certain contents. The concept of adaptability in education is defined due to acquirement of some variant of educational content, and the content itself is a certain invariant construct. Individual acquirement of the content invariant lets us speak about the acquirement of the content to the full, which includes different possibilities, which allow achieving internal content adaptation. In this case, first, the aims of education don't lose their meaning; second, we can control the results of education because there is certain invariant construct of educational contents; third, adaptation is regarded as a category defined individually, not as an averaged category. However, there are difficulties of implementation adaptive educational process in practice. Pedagogical reflexion by means of which the teacher achieves adaptation of educational contents to the abilities and inclinations of the student plays an important part in the process of educational

content construction. Pedagogical reflexion of the teacher is the basic tool for solving the pedagogical task to adapt the contents of education to the peculiarities of the student following the requirements of the standard. At the same time pedagogical task consists of two main elements: on the one hand, the contents of education, on the other hand, personal abilities of the student. Pedagogical reflexion is a professional ability of the teacher to take both elements into account in the course of educational process. Thereupon we should remark that the main problem of adaptive educational system management is lack of competent teachers who had special training in the field of theory of constructing pedagogical constructs, especially building the constructs with adaptive contents of education. It is obvious that students are not able to solve the problem of choice of their own education contents on their own because of their incompetence. That is why the provision of educational institutions with trained specialists in the field of pedagogics able to construct contents for individual education. Thus, the implementation of the idea of constructing the individual educational process made us solve the problem of adaptation in education, which we think is realized by means of management in the field of professional constructing of adaptive educational contents, which is achieved due to reflexive teaching.

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The work is submitted to III Scientific Conference "The Problems of International Integration of Educational Standards", Czechia (Prague) – Luxembourg – France (Paris), April, 20-27, 2008. Came to the Editor's Office on 29.12.2007.

FIRST AUTHORS' SCIENTIFIC SCHOOL OF TEACHERS IN POSTGRADUATE EDUCATION

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The quickly changing social reality has called into being new objectives, content, methods and technologies of education. It resulted in other requirements for the educator. Nowadays the educator has to perform functions not only of a teacher, instructor, advisor, tutor, but also an *investigator*, who discovers new principles and modes of training and upbringing, combines traditions with innovations, strict algorithms and rules with creative research, uses new information and communication technologies in his activity.

Only he, who will acquire the scientific cognition methodology, will be ready for scientific-pedagogical research and will be able to use the obtained result in his practical activity, can achieve this work and conform to modern requirements. In present-day education there appeared a demand for purposeful research activity of the pedagogue.

The necessity of investigative position formation in the teacher able to realize his research activity orients the system of continuous professional education to new forms of scientific-methods work with teachers.

In January 2007, under our scientific supervision, an authors' scientific school of pedagogues "ACME", the objective of which is to assist the professional-personal enhancement of teachers and their mastering of scientific cognition methodology was opened. The school united the pedagogues engaged in initiative scientific research. The integration of educational processes with innovative and research work has become an activity norm of the pedagogic workers having joined the scientific school and developing new educational areas and new technologies.

The authors' scientific school opening within the advanced training system is conditioned by the following purposes:

- the promotion of scientific-pedagogical and scientific-research activity of various educational institutions pedagogues;
- the performance of research activities on professional, pedagogical and managerial activity actual problems;
- the introduction of scientific findings and developments into pedagogical practice;

In this case we use the approach to the consideration of the scientific school as an objectively conditioned organization form of the scientific activity of a group of pedagogues-investigators. The scientific school of pedagogues "ACME" – is a study personnel, within the framework of which the scientific communication culture becoming takes place, the norms of professional and social behavior of an academic community are accustomed.

The main function of the school in science (as any school) – is an educational one and oriented to the reproduction of scientific personnel through teaching pedagogues scientific work foundations, expansion of their scientific outlook. Together with that, performing the educational function, the authors' scientific school of pedagogues "ACME" is oriented to a joint research activity on the leader's program and under his supervision.

In the scientific school of pedagogues "ACME" the work is performed on the *unitary scientific and research program*. The theme – is "**Acmeology of Education**". The research control function is performed by the research manager.

Why is the scientific school of pedagogues called "ACME", and the research program is denoted as "Acmeology of Education"?

The pendency and complexity of many problems of theory and practice of education at the turn of XXI century under new conditions of socio-economical and informative-culturological development of the society are known to have become the appearance cause of a variety of integrative sciences about education. It is natural, because education as a social sphere of human life activity mustn't be considered as a pedagogical reality only and lead educational problems only to pedagogical aspects. The system of education has various associations with science and society and the education itself is the sphere of scientific knowledge *system* functioning. Every educational problem represents a complex interdisciplinary problem demanding the integration of knowledge for its analysis and solving. Educational problems are becoming the core of new scientific disciplines; the "dual subordination" academic fields design principles.

One of such academic fields the acmeology of education has become. It is a psycho-pedagogical science, which integrates the knowledge about human development in educational milieu, about tops reaching in human life activity and development in conditions of educational activities in various types and kinds of educational systems. It is the acmeological approach that puts forward an integral human development as the purpose of education and considers individuality as the top value.

For the purpose of objective and thorough investigation and development of education it is necessary to use the integrated methodology, within the structure of which the acmeological approach occupies a worthy place as a new methodology of education. The acmeological approach forms a high achievement motivation, need for success, high results, human inner life revival, his intellectual development and spiritual maturity. In connection with this, the social role of the pedagogue's post-graduate education system, its strategic ideological function grew significantly. The teacher's consciousness reorganization, the disclosure of his individuality formation and

self-development mechanisms at the post-graduate stage appear especially important in the aspect of solving new educational tasks.

In accordance with this, the participants of the scientific school "ACME" work out nowadays such significant problems for education as the professional-personal enhancement of the pedagogue in conditions of innovative activity of an educational institution, the arrangement of conditions for creative activities of teachers, the provision of effective interaction of teachers and learners by means of the acmeological approach implementation, the formation and development of an acmeological position of the educational process subjects, the creation of acmeological space of the lesson.

Let us name the principal positions defining the essence and main activity characteristics of the scientific school of pedagogues "ACME" (SSP):

- the purpose of the SSP educational activity is not the transfer of knowledge and skills as it is, but teaching scientific creativity;
- the systematic nature and training content mastering consistency is defined by the logic of research and evaluation on the higher priority subject;
- the educational process in the scientific school isn't restricted by time frames, they are individual for every SSP participant;
- the educational process in the SSP "is deepened" in the process of the scientific and research activity itself on the priority subject;
- the SSP participants are offered a free hand at the research subject choice within the framework of the school scientific and research program;
- the training individualization is combined with the research and evaluation collective character;
- the educational interaction result is the becoming of the teacher as a scientist and obtaining objectively new scientific knowledge.

The formation of professionally personal readiness of the teacher for independent scientific and research and scientific educational work is performed by means of the following functions realization by the scientific school:

1. **Information-training:** constant field extension and deepening of knowledge in the area of cognition theory and methodology occur; teachers get current information in panel sessions, lectures and seminars.
2. **Consultative:** in the process of professional experience and self-educational activity apprehension teachers can take opinion of the research manager immediately, if there is a need.
3. **Organization-communicative:** an ongoing psycho-pedagogical seminar, which satisfies the need of teachers in professional communication, research and evaluation experience interchange, provides the acquaintance with current achievements in its academic field and fringe areas of the scientific knowledge, is created.

4. **Motivation inspiring:** the activation of self-education, self-improvement, self-actualization in the professional and scientific-pedagogical activity, personal development, and also the teacher's research methods mastering conation activation.

5. **Axiological-organizational (conceptual-ideological):** the actualization of professionalism and creativity value; conceptual framework apprehension of professional (pedagogical) and scientific and research activity, perspectives of professionally personal development.

