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Contents

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Cell biology

Ganglioside changes of rabbit spinal cord in early phases of experimental allergic encephalomyelitis

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Rabbits immunized with total brain gangliosides developed a chronic experimental allergic encephalomyelitis (EAE) with clinical and pathological features resembling multiple sclerosis in man. The total concentration and the relative distribution of major gangliosides (GM1, GD1a, GD1b and GT1b) isolated from spinal cord of EAE rabbits before the appearance of gross clinical signs and from controls were determined. The existence of biochemical changes of these gangliosides is especially remarkable in the lumbal part of the spinal cord. The data obtained show an evident decrease of GD1b content in parallel with high level of GM1 in EAE rabbit spinal cord during the early phase of the disease.

Key words: brain gangliosides, EAE, rabbit.

Experimental allergic encephalomyelitis (EAE) is an autoimmune disease of the central nervous system (CNS) induced by sensitization of various animal species to CNS tissue or myelin components. It is a widely studied animal model because it shares many clinical and histological features with the human demyelinating disease multiple sclerosis (MS) [1]. For long time myelin basic protein has been considered as the main encephalitogenic autoantigen [7]. However, it was recognized that other myelin components, the proteolipid protein and the gangliosides, may give rise to an encephalitogenic response, which leads to a chronic EAE in rabbits [2, 3, 4, 5, 11].

Gangliosides are acidic glycosphingolipids which are highly enriched in the central nervous system of vertebrates. They have been implicated in a wide range of processes including induction of experimental demyelinating diseases. Injected under special conditions, brain gangliosides cause an autoimmune multiple sclerosis-like disease. Gangliosides undergo characteristic changes in composition during the development of the illness and in MS plaques [13, 14]. However, the ganglioside alterations in early phases of multiple sclerosis and EAE have received little attention.

The present study was undertaken to evaluate the ganglioside composition of the spinal cord of rabbits with EAE induced with gangliosides before gross clinical signs were evident.

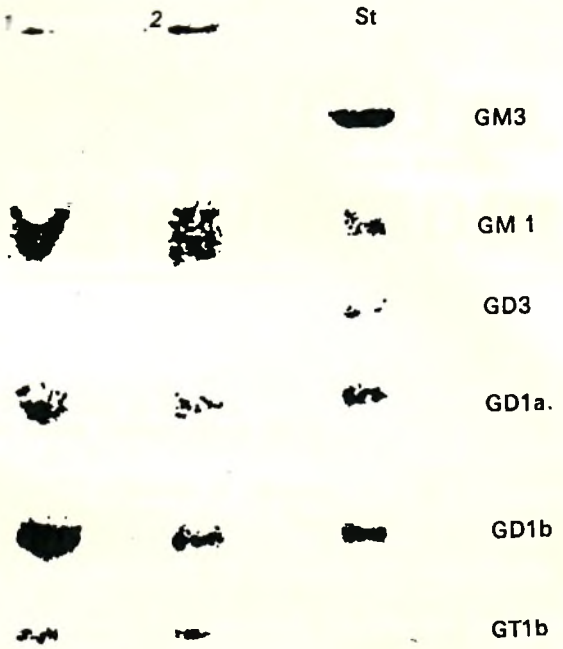

Fig. 1. Thin-layer chromatogram of total spinal cord gangliosides
1 — control animals; *2* — EAE rabbits; St — standard mixture gangliosides (Calbiochem)

Materials and methods

Twelve rabbits of the Chincilla strain, weighing 2,2-3 kg, were immunized with 5 mg highly purified bovine gangliosides prepared as described previously [6] following the scheme of C o h e n et al. [3]. Two control rabbits received 5 mg methyllated bovine serum albumin in complete Freund's adjuvant without gangliosides. Animals were examined daily for clinical signs. Clinical disease was graded on a scale from 0 to 4 as follows: 0 — no clinical signs; 1 — ataxia; 2 — hind or forelimb paresis; 3 — hind or forelimb paralysis; 4 — limb paralysis plus incontinence.

The major brain gangliosides (GM1, GD1a, GD1b and GT1b) were extracted from all parts of the spinal cord of nine EAE rabbits (disease grade 1) and from two control animals by the method of I l i n o v et al. [6].They were submitted to thin-layer chromatography (TLC) fractionation and identified by comparison to standards and named according to S v e n n e r h o l m and F r e d m a n [12] (Fig. 1). GM1, GD1, GD1b and GT1b were quantified by densitometric scanning of the plates at 580 nm. A typical densitogram of these gangliosides from EAE rabbit spinal cord is shown in Fig. 2. The Student test was used to determine statistical differences between the groups using $p<0,05$ as the level of confidence.

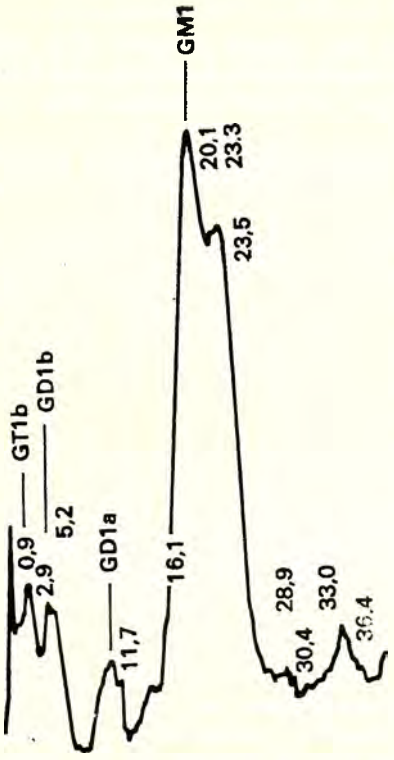

Fig. 2. Typical densitogram of the major gangliosides from spinal cord of EAE rabbits

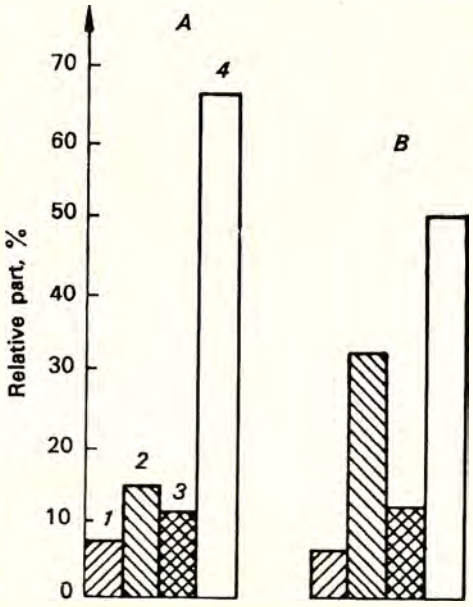

Fig. 3. Percentage distribution of the four major gangliosides — GT1b (*1*), GD1b (*2*), GD1a (*3*) and GM1 (*4*), in spinal cord of EAE (*A*) and control rabbits (*B*)

Results

Nine test rabbits (75%) developed clinical signs of neurological dysfunction, most frequently beginning 20-25 day after inoculation. None of the control rabbits showed signs of illness.

Regional differences in ganglioside patterns of the various spinal cord areas of the nine rabbits with ataxia (disease grade 1) have been recognized. The existence of biochemical changes of the GM1, GD1a, GD1b and GT1b in the lumbal part of the spinal cord is especially remarkable.

The percentages of GM1, GD1a, GD1b and GT1b in the spinal cord were recalculated on the basis of the densitograms (Fig. 3). The data obtained show that GM1 predominates in spinal cord of EAE rabbits (66%) and in control animals (50%). Another interesting finding is an evident decrease of the GD1b content in EAE rabbits (14%) in comparison with controls (32%).

Discussion

Ganglioside-induced chronic EAE in rabbits has several features that make it particulary useful as a model of multiple sclerosis. In our previous investigations we have described some clinical, immunological and morphological data concerning this EAE [4, 5].

In the present study we have performed biochemical analysis of ganglioside changes in the spinal cord during the early development of the disease, when the rabbits first showed clinical symptoms (ataxia).

The typical purified ganglioside preparations obtained from rabbit spinal cord contain four major gangliosides in the following average proportions: 1) GM 1 — 50%, GD1b — 32%, GD1a — 12%, GT1b — 6% (control animals); 2) GM1 — 66%, GD1b — 15%, GD1a — 11%, GT1b — 8% (EAE animals). There was an evident decrease of GD1b content in parallel with high level of GM1. The decrease of GD1b suggests that a partial block may exist in the conversion of GD2 to GD1b as it was found in amyotrophic lateral sclerosis [10].

As mentioned before there are no data concerning the early ganglioside changes of brain and spinal cord in EAE and MS. In the disease a decrease of GM1 was found in MS plaques in brain and spinal cord [13, 14]. As in multiple sclerosis, EAE is characterized by inflammation and demyelination [8]. Merril et al. [9] have shown that inflammation in brain and spinal cord preceded clinical signs of EAE. Therefore the evaluation of early disease changes in brain and spinal cord are of great importance.

Summarizing the present knowledge on the pathology of EAE, the similarities to the alterations in human inflammatory demyelinating diseases are striking. This indicates that very similar pathogenetic mechanisms are responsible for the initiation and propagation of the lesions [8]. Thus our knowledge of the pathogenetic mechanisms involved in the animal models may be relevant for the understanding of the human diseases.

This work is supported by a grant from the National Fund "Scientific Research".

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Morphological and quantitative analysis of prepubertal rat germ and Sertoli cells in conditions of vitamin A-deficiency

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Control and vitamin A-deficient (VAD) rat testes were investigated on days 3, 6, 12, 20 and 25 after birth. The germ cell counting on day 3th p.p. showed nonsignificant differences between VAD and control testes. The quantitative study during prepubertal period demonstrated a reduction of germ cell number and their ^{3}H-Thymidine labelling index. In- and B-spermatogonia were considerably reduced whereas A-spermatogonia decreased in a lesser extent. The Sertoli cell number and their labelling index were unchanged. Ultrastructural observations revealed normal structure of Sertoli cells while germ cell showed varying degenerative features. The obtained results suggest that during prepubertal period when significant changes occur in testicular cell populations, VAD severely affected germ cell structure and proliferation.

Key words: vitamin A-deficiency, germ cells, Sertoli cells.

Introduction

Recently it has been established that in addition to the hormonal regulation (FSH, LH and testosterone) spermatogenic function is controlled by an intragonadal paracrine system. Interactions between germ, Sertoli, Leydig and peritubular cells have been demonstrated by many authors [14].

An approach in the investigation of paracrine regulation of spermatogenesis is selective in vivo alterations of seminiferous epithelium using various experimental models and the study of different parameters of principal testicular cell populations. Vitamin A-deficiency is a good model for the study of interactions between germ and Sertoli cells.

Vitamin A in the form of retionol has been known to be essential for normal male reproduction. A deficiency of this vitamin in adult rats results in a degeneration and loss of germ cells which leads to an arrest in spermatogenesis at preleptotene stage [7]. Quantitative studies on adult VAD rat testes by Mitranond et al. [12] showed a decrease of the number of different types germ cells, while Unni et al. [19] demonstrated a reduction of mitotic activity of spermatogonial cells. According to the recent autoradiographic studies [10, 12] there appears to be a mitotic division arrest of A-spermatogonia in adult VAD rat testes. This type spermato-

gonia is responsible to reinitiation of spermatogenesis in synchronized manner after vitamin A-replacement [4].

The ultrastructural study of above-mentioned authors showed different degrees of degeneration of germ and Sertoli cells and their intercellular contacts.

The exact mechanism by which vitamin A-deficiency acts on the process of spermatogenesis is still unknown. Whether this effect is direct on germ cells or mediated by Sertoli cells is under discussion. On the base of biochemical studies about retinol esterification and accumulation in Sertoli cells S h i n g l e t o n et al. [17] postulated a hypothesis that Sertoli cells are central place in retinol metabolism of seminiferous tubules. E s k i l d et al. [3] demonstrated maximal levels of mRNAs for cellular retinol binding protein (CRBP) in early postnatal period.

There is no information about the effect of VAD on male gametogenesis during prepubertal period when important and significant changes occur in testicular cell populations that precede the oncet of spermatogenesis and determine the fertility in the adults. The aim of present work was to investigate the quantitative and morphological changes in principal cell types of prepubertal rat testes in conditions of vitamin A-deficiency.

Materials and methods

Female Wistar rats were fed on diet without vitamin A (AIN-76™ Purified diet for rats and mice [1]) at first day of pregnancy (when spermatozoa were found in the vaginal smears in the morning). New-born male rats continued to receive the same diet. The water was given ad libitum. Control and VAD male rats were killed on days 3th, 6th, 12th, 20th and 25th after birth. For electron microscopy (EM) the testes were fixed in 2,5% glutar aldehyde, postfixed in 1% OsO_4 and embedded in Durcupan. EM observations and microphotos were made on Opton EM 109. The selective 6-day-old animals were injected i. p. with ^{3}H-Thymidine (1 μCi/g body weight) and were killed after 3 hours. The testes were fixed in Serra's fixative and embedded in paraffin. Deparaffined sections 5 μm were dipped in Ilford-K_2 emulsion and processed for autoradiography. The quantitative analysis on 3, 6, 12 and 20 day p. p. was made on semithin Methyleneblue-Azur II-basic Fuchine stained sections. The general germ and Sertoli cell number and the number of different types germ cells were established in circular cross-sections of seminiferous cords and tubules. The per cent of labelled germ and Sertoli cells was counted in autoradiograms. For statistical analysis Students's *t*-test was used.

Results

On day 3th p. p. the counting of germ cells per cross-section of a seminiferous cord showed nonsignificant differences between VAD and control testes. The quantitative study during prepubertal period (6, 12 and 20 day) displayed a rapid decrease in general germ cell number in VAD animals comparing with the controls (Fig. 1). It is obviously that the tendency of several times multiplication of normal germ cells number during prepubertal period was absent in VAD specimens where a slight increment of that parameter was established. The Sertoli cell number per cross-section of a seminiferous cord and tubule during investigated period showed nonsignificant differences between VAD and control rats (Fig. 2).

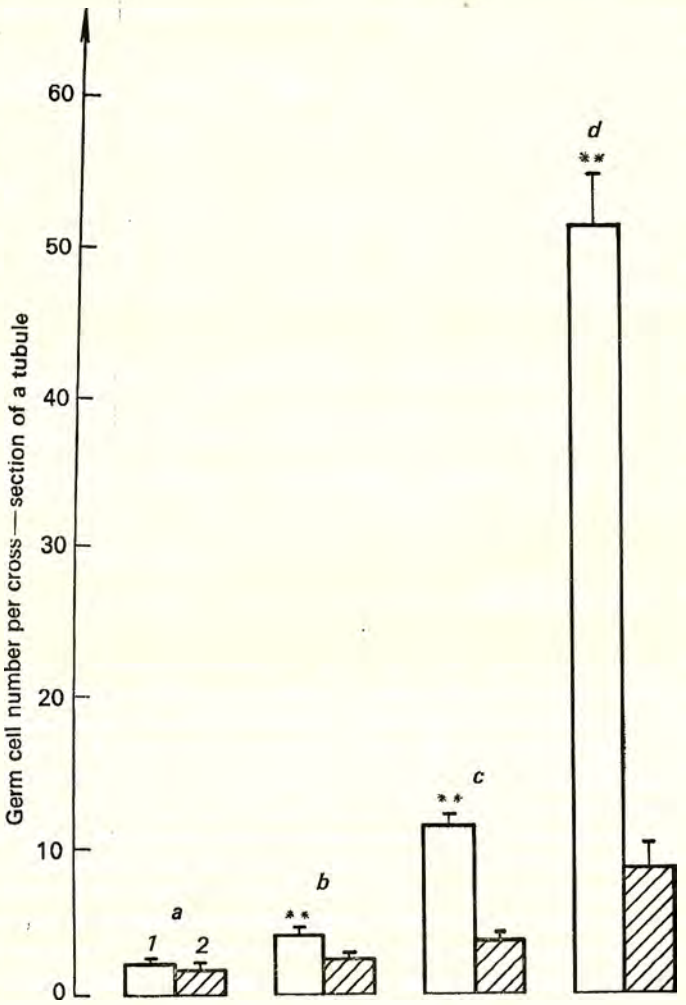

Fig. 1. Germ cell number per cross-section of a seminiferous cord and tubule of 3- (*a*), 6- (*b*), 12- (*c*) and 20- (*d*) day-old control (*1*) and VAD (*2*) rat testes. Data represent the mean $\pm$SD; ** — $p<0{,}01$

Autoradiographic data on day 6th p. p. demonstrated 25 % decrease of ^{3}H-Thymidine germ cell labelling index in VAD testes. The differences of Sertoli cell labelling index in VAD and control specimens was not significant (Fig. 3).

The quantitative study of different types spermatogonia on day 12th showed a reduction of relative number of differentiated spermatogonia (A-, In- and B-sg) in VAD rats whereas the number of undifferentiated spermatogonia increased (Fig. 4). At this age only in VAD animals a considerable number of degenerating germ cells in division was established.