6. **Developing:** various forms and methods of scientific and educational interaction of pedagogues at the post-graduate stage, self-educational activity are oriented to the development of all sides of the teacher's personality – the need-motivation sphere, value system, conceptual thinking, operational-technological components of the pedagogical activity, ability and readiness for scientific activity, etc.

7. **General-cultural:** widening of the teacher's general-cultural horizon, the promotion of their human culture and ability to creative self-actualization.

The education at the scientific school of pedagogues should result in the formation (becoming) of an independent scientist, the definition of his own professional position in the scientific and research activity, the defence of doctoral dissertations in pedagogy and psychology.

The work is submitted to the Scientific International Conference "Higher School Scientific Research in Priority Orientations of Science and Technology", June, 16-23, 2008. Came to the Editor's Office on 28.01.2008.

CREATION OF NATIONAL EDUCATIONAL STANDARD "SCHOOL – HIGHER EDUCATIONAL INSTITUTION (HEI)" WITHIN CONTINUOUS CHEMICAL EDUCATION SYSTEM OF TVER STATE TECHNICAL UNIVERSITY

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The becoming of a new pedagogical system oriented to the entry into the world's educational space is attended by essential modifications in the theory and practice of the educational process. A change of educational paradigm takes place: another content, other approaches to teaching, other relations between the participants of the educational process, another pedagogical mentality are offered. An important role here is played by the development of relations between the links of the continuous education system, the adequate performance of which is impossible without close collaboration of pedagogical workers of secondary and higher school.

For the purpose of chemistry teaching succession assurance in the Tver State Technical University (TSTU) the system of continuous chemical education of youth has been created, the realization of which is performed in the following basic directions:

- the intensification of research and cognitive activity of school children in Chemistry by means of the enlargement of senior high school students' workshop conferences system in the Tver Region; the assistance to municipal educational institutions in carrying out research works;

- the consulting and methodical support of the preparation for the Uniform State Exam (USE) in Chemistry both for the 11th form students and school teachers of the Tver Region;

- the organization of an experimental ground on the continuous chemical education system development on the basis of one of Tver schools;

- the promotion of chemical academic competitions of school children in the Tver Region;

- the individual work with the most talented students able to in-depth Chemistry study;

- the professional orientation of school leavers.

The enumerated areas of activities imply the educational process modernization and Chemistry knowledge quality improvement as the result of the education content perfection and modern pedagogical technologies introduction, and also the creation of conditions for the assurance of the national educational standard "school – HEI".

The increasing spread between the scientific work current state and the academic activity makes search for educational technologies for early attraction of learners to research activities. The lecturers of chemical departments of the TSTU annually are the research managers of seniors' research works; take part in the workshop conferences "Step into the Future" together with them. Year by year the number of experimental research works carried out by school children on the basis of the university including those carried out in partnership with post-graduates and students grows up. To our opinion, such joint scientific projects are an exclusively useful activation form of the cognitive activity both of school children and students, as they promote the preparation of either for serious scientific investigations.

A great work among the future enrollees is carried out in specialized chemical and chemical-biological classes of a range of secondary comprehensive and grammar schools of Tver. The activity of these classes supposes the correction of school curricula and programs for the purpose of their approach to HEI requirements, the development of interest in academic disciplines of the University in school children, their in-depth study with the attraction of leading TSTU lecturers.

It is our opinion that the adaptability of the educational system should be performed through an absolute succession of educational programs of second

dary and higher schools, the strategic renovation of the educational content and technologies used with due account for changing time demands. The succession degree at that should be determined by both search for studied disciplines' desired depth (the fundamental knowledge and practical work experience volume) and balance between the obligatory and variable parts of national educational standards.

For the experimental work on the development of the continuous chemical education system a treaty about the cooperation of the Tver State Technical University and the municipal educational institution of "Secondary comprehensive school №45 of Tver" has been made. The pedagogical essence of the cooperation between the school and University is manifested in the interrelation of the educational process, in the formation of general and special knowledge, abilities and skills in the course of initial professional training, in the cooperation of pedagogical collectives with learners' parents, in the scientific supply of the school's experimental work.

For the organization of the education succession the available school curricula analysis has been carried out and Chemistry, Physics and Mathematics courses in-depth study programs have been developed, the last including all basic HEI forms of classes carrying out – lectures, seminars, colloquiums, laboratory courses, etc. Specialized physio-mathematical and vocational classes, the acquisition of which takes place on a competitive basis from the number of all city educational institutions learners, have been created at the school. A scientific-methodological assistance to pedagogues teaching the profile comprehensive subjects has been organized. A part of the classes on these subjects are conducted by the University lecturers. Meetings of leading TSTU specialists with the profile classes learners and excursions in the HEI labs are regular.

A scientific society has been created at the school. An in-depth preparation of the scientific society members for independent scientific work skills acquirement is performed. The University employees render assistance in the scientific investigations organization, individual work with the most talented school children at the Chairs and in the labs of the TSTU. A program of joint internship of students and senior school children has been launched. A joint participation of school children and students in training research work, in theoretical and practical, scientific and technical conferences of the TSTU has been organized. The educational guidance and control over the professional education of the learners is performed by both the school and the HEI.

At the methodical support of the TSTU a program of the propaedeutical course for the fifth grades "Introduction into Chemistry" has been launched at the school №45. The course is based upon the idea of Chemistry and other natural disciplines (having been studied earlier or studied in parallel) intersubject

communications realization and that is why allows making the learners' chemical and ecological knowledge got at the Nature Study, Biology, Geography classes actual. The course objectives are: the integration of Chemistry into the system of natural-scientific knowledge, the formation of the chemical world view as an element of the natural-scientific world outlook, the formation of a sustained interest in the subject, the implantation of creative attitude towards scientific facts understanding. The application of the research method in education is an effective means of the self-dependence ability and creativity development, that is why propaedeutical course introduction in the fifth grades allowed us to begin the chemical thinking formation in school children at the early stage of learning.

The active cooperation of the TSTU with the school №45 gives its results. Thus, in 2007 a leaver of the school Ekaterina Puklina became the only school-child having gained the maximum possible score – 100 points, at the Uniform State Exam in Chemistry for the whole history of the Tver Region. From the number of the eleven-graders having attended classes with the TSTU lecturers, 80% of the school children passed the USE in Chemistry with the mark “excellent”, and 10% - with the mark “good”. According to the results of the USE passing in Chemistry in 2007 the school №45 has fallen into the best five schools of Tver. In November, 2007, the school №45 won the victory in the interscholastic methodical center creation competition in Chemistry in the Tver Region at the active support of the TSTU employees. No doubt, that such cooperation turns out to be exclusively useful for the secondary and higher natural-scientific education succession guarantee.

The chemical academic competitions among school children represent a harmonic multistage system of seniors' intellectual competitions aiming at the discovery of talented learners able to in-depth study of Chemistry. School academic competitions – is a powerful means of the development of interest in Chemistry.

The Chemistry Department of the Tver State Technical University has been a methodical center on chemical academic competitions among school children in the Tver Region for the last fifteen years. Annually at the TSTU basis an experimental round of the Regional Academic Competition among School Children in Chemistry is held, the Department lecturers work in the judge panel of the city and Regional stage of the competition.

In 2005-2007 the Tver Region became the place of holding of the IV (district) stage of the All – Russia Academic Competition in Chemistry in the Central Federal District. It came as a service acknowledgement of the Tver region in chemical education development (no any other region of the Russian Federation has ever been dignified like that thrice). In the district Academic Competition about 70 school chil-

dren from 17 regions of the Central zone of Russia – winners of the III (regional) stage of the Competition in their own territories, participate. The TSTU Chemical Departments lecturers work annually in the judge panel of this Competition, and its experimental round traditionally takes course on the basis of the Polymer Materials Technology and Chemistry Departments. The experimental capability and staff resources of the TSTU were highly praised by the representatives of the Federal Education Agency and Central Methodical Commission. The objectivity of school children's works evaluation, the professionalism and goodwill of the jury have deserved favourable report of the Competition participants and the teams leaders. The work oriented to the District stage of the All-Russia Academic Competition of school children in Chemistry to be traditionally held in Tver on the basis of the TSTU is being carried out.