In 20-day-old control and VAD testes meiotic germ cells were present but the most advanced in spermatogenic cell differentiation were the pachytene spermatocytes. The seminiferous tubules of VAD testes contain Sertoli cells, spermatogonia and few spermatocytes. The relation of spermatocyte to spermatogonial

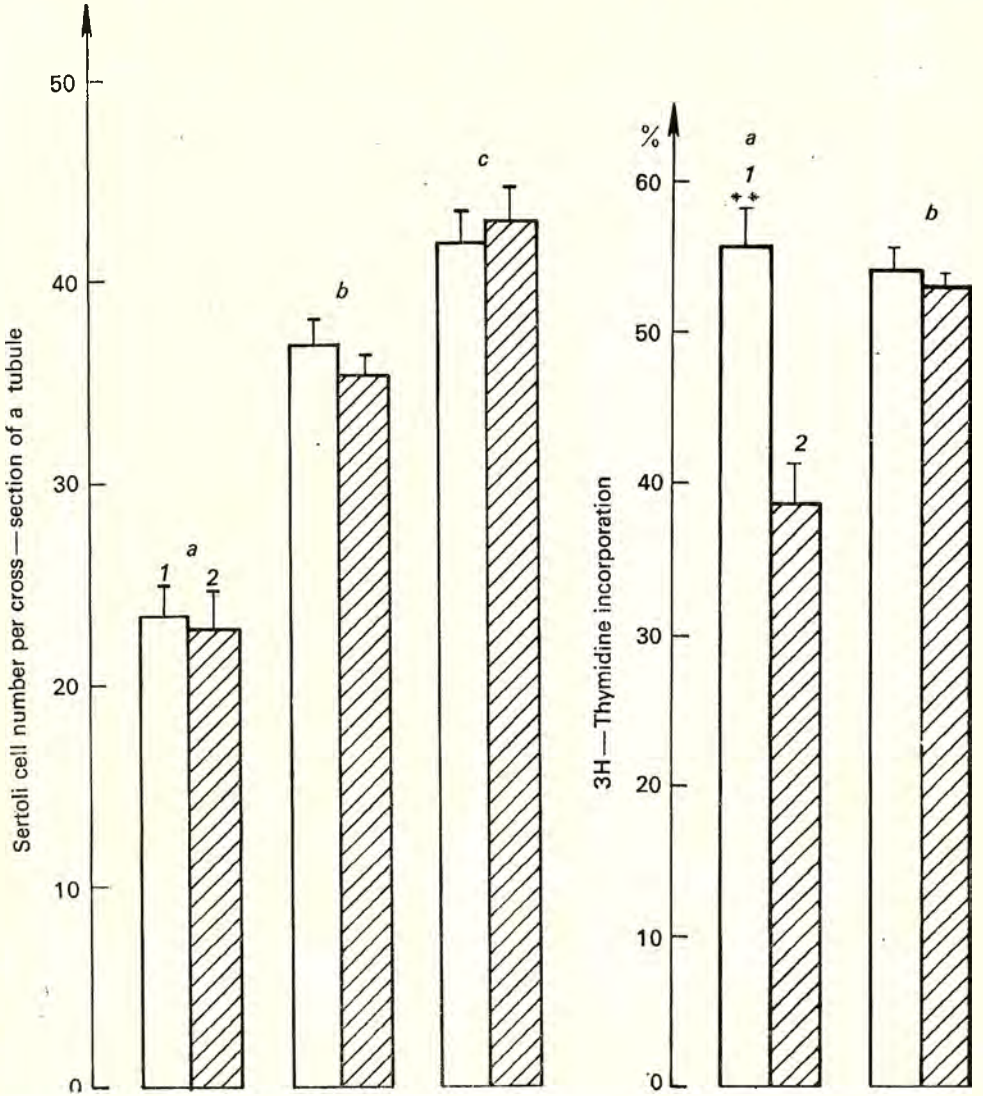

Fig. 2. Sertoli cell number per cross-section of a seminiferous cord and tubule of 6- (*a*), 12- (*b*) and 20- (*c*) day-old control (*1*) and VAD (*2*) rat testes. Data represent the mean±SD. The differences between VAD and control means are not significant ($p<0,1$)

Fig. 3. ^{3}H-Thymidine labelling index of germ (*a*) and Sertoli (*b*) cells of 6-day-old control (*1*) and VAD (*2*) testes. Data represent the mean±SD; ** — $p<0,01$

number showed that in controls spermatocytes predominated as a result of normal germ cell differentiation whereas in VAD specimens spermatogonia prevailed. A considerable reduction of different types germ cells in experimental animals at this age was established. With several time decrement in spermatocytes number, that of In- and B-spermatogonia was rather reduced whereas A-spermatogonia decreased in a lesser extent (Fig. 5).

Ultrastructural observation on 6-day-old VAD testes showed a normal structure of seminiferous cords — Sertoli cells lay on the basement membrane and

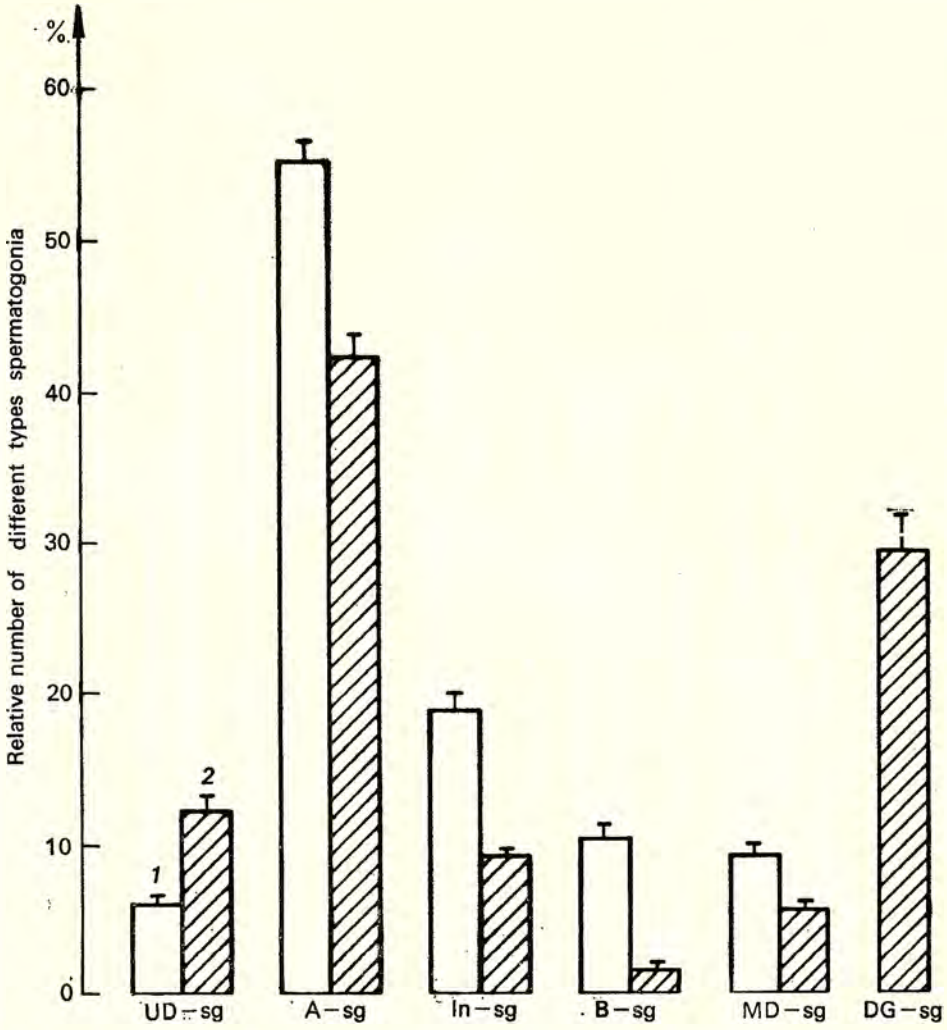

Fig. 4. Relative number of different types spermatogonia of 12-day-old control (*1*) and VAD (*2*) testes

UD-sg — undifferentiated spermatogonia; A-sg, In-sg and B-sg — types A-, In- and B-spermatogonia; MD-sg — mitotic dividing spermatogonia; DG-sg — degenerating spermatogonia. Data represent the mean ± SD; $p<0,01$

prespermatogonia were centrally situated. At this age a normal Sertoli cell structure was visible. Simultaneously with intact prespermatogonia at some places degenerating germ cells connected with wide cytoplasm bridges were observed (Fig. 6). In their cytoplasm many vacuoles were found. In 12- day-old VAD testes the intact Seroli cells and great number of degenerating dividing germ cells was established. They ultrastructurally were visualized as large spherical cells in which cytoplasm numerous vacuoles were seen (Fig. 7). Degenerated germ cells were fagocytozed by Sertoli cell cytoplasm.

In 20-day-old VAD seminiferous tubules spermatocytes were rarely found and some of them showed different degrees of degeneration. Spermatogonia for-

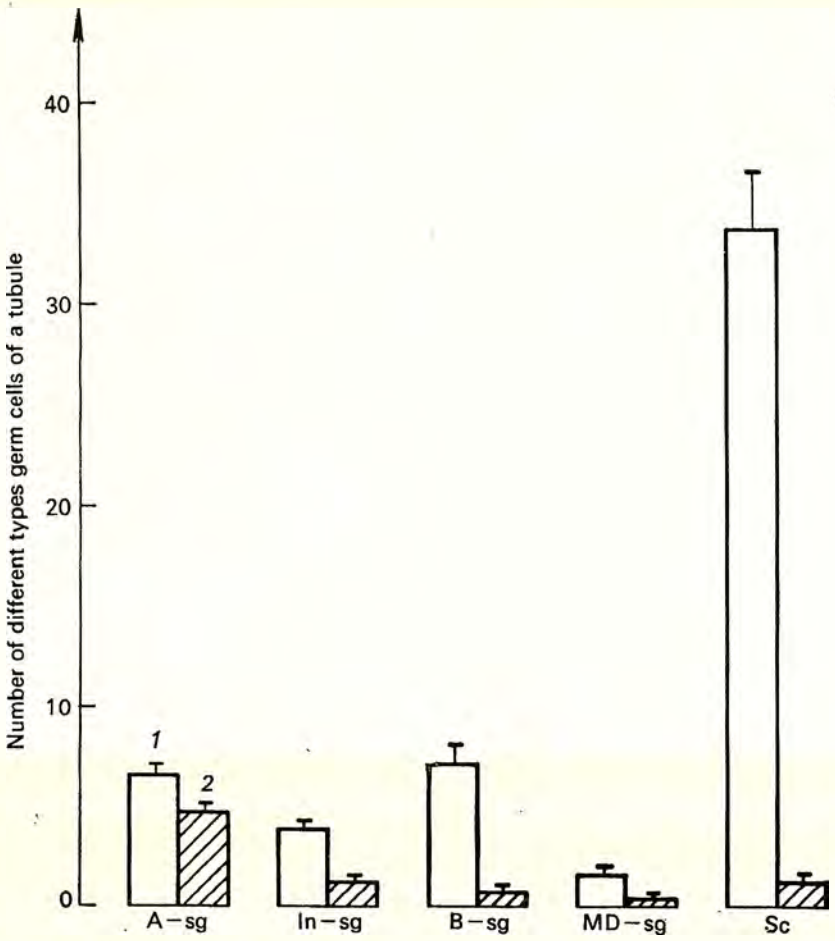

Fig. 5. Number of different types germ cells per cross-section of a seminiferous tubule of 20-day-old control (*1*) and VAD (*2*) testes

A-sg, In-sg and B-sg — types A-, In- and B-spermatogonia; MD-sg — mitotic dividing spermatogonia; Sc — spermatocytes. Data represent the mean ±SD; $p<0,01$

med a heterogeneous population. Certain cells showed varying degenerative features such as irregular nuclear contures, disruption of nuclear envelope, picnotic nuclei with condensed chromatin and cytoplasm vacuolization (Fig. 8). At some places binuclear germ cells with abnormal nuclear shape and contures (probably spermatogonia) were seen. At this age simultaneously with germ cells in process of degeneration a considerable number of intact spermatogonia situated on basal membrane were observed. Most of them were type A-spermatogonia whereas In- and B-spermatogonia were infrequently seen.

On 25th day in control testes clearly formed tight junctions between neighbouring Sertoli cells were visualized while in VAD gonads a typical morphology of these contacts was not seen and desmosome-like structures were found. A disruption of normal seminiferous tubule structure was observed and some spermatogonia which normally lie on the basement membrane were displaced inside to the lumen area. An uncomplete cytotomy of several spermatocytes was demonstrated by wide cytoplasm bridges (Fig. 9).

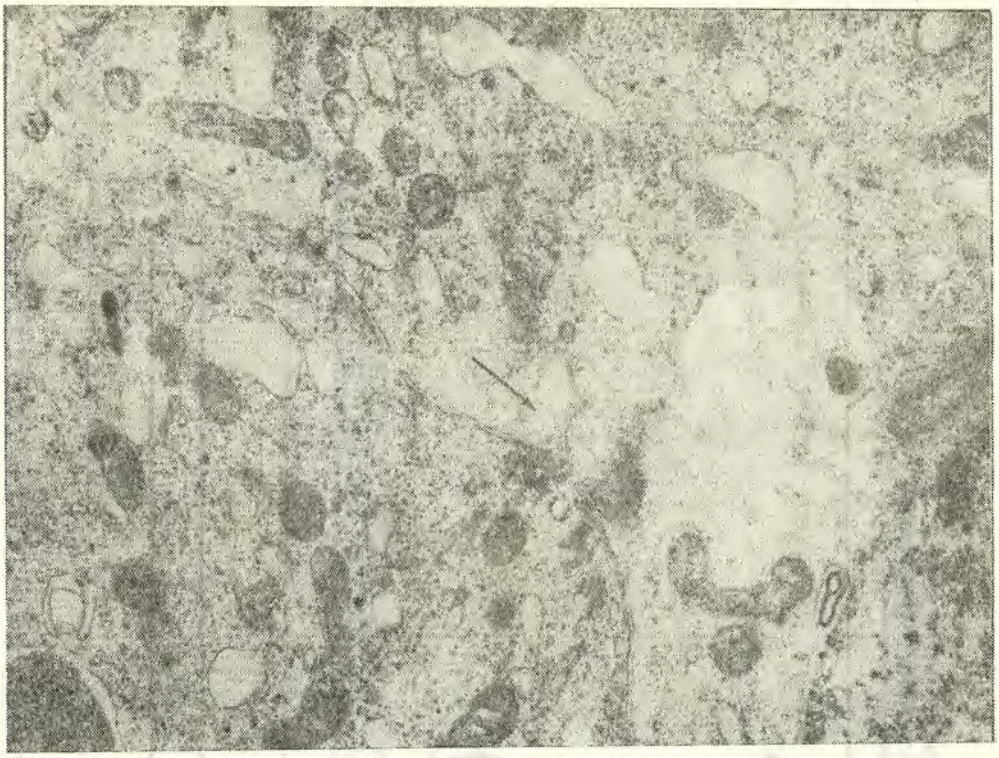

Fig. 6. Electron micrograph (EM) of 6-day-old VAD rat testis. Two degenerative germ cells connected by cytoplasm bridge (arrow), ×28 575

Discussion

During prepubertal rat period on day 4,5 p.p quiescent T_1-prespermatogonia reinitiate their mitotic division and give rise to T_2-prespermatogonia [5]. The latter reduce their size by some consecutive divisions, migrate from seminiferous cord center to the basement membrane and give rise to A-spermatogonia. Our data showed that on day 3th p.p. the number of quiescent T_1-prespermatogonia was unchanged and the decreased prespermatogonial number in 6-day-old VAD testes most probably due to retention of entering mitosis of T_1-prespermatogonia. This suggestion was confirmed by autoradiographic data on day 6th that demonstrated 25 % decrement in ^{3}H-Thymidine germ cell labelling index.

Our investigations on VAD rat testes during prepubertal period (6, 12 and 20 day) showed a considerable reduction of germ cell number in VAD animals comparing with the controls. The extent of this reduction increased with ontogenesis and could be explained with more continuous action of VAD on germ cell proliferation. Our data are in addition to quantitative study of M i t r a n o n d et al. [12] that demonstrated strongly decrease of germ cell number in VAD adult rat testes.