The stimulation of an independent search activity in learners by means of gradual sophistication of assignments from reproductive to creative ones allows guaranteeing the differentiation of the Academic Competition participants on the preparation level. The most talented learners able to in-depth Chemistry study become apparent on the results of Academic Competitions. An individual work is carried out with them at the departments and in the labs of the University. Various perspective forms of such cooperation are offered in the TSTU. The idea of the “Young Chemist School” creation on the basis of the University scientific-educational park turned out to be rather productive. An analogous structure – “Small Chemical Academy” – has been functioning successfully on the basis of the Polymer Materials Department for years. The educational process in them is performed on the basis of the learner-centered education technology and differentiated approach to school children with various intellectual activity degrees. Some school children having attended these classes became later the All-Russia Academic Competitions winners, many of them entered chemical specialties of the TSTU. Such practice of work with the enrollees gives an opportunity to raise the knowledge level of school children in Chemistry, implant professional skills to them and expand their outlook using the variability of teaching forms and methods.

The realization of the developed system of Chemical Academic Competitions in the Tver Region stimulates the Chemistry teaching level at schools, promotes the secondary and higher educational institutions' educational programs integration and, helping providing the University with talented enrollees, allows maintaining traditionally high quality of chemists-specialists' training.

Within the framework of annual “Educational Services Fairs” organized by the Federal State Employment Service together with the Regional Educational Office, the TSTU lecturers work on the attraction of learners from cities and districts of the Tver

Region for continuing education at the TSTU chemical specialties.

A great attention is paid to the coordination of the University chemical departments', education regulatory bodies' and city methodical center's activity. Working closely with the Tver Regional Teachers' Extension Course Institute the Chemistry instructors regularly render methodical and consultative aid to pedagogical collectives of Tver and Tver Region schools on the questions of school children's preparation for the USE, solution of advanced complexity problems, entering and studying at the TSTU.

School as a social institution is leading among other educational institutions and kinds of pedagogical systems. It is possible, however, to take up the position that only on the basis of school and HEI cooperation in the present-day world one manages to build such a system of continuous education, which reacts actively to a quick change of life demands and makes the effective connection with the perspective labour market possible.

The work was submitted to VII international scientific conference «Strategy of scientific education», Egypt, February 22-29, 2008, came to the editorial office 16.01.2008.

USE OF NEW TRAINING METHODS IN TEACHING BRANCHES OF JURISPRUDENCE

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Whatever training methods be applied for the professional education efficiency upgrading it is important to create such psychological-pedagogical conditions, in which the student can occupy an active personal position and manifest himself as an academic activity subject to the full extent. The didactical principle of personal activity in training and professional self-determination conditions the system of requirements for the student's academic activity and the teacher's educational work in the integrated learning process. Internal and external factors, needs and motives are involved into the system. The correlation of these characteristics determines the choice of the education content, concrete forms and methods of training, conditions of organization of the entire process of active creative personality formation. There are no universally effective or ineffective methods.

All training methods have their own strong and weak sides, and that is why, depending on purposes, conditions, time available, it is necessary to combine them in the optimal way. The quality of education is made of training quality and quality of upbringing. The training quality can be achieved only as the result of the efficiency of every training stage. I.e. the whole process of training is built on the scheme: to conceive

– to understand – to remember – to use – to control. To gain the training quality it is necessary to get through all these stages of cognitive activity consistently. The use of various forms and methods in the process of training guarantees the training quality upgrading.

The main forms and methods of training acting to raise the training quality are: role-playing games, business games, conferences, forums, dialogues, problem training, solitary work, abstract defence, individual performances, creative compositions, reports, messages; tasting, programmed control, research work, etc. All the numerated training technologies promote the training quality problems solution.

At a higher educational institution at verbal presentment of training material on jurisprudence branches wordly training methods are generally used. Among them an important place is occupied by a lecture. The lecture acts as a leading part of the whole training program and represents a means of extensional theoretical material presentment, providing the integrity and completeness of the students' perception of it. The lecture must give systematized foundations of scientific knowledge on a discipline; disclose the state and development prospects of the correspondent domain of science and technology; concentrate the learners' attention on the most complex, topical points; stimulate their active cognitive work and promote the formation of creative thinking. However, a traditional lecture has a series of disadvantages, which are conditioned by the following:

1. The lecture teaches to conceive other opinions passively, restrains learners' independent thinking.
2. The lecture spoils the wish to work independently.
3. Lectures are needed, when there are no textbooks or there are few of them.
4. Some students have time to comprehend the material, others – manage only to write the lecturer's words down mechanically. It goes against the principle of the training individualization.

However, the higher school experience testifies that lecture refusal decreases the scientific level of learners' training, breaks the consistency and uniformity of their work during a term. That is why the lecture still remains both guiding jurisprudence branches training method and guiding form of the academic process organization at a higher educational institution. The specified disadvantages can be largely overcome with the help of correct methods and rational composition of the material studied.

To some extent the acuteness of the contradictions named dismisses the opportunity to use non-conventional kinds of delivering lectures in the academic process. The present-day methodology numbers over 250 various methods. These methods result in the change of the teacher's role, new instruments of learners' achievements estimation.

One of the effective training methods, especially in jurisprudence branches teaching, is the method of problem solving (problem training), as long as the apprehension of a large scale normative legal material is required just for the solution of one or another practical case. Instead of “translating” facts and their interrelation for students one may offer them to analyse the situation (problem) and make a legal analysis and search for its solution.

In a traditional lecture such means as the explanation, illustration, description, giving examples, and in a problem one – the all-around analysis of events, scientific search for truth are mainly used. The problem lecture rests on the logic of consistently simulated problem situations by means of putting problem questions and setting problem tasks. The problem situation – is a complex contradictory atmosphere created at the lectures by putting problem issues (introductory), requiring active cognitive activity from learners, for its correct estimation and solution. The problem issue contains a dialectical contradiction and requires not a reproduction of the knowledge got, but a speculation, comparison, search, acquirement of new knowledge or application of the earlier got one. The problem task unlike the problem issue contains some additional introductory information and, if necessary, some orienting search points for its solution. The notions “problem issue” and “problem task” are divided only nominally as problem issues can grow into tasks and tasks – be divided into issues and sub-issues.

The complexity level and problems’ character depend on the learners’ preparedness, the studied subject and other circumstances.

The problem tasks solution and answers to problem issues are performed by the teacher (sometimes falling back to listeners’ help, organizing dialogues). The teacher should not only adjust differences, but also show the logic and methods, demonstrate techniques for brainwork coming from the dialectical method of complex events cognition. It requires considerable time, that is why a preliminary work on the training material selection and lecture “scenario” preparation are required from the teacher.

The skill to solve problems is the most important key competence necessary for a human in any sphere of his activity and everyday life. If learners possess the skills to solve problems, their worth for the organizations, where they will work, will increase manifold; besides, they will acquire the competence, which will be useful for them as long as their life endures.

In the course of the problem’s evaluation learners: deepen their knowledge on specific issues, develop their abilities to solve problems using principles and procedures (theory); develop social and communicative skills. Thus, at the lecture of problem character students are in a constant process of “co-thinking” with the lecturer, and finally become the co-

authors in problem tasks solution. All this does much good, because, first, the knowledge acquired in such a way becomes the property of students, i.e. to some extent knowledge-opinions; second, acquired actively, they are remembered more deeply and easily become actual (training effect), are more flexible and possess the property of transferring into other situations (the effect of creative thinking development); third, the problem tasks solution appears as a peculiar simulator in the development of intellect (the developing effect); fourth, a such-like lecture promotes interest to the content and enhances professional training (the effect of psychological preparation for the future activity).

The work was submitted to III international scientific conference “Problems of International Integration of National Educational Standards”, scientific 23-27, April, 2008, Czechia (Prague) – Luxemburg – France (Paris), came to the editorial office 08.02.2008.

ENGINEER’S SELF-EDUCATION IN TERMS OF NATIONAL EDUCATIONAL STANDARDS

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One of the problems to be solved within the framework of the Bologna Process is the attraction of a great amount of learners and highly qualified technicians into Europe from other countries of the world. At that, an important stage in the quality assurance cooperation development has become the year 1998. The recommendation of the Council about the higher education quality assurance European collaboration was accepted in September. Having signed the Bologna Agreement Russia passed to the training of specialists, who would correspond to the European Community quality criteria. The given change-over can hardly be considered an ultimate one, a serious work on the introduction of teaching methods and education quality control over the period of a series of years lies ahead. Great discussions on these and other problems associated with the higher educational institutions students’ training are being conducted for years. At that, the most important Quality Assurance Systems’ features stand out: the autonomy of the structures responsible for the quality assurance in member countries in the context of procedures and methods choice; their adequacy to the profile and purposes of concrete institutes; the dedicated use of internal and external procedures of valuation. At the given historical stage everything concerning the students’ training has become clear enough. The problem of training of highly qualified specialists, of engineering specialties, first of all, from the number of people graduated from higher educational institutions 10-15 years ago, appears to be rather more complicated. Within the framework of mobility and cooperation programs in the sphere of education and professional

training the integration of the programs SOCRATES, LEONARDO DA VINCI, TEMPUS III has been offered. The new integrated program includes: a comprehensive program of mobility and cooperation in education for the EC member-states, the European Economic Area member-countries / European Free Trade Association (EEA / EFTA) and candidate countries as long as life endures, including both higher education and professional training of other levels; the new program TEMPUS PLUS for the cooperation between the countries bordering the EC and participating in the program TEMPUS devoted to education and professional development; the program ERASMUS MUNDUS. The comprehensive program for lifelong learning has been essentially enhanced in the part of decentralized actions and is aimed at the support of individual mobility of people and partnership between organizations. Its tasks include: the participation of not less than 10% of school children and teachers in the program COMENIUS for the period of 2007-2013; the participation of not less than 3 million students in the program ERASMUS by 2010; not less than 150 thousand listeners to qualification programs per year up to 2013; not less than 50 thousand adults taught and teaching abroad per year up to 2013. As one can see – the figures are impressive. Which will be Russia's part in these figures? Two cornerstones of education – language knowledge and financial problems, as always, will be in the path of “individual mobility of people and partnership between organizations”.

The work is submitted to III Scientific Conference “The Problems of International Integration of Educational Standards”, Czechia (Prague) – Luxembourg – France (Paris), April, 20-27, 2008. Came to the Editor's Office on 24.01.2008.

PROBLEM-BASED METHODOLOGY- LEARNING OF PHARMACEUTICAL EDUCATION

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An important role in the international integration of national educational standards in Russia is played by the international projects coordinating the efforts of various nations in solution of global problems of health care, the concepts determining the strategy and tactics of the nations' health care systems perfection. The “Rational pharmaceutical management” and the concept of “Rational use of medicaments” should be referred to such projects. Their realization in Russia has changed the native health care national policy essentially, presented new requirements to the professionalism of all levels specialists working in the area of medicine and pharmacy. It

couldn't help affecting the medical and pharmaceutical education in Russia.

A certain influence on the integration of medical and pharmaceutical education has had the activity of the European regional Bureau of the World Health Organization. On its initiative the international seminar “Implementation of Rational Medication into Academic Programs of Medical Higher Educational Institutions of the CIS” (Russia, Moscow, May 21-23, 1997) was held and the internship of the Russian medical and pharmaceutical HEI faculties in the international courses “Teaching Rational Medicinal Treatment for Medical HEI students (Netherlands, Groningen, August 21-29, 1997). A major landmark in the international integration of national educational standards has become the “International Conference on the pharmaceutical education strategy in Europe” organized within the framework of the program of cooperation between the Ministry of Health Care and Social Development of the Russian Federation and European Regional Bureau of the World Health Organization (Russia, Moscow, October 11-12, 2005).

The rational use of medications – is the carrying out of medicinal treatment adequate to the clinical state of the patient in the dosage corresponding to his individual features for a proper time period and to the keenest price. The analysis of the “Rational use of medicaments” concept testifies that there is much in common in the integration of medical and pharmaceutical education. In accord with current trends of medical and pharmaceutical help development from the position of evidence based medicine both the doctor and the pharmacist focus their professionalism on the patient, they solving the patient's problems by means of medicaments. It is the patient's problems that are the motivation of the professional communication of the doctor and the pharmacist, the competence of who will be determined by the knowledge and skills level acquired while studying at a basic and pharmacology HEI.

Present-day achievements of science and practice testify to the impetuous expansion rate of the extent of knowledge on pharmacological disciplines, rapid development of the pharmaceutical market and ever-increasing part of principally new medicaments. There is hardly a dare-devil, who will dare to maintain that the decades existing educational system meets modern trends of medicine and pharmacy development. The education basing on facts cognition is becoming unreasonable. The medical and pharmaceutical education reforming expediency emerges objectively.

The main principles of medical and pharmaceutical education reforming become the international community statements, one of which is that “the pharmacist occupied in a retail drugstore – is not a seller, but, first of all, a bearer of specialized knowledge, advisor of the doctor and patient” (WHO, GPP rules). Thus, the knowledge and skills level on phar-

macological disciplines in a pharmacist cannot be lower than that in a doctor.

The pharmaceutical education reforming supposes the modernization of education programs, the keystones of which are:

- the correspondence to present-day practice of rational use of medicaments;
- the correspondence to the knowledge level, national health care problems;
- the teaching of problem solving skills (which do not depreciate in the course of time);
- the use of preparations, included into the national instruction for medicaments use, as the basis for pharmacists;

In spite of the fact that all these principles were put across pharmacologists and clinical pharmacologists in 1997 already at the international seminar in Moscow (1997), unfortunately, the program on pharmacology, which pharmaceutical HEIs follow, has not been modernized and doesn't meet the international integration principles. The same problems in teaching clinical pharmacology/medicinal treatment (the Problem Committee Chairman is the Academician of RAMS Kukes V.G.) are being solved more progressively.

One of the key regulations for the rational use of medicaments is teaching pharmaceutical HEI students medicinal treatment by means of problem-based method. The HEI faculties of Moscow, Smolensk, Ryazan, Kazan and Perm have passed the internship at the Gronningen University under the auspices of the European Regional Bureau of the World Health Organization. This method has been used for 10 years at the Pharmacology Department of the Perm Pharmaceutical Academy.

The introduction of rational medications use practice into the pharmaceutical education in Perm took place in a stepwise manner. Originally it included:

- the organization and holding an interuniversity seminar for teachers on the rational medications use methodology;
- the modernization of clinical pharmacology/medicinal treatment education program;
- the development and publishing a rational medications use principles-based study guide for pharmaceutical HEI students;
- the development and publishing a clinical pharmacology/medicinal treatment study guide containing tests and problem-based assignments for pharmaceutical HEI students. The purpose of the study guide is to promote the acquisition of skills of rational medications choice at various diseases on the efficiency and safety criteria, carrying out the pharmacoeconomic analysis of advantages of a series of medicaments over the other ones, the inclusion of medicaments into logbooks of various levels, the by-

effects monitoring and forecasting, the exercise of competent consultation of the preparations consumers;

- the organization of problem-based learning method training for the Pharmacology/Clinical Pharmacology Department faculty instructors;
- the organization and development of rational medications use research, holding conferences, symposia of regional and federal levels;
- the organization and carrying out the knowledge and skills quality control of rational use of medications included into the federal formulary system in students.

At the following stage the practice of rational medications use is introduced into qualified pharmaceutical chemists' education at the extramural department, internship and the department of postgraduate professional pharmaceutical education. It should be noted that the academic process correction touched mainly the material volumes that is connected with the difference in hours. The objectives and teaching methods are identical to those defined at the intramural form of pharmaceutical education.