The increased relative number of undifferentiated spermatogonia in 12-day-old VAD testes is probably due to retention of mitotic division of T_1-prespermatogonia and their differentiation into A-spermatogonia. The reduction of absolute and relative number of three types differentiated spermatogonia (according to

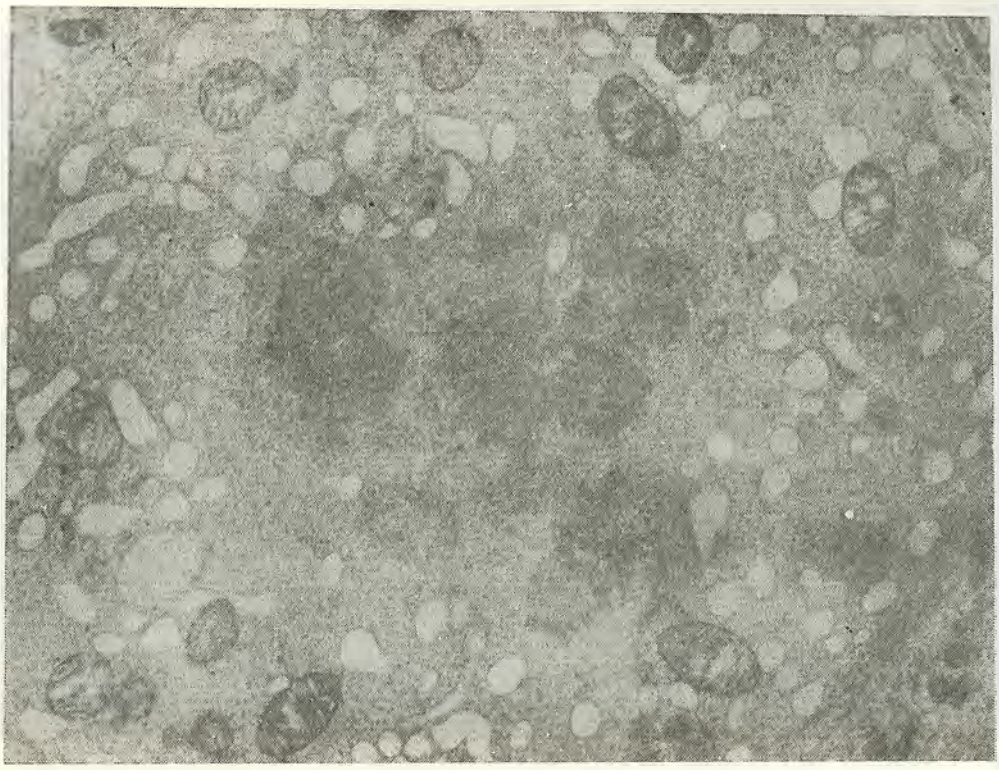

Fig. 7. EM of 12-day-old VAD testis. Cytoplasm vacuolization of a degenerative dividing germ cell, ×16 425

Clermon and Bustos-Obregon [2]) is significant. At this age only in VAD gonads a considerable per cent of degenerating dividing germ cells was established. It is known that in rat by day 12th B-spermatogonia after dividing give rise to preleptotene spermatocytes, which perform the last DNA synthesis and enter meiotic prophase I. That is the second crucial moment (after the first proliferating wave of prespermatogonia on day 5th) during rat prespermatogenesis. Probably at this age the germ cell division at given VAD conditions is rather sensitive stage of germ cell differentiation.

The germ cell counting on day 20th showed that in VAD testes spermatogonia prevailed upon spermatocytes unlike the controls. The considerable reduction of spermatocyte number could be interpreted as a maturation depletion fenomenon that was discussed by Ismail and Moales [10] to explain severe germ cell loss during vitamin A-deficiency. Our quantitation of the three types differentiated spermatogonia demonstrated that in VAD testes on 12th and 20th day A-spermatogonia decreased in a lesser extent comparing with the In- and B-spermatogonia. These data are coincident with results of Ismail et al. [9], Van Pelt and De Rooij (21) that demonstrated a selective mitotic arrest of A-spermatogonia in adult VAD rat testes. This type spermatogonia survives avitaminosis-A and is responsible to reinitiation of spermatogenesis after vitamin A replacement.

The Sertoli cell number per cross-section of seminiferous cord and tubule and their ^{3}H-Thymidine labelling index in prepubertal VAD gonads were unchanged comparing with the controls. It is known that rat Sertoli cells mitotically divide

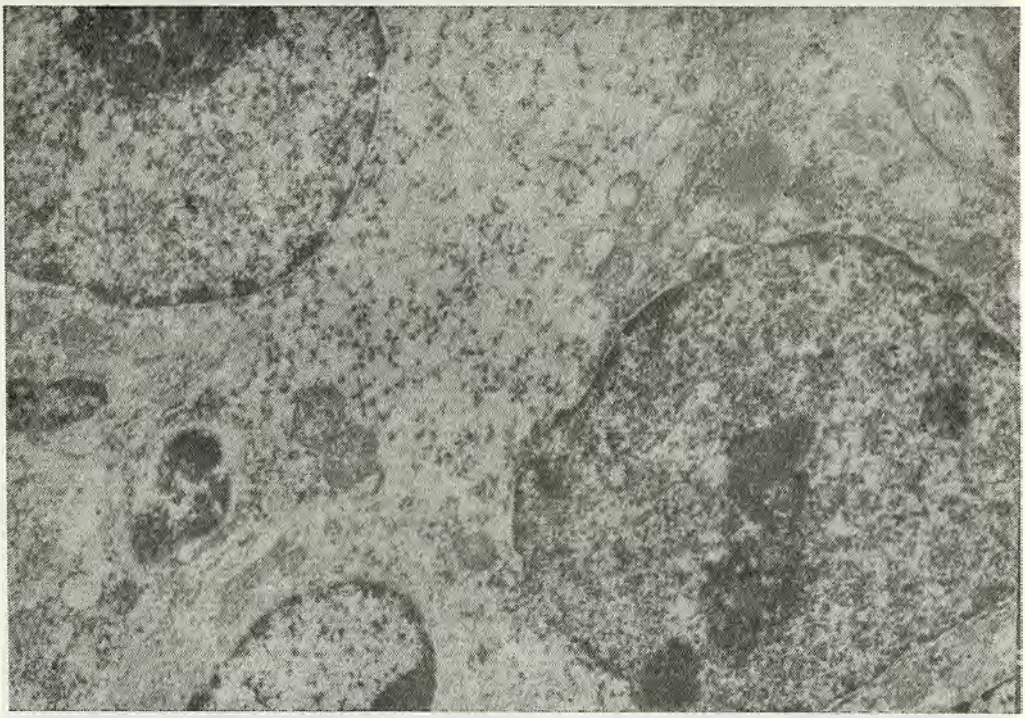

Fig. 8. EM of 20-day-old VAD testis. Cytoplasm vacuolization and onset of degenerative nuclear changes of two B-spermatogonia, × 14 700

by day 15th p.p. after that they stop proliferating and form a constant cell population in the testis [15]. Probably vitamin A-deficiency had no effect on Sertoli cell proliferation during prepubertal period but the question about Sertoli cell functional alterations in that conditions is under discussion.

The ultrastructural observation are in addition to quantitative results and showed destructive changes in germ cells. Similar alterations in germ cell structure were demonstrated by S o b h o n et al. [18] and U n n i et al. [19] in adult VAD rat testes. The absence of typical structure of inter-Sertoli cell tight junctions in 25-day-old VAD testes could be discussed with results of H u a n g et al. [7] demonstrating disruption of these specialized contacts. The recent study of I s m a i l and M o r a l e s [10] showed intact Sertoli cell tight junctions during vitamin A-deficiency. This discrepancy between both investigations is probably due to some differences in VAD status and its effect on Sertoli cell function to form tight junctions.

The decreased germ cell number and ultrastructural changes in VAD testis during prepubertal period could be discussed in the aspects of the functional coupling of immature Sertoli and germ cells and the modulating action of Sertoli cell secreted mitogenic factors. The study of O r t h and B o e h m [16] showed in neonatal rat testes presence of gap junction-mediated communication between Sertoli and germ cells that is of great importance for germ cell development. In earlier study [11] we established that prepubertal rat Sertoli cells secreted mitogenic factors which modulated germ cell proliferation. It is of great interest for us to study whether this Sertoli cell function will be affected by vitamin A-deficiency. According to U n n i et al. [20] VAD caused a great reduction in

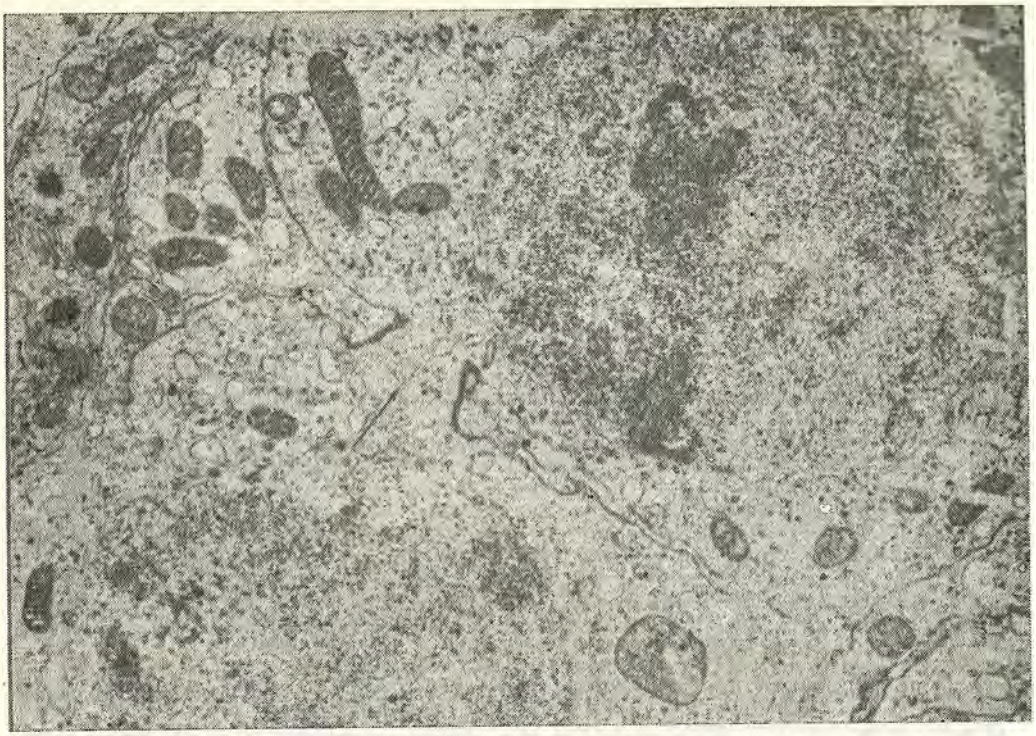

Fig. 9. EM of 25 day-old VAD testis. Two spermatocytes connected by wide cytoplasm bridge (arrow), × 16 425

mitogenic activity of adult testis homogenates. The investigations of Hugly and Griswold [8] and Morales and Griswold [13] demonstrated a significant decrease in the levels of both transferrin and SGP-2 mRNAs in adult VAD rat testes. The contemporary interpretation of our results in terms of Sertoli cell-germ cell interactions requires investigation of Sertoli cell mitogen synthetic function during prepubertal period in conditions of vitamin A-deficiency.

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Effect of Lindane on the differentiation of embryonic chick gonads in culture

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The embryonic chick ovary and testis in organ culture were used to test the direct effect of a chlorinated organic pesticide, Lindane. The ultrastructural alterations in the three main cell populations allowed the authors to characterize the Lindane action and its basic mechanism in affecting the germ and somatic cells in the gonads.

Key words: Lindane, ovary, testis, organ culture, chick embryo, ultrastructure.

During the last decade the agricultural intensification was connected with the administration of various kinds of herbecides and insecticides which resulted in entrance and accumulation in biosphere of stable chemical compounds noxious for humans and animals. Lindane, the gamma-isomer of benzenehexachloride, holds an unique place among chlorinated organic insecticides. It is widely used because of its good solubility in water and lipids and because of its wide spectrum of action.

The uptake of Lindane into the body can occur mainly with water and food of animal and vegetable origin. It is known that the Lindane is quickly metabolized in the liver and the main excretion products are water-soluble conjugates of glucuronic acid and of sulphuric acid, free phenols etc. [6]. The metabolism of Lindane counteracts the accumulation of residues, and may be considered an important factor for maintaining an equilibrium between the uptake of Lindane and the excretion of its metabolites.

In warm-blooded animals, pesticides and their metabolites affect almost all organs and systems especially liver and reproductive system. Some data point to an augmentation of abortions in pregnant animals as well as of mortality of the offspring in regions with intense chimization [12]. A delayed sexual maturity and a diminished fertility in laboratory animals were also observed. Experimental studies revealed a gonado- and embryotoxic effect of some pesticides such as Lindane and sevin [9, 10].

In Mammals, Lindane is able to cross the placenta into the embryo [3]. In Birds, Lindane is accumulated in egg yolk, the 80-85% of the total quantity being detected in its lipovitellin fraction [3].

On the basis of these observations, the question of harmful influences on the fetus and on the reproductive system is of particular interest. It is very important to know if

Lindane effects directly developing germ cells and gonads or its metabolic products induce the histopathological alterations.

The present study was undertaken with aim to investigate the dirrect effect of Lindane on embryonic gonads during ontogenesis. The chick embryo was chosen as an experimental model, taking into consideration the fact that avian egg develops outside the influences of hormonal and other controlling factors coming from the maternal organism, in natural conditions as those neighbouring humans and animals.

Material and methods

Male and female gonads explanted in organ culture, were used. Our experiments concern 2 critical periods in chick embryo development: 1) 9th embryonic day (ED), immediately after the gonadal sex differentiation (stage of 7,5-8th ED) when indifferent gonad development is already oriented in male or female directions; 2) 15th ED, a stage when considerable differences in the development of male and female germ cells are observed: in the ovary, cortical oogonia enter the meiotic prophase, but in the testis an active spermatogonial proliferation is present.

When Lindane was given orally in rats, LD_{50} was 125 µg/g body weight but during longterm administration, doses between 25 and 50 µg/g did not have pathological effect [8]. On the basis of these results, in our experiments the action of 3 doses of Lindane was studied: high dose of 100 µg/ml of culture medium, a concentration near to the LD_{50}; middle dose of 60 µg/ml, and low dose — 40 µg/ml (the latter is effectless during in vivo administration).

The left testes and ovaries, explanted from 9- or 15-day old chick embryos, were cultured on celloidin membranes covered with a thin layer of 1% agar [5]. A culture medium containing yolk dialysates [4] and 25% calf serum, was applied. Lindane was added in final concentrations of 100, 60 and 40 µg/ml. A part of explants served as controls. After a 24h incubation, the organs were fixed in Carnoy's fluid or in 2,5% glutaraldehyde — 1% osmium tetraoxide, and embedded in paraffin or epon, respectively. The observations were carried out on a light microscope Opton, as well as on an electron microscope Opton 109.

Results and discussion

Using the mentioned above experimental technique for organ culture, we observed, as described previously [5], that the development of ovarian and testicular explants corresponds to the same process in vivo.

Administration of the high dose of Lindane (100 µg/ml) resulted in intense degenerative changes of the three main cell populations in the gonads: germ cells, satellite cells (Sertoli cells in the testis and prefollicular cells in the ovary) and interstitial steroidogenic cells. Histopathological alterations were observed on the two stages examined (9th and 15th ED) which point's to the fact that the embryonic gonad and the differentiating cells are quite sensitive to toxic action of Lindane. In the most cases germ cells are absent, a disturbed structure and almost full degeneration of the gonads were visible.

Treatment of the cultured gonads with a concentration of Lindane at the middle rate of 60 µg/ml of culture medium resulted in more diverse alterations in the ovary and testis. In earlier ontogenic stages (9th ED) the histological structure of the gonads was disturbed. In the testis, a desorganization of the seminiferous

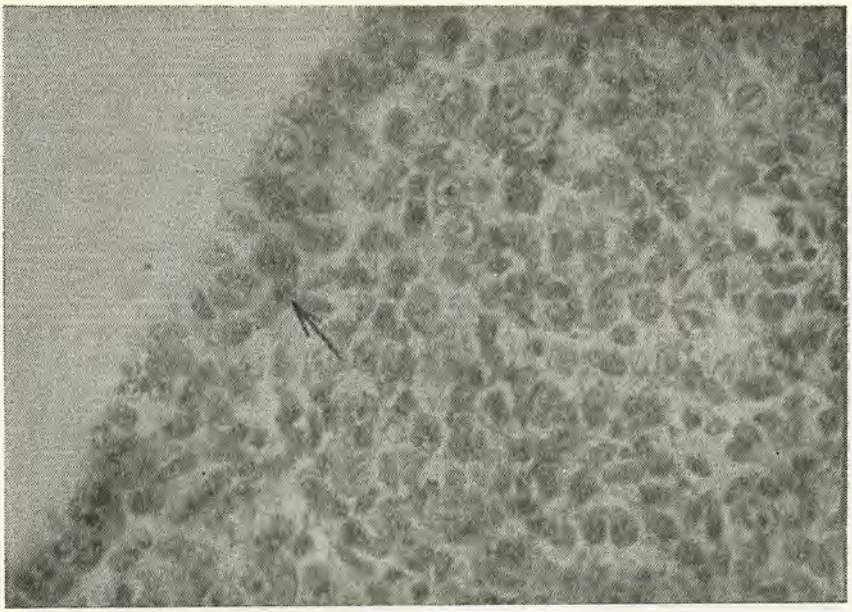

Fig. 1. A 9-day old testis incubated 24 hours with Lindane (60 μg/ml). Pseudocorticalization of the testis including the presence of a high germinative epithelium and a desorganization of seminiferous cords (↑), × 500

cords in the periphery occurred thus resembling a "pseudocorticalization" (Fig. 1). In the ovary, sometimes the typical orientation of the satellite cells around oogonia and the formation of the so-called "prafollicle" [2] was disturbed. At the beginning, degeneration affected the germ cells; later, the prefollicular cells were also damaged. Alterations in spermatogonia and oogonia were analogous; they were expressed in pyknosis and karyorrhexis, as well as in cytoplasmic vacuolization and lipid dystrophy. Ovarian interstitial cells and testicular Leydig cells were less damaged: the structure of some cytoplasmic organelles was affected, irregular lipid vacuoles and secondary lisosomes were observed.