The experience of rational medications use concept introduction into the academic process of the Perm Pharmaceutical Academy was reported in panel sessions of the Russian Problem Educational Methodological Committee on clinical pharmacology, the International Conference on the pharmaceutical education in Europe (Moscow, 2005).

Our experience has showed that the international integration of national educational standards opens manifold possibilities for the introduction of new educational technologies, leads the pharmaceutical education quality to a principally new level, raises the prestigious value of the profession, guarantees its socially significant and economically effective realization.

The constraint of the international integration of national educational standards, in our opinion, is the teaching staff, as it is little informed about the processes taking place within the training system of high qualification cadres, and therefore, not all its representatives are ready for the pharmaceutical education modernization.

It is advisable to ease the availability of information about the features of national educational standards, new technologies of education for the teachers' body, to make the educational internet-resources free. All this will allow understanding the essence of the education reforming, entering actively the processes of educational standards' and technologies' international integration.

The work is submitted to III Scientific Conference "The Problems of International Integration of Educational Standards", Czechia (Prague) – Luxembourg – France (Paris), April, 20-27, 2008. Came to the Editor's Office on 31.12.2007.

*Materials of conferences***PROSPECTIVES OF THE POWER SECTOR REFORMATION**

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Over the last decades there were many debates about suitability and reconstruction ways of those sectors of national economy which in some ways have signs of natural monopoly. A similar situation is taking place in Russia; a special attention is paid on power industry reformation – a classical model of natural monopoly.

At the present time the power grid is a rod of Russian power sector, which involves a complex of economic relations, occurred at the production process (including complex production of power and heat energy), power energy transmission, operative-dispatch management at electric power engineering, distribution and usage of power energy.

Russian power complex is an engine of the country's development. Not only future of the power sector directly depends on its reformation results but also Russian economy prospects in whole – the place and the role on the world arena.

The main aim of the power sector reconstruction is to form market relations at power industry. It's specified by the fact that Russian power sector directly and seriously depends on governmental and political institutions and large private conglomerates, it means that power sector is a vertically integrated monopoly with a public form of ownership. According to many experts opinion, monopolistic structure saving at Russian power industry encourages ineffective funds and natural recourses usage that finally affects not only price for the provided service but also quality decrease and leads to consumption rights infringement.

Competitive conditions, created during reform, will let to optimize Russian power grid work and raise efficiency of generating capacity charging. It's connected with the fact that separation of the present monopoly into independent companies, according to the activity kinds will let to consolidate uncoordinated assets management; it will raise managing ability of the new power sector subjects (operating companies), simplify realization of the single development strategy and involve considerable financial resources. In addition, competitive market creation will raise investment attraction of the network infrastructure; it will create additional abilities for power energy transmission and extend its volume. Due to this, geography of the power energy trade will expand and become more sophisticated, including Export-Import operations. As a result, development of energy-saving technology and energy consumption schemes will be stimulated; it will considerably reduce power inputs per unit of a generated item. The reform will also let to solve the

problem of consumers powering reliability, eliminate local electric energy deficiencies which are typical for many Russian regions. Eventually, injected into sectors competitions will let to stabilize prices for electric energy at acceptable level and give new abilities for a schedule and purchases' value optimization as well as risk compensation.

It's obvious that power industry reformation will change sector's structure. New principles and mechanism of control and development of the power industry at the modern market economy should be developed as well as market criterion of estimation of the reform efficiency.

The work was submitted to III international scientific conference «Basic Research», Dominican Republic, April, 10-20, 2008, came to the editorial office 09.02.2008.

EFFICIENCY OF ENTERPRISES' BUSINESS PROCESSES AS PART OF MULTI-INDUSTRY INTEGRATED STRUCTURES IN TERMS OF INNOVATIONS DEVELOPMENT

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The world's experience shows that the stable economical growth and the gross domestic product increase are possible only on the innovative basis with active use of modern scientific and technological achievements and on the ability to innovative activities and introductions.

In present-day competitive activity the struggle for the ability to innovative introductions and not for the resource and material values possession takes place.

This problem is especially burning for our country, which is in the period of transition to market relations. Nowadays, for the majority of industrial organizations the adaptability to quickly changing economical and political situation is a pressing task.

In connection with this the choice of the enterprise's most effective innovative activities management instrumentation, the innovation potential estimation and the ways of innovation activity promotion development emerge. It will allow providing the competitive edge of the enterprise, defining the innovative activities' internal possibilities swiftly, detecting hidden reserves of the organization's development for the purpose of its commercial activity's efficiency upgrading.

The innovative activity's and its results' high level at the industrial organizations of developed countries is conditioned, to a large extent, by the availability and constant development of the complex of multi-industry relations formed on the one hand –

under the pressure of competitive conditions, and on the other hand – at the governmental active assistance.

Lately an increased attention to the role of innovations at the state's economic policy implementation has been paid in Russia. A package of measures on the innovation infrastructure in the form of special economic zones and technology parks is carried out; competitions on the innovation programs' financial backing within the framework of the National Project are held among educational institutions. A significant attention is paid to the discussion of the science development strategy and its reforming. However, all these measures are of fragmentary character and do not take into account the features and tendencies formed in the innovative activities subjects.

It becomes apparent that to preserve and develop the scientific and industrial potential of the country there should be doubled the guiding and regulating role of the government in the strategy and tactics of reforms, especially if it concerns the innovative activities in the sphere of higher education. So, for example, the higher education sector percentage in internal research and development costs in Russia makes 6, 8%, for the comparison in Japan – 13, 9%, in Germany – 16, 9%, in the USA – 15, 9% and average over the EC – 21, 5%.

Among other guidelines of the governmental scientific and technological policy there outstands the flight to strengthening of science and education interrelations, and also the principles of guaranteeing the priority development of basic scientific research and the integration of scientific, scientific and technological and educational activities on the ground of various forms of participation of workers, post-graduates and students of educational institutions of higher professional education in scientific research and experimental developments by means of creation of educational and scientific complexes on the basis of educational institutions of higher professional education, scientific organizations of Science Academies having the governmental status, and also scientific organizations of national departments and other federal executive bodies.

Nevertheless, in present-day economics there is no other alternative in our enterprises except searching and using swiftly any potential to enhance the competitiveness on the ground of innovations using the like interest of other native economy branches' subjects.

Today even strongest enterprises have no opportunity to provide a high state level of all the links of their value chain simultaneously using their own resources that is required for a rival product offering. This makes them include other organizations' value chain separate elements, which allow implementing this task, into their business processes.

In the necessity perception, and also the combinative processes realization itself, the integration participant should not treat them formally following in

this connection the principle of pragmatism and final objective distinct vision. In general, the integration activity in the microlevel (organization level) makes sense in the horizontal level, if it brings the participant organization to the following moments:

a) the promotion of efficiency and effectiveness of its own business processes carried out on the most modern basis – intensive penetration, introduction and diffusion of innovations;

b) the access to those partner's resource categories, which are critical for the development of its own business processes and reaching by them the required results;

c) the access to the partner's business processes' disconnected elements, which are critical in terms of their "inclusion" into its value chain;

d) the enhancement of the joint with the partner access to the sources of financial and other third parties' resources at the expense of increased investment appeal of the integrated structure both for the government and non-governmental organizations.

In the enumerated cases the integration processes really can give the IC participant a significant effect, and the expenditures connected with their implementation are feasible taking into account the long-term effect of integration.

The joint efforts on the integration provision, in spite of their complex character, become easier because the potential partners treat them from the same positions, and pursue the same objectives, that allows reaching a consensus on many complex questions and final effect of synergy.

It is especially indicative for the innovation role growth conditions in achieving competitive success of organizations from any branches, and thus, even according too the given above points b) and c), it is their innovative components, making partner structures consider the integration as an opportunity to develop their own innovative activities, that are meant more often.

To achieve the output goods' market novelty high degree, taking into account the objective necessity of the innovation "transition" through special phases from its ideal to product form, enterprises have to integrate with those organizations, first of all, without the participation of which the success probability of such a "transition" diminishes sharply.

For our country such basic innovation process participants are the following organizations: Research and Development Institutes (academic and industrial), higher education institutions (polytechnical) and enterprises (small and large). Their role in the innovation cycle conditioned by the ability to "cover" its various phases by their activities is reflected in the picture 1.

The availability of a peculiar resource, business process and product collection within the limits of its branch belonging in every basic integration participant means the possibility of their cross use by other participants of the integrated structures pursuing

their own and general ends. In other words, the practical implementation of the participants' voluntary integration idea is based on their very motivation to perform product-resource exchange among themselves.

It follows from that the estimation of the state and integrated structures participants' effective economic contacts formation perspectives can be based on the analysis of their interaction level consisting in every participant's resources and final output degree of utilization by other participants. Being compared to the achieved level of final activity factors of the integrated structure itself, the participants' interaction degree will reflect the integration efficiency. The similar comparison will allow shedding light on the integrated structures participants' functioning peculiarities both individually and in the aggregate, giving its estimate, making the perspective forecast and developing managerial solutions adequate to the situation at various levels of their control. In such a conception the development of integrated structures is directly tied in with the maximization of industrial product innovation cycle successful completion possibility by means of strenuous integration of the innovation infrastructure relevant elements actualizing these phases.

Let us note that within the framework of the meaningful integration the probability of the fact that the product of one of the participants will be a re-

source inlet for business processes of other participants rises sharply.

It will not in word, but in fact make the integrated structures organizations critically comprehend their own and partners' activity selecting only the most effective participants for the integration, establish more and more closer product-resource contacts among themselves finally striving for the effect of synergy.

To our opinion, only on this ground native enterprises and organizations of different industries can produce competitive goods and have a chance of corresponding to severe requirements of present-day economics, that defines a high relevance of the development of strategies and concrete measures on the IC formation in our country.

In this connection we should note that the NSTU Business Department research team has been performing investigations in this direction for some years already and has got definite results, which will be aimed to solve the problems described in the presented report; the investigations being supported by the grant of the RF Department of Education and Science.

The work was submitted to international scientific conference "Innovation technologies», USA, (New York) December, 19-27, 2007, came to the editorial office 26.10.2007.

*Materials of conferences***SYSTEM MODELS OF ARMED CONFLICTS**

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In our comprehension a *system model* means an abstract and logically closed (“ringed”) description of a phenomenon represented in the form of autonomous organization of elements and relations. The construction of an armed conflict system model implies that there is no and *cannot be* any armed conflict, which couldn’t be consistently described within the framework of the specified system model [1]. In other words, a *system model* has no (in our comprehension mustn’t have) variants and versions.

Any system object, in specialists’ opinion, must satisfy certain indispensable principles of consistency, that is: to consist of several interconnected components, to have a relative insularity from other objects, i.e. a particular autonomy, and, finally, to possess a minimal internal integrity (that means that the integer is not organized into a sum of elements) [2].

The construction of armed conflicts system models cannot be implemented out of the field of the following axiomatic statements:

- all armed conflicts have one causal basis at their root;
- all armed conflicts have one mechanism and logic of development (but not one dynamics – it is important not to confuse these two notions).

From these considerations, the simplest system model can exist, where in the limited space with scarce resources the role of components is performed by political actors with “vital interests” dynamically changing their force and direction. This dynamics of basic interests can be easily brought in balance, and even mathematical one, with the dynamics of armed confrontations. The curves of dependence of the probability of its entering into an armed confrontation on the direction or length of the “vital space” enlargement vector can be easily constructed for every component (that corresponds to the direction and intensity of the interest). At that, other dependence parameters, like the geopolitical position (and the correspondent claims for resource zones and areas of influence), the internal dynamics of the actor (the political system evolution, change of technology, etc.), the peculiarities of global processes (the climate, demography, etc.) can be introduced into the specified model. The simplest conditions can be introduced as well; for example, to preset obligatory power balance vectors or vectors of “land” and “sea” fight. Here the simplest dependences can be controlled.

All this can be pictured in the form of forces or vectors, when thinking schematically, directionally working in the limited space. It is clear, that the sys-

tem on its own account, at any level, moves in the total force direction in the investigated space. The conflicts within the system are caused by the polarization of forces in such a point of the system space, when at maximal expressiveness of the component vectors (mathematically – at maximum tendency to vector module increase) the resultant of political forces tends to zero. When drawing a closer parallel with classical mechanics, the political process actors should be pictured not in the form of simple physical bodies, but as similar charges bearers, the potential of which, at that, is constantly changing dynamically. The force polarization on a definite and limited section of the system leads to an over-strain and a tendency to get rid of an extra potential either by means of an essential reorganization of forces (say, political decision of the problem) or by a direct drop of the potential through an armed exterminatory conflict, like, for example, the Thirty Years’ War of 1618-1648.

The dialectical approach brings to this model definite rules or, in other words, structures the course of endogenous processes. The central idea here is a bi-polarization of the system; this polarization bearing not only structural, but substantial character. Within the framework of the dialectical approach many specialists confine themselves with structural oppositions’ statements; for example, democracy in some countries – totalitarianism or autocracy in others. Capitalism and communism, West and East, rich North and poor South, etc.... The bi-polarization conditions the rise and development of a variety of contact zones, where political actors’ interests collide. If we turn to a vector scheme, we can formulate “political” laws of dialectics, that is – first, there are conditions for every pair of political subjects, where the resultant of their interests’ vectors tends to zero, *by all means*; second, for any point of a political system there is *one* pair of subjects as a minimum, the resultant of interests of which tends to zero, by all means. These laws irrationalize the bi-polarity and define the original dialectical contradictoriness of the system. The effects of these laws will be the statements, according to which, first, between any two political actors at corresponding conditions an armed conflict is possible, and, second, there is not a point of the political system space, where the interests of any pair of subjects could meet each other. The state of “zero resultant” can be defined by us as a contradiction of the contradiction subjects – the oppositions, which form a dialectical unity in struggle; the struggle itself being aimed at the contradiction settlement, that results in quality modification and progress of the entire system.

The power balance in this model plays a dual role. On the one hand, it naturally counterweighs the opponents’ potentials, that leads to containment or keeping the opponents from extremes – an armed conflict in our situation. On the other hand, the power

balance magnifies the strain due to the same counterweight of the forces and, as a consequence, the formation of well-defined poles equally naturally with the result that the magnifying strain can exceed a certain threshold and find expression in a bloody conflict. While the dictate of the hegemon naturally structures the system rigorously in the unbalanced system. It is what Modelsky means, when speaking of non-anarchy within the system of global leadership. However, the artificial concealment of the *hegemonic dictate* with the idea of the *world's leader* makes his scheme scientifically false conceptually.

Unbalanced systems exist for a little time irrespective of either the matter concerns the power balance within the polity or it is referred to regional or global relations. The peculiarity is that the power balance can never be absolute either, as the subjects are dynamic and the "equilibrium point" always tends to zero on a certain imaginary bidirectional number axis reflecting the power level of the opponents. Though a multi-ray (multiaxial) scheme of the balance, where the number of rays is identical to the number of power "absorbers" would be a more accurate one. It is clear that the ideal variant will be the one with the balance point position in the zero point; actually it is constantly migrating.

The velocity and offset value of the power balance point is of a far greater importance, than even its position and distance vector directivity. The higher the velocity and the more the balance point offset value, the more probable the development of an armed conflict. As a matter of principle, there is, however, a non-calculated purely mathematically velocity and balance point offset magnitude threshold, the exceeding of which with maximal probability gives rise to the development of an armed conflict. Moreover, one can with good reason suppose that for every culturally specific zone this threshold will be peculiar. Furthermore, these threshold values dynamically change with the course of time, depending on political environment dynamics. Theoretically, it is possible even to study the dependence of the specified thresholds change on various universal and unique factors for every culturally specific zone.