At a dose of 40 μg/ml, ovaarian and testicular morphological changes were less expressed especially on day 9 of the embryonic development. The integrity of the membranous structures in the spermatogonia was affected. An irregular dilatation of the perinuclear space (Fig. 2) as well as of the endoplasmic reticulum was present. A destruction of the mitochondrial cristae or of the mitochondria, and a formation of myelin figures were observed. In the cytoplasm, secondary lisosomes and residual bodies were visible. The Leydig cells were more or less preserved, in some places cytoplasmic vacuoles were formed (Fig. 3). On the 15th ED the degenerative changes were more advanced. Cells more or less preserved as well as Leydig cells with damaged mitochondria and agranular endoplasmic reticulum, with myelin figures and irregular lipid inclusions were present.

In the ovary on the 9th ED lipid inclusions, destroyed mitochondria, lisosomes and myelin figures in the cytoplasm were observed. Fragmentation of nucleoli and irregular chromatin distribution (condensation or dispersion) in some oogonia were found (Fig. 4). Similar degenerative changes in prefollicular cells were also present. Interstitial cells were less damaged (Fig. 5).

On the 15th ED degenerative alterations in the female gonads were more ex-

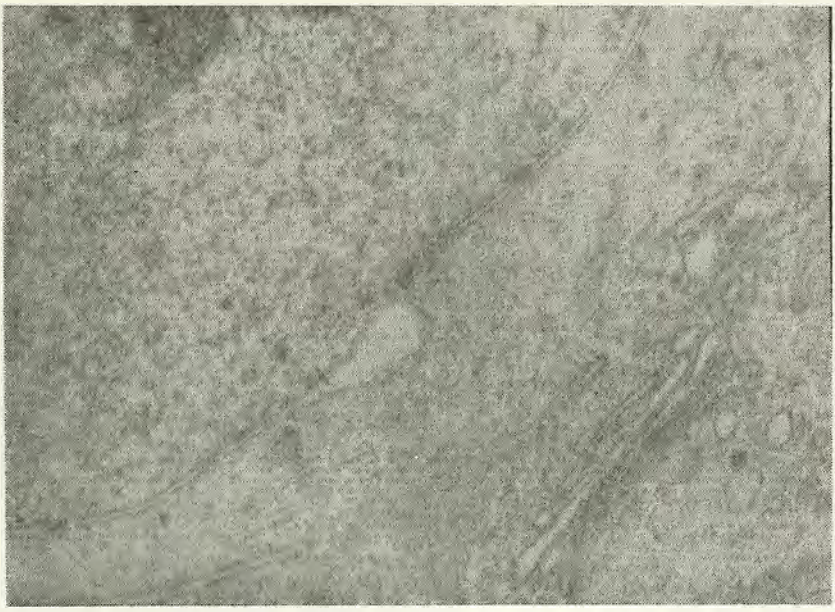

Fig. 2. Spermatogonium in a 9-day old testis treated with Lindane (40 µg/ml). Irregular dilatation of the perinuclear space is observed, × 20 000

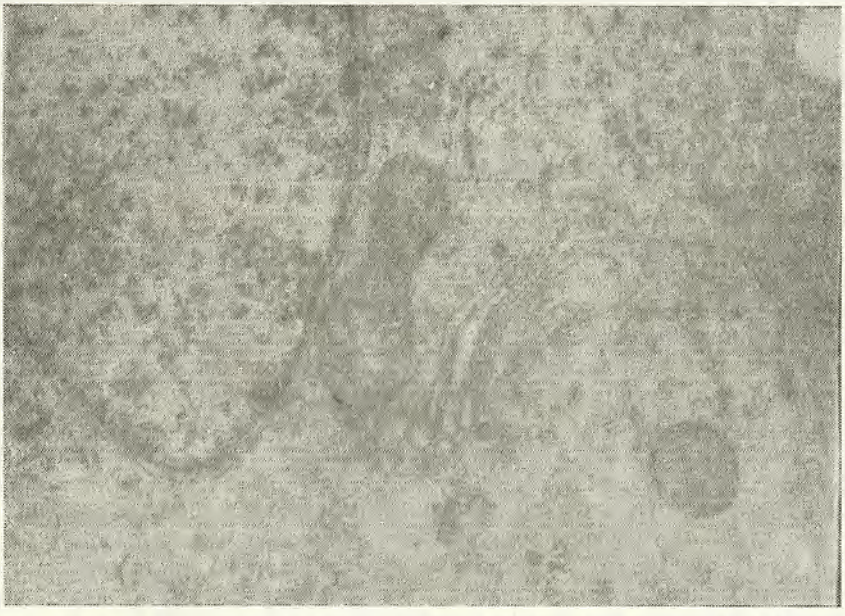

Fig. 3. A 9-day old testis is incubated 24 hours with Lindane (40 µg/ml). Irregular mitochondrial configuration and a cytoplasmic vacuole in a Leydig cell. × 20 000

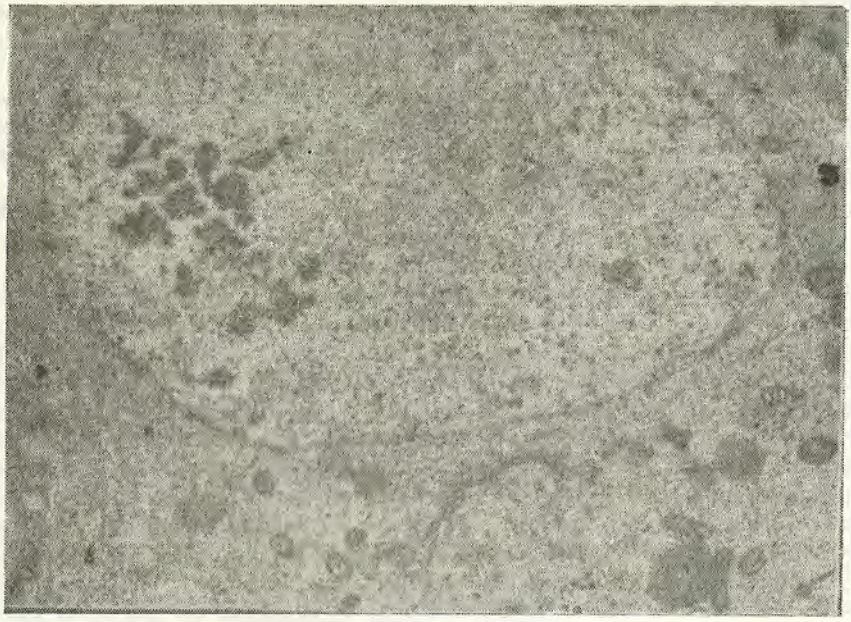

Fig. 4. Ovary from a 9-day old embryo after a 24 hours culture with Lindane (40 µg/ml). Nucleolar fragmentation and lipid inclusions in the cytoplasm of a cortical oogonium, × 20 000

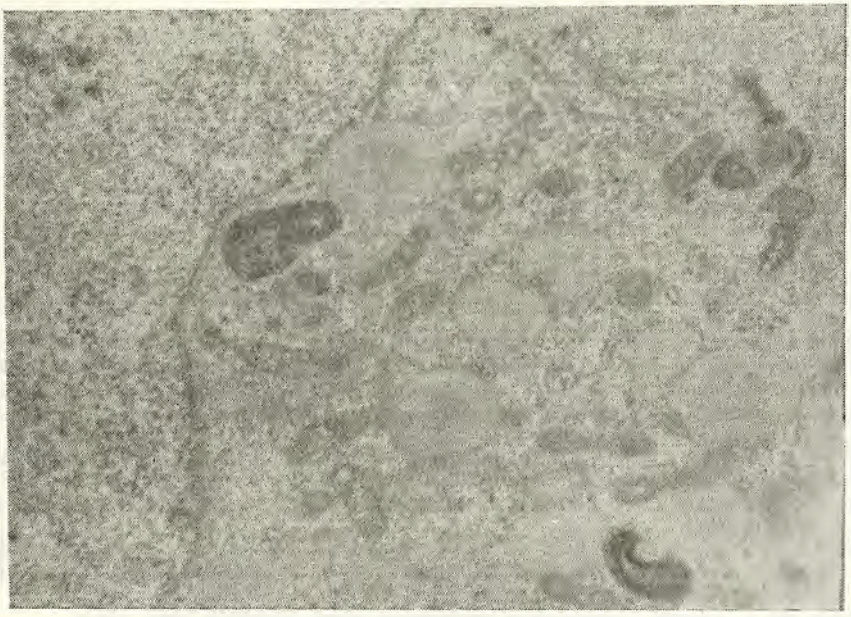

Fig. 5. Interstitial cell in a 9-day old ovary treated with Lindane (40µg/ml). Degenerative changes in mitochondria and myelin figures in the cytoplasm are present, × 12 000

tended. Some cortical oocytes were almost fully destroyed but other oogonia, prefollicular cells and interstitial cells with normal structure were also present.

The results obtained by us allow the following conclusions to be made:

1. The germ cells in both ovary and testis are the most sensitive ones to the direct effect of Lindane. The lower dose induced histopathological alterations of the membrane components and mitochondria, as well as of the genetic apparatus of the cell. When higher doses were administrated, both the germ cells and the somatic cells in the gonads were damaged.

2. During earlier stages of ontogenesis, the histological structure of the gonads is also affected (for instance, pseudocorticalization of the testis).

3. The induced changes are dose-dependent.

Our investigations supplement and extend the previous communication of Baleva et al. [1]. They present a more complete characterization of the Lindane action on the main gonadal cells populations and their ultrastructure. It was established that even in low doses, the pesticide induce pathological ultrastructural changes in differentiating germ and somatic cells. The observed alterations in the membranous structures are morphological characteristics of the toxic Lindane action. Recently, it was demonstrated that the interaction of pesticides with protein components of the interne mitochondrial membrane which causes an inhibition of electron transport and a decrease of the ATP synthesis in mitochondria, is a basic mechanism of toxic pesticide effect [11]. Our results confirm these data. The observations mentioned above suggest that the in vivo induced alterations in gonads are also due mainly to direct action of Lindane. On the other hand, the effect of Lindane on spermatogenesis, oogenesis and during the perinatal stages of development indicates the possibility of the induction of chromosomal abberations [7], which are also connected with the affected embryo development and survival.

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Fluorescent and flow cytometric analysis of cellular biochemical content of basic (cationic) cytoplasmic proteins in granulocytes

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Content of basic proteins in the cytoplasmic granules of granulocytes from human blood and mouse bone marrow is achieved by labelling with five sulfonated fluorescent dyes. Three of them — brilliant sulfoflavine, primuline and lucifer yellow are bound specifically to basic proteins in cytoplasmic granules of granulocytes — neutrophils and eosinophils. The staining is stoichiometric and the fluorescence intensity reflects quantitatively the content of basic cytoplasmic proteins in granulocytes. The exitation, wavelength, color of emitted fluorescence and histogrammes are characterized for each staining method. The best results are obtained with a new fluorescent dye lucifer yellow. This fluorochrome applied in our flow cytometrical studies gives basis for development of quantitative cytochemical method for flow cytometrical diagnosis of basic cytoplasmic proteins in cells of myeloid series (granulocytes from peripheral blood and their precursors from bone marrow) — in normal and pathological conditions.

Key words: basic cytoplasmic proteins, myeloid cells, granulocytes, fluorescent cytochemistry, flow cytometry, sulfonated fluorescent dyes (brilliant sulfoflavine, lucifer yellow, primuline).

Basic (cationic) proteins in the cytoplasmic granules of neutrophilic and eosinophilic granulocytes are good markers of myeloid cells from the peripheral blood and bone marrow [1, 3, 8, 13, 14]. The content of these proteins, which are also bactericidic substances, changes in cases of bacterial, viral and fungal infections, leukaemias, autoimmune diseases, cancer etc. [4-7, 9-12].

The purpose of the present study is the fluorescent and flow cytometrical analysis of basic cytoplasmic proteins in myeloid cells (neutrophils, eosinophils and their precursors) — from the human peripheral blood and mouse bone marrow, after fluorochromation with some sulfonated acid dyes. The results obtained are compared with these of our previous cytological method for staining of basic proteins by fast green, which is also acid sulfonated dye [8, 9].

Material and methods

I. Fluorescent cytochemistry

Human leukocytes from the peripheral blood smears and haemopoietic cells from mouse bone marrow smears were fluorochromated by acid sulfonated dyes (brilliant sulfoflavine, lucifer yellow, primuline, sulforodamine B or acid red 52, acid blue 93 or methylblue etc., obtained by Merck, Aldrich and Sigma). The staining was performed by different concentrations of fluorochromes — from 0,1 % to 0,001 % solutions in PBS or borate buffer (pH 7,2—8), without or after fixation of smears. After 5 min staining smears are rinsed in the same buffer, air-dried, cleared in two xylenes (30s each) and embedded in Fluormount. Fluorescentmicroscopical studies were carried out with a Zeiss universal microscope with suitable filter combinations.

II. Flow cytometry

Human leukocytes were isolated from the heparinized venous blood in suspension, rinsed in PBS and stained by the same acid sulfonated dyes. In each case 250 µl of leucocytes' suspension in PBS were mixed with 5 µl cocktail of sulfonated acid dye (1 mg/ml) and 5 µl propidium iodid (2 mg/ml) — for nuclear staining of death cells, rinsed in PBS, centrifuged and measured with FITC filter combination by techniques of flow cytometry (measurements were performed in the Max Planck Institute of Biochemistry — Munich, Germany, by Fluvo-Metricell flow cytometer).

Results and discussion

The analysis of the results from fluorescent-cytochemical studies gives the basis to conclude that three of sulfonated fluorescent dyes applied — brilliant sulfoflavine, primuline and lucifer yellow bind specifically to basic cytoplasmic proteins in myeloid cells. The chemical structures of two of these compounds are shown in Fig. 1.

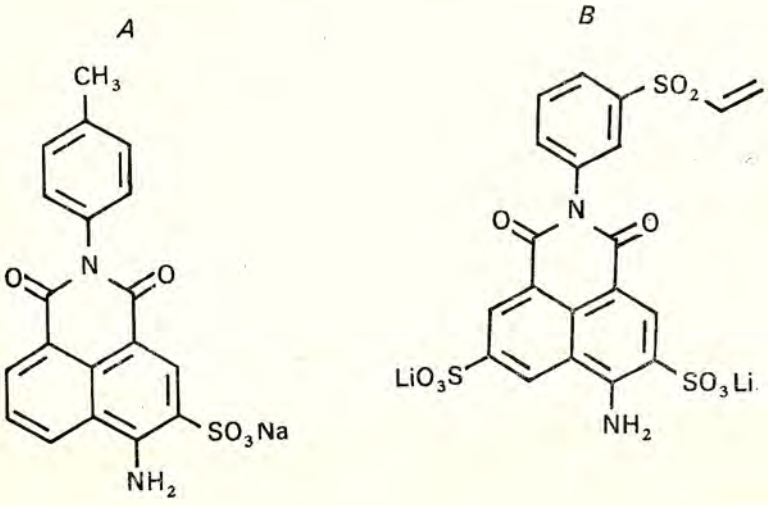

Fig. 1. The chemical structures of two sulfonated fluorochromes
A — brilliant sulfoflavine; B — lucifer yellow

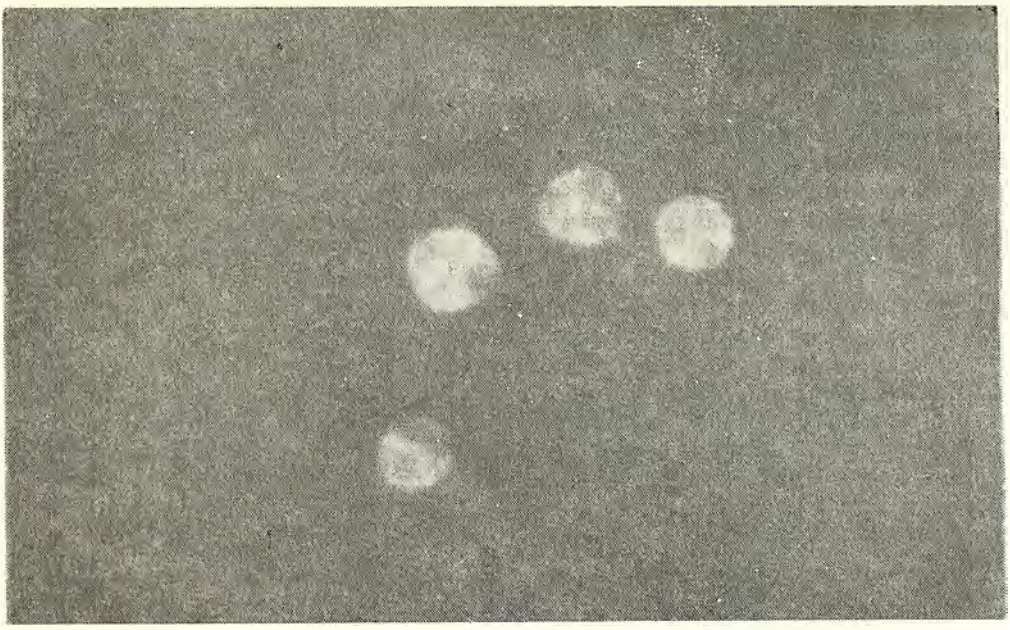

Fig. 2. Granulocytes from human peripheral blood, after fluorochromation by lucifer yellow. One can see brightly fluorescent cytoplasmic granules in neutrophils and eosinophil. Only nuclear areas are non fluorescent, × 1000. Immersion

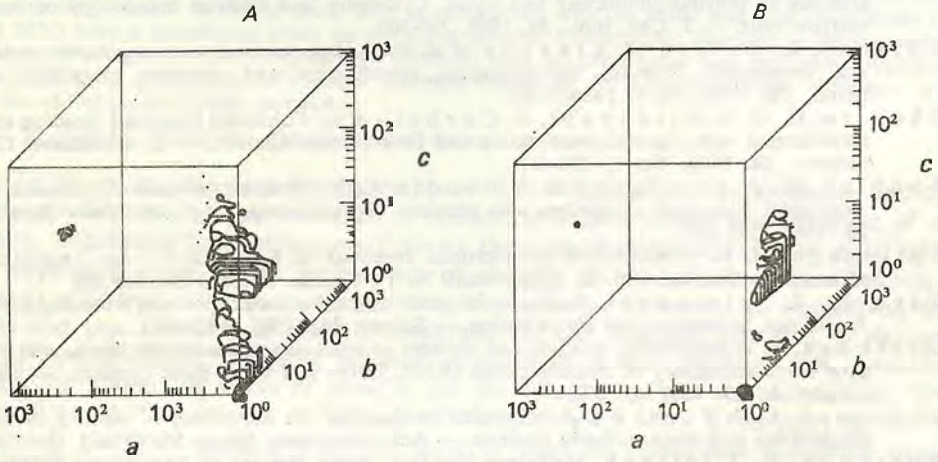

Fig. 3. Three-dimensional histogrammes of 366-610 nm fluorescence for the human leukocyte population

A — green versus red fluorescence and cell volume, after fluorochromation by lucifer yellow (LY); *B* — green versus red fluorescence and cell volume, after fluorochromation by brilliant sulfoflavine (BSF). Fluvo-Metricell flow cytometer; *a* — green fluor; *b* — volume; *c* — red fluor

The colour of fluorescence is green-yellow after fluorochromation with brilliant sulfoflavine and primuline and golden-yellow — after lucifer yellow. The fluorescent cytoplasmic granules are localised in different quantity in mature peripheral blood granulocytes — neutrophils and eosinophils (Fig. 2). The intensity of the fluorescence is more strong and fluorescent cytoplasmic granules are largest in eosinophilic cytoplasm. The staining is stoichiometric and the intensity of fluo-

rescence reflects quantitatively the content of basic cytoplasmic proteins not only in the mature blood cells from myeloid origin, but also in their bone marrow precursors.

The best cytochemical and flow cytometrical results were obtained with a new fluorescent dye lucifer yellow. The application of this fluorochrome in flow cytometrical studies (Fig. 3) gives basis for development of new quantitative method for flow cytometrical diagnosis of basic cytoplasmic proteins in leukocytes from myeloid series (granulocytes — neutrophils and eosinophils, as well as their bone marrow precursors), in normal and pathological conditions.

The results obtained are also in reasonable agreement with these of other fluorescent studies on basic proteins [2, 3, 4, 12], as well as with our previous results by the methylene blue-fast green staining method [7-11].

The conclusion is that the exitation, wavelength, color of emitted fluorescence and the histogrammes obtained with a new dye lucifer yellow, give a possible new way for cytochemical and flowcytometrical studies of basic cytoplasmic proteins in leukocytes from myeloid origin, in different states of maturation and differentiation. Cytoplasmic fluorescence values may be further analyzed to obtain the ratio of different leukocytes subtypes — e. g. neutrophils, eosinophils and their precursors.

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Histological and ultrastructural characteristics of the thyroid of rats treated with ethanol

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Histological and ultrastructural changes in thyroid of rats after treatment with different doses of ethanol were investigated. The animals were given for 30 days a solution of 10, 20, 30 and 50% of ethyl alchohol instead of drinking water. At the end of the 4th week the body weight of rats treated with 50% ethanol decreased about 30% in comparison with the initial one. The lower doses (10 and 20%) have a stimulating effect on the gland activity. Considerable damages in the thyroid of animals receiving a 50% of ethanol were observed both on histological and electronmicroscopical level. The main ultrastructural changes included reduction of the endoplasmic reticulum, lysis of mitochondria and Golgi apparatus.

Key words: thyrocyte, ethanol, ultrastructural changes.

In the recent two decades there appeared new data about the character and the dynamics of the changes in different endocrine organs under the influence of alcohol. According to contemporary views the hypothalamic-hypophisal axis takes an important place in the mechanism of chronic alcoholism. In this direction a special attention is paid to the investigation on the influence of alcohol on the structure and function of the biomembrane cellular systems resulting in changes of cell metabolism, energetic balance, regulation of cell activity. Still, however, is known about the effect of alcohol on the integrity of the neuroendocrine system. First in this respect is lacking of morphological data. Especially, the investigations on the morphofunctional state of such an important endocrine organ as the thyroid, are poor. There is some information on the basis of clinic biochemical data about the contents of thyroid hormones in blood. According to Israel et al. [10] in cases of alcoholic liver disease the concentration of T_3 and T_4 in blood serum significantly decreases. Lower is the level of TSH too [11]. An expressed hypothyroidism and a decreased autoimmune response is observed in 64% of the patients with chronic alcoholism [5].

The purpose of this study is to analyse the histological and ultrastructural changes in the rat thyroid after treatment with ethanol during 30 days.

Material and methods

The experiments were performed on white 2 months old rats Wistar. The animals were given a solution of ethanol in different concentrations instead of drinking water. They were divided into the following experimental groups:
1st group — rats treated with 10% solution of ethanol;
2nd group — rats treated with 20% solution of ethanol;
3rd group — rats treated with 30% solution of ethanol;
4th group — rats treated with 50% solution of ethanol.
Rats of corresponding age bread in normal conditions were used as controls. The body weight of the animals was measured daily and at the end of each week the average values were calculated. At the 30th day all the animals were killed and pieces of the thyroid were taken for histological and electronmicroscopic studies. The material for histological observation was fixed in Bouin's mixture and included in paraffin. On serial sections of the gland, stained with haematoxilin-eosine, the following morphometric investigations were carried out: in a 100 follicles the height of 10 opposite thyrocytes was measured by an ocular micrometer and the average value was calculated. The percentage ratio of the theree basic structural components of the gland: thyroid epithelium — colloid — connective tissue was measured and the results were statistically processed. For the transmission electron microscopy (TEM) small pieces of the glands were fixed in 2,5% glutaraldehyde, post-fixed in 1% OsO_4 and then processed further by the routine method. The observations were made with EM "Opton-109".

Results and discussion

On Table 1 the average values of the body weight of the animals of the corresponding groups were shown. In rats treated with 10, 20 and 30% ethanol the weight similar to that in control animals increases progressively in the course of the experiment. Differences were found only for the animals treated with 50% ethanol. From the second week a diminution in the weight of these rats began and at the end of 4th week it decreased with 30% in comparison with the initial one.

Histological study. The histological analysis of the thyroid sections of the control animals (Fig. 1 — *A*) showed the domination of follicles of middle size with cubic thyrocytes. The larger follicles were situated peripherally. The average height of the thyroid cells is 4,09 μm. The percentage ratio of thyroid epitelium: colloid: connective tissue is respectively 41,16: 49,83: 8,66 (Table 2). The colloid is of a pale pink colour. All these morphological characteristics suggested a moderate functional activity of the organ. The study of the thyroid from the experimental rats treated with a 10% alcohol revealed the following changes. In most of the follicles the colloid was more clear with the appearance of the zones of re-

Table 1. The average values of the weight of the rats, g

Group	S_0	S_1	S_2	S_3	S_4
1st—10%	77,25± 5,90	90,35±13,93	117,65±20,83	151,25±21,11	178,20±19,64
2nd—20%	83,60± 4,91	88,47± 6,18	102,95± 9,29	125,12± 4,66	151,20±14,85
3rd—30%	96,83±15,57	95,50±16,48	104,46±19,71	121,90±25,83	143,10±34,59
4th—50%	118,16± 8,26	103,93±10,27	92,26±14,68	85,30±15,30	84,30±17,93
Control rats	68,40± 2,27	84,45± 7,57	95,17±12,36	131,82±13,57	155,80±20,49

Note: S_0 — inital weight; S_1—S_4 — body weight at the end of the 1st to 4th week respectively.

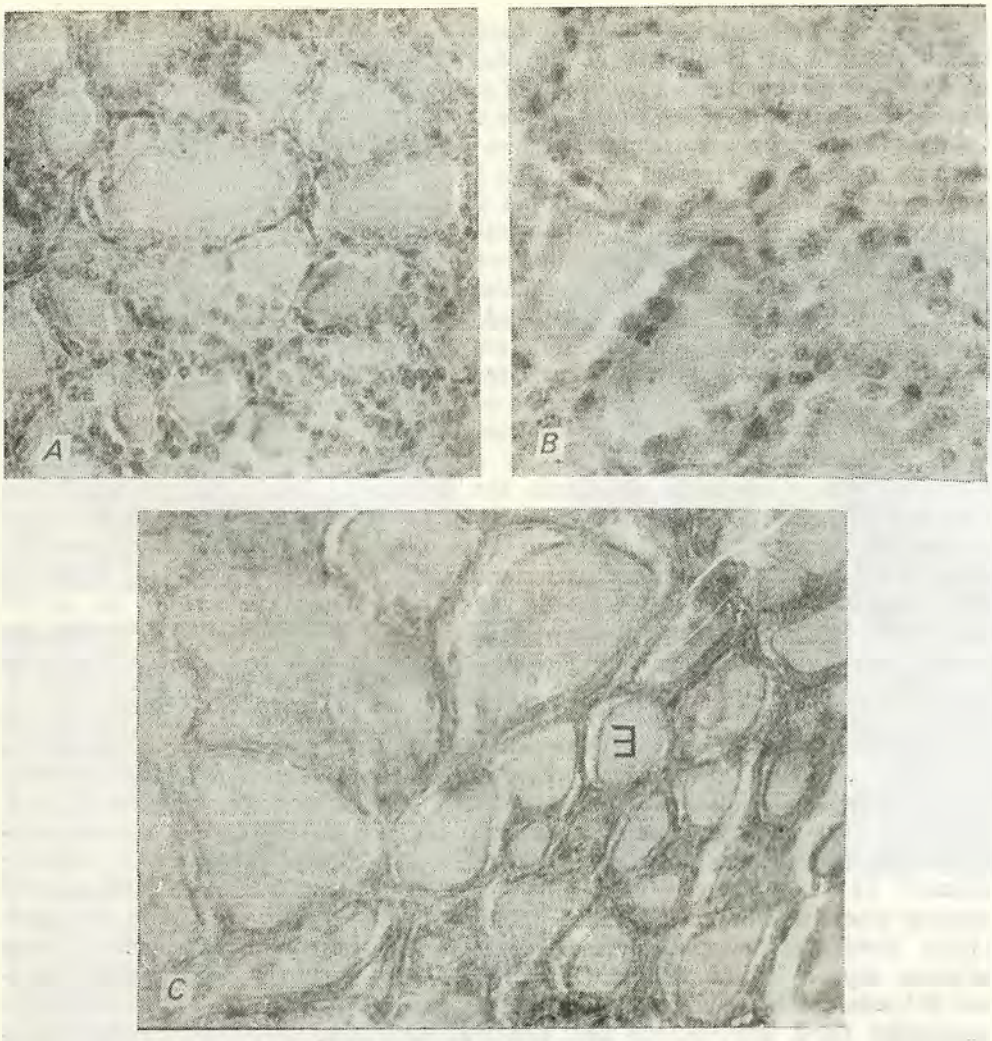

Fig. 1. Histological sections of the rat thyroid. Haematoxilin-eosine staining
A — control rat, × 250; *B* — rat treated with a 30% solution of ethanol, × 300; *C* — rat treated with a 50% solution of ethanol (Э), × 250

Table 2. The percentage ratio of thyroid components

Group	Thyroid epithelium	Colloid	Connective tissue
1st—10%	57,00±7,93	33,33±10,25	10,22±3,48
2nd—20%	57,11±4,09	36,00± 4,32	7,33±1,33
3rd—30%	58,00±3,16	33,00± 2,44	9,00±3,54
4th—50%	33,22±2,18	57,77± 2,65	9,00±2,29
Control rats	41,16±7,38	49,83± 6,84	8,66±6,54

sorption. The thyrocytes, prismatic in form, had a light apical part. Their average height was 5,11 μm. Similar is the morphologic picture of the thyroid of rats treated with 20% ethanol. Follicles in a state of an increased functional activity were found even in the periphery of the organ where resting follicles are usually situated. The values of the morphometric measurements of the three components were near to those of the group treated with a 10% (Table 2), they are another proof on the active resorption of the stored thyroid hormones. The alterations began more demonstrative in the thyroid of the animals treated with 30% ethanol (Fig. 1—*B*). The percentage of the thyroid epithelium reached its maximim — 58% and the colloid was 33%. In some follicles an invagination of the thyrocytes and a modification of the follicle wall were found. The histological picture in the thyroid of the rats treated with 50% ethanol was very different that of the other experimental animals. Most of the follicles were in an inactivated functional state. The thyrocytes were low to flat (Fig. 1—*C*). Their height was significantly decreased — 3,21 μm; the thyroid epitelium — 33,22%. Follicles with dense stored colloid were dominant. In some cases a desquamation is observed — e. g. a penetration of fragments or whole epithelial cells into the lumen. A destruction of the follicle wall and of entire cytoarchitectonic of the gland was preset. The comparison of the morphometric parameters (Table 2) showed essential changes in the percentage ratio of the thyroid epithelium. It is significantly higher in the group, receiving 10%, than in the control group. These values were almost the same in the second and in the third experimental group — 57% and 58% respectively. For the animals, treated with 50% they were lower — 33,33%. The parameters of the colloid were reciproc.

Electronmicroscopic study. The thyrocytes of the control rats (Fig. 2—*A*) were characterised by an well-expressed polarity, a great number of microvilli of the apical surface and an ultrastructure, similar to that of the most secreting cells (developed granular endoplasmic reticulum, presence of free ribosomes, Golgi apparatus, secretory granules of different size and density and numerous mitochondria). The nucleus, spherical in shape, with 1-2 small nucleoli was centrally situated. The ultrastructure of the thyrocytes of rats treated with 30% ethanol showed essential differences related to the plasmolem, as well as to the cytoplasm itself. On the apical surface the microvilli were short, fragmented, most of them dipped in the colloid (Fig. 2—*B*). Thyrocytes in the animals received 50% ethanol showed more expressed disturbances (Fig. 2—*C*). The cytoplasm is vacuolized, filled with electron light granular substance. The elements of the granular endoplasmic reticulum and Golgi apparatus, as well as the mitochondria were damaged. The nucleus is also deformed. The chromatine is more condensed, the nuclear membrane — disrupted at same places. In the animals treated with a 30% solution of ethanol single cells were damaged, whereas in case of 50% they were more numerous.

The ethyl alcohol has many-sided effect on the organism, especially on the endocrine organs [2, 7, 9]. The degree of the caused damages are dose-dependent. On the other hand, the duration of the treatment is also an important factor. The alterations are based on two phenomena: the direct influence on the cellular biomembranes and the action of neurotransmittors such as acetylcholin and biogenic amines (catecholamines, serotonin, etc.) [1, 3, 4]. Ethanol is able to modify the chemical content, as well as the physical state of the biomembranes [6, 8].Being a liposolvent, its molecules are included in the phospholipid layers of the membranes thus desorganising and modifying their viscosity and permeability. Besides it has a direct influence on the membrane proteins, especially the membrane bound enzymes [8]. For example the K^+ — Na^+ dependent ATF-ase is activated by small doses ethanol, but it is inhibited by the bigger ones [1].

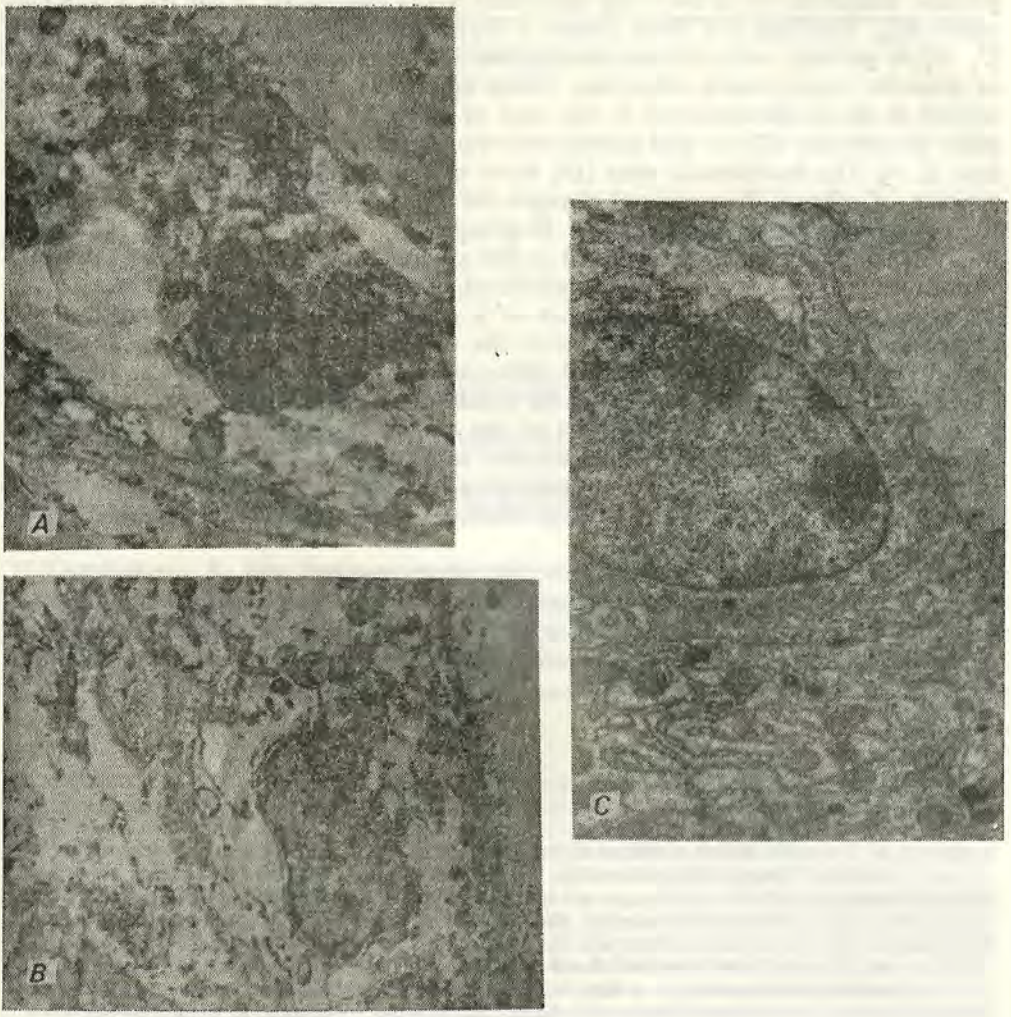

Fig. 2. Electronograms from rat thyrocytes
A — control rat, × 10 000; *B* — rat treated with a 30% solution of ethanol, × 7000; *C* — rat treated with a 50% solution of ethanol, × 7000

The pathogenesis of the alcohol damage of the thyroid is a multifactor process, which includes the direct or indirect participation of the other endocrin organs and regulating mechanisms [11, 16]. The comparison of the histological picture with the morphometric parameters, obtained in the present study shows that the lower doses (10, 20%) administrated in our experiment, have a stimulating effect to a certain degree, on the functional activity of the gland (an increase in the height of the thyrocytes, an activation of the colloid resorption). However, according to results concerning the prolonged application of a diet of 20% ethanol during a 5 months period (from the 10th day on after birth) leads to a functional exhaustion of the rat thyroid, as well as to an essential destruction of almost all cellular organoids. The ultrastructural picture is the same as that observed in the animals receiving a 50% ethanol. Our findings (damage of the granular endo-

plasmic reticulum and of the Golgi apparatus which participate in the thyreoglobulin synthesis), confirm the data about a thyroid hormones defficiency in the blood [5, 10] in patients with chronic alcoholism. Considerable ultrastructural damages of granular endoplasmic reticulum, Golgi apparatus and mitochondria were observed in the cardiomyocytes in the case of alcoholic cardiomiopatia [13, 15]. Similar destruction of liver and kidney cells was reported at acute alcoholic intoxication [2, 9]. The biochemical data [14] show that the acetaldehyde (an intermediate product of ethanol dissociation) changes the protein synthesis by blocking the cellular enzymes interacting with the H-groups.

The colloid resorption, observed in this study, is activated during the treatment with 10 and 20% ethanol, but an inhibition of the process appeared in animals receiving 50% solution. The existence of a colloid-like substance in most of the thyrocytes is probably connected with the alteration of the permeability of the apical plasmolem and with a colloid diffusion into the thyrocytes. From the other side the structural degeneration of the mitochondria decreases the cells energetic potential and disturbs the transport of the colloid which remains stocked in the cytoplasm. Besides, the high concentration of alcohol in blood has a toxic effect on the big blood vessels of the thyroid thus breaking the follicles [5]. The desquamation of thyrocytes and the desorganisation of the follicle wall observed by us supported this data.

On the base of the investigations previously reported and of our results [12], the conclusion that the ethanol causes important structural and metabolic changes in thyroid, can be made. Both similar type of studies and biochemical analysis can help to elucidate the molecular and cellular mechanisms of the alcoholism and its eventual treatment.

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Anthropology

Preliminary data of the investigation of the national programme "Anthropological characterization of the Bulgarian population"

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The anthropological characteristics of the population in separate countries gives a rich information about the physical development of the modern man in connection with his concrete manner of life and work. Representative anthropological data about the population in different countries at the end of the present century can be a solid base for comparative studies with similar data from passed generations and can be a foundation prognosticating the anthropological characteristics of the coming generations. Through the collected anthropological data, the morphological status of the Bulgarian population at the end of the 20th century is characterized. Grouped together and compared on the ground of the natural-geographic, ecological, socio-economic, professional and other factors, the data can give a basis for evaluation and prognostication the influence of that factors on the morpho-functional characteristics of modern man. The bone remains' data from the archaelogical excavations in Bulgaria (Paleolith — Late Middle Ages) give an idea of the epochal tendences in the development of the modern man on the territory of the country.

Key words: anthropology, physical development, cephalo- and somatometry, cephalo- and somatoscopy, physiometry, odontoscopy, dermatoglyphics, socio-economical factors, professional factors, demographic processes, paleoanthropology, brachycephalization.

The population of every country is an unique except of the world population which has no analogue, not only with its historical development, but also because of the specificity of its natural-geographic and socio-economical conditions of life. As a peculiar biological reflector, the anthropological characteristics of the population in different countries gives a rich information of the physical development of the modern man in connection with the concrete manner of life and labour. Being traced towards the centuries and grounded in different territories, the anthropological investigations give possibilities to be found and studied the specific regularities in the physical development of the man in our times. Data for analysis and revaluation of the acceleration and retardation processes, as well as the biological maturation and growing older (senescence) can be received. Moreover, the representative anthropological data about the population in different countries

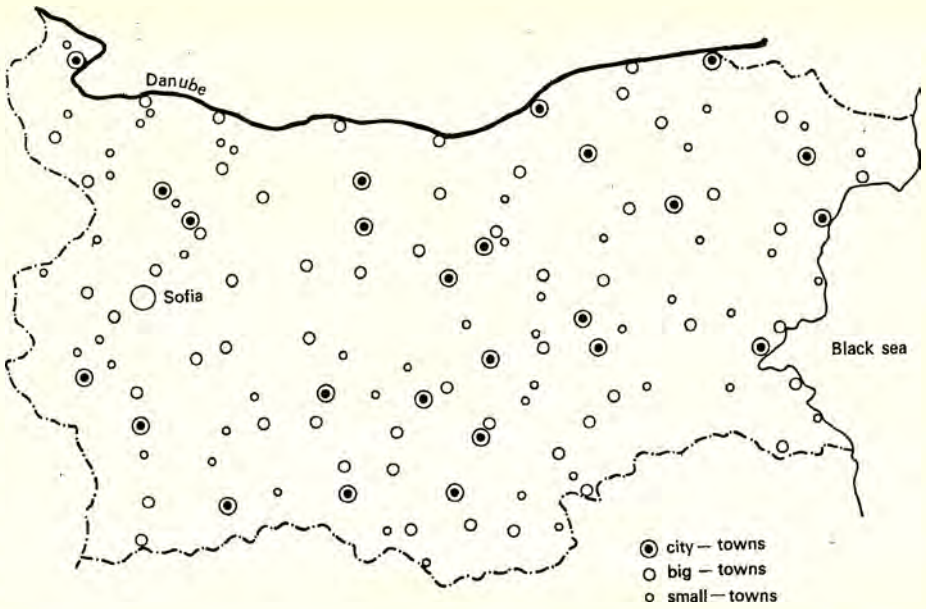


Fig. 1. Bulgarian national anthopological survey. Investigated settlements

at the end of the century can be used for a solid base of a comparative studies with similar data of past generation [22, 23, 32, 48], and for a prognostic base of the coming generation anthropological characteristics.

Subject of the anthropological investigations are the individuals or representative groups of the population from different geographic regions and states, being various occupated, having varied socio-economical and family status, or grouped in some other criteria. In the period 1989-1992 years commonly 5171 adults at the age of 30-39 years from which 2412 men and 2759 women from 118 setlements (administrative-towns, small towns and villages) are studied (see the enclosed map, Fig. 1). The anthropological programme by which is taken the survey of the population consists of 2 basic parts (see enclosed anthropological card): *questionnaire form* by which information about social, professional, demographic and health-biological status of the investigated is taken (commonly 40 questions); *metric-scopical part* which gives data about the anthropological characteristics of the individual (30 cephalo- and somatoscopical features, 20 cephalometric features, 68 somatometrical features — 26 measured bilaterally to be rendered the body asymmetry, 5 basic physiometric features, 26 odontoscopic features, palm and finger dermatoglyphic prints of both hands, and fullface and halfface photographs).

The bone remains from the archaeological excavations are studied after the conventional anthropological methods [11]. They cover 4000 skeletons from 150 necropolis dated from the Palaeolith (40 thousands years B. C.) up to the end of the XVIII century (see the enclosed map, Fig. 2).

The more important directions in which fundamental and practical-scientific results from the national anthropological programme can be expected are:

1. Evaluation of the influence of the following factors on the physical development of man:

1.1. Natural-geographical and ecological conditions of living;
1.2. Urbanization degree of the settlements;
1.3. Socio-economic factors;

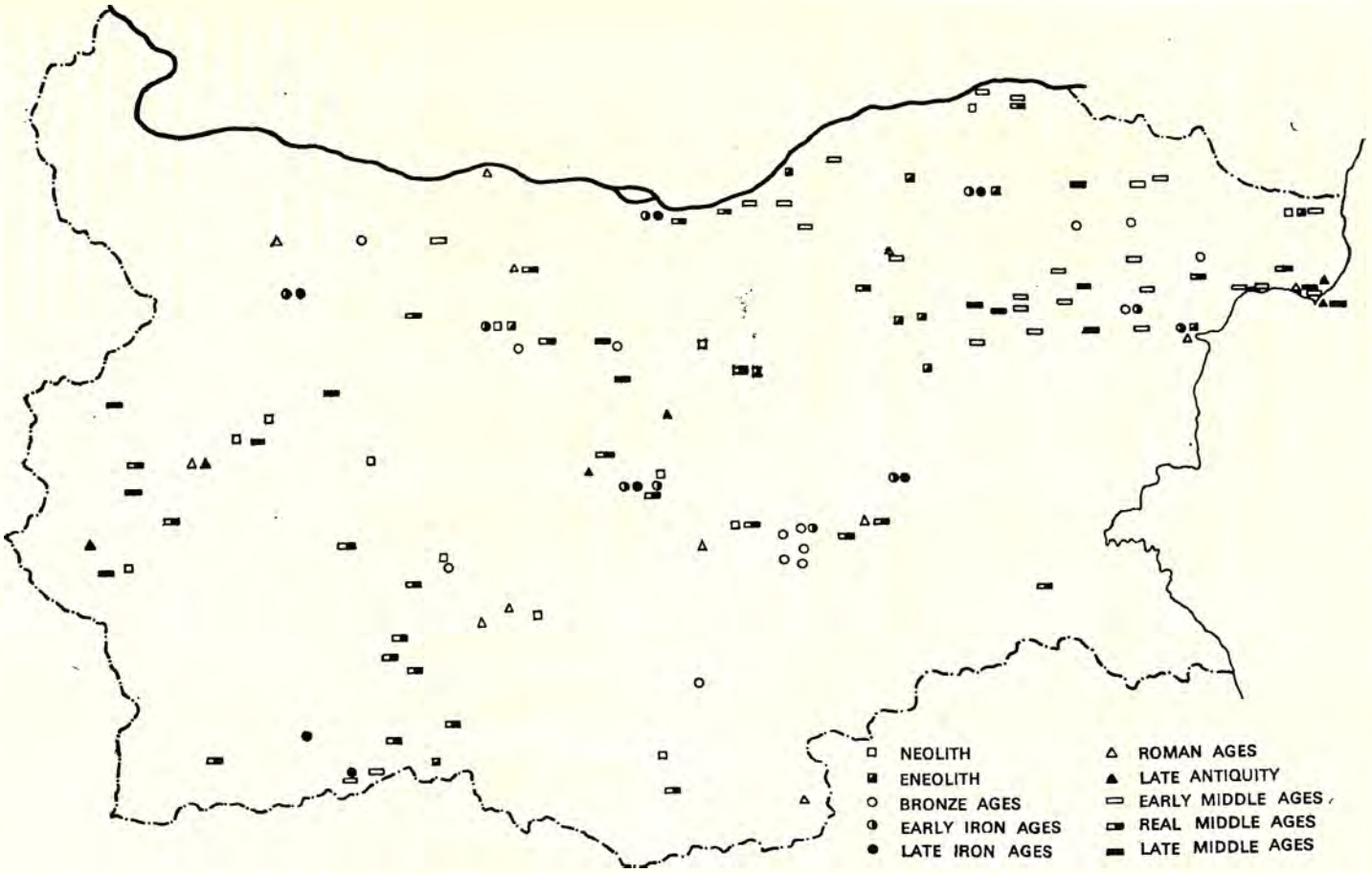


Fig. 2. Bulgarian national anthropological survey. Investigated necropolis

1.4. Living conditions, nutrition and manner of living;
1.5. Type of physical activity of the person connected with his profession — evaluating 4 professional categories — clercs, physical workers in the light industry, physical workers in the heavy industry and agricultural workers.

2. Assessment of the demographic processes on the territory of Bulgaria studied in the last three generations.

3. Frequency and characteristics of the multiple births in Bulgaria studied in the three generations.

4. Age of menarche appearance in the investigated women and their daughters.

5. General anthropological characterization of the up-to-date population in Bulgaria:

5.1. Cephalometric and cephaloscopic characterization;
5.2. Somatometric and somatoscopic characterization;
5.3. Somatotypological (kinanthropometry) characterization;
5.4. Odontoscopic characterization;
5.5. Dermatoglyphic characterization.

6. Making anthropological standards for the industrial design.

7. Invention of anthropological norms for the needs of the professional medicine and health protection.

8. Paleoanthropological characteristics relevant to the origin and the development of the anthropological types in the Bulgarian lands; paleodemography and paleopathology.

The results given in this study have both national and general biological importance. Grouped together and compared with different factors, they can give a good possibilities to estimate and to prognosticate the influence of the socio-economical, the ecological, the professional and other specific factors upon the morpho-functional characteristics of the modern man, as well as to be used for a comparative analysis and assessment of the epochal tendences in the physical development of *Homo sapiens sapiens.*

At the end of november 1992 are finished the field-study of the up-to-date Bulgarian population.

In the present work are given some of the preliminary data of this investigation.