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The work was submitted to III international scientific conference "Human and noosphere", scientific heritage of Vernadsky V.I., Global problems of present-day civilization, Italy, Sicily, 15-20, July, 2007, came to the editorial office 07.02.2008.

METHODS OF SOCIAL-ECOLOGICAL DIAGNOSTICS: ESSENCE AND REALIZATION FEATURES (EDUCATIONAL ASPECT)

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The most important problem of present-day higher education is the one of students' training and mannerliness level quality. It is referred to all the orientations of professional training, including the aspect of students' interaction with the environment, and education in this sphere. The last is conditioned by the necessity to form the readiness of all people to the establishment of optimal social-ecological relations of the corresponding competence.

A constituent part of the quality determination process is the examined object's state diagnostics. Our research has allowed finding out the essence and features of one of its kinds considered. In general, the **diagnostics in the sphere of learning youth's social-ecological education** supposes a purposeful determination of the learners' state of readiness to the optimal interaction with the environment, education in the sphere of social ecological relations conditioned by pedagogical, psychological, social and social-ecological contexts. The pedagogical context supposes the maturity of necessary social-ecological knowledge, social-ecological skills, creative and emotionally axiological attitude to the environment; common pedagogical principles. The psychological context means the maturity of knowledge and skills in the area of general and developmental psychology; the directivity in the subject of psychology of attitude to nature (Yasvin V.A., Deryabo S.D.); the behavior and activity in it. The social context involves the maturity of knowledge, abilities and skills aimed at the identification of social conditions affecting the character of the interaction of personality and natural habitat; peculiarities of macro- and microenvironment creating a foundation of social-ecological culture. The ecological context supposes students' preparedness for the environment quality assessment implementation (monitoring, control and modeling).

The students' training level diagnostics in the area of social-ecological education of learning youth is a complex one, supposes the availability of academic knowledge of natural and human sciences, integrated science areas. The following should be referred to them in this case: ecology, geography, ecosystem exploitation, pedagogy, psychology, social ecology; the idea of research methods of these sciences, and also specific diagnostic methods. The *purposes* of the developed *diagnostics* are pedagogical ones, the implementation methodology has a sophisticated, complex character, supposes the use of methods of all the sciences necessary in this case.

The *diagnostics object* – is the students' education in the area of interaction of the society and na-

ture in the process of their chosen speciality acquirement. The *diagnostics subject* – is the state of students' preparedness for social-ecological education of learning youth. The *diagnostics subject matter* is various aspects of both students' social-ecological education proper, and the future pedagogical specialties students' preparation for the education in the area of social-ecological relations under the conditions of educational institutions. Thereby, the subject matter of the diagnostics developed by us involves two principle blocks differing from each other by a specific filling.

The first block – the main one – includes the content of the social-ecological science and school children education in the area of the environment. It is focused on the definition of social-ecological knowledge and skills, creative and emotionally axiological attitude of students themselves, as it expands the basic culture of a personality in mutual relations with natural habitat, obtained at secondary school; strengthens the formative position in social-ecological interrelations; forms the persuasion of the necessity of further society-nature relations' harmonization. The competence in the social ecology problems will let students form the correspondent knowledge in conditions of comprehensive educational institutions. It will also be promoted by the second component of the main block – the pedagogical one – revealing the foundations of the social-ecological education of school children: its purpose, informative and processual elements. In connection with it, the definition of student's level of the essence and subject matter assimilation of these elements appears to be necessary.

The other block of the diagnostics considered by us is an additional one and is represented by knowledge and skills in the area of general and developmental psychology, geography, history and other particular methodics, problems of ecological pedagogy and psychology. The social knowledge, uncovering the functions of the society towards nature, the legal aspect among them, and also the regional knowledge and skills reflecting the peculiarities of the local population and the nature around it, is included into this block as well.

Summing up, let us emphasize that the diagnostics in the area of social-ecological education is a complex, many-component-in-matter process of defining of many-sided readiness of learning youth to the interaction with the environment, education of other population strata in this area, self-education. Various characteristics of learners at cognitive, activity and personality levels are identified in the social-ecological diagnostics; the attitude of the society to the problems of the society and nature interaction in global, regional and local scale is determined; the pedagogical process peculiarities, aimed at the formation of social-ecological readiness, are found out; the necessary conditions, affecting the specified parameters, are established. For example, defining the preparedness of students for social-ecological education of

school children the state of their theoretical preparedness, the attitude to natural habitat and its optimization conditions, the relations with different competent social structures, the personal involvement of every student into practical workmanship in nature should be found out.

The diagnostics includes not only the content, but the corresponding methods as well. By the **methods of social-ecological diagnostics** we mean the ways of finding out its content's principle elements, the defining of the essence and peculiarities of every element according to the predetermined criteria, factors and levels. The composition of social-ecological diagnostics methods is represented by the methods of social, psychological, pedagogical and ecological diagnostics. At that, the sought methods selection from these groups was performed by the way of revelation of the overall representation of any of the methods in every aggregate.

The specified diagnostics, particular methods of every of them have allowed defining the aggregate of the students' social-ecological education diagnostics methods. They are represented by four main groups: monitoring, inquiry, mathematical methods, modeling. The monitoring includes the observation, valuation, content analysis, forecasting; the inquiry – the questionnaire, conversation, interview, testing; mathematical methods – statistical ones, the aggregate definition; the modeling – the analogue and logical ones, mental experiment.

Our further research are connected with finding out specific peculiarities of every of the methods, the conditions of their application in the pedagogical process of higher and general educational institutions.

The work was submitted to international scientific conference «Present-day problems of science and education», Russia, (Moscow), May, 13-15, 2008, came to the editorial office 10.01.2008.

INNOVATIVE TECHNOLOGY DEVELOPMENT IN THE SPHERE OF SOCIAL MANAGEMENT

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At present time in Russia some directions of social work with the population are generated.

Apart from traditional for the country technologies of social protection, state guarantees and classical forms of training, innovative technologies of social management aimed at development of creative skills and self-keeping behaviour of Russians are formed.

Author's development in this direction are presented in the given message. They concern substantia-

tions of social mission of the ecological tourism which is carried out in a mode of continuity of generations and social-and-pedagogical technologies of a healthy way of life forming among students. We believe, that in a modern quickly varying society, the social role of tourism as of a tool for people communiti consolidation is unique, highly effective and demanded. It is possible to classify many forms of tourism as socially significant (cognitive, scientific, ecological, ethnographic, pilgrim, historical, excursion, scientific expeditions, sport-and-health, improving, sports, family). All of them are not only profitable, but also socially significant. They bring an essential contribution to improvement of stability in a society, consolidation of family, formation of a world-view and the outlook of rising generation, provide continuity of generations in the way of their life and scripts of group behaviour. They are characterized not only by the high heat of interpersonal dialogue, develop everyday communication between generations. Besides it is a high-speed channel of the information about appropriate and the most effective ways of social adaptation and strategy of tolerant behaviour transfer to posterity, it allows to form simultaneously and synchronously useful life experience for two, three and more generations of people. An example of rising interest of scientists to social forms of tourism is scientific tourism which now actively revives in Russia after a period of lull. Scientific schools formation by a method of scientific tourism is rather effective. It includes active psycho-technics in the form of carrying out of seminars, debates and brain storms in the informal conditions strengthened by landscape, climate and esthetic effects. This direction of tourism successfully continues the best traditions of The Institute of Plant and Animal Ecology UrD RAS, that have been founded by the famous geneticist N.V.Timofeev-Resovsky, and are known among the scientific world as annual seminars in Miassovo.