I. Basic somatometric features

Three basic somatometric features — height, weight and chest circumference at pause have been chosen for primary processing with regard to the creation of general notion about the physical development of the population in Bulgaria. 1256 men and 1405 women were investigated in the 30-39 years of age group from the capital of the country Sofia and 56 settlements situated on the territory of North Bulgaria. The data processing and the following comparative analysis have been considered only in the context of the geographic situation of the regions and the administrative significance of the investigated habitats. The in-detail analysis according to foci, type of labour and a number of other factors will be performed after the final processing of the data. Despite the preliminary character of the study clear cut tendencies are being outlined in the differences according to all three chosen features. In most cases the changes are related to a considerable correlation among the different features which, however, is not of an identical direction in both cases. The summed up mean values for whole North Bulgaria respectively about height, weight and chest circumference are 170,9 cm, 76,6 kg and 95,4 cm in the male group and 158,3 cm, 65,6 kg and 82,5 cm in the female one. After the height categories of M a r t i n and S a l l e r [11] the men are tall and the women are

Table 1. Stature (North Bulgaria and Sofia-town)

Region	Men				Women			
	n	$\bar{x}$	min	max	*n*	$\bar{x}$	min	max
Vidin	103	170,9	155,3	183,8	78	158,9	144,4	178,9
Mihaylovgrad	109	171,5	157,6	188,8	121	159,7	146,1	170,7
Vratza	129	171,2	157,3	189,4	137	158,1	144,3	172,1
Pleven	62	171,7	153,2	183,0	89	157,9	138,9	175,8
Lovech	68	170,9	159,7	188,9	55	158,4	144,1	175,2
Gabrovo	66	172,0	158,1	185,0	70	158,4	147,1	170,0
Veliko Tarnovo	73	171,3	152,5	188,0	103	158,5	142,8	177,2
Rousse	50	172,7	156,0	183,4	61	158,7	145,5	174,8
Razgrad	64	169,0	152,0	185,5	70	157,4	139,1	169,4
Targovishte	27	169,2	159,0	178,3	33	157,8	143,6	171,1
Shoumen	54	171,5	162,2	184,6	73	158,0	146,3	170,8
Silistra	79	168,5	150,5	181,8	52	156,9	142,7	167,6
Dobrich	77	169,3	152,3	187,4	122	157,4	135,4	173,4
Varna	60	172,7	155,6	186,0	63	158,9	145,2	169,5
Sofia-town	235	173,1	157,0	190,4	278	160,8	148,0	173,7

Table 2. Weight (North Bulgaria and Sofia-town)

Region	Men				Women			
	n	$\bar{x}$	min	max	*n*	$\bar{x}$	min	max
Vidin	103	76,5	50,0	106,0	78	67,6	39,0	112,0
Mihaylovgrad	109	79,6	54,0	125,0	121	67,0	48,0	94,0
Vratza	129	79,0	50,0	121,0	137	67,3	42,0	115,0
Pleven	62	77,3	52,0	115,0	89	65,5	45,0	90,0
Lovech	68	78,9	58,0	125,0	55	64,6	46,0	92,0
Gabrovo	66	75,9	55,0	107,0	70	67,4	46,0	120,0
Veliko Tarnovo	73	77,1	49,0	120,0	103	64,8	44,0	105,0
Rousse	50	75,4	56,0	99,0	61	64,7	46,0	95,0
Razgrad	64	72,2	51,0	100,0	70	64,7	45,0	100,0
Targovishte	27	78,0	57,0	97,0	33	65,6	47,0	88,0
Shoumen	54	76,3	53,5	101,0	73	64,5	48,0	96,0
Silistra	79	71,6	49,0	100,0	52	63,1	46,0	96,0
Dobrich	77	76,2	55,0	111,0	122	63,5	41,0	102,0
Varna	60	75,5	52,0	108,0	63	66,6	46,0	100,0
Sofia-town	235	79,1	55,0	133,0	278	65,5	42,0	150,0

of a height "above the average" one. In the intergroup analysis the foci under study were so assembled as to represent 14 districts the Vidin, Mihaylovgrad, Vratza, Pleven, Lovech, Gabrovo, Veliko Tarnovo, Rousse, Razgrad, Targovishte, Shoumen, Silistra, Dobrich and Varna ones. As a separate group the population of Sofia-city has shown markedly higher values according to all three basic features. The height in the capital of both sex groups reaches highest mean values of 173,1 cm in the males and 160,8 cm in the females, compared to all investigated regions (Table 1). Closest to these values display men from Rousse and Varna districts followed by the ones from the Gabrovo, Pleven and Mihaylovgrad ones. In the female group the height is of higher values in the Mihaylovgrad, Vidin, Varna and Rousse districts. In the comparison according to geographical criteria between the population of the western and the central parts of North Bulgaria it is noteworthy that the height difference is minimal, the males from the Vidin, Vratza and Mihaylovgrad regions are only by 0,3 cm shorter and the women are with

Table 3. Chest circumference in pause (North Bulgaria and Sofia-town)

Region	Men				Women			
	n	$\bar{x}$	min	max	*n*	$\bar{x}$	min	max
Vidin	103	96,0	81,2	113,5	78	84,8	66,7	115,0
Mihaylovgrad	109	98,0	75,2	123,0	121	84,0	69,4	105,0
Vratza	129	97,3	78,0	118,9	137	85,1	65,0	120,0
Pleven	62	94,1	81,0	110,5	89	81,4	68,7	103,5
Lovech	68	95,9	83,3	120,0	55	81,0	66,7	97,3
Gabrovo	66	94,2	81,0	109,0	70	82,3	69,2	110 8
Veliko Tarnovo	73	96,3	72,4	130,5	103	82,1	66,5	105,0
Rousse	50	93,9	80,0	111,0	61	80,4	67,7	100,5
Razgrad	64	92,5	80,0	106,0	70	81,7	68,5	113,5
Targovishte	27	95,2	83,8	105,5	33	81,5	71,2	100,2
Shoumen	54	94,5	82,0	111,0	73	81,0	70,0	100,5
Silistra	79	92,4	78,0	112,5	52	80,1	68,0	103,0
Dobrich	77	96,4	81,0	115,0	122	82,0	68,1	103,0
Varna	60	94,3	79,0	108,0	63	82,1	62,8	112,7
Sofia-town	235	99,1	82,5	127,6	278	82,8	68,1	124,4

0,5 cm taller than the ones in the Pleven, Lovech, Gabrovo and V. Tarnovo districts. Markedly lower values for the height are found in the east regions (Rousse and Varna excluding). The inhabitants of the Silistra and Razgrad regions are the shortest. The lowest values of the weight and chest circumference are found on the territory of the Silistra district as well (Tables 2 and 3). The height of the population from Targovishte and Dobrich is also low but the values of weight and chest circumference are relatively higher especially in the men. Comparing the inhabi-

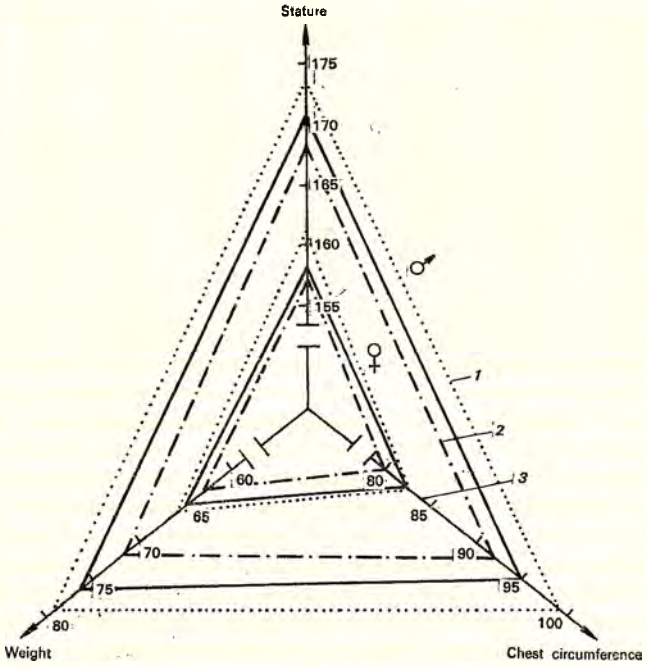


Fig. 3. Relationships between the three basic somatometric features
1 — Sofia; *2* — Silistra; *3* — North Bulgaria

Table 4. Stature (administrative centres)

Administrative centre	Men				Women			
	n	$\bar{x}$	min	max	*n*	$\bar{x}$	min	max
Sofia	235	173,1	157,0	190,4	278	160,8	148,0	173,7
Vidin	34	172,8	159,8	183,8	22	160,4	153,1	169,1
Mihaylovgrad	34	172,5	159,2	188,8	33	159,0	146,1	169,4
Vratza	26	172,0	162,7	182,6	42	159,1	144,3	172,1
Pleven	38	172,4	161,0	183,0	45	159,2	140,8	175,8
Lovech	42	170,4	159,7	185,4	27	159,4	148,6	175,2
Gabrovo	31	170,5	158,1	183,1	39	157,8	147,1	169,4
Veliko Tarnovo	22	172,5	152,5	178,8	20	160,6	147,4	170,4
Rousse	38	172,3	156,0	183,3	37	160,1	148,1	174,8
Razgrad	36	170,4	152,1	185,5	40	156,8	139,1	169,3
Targovishte	10	171,2	161,2	178,3	14	160,3	153,4	171,1
Shoumen	29	172,3	163,6	181,0	45	157,7	146,3	170,8
Silistra	21	168,6	150,5	177,3	26	158,4	145,2	167,6
Dobrich	24	169,3	154,2	187,4	52	157,5	146,2	170,2
Varna	33	174,3	160,2	185,0	38	158,8	145,2	169,5

Table 5. Weight (administrative centres)

Administrative centre	Men				Women			
	n	$\bar{x}$	min	max	*n*	$\bar{x}$	min	max
Sofia	235	79,1	55,0	133,0	278	65,5	42,0	150,0
Vidin	34	77,4	57,0	101,0	22	70,2	51,0	98,0
Mihaylovgrad	34	82,1	60,0	117,0	33	65,5	48,0	90,0
Vratza	26	79,5	58,0	107,0	42	68,3	45,0	115,0
Pleven	38	77,8	60,0	110,0	45	64,2	46,0	90,0
Lovech	42	79,9	60,0	125,0	27	63,9	46,0	90,0
Gabrovo	31	73,7	55,0	90,0	39	67,2	46,0	105,0
Veliko Tarnovo	22	73,0	50,0	85,0	20	62,1	44,0	85,0
Rousse	38	75,8	56,0	96,0	37	64,0	48,0	95,0
Razgrad	36	72,8	53,0	100,0	40	61,4	45,0	85,0
Targovishte	10	80,0	65,0	97,0	14	66,0	50,0	88,0
Shoumen	29	76,0	53,5	101,0	45	64,2	49,0	96,0
Silistra	21	70,9	57,0	83,0	26	64,4	46,0	96,0
Dobrich	24	72,3	55,0	89,0	52	62,0	41,0	95,0
Varna	33	74,7	60,0	93,0	38	61,8	49,0	93,0

tants of the Dobrich and Silistra regions with insignificant differences in the height the men from the first district are heavier and with a larger chest circumference while in women the weight is almost identical and chest circumference shows a slight prevalence for the first ones.

Interesting intersexuel differences were established in the three features upon comparing the average of the capital population with the mean values for the whole North Bulgaria ones (Fig. 3). In the males the distances are almost equal in all three peaks while females at a considerably higher value of the height have a chest circumference only 0,4 cm greater and even a lower weight (by 0,1 kg). Greater intersexual differences for weight and chest circumference are typical for separate regions in North Bulgaria too. For example, the maximum weight for the males is found in Mihaylovgrad followed by the men from Sofia, Vratza and Lovech. Its greatest mean value in women is registered in Vidin, Gabrovo, Vratza and just then it is followed by Mihaylovgrad and Sofia. With markedly greater chest circumference are the men from Sofia and Mihaylovgrad as are the women from Vratza and Vi-

Table 6. Chest circumference in pause (administrative centres)

Administrative centre	Men				Women			
	n	$\bar{x}$	min	max	*n*	$\bar{x}$	min	max
Sofia	235	99,1	82,5	127,6	278	82,8	68,1	124,4
Vidin	34	95,2	83,7	110,0	22	85,4	72,0	102,4
Mihaylovgrad	34	100,3	75,2	117,8	33	81,9	69,4	93,5
Vratza	26	97,2	83,8	118,9	42	84,8	70,5	120,0
Pleven	38	94,3	81,0	108,0	45	79,8	68,7	103,5
Lovech	42	96,9	83,5	120,0	27	79,6	66,7	93,0
Gabrovo	31	93,6	81,0	109,0	39	82,4	69,5	109,3
Veliko Tarnovo	22	92,2	72,4	100,5	20	80,0	66,5	101,0
Rousse	38	93,7	80,0	111,0	37	79,4	67,7	100,5
Razgrad	36	92,1	82,2	103,0	40	78,9	68,5	94,0
Targovishte	10	96,6	88,5	105,5	14	81,8	71,2	100,2
Shoumen	29	93,9	82,0	111,0	45	80,8	70,0	100,5
Silistra	21	91,2	79,0	99,0	26	80,8	68,0	103,0
Dobrich	24	94,5	83,0	107,0	52	80,3	70,4	100,1
Varna	33	92,8	79,0	108,0	38	79,0	66,7	103,6

din (Table 3). Naturally, there exist also local deviations related to the size and administrative importance of the studied settlements which are cleary demonstrated upon comparison of the population from the district centres. The mean values calculated about them are (Tables 4, 5, 6) in almost all cases higher than the ones summarized for the region. The greater height of men from Varna, Vidin and V. Tarnovo is readily standing out while in Dobrich and Silistra the values remain unaltered. In women from Silistra the height measured in the administrative centre is 1,5 cm greater.

The data presented tracing the variations according to the three basic somatometric features make the tendency towards decrease of height, together with the diminution of weight and chest circumference in direction from the west to the east

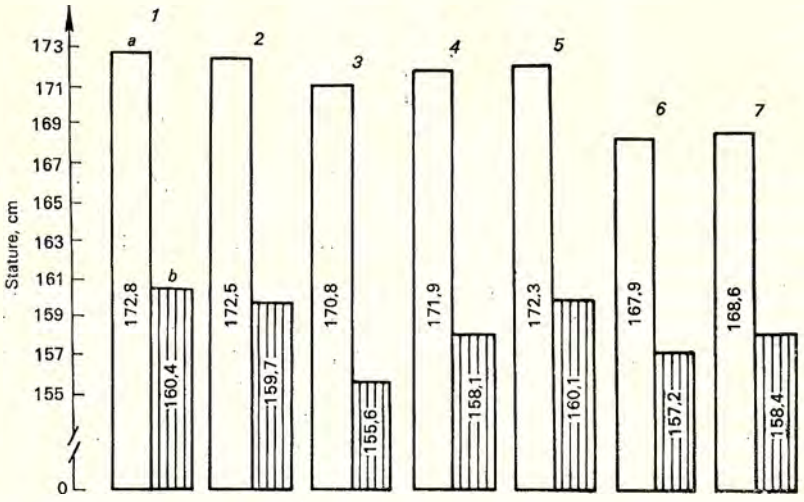


Fig. 4. Stature of the population in the Danube Plain region
a — men; *b* — women; *1* — Vidin; *2* — Lom; *3* — Nicopol; *4* — Svishtov; *5* — Russe; *6* — Tutrakan; *7* — Silistra

easily discernible. The same tendency is observed, as well in tracing the changes of the values for the height, weight and chest circumference in the settlements situated in the Danube Plain region. Especially pronouced it is found in the height of men where the difference between the extreme west (Vidin and Lom) and extreme east (Tutrakan and Silistra) is over 4 cm (Fig. 4).

The results obtained though unfinished give a clear picture of the variations of the height, weight and chest circumference found on the territory of the whole North Bulgaria. They outline the main trends in the alterations of the physical development of the population from the given regions unveiling the factors whose influence is to be sought and discussed in the final analysis of the data from the research programme.

II. Subcutaneous fat tissue

One of the priorities of the national anthropological programme is by studying the specificity of human physical development to provide information about the ratio between the hereditary component and the ecosensitivity in the anthropometric characteristics. It is anticipated that through unraveling the features of greatest reactivity important sides of the morpho-functional adaptation capacities of the organism to be shed light on under concrete conditions of life and labour. Ensuring such an information is in itself an opportunity for morpho-functional control upon

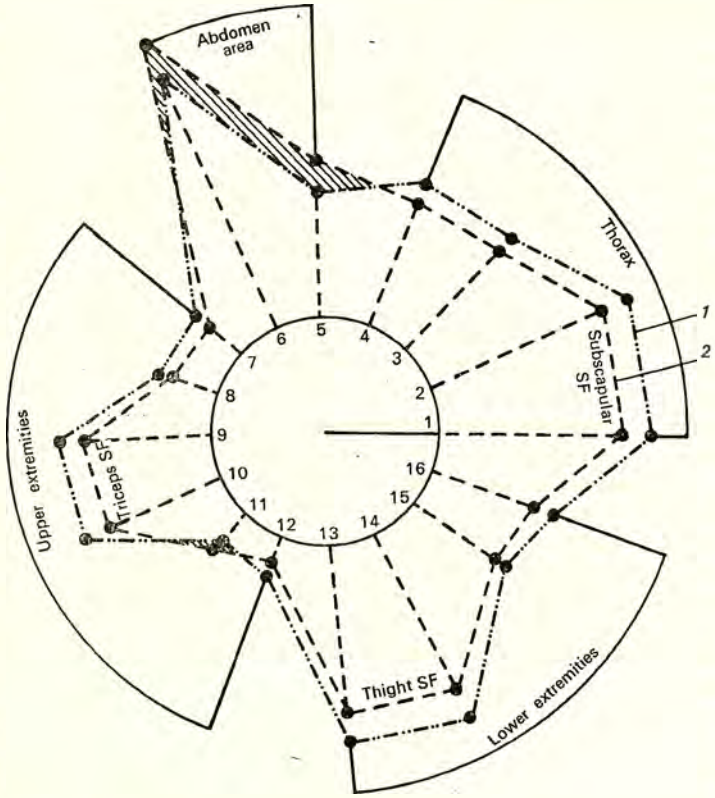


Fig. 5. Morphograms of the investigated skinfolds
1 — Sofia town; *2* — Vratza region
* The numbers in the circle correspond to the serial numbers of the skinfolds in Table 7

Table 7. Metrical data of investigated skinfolds, mm

	Skinfolds (SF)	Laterality	Sofia-town		Vratza region	
			$\bar{x}$	σ	$\bar{x}$	σ
			Thorax			
1	Subscapular	right	21,28	10,15	19,33	7,45
2		left	21,64	10,20	19,65	7,36
3	Xth rib	right	16,65	8,25	15,50	6,59
4		left	16,88	8,05	15,11	6,54
	Sum of 4 SF		76,25	35,48	69,66	26,35
	Mean SF		19,06	8,87	17,41	6,59
			Abdomen area			
5	Suprailiac		14,82	7,15	17,84	6,70
6	Abdomen		27,55	11,03	30,86	10,14
	Sum of 2 SF		42,33	17,39	48,63	15,77
	Mean SF		21,26	8,62	24,32	7,88
			Upper extremities			
7	Biceps	right	8,18	4,42	6,94	3,26
8		left	8,20	4,49	7,64	3,52
9	Triceps	right	15,98	6,75	13,96	5,03
10		left	16,47	6,72	14,43	5,18
11	Forearm	right	7,08	3,81	7,29	2,78
12		left	7,15	4,35	6,88	2,64
	Sum of 6 SF		62,70	26,63	57,17	20,08
	Mean SF		10,45	4,44	9,53	3,35
			Lower extremities			
13	Thigh	right	21,25	7,83	18,58	5,96
14		left	21,42	7,82	18,83	6,12
15	Calf	right	12,80	5,39	12,36	4,68
16		left	13,35	5,31	12,97	4,82
	Sum of 4 SF		68,73	25,32	62,64	20,21
	Mean SF		17,18	6,33	15,66	5,05
			Total			
	Sum of 16 SF		250,70	111,72	237,72	80,31
	Mean SF		15,67	6,98	14,86	4,85

Table 8. Percentage distribution of subcutaneous fat tissue

Settlement	Total $4\bar{x}$ SF	Thorax		Abdomen		Upper extremities		Lower extremities	
		$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%	$\bar{x}$	%
Sofia	67,95	19,06	28,05	21,26	31,29	10,45	15,39	17,18	25,28
Vratza region	66,92	17,41	26,02	24,32	36,34	9,53	14,24	15,66	23,40

the physical development and health condition of various population groups with regard to the factor according to which these groups are differentiated [20, 21, 35, 36, 38]. As an illustration for obtaining biological information of this type we show preliminary results from a comparative study on the subcutaneous fat tissue (SFT) in the present work. It is known that this body ingredient is one of the most ecosensitive characteristics of the human body. In comparative investigations of SFT (interpersonal, intergroup and intersexual ones) differences can be recorded in its reactivity in two aspects — as differences in its total amount and as differences in its topic distribution over the body and extremities. Both types of differences reflect the specificity of the way and type of nutrition, type of physical activity during work, everyday life and sports, type of climate and geographical peculiarities of the environment, etc.

The comparative analysis of SFT in the present study is performed on the background of the natural geographic and urbanization differences in life conditions between two investigation sites in the country. Metric data about 9 standard skin folds (SF) along body and extremities in 234 men from the capital and 125 men from the Vratza region were used. Seven of the SF were bilaterally investigated to account for the asymmetry in the SFT topical distribution. The caliperometry is carried out after B r o z e k and K e y s [3] by the help of an original Holtain [14] caliper. The common mean SF, the averaged SFT and their per cut distribution of the SFT measured over the chest, abdomen, upper and lower extremities have been additionally calculated.

For the purpose of carrying out an objective comparative estimation of the asymmetry in the SFT accumulation in both male groups the calculation of sizeless standardized measures called units of asymmetry (UA) was introduced. They represent the difference between 100 and per cent ratio between the right side and left side value of every bilaterally measured SF [14]. UA gives the opportunity for comparing the values of the asymmetry displayed excluding the possibility for influencing the estimation through the dimension and sizability of the initial metric data. The positive values of UA characterize quantitatively the right-side asymmetry while the negative ones the left-side one.

The analysis and estimation of the intergroup differences in the quantity and topical distribution of SFT studied are carried out in several directions. With regard to quantity of SFT measured most generally, the men from Sofia have more SFT than the ones from the Vratza region (Table 7). This is corroborated both by the addition of a total 16 SF and by the SF common middle value, which is found to be 15,67 mm in their group while in the men from the Vratza region it is 14,86 mm. The morphograms constructed give a summarized demonstrable notion about the intergroup quantitative differences and the differences in the SFT distribution over body and extremities (Fig. 5). Generally, the thickness of SF in the men from Sofia is predominant in 13 out of the 16 topical radii of the morphogram in one of them being relatively identical and the two topical radii of the adbominal area show a markedly thicker SF for the males from Vratza region. For objectivization reasons of the established intergroup differences, the per cent ratio between the averaged SF for the chest, abdomen, upper and lower extremities was evaluated in such a manner, that the estimation of the metric differences between them are drawn out of one and this same initial basis (Table 8). As is seen from Fig. 6 the most considerable intergroup difference is found in the thickness of abdomen SFT. This is a body area where accumulation of SFT strongly depends on the dietary habits, active sports engagement, and the will-power of the individual.

The results obtained up to this moment clearly show that both male groups distinctly differentiate between themselves by the predilection topics for amassing greater quantities of SFT. It is obvious that this fact reflects the differences between

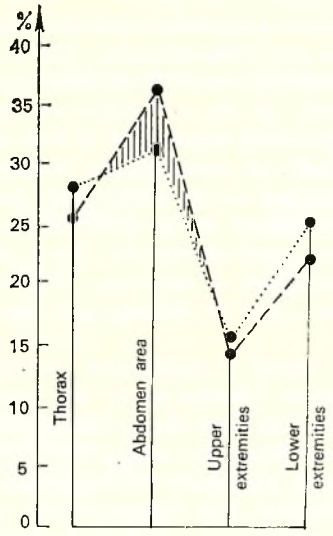


Fig. 6. Percentage distribution of the subcutaneous fat tissue
(. . .) — Sofia;
(— — —) —Vratza

the two studied foci in view of the dietary habits, the type and characteristics of the physical activity of the individuals in their professional labour, everyday-life labour and sports activities. An explanation of the established intergroup differences is provided by the significant ecosensitivity of the fat tissue in general which is namely defined as a functionally dependent feature. With even greater validity this explanation is true for the SFT, the latter being an important energy source for the work of the underlying musculature. An illustration of the functional dependence of SFT of the work specificity of the underlying musculature are the obtained in the present study topical differences in the SFT distribution in both male groups under investigation. As is distinctly seen from the morphogram (Fig. 5), the intergroup differences in the SF thickness are more significant in the areas covering muscles and muscle groups with a greater differentiation in the various types of physical activity (motoric activity, static efforts, weight lifting, mixed types, etc.). These are musculus triceps, the back musculature and the muscles of the thigh. In the areas over muscles and muscle groups which have a more unified and obligatory participation in human physical activity in general, such as the musculature of the forearm, partly muscules biceps, and the tibial musculature, the intergroup differences in the SFT thickness found in them are significantly smaller.

Still richer and more interesting is the information about the specificity of the intergroup differences in the distribution of SFT yielded by the assessment of the asymmetry in the thickness of the skin folds studied (Table 9). In the males from Sofia the left side values predominate in all SF i. e. the asymmetry in them is left-sided and a significant one as is observed in the corresponding UA (Fig. 7). In the males from the Vratza region the skin folds over the X-th rib and over the forearm demonstrate a right-sided asymmetry. The rest of the skin folds have displayed a left-sided asymmetry again as it is in the men from Sofia only differing by metric characteristics. In the subscapular skin folds and in the skin folds of the thigh the left-sided asymmetry is comparatively less expressed while in the skin folds of m. biceps, m. triceps and the thigh it is much better pronounced than the ones in the men from Sofia.

Table 9. Manifestation of asymmetry of the investigated skinfolds

Regions	Unit of asymmetry (UA)						
	subscapular SF	Xth rib SF	biceps SF	triceps SF	forearm SF	thigh SF	calf SF
Sofia	—1,67	—1,36	—0,24	—2,97	—0,98	—0,79	—4,12
Vratza region	—1,63	+2,58	—9,16	—3,26	+5,96	—1,33	—4,70

Note : (+) — right side; (—) — left side asymmetry.

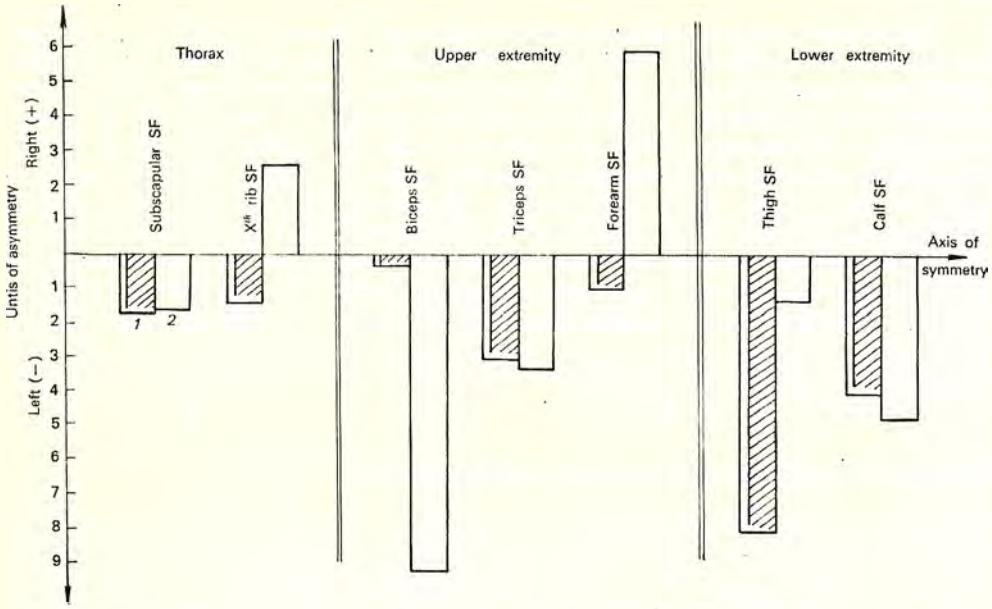


Fig. 7. Manifestation of the asymmetry of the investigated skinfolds
1 — Sofia town; *2* — Vratza region

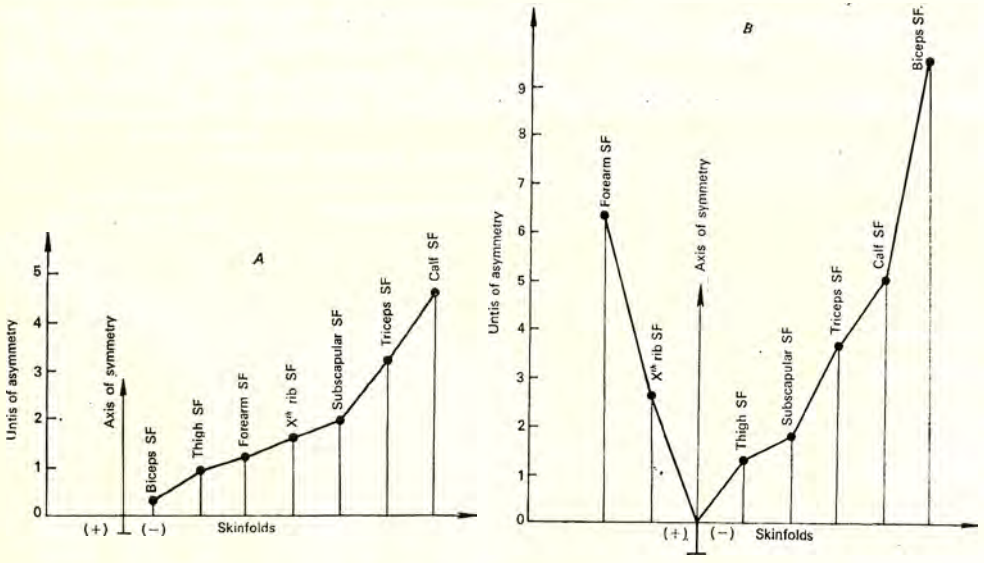


Fig. 8. Curves of the manifestated asymmetry
A — Sofia; *B* — Vratza region

The asymmetry profiles of both male groups render a summarized notion about the intergroup differences in the cases of asymmetry (Fig. 8). They have been constructed by the descending right arrangement, and the ascending for the left one of the features in the coordinate system according to the values of their

UA. The curves obtained define a marked left-sided asymmetry profile for the men from Sofia and a right-left sided one for the males from the Vratza region. This summarized result illustrated in the best way the quantitative-qualitative differences in the distribution of SFT in both male groups. A reason for the specificity of the asymmetry profile in the males from the Vratza region can be traced evidently in their more energetic physical activity not only in the labour process but also in the everyday-life activity at home, and the private land estate.

The results from the present study definitely show that the established quantitative and topical differences in the SFT studied in both male groups clearly reflect the urbanisation and socio-professional differences in the living conditions between the two investigation foci. Results of this kind give an important medico-biological information necessary for a more precise evaluation of the physical development of the modern Bulgarian population on the background of the concrete living and labour conditions.

III. Basic cephalometric and cephaloscopic features

Determinating the anthropological pecularities of a certain population, of a great importance is the investigation of the cephalometric and the cephaloscopic features.

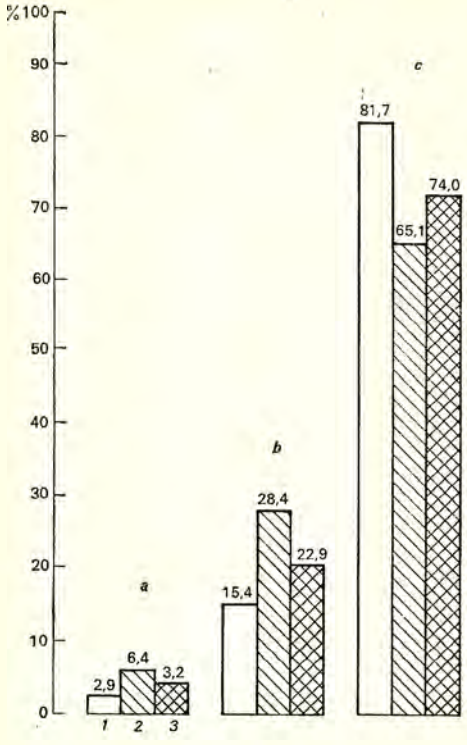
677 persons of both sexes in North-West Bulgaria — 340 men and 337 women at the age of 30-39 years are studied after the conventional anthropological methods [11, 30, 31, 48, 50]. The study includes Vidin, Mihaylovgrad and Vratza region. The following cephalometric features are examined: head length and head breadth; bizygomatical diameter and morphological face height on which basis are calculated the head index and the morphological face index (Table 10). The percentage distribution of the skin colour, hair colour and eye colour is calculated. Comparison-anthropological analysis between the population of Vidin, Mihaylovgrad and Vratza regions, as well as the population from Vratza region studied by M. P o p o v [48] after the administrative division of the country at 1948 which is significant coincided with the up-to-date Mihaylovgrad region is made.

T a b l e 10. Cephalometrical data about the North-West Bulgarian population

Features	Sex	North-West Bulgaria		Vratza region (M. P