We offered a creative way of development in this direction: a form of family cognitive tourism in "grandparents and grandsons" format. The given kind of social tourism is actual for modern Russia. It is connected not only with free time availability among the grandparents, its absence among parents and youngers' desire for interactive dialogue. It is supported by new economic realities: development of a financial system in the country, improvment in pension funds and social protection services. Today there are all conditions for social society consolidative mission of tourism development. We analysed the tendencies of self-keeping behaviour among students of innovative Urals high schools, the ability of experienced teachers to work with modern interactive health saving technologies, and established a mismatch between expected results and the real situation in the given sphere.

The found out disbalance in a "teacher-pupil" system was the stimulus for us to develop new social-and-pedagogical technologies.

It is established, that self-keeping behaviour among the students of innovative Urals high schools is not homogeneous in its manifestation. Three variants of self-keeping behaviour of students may be described: students that admit the popularity of healthy way of life (HWL) in society and among their contemporaries, those who do not admit this popularity, and those not yet defined with their attitude to HWL. The proportion of representatives of the above specified types of behaviour among the polled students has made 1:1:2. It is found out, that the declared and the real way of life of a significant part of respondents differ essentially. It is revealed, that among the persons declaring popularity of HWL, only 1/2 of respondents practise HWL in everyday life.

Among the persons denying popularity of HWL, the number of persons, having healthy way of life in practice is 1/3. The minimal number of the persons practising HWL, (1/4 from the number of respondents in the group), is observed among those not defined in their attitude to HWL, maiking "social drift" and have not generated the script of life.

Specificity of self-keeping behaviour of students with different types of attitude existential and rational is formalized. It is fixed, that at the first group the values of interpersonal dialogue (love, friendship) prevale in their world-view, at the second group material equivalent (money, career, social prestige) predominate. The first prefer to use moderate physical activities (walks, sauna, sports) as antistress actions; the second use chemical means (alcohol, smoking, etc.) The found out features of self-keeping behaviour, to our opinion, must be considered when developing technologies of HWL formation among youth.

It is established also, that the majority of experienced teachers of HIGH SCHOOLS and schools undergo serious psychological difficulties when working with interactive health-keeping technologies. They prefer those variants of self-keeping behaviour formation among youth which are presented by medical information about negative influence of bad habits and appeals to refrain from the last. The given type of HWL formation, as it is known, is many times less effective, than its antagonists - tempting advertising of consumer behaviour and the latent advertising of bad habits (beer, alcohol, etc.). In the described situation search of new methods of HWL formation which would be simultaneously effective for youth and comfortable for teachers is necessary.

In view of the described facts a compromise social-and-pedagogical innovative technology is developed. It is offered to replace "frightening-and-indifferent" style of communication in a "teacher-pupil" system by "optimistic-and-constructive" one. The given technology is introduced into educational process in USTU-UI for students-sociologists.

More detailed results of innovative social-and-pedagogical technologies creation are presented in author's publications, published at www.talalaeva.uran.ru (a server of Institute of Mathematics and Mechanics UrD RAS).

The work was submitted to international scientific conference "Innovation technologies", USA (New York), December, 19-27, 2007, came to the editorial office 15.11.2007.

*Materials of conferences***CORRELATION METOD IN HISTORIC RESEARCHES**

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The correlation method of static series reflecting social and economical processes has a wide enough spread occurrence. The attributive series correlation is used to the lesser extent, though in historical studies the quantitative formalization of politico-social events can be most often expressed in them particularly. At the Mordovian State University the experience showing that the application of methods, in particular, of rank correlation can work well in the politico-social sphere, has been collected. As an illustration it is possible to quote the investigation of agrarian waves of peasant unrest in Mordovia in 1917 and 1905-1907. It is usually thought that both disorders were determined by the peasants' lack of land and the authorities' weakness. In the documents of the Provisional Government bodies the peasant-

participants of the agrarian movement are differentiated on historical and national features. The peasants of Mordovia were referred mainly to 11 national-historical groups, however, there are villages belonging to 7 groups only there in the records on the topic. Finally, in October-November, 1917, at the peak of the movement, there are only "mass massacres" often spoken about in them. All this gives evidence of the quantitative data inaccuracy and that the rank correlation is the most appropriate method to use here. The maximum energy in the movement was demonstrated by the Russian former manor peasantry (Rus. Man.) – I rank, then – Russian former demesne one (Rus. Dem.) – II rank, Mordovian former manor peasantry (Mrd. Man.) – III rank, etc. Let us place them in series in a table with the correspondent ranks of land amount per host. In this case we shall obtain the Spearman's rank correlation coefficient $\rho_1 = -0.93$. I.e. between the participation of Mordovian peasants in the agrarian movement of 1917 and their provision with land there was a rather close feedback.

| Peasantry | Rus. Man. | Rus. Dem. | Mrd. Man. | Mrd. Dem | Rus. St. | Mrd. St. | Tat. St |
|----------------------|-----------|-----------|-----------|----------|----------|----------|---------|
| Activity | I | II | III | IV | V | VI | VII |
| Land amount per host | VII | VI | IV | V | III | II | I |

The coefficient of Kendall $\tau_1 = -0,81$, though more carefully, but also shows the high closeness of this relation. If we carry out an analogous investigation for 1905-1907, we shall obtain accordingly $\rho_2 = -0,57$, $\tau_2 = -0,33$. I.e. the unrest waves of 1905-1907 were conditioned by the lack of land to a lesser extent than those of 1917, and - to a greater extent turned up to be the reflection of All-Russia po-

litical crisis. And in 1917 the specified closeness of the relation was stimulated by the Land Decree, which had provoked the communities to capture outside lands.

The work was submitted to international scientific conference «Innovation technologies», USA (New York), December, 19-27, 2007, came to the editorial office 14.01.2008.

*Materials of conferences***CONTINUOUS ECOLOGICAL EDUCATION –
PLEDGE OF ECOLOGICAL SITUATION
STABILIZATION**

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The ecological situation in the world becomes more strained year after year. And, in spite of the fact that there is an active propaganda on the effect of the ecological world outlook formation in the MSM and scientists have been beating the alarm, the situation appears impossible to alter.

The reason, most likely, lies in the very lack of ecological culture, the base of which is made up of so-called ecological world outlook. And, as it is known, the outlook starts shaping together with the first steps of the baby, and therefore, to reckon on the result is only possible at the organization of continuous ecological education, starting with the earliest age.

The many years' experience of our work allows making the following conclusions:

1. The ecological education, first of all, should rest on the foundational nature laws (mathematical, physical, chemical and biological) and the laws formulated by the scientist Commoner B., which rest on the main nature laws, as well, and, together with that, allow evoking a response from the human consciousness.

2. It is necessary to check into the place of the human being on this planet. And, if he is a "part of Nature", he loses the main human dignities, and more specifically:

- the ability to be charged (with those who are by your side, with "Friars Minor", with the old and small, etc.);

- to live on the laws of the "house", which he is happened to be born in (to remember about the interconnections in nature, not to collect garbage, to regulate his own wants and invent such technologies, which do not cause damage to the outward things);

- to organize life in conformity with human society laws as distinct from animal communities (herd, flock, etc.).

3. Throughout all the aspects of human culture (music, painting, architecture, education, etc.) it is necessary to strive for the formation of the sense of harmony, which is the only to help groping for the life harmony of one's own accord, the relatives and surroundings, both natural and created by human hands.

4. The acquaintance with the world, especially at the early age should be built upon positive principles: the world is still beautiful, and to inspire the sense of love and care towards it in the child, the last should admire this world, at first.

The authors, trying a shot to embody those ideas, which they had come to, have created a concept of nursery educational literature – "First steps in the outworld", meant for 12 albums in accord with the year months, have published an "Ecological album for preschoolers", have developed a program on the school subject "Environment" and printed exercise books for 1-4 grades' learners. Every publication for children is attended by detailed methodical recommendations, where the aspects aimed at the formation of the ecological world outlook are specially emphasized.

The work was submitted to III international scientific conference «Actual problems of science and education», Cuba, March, 19-29, 2008, came to the editorial office 16.01.2008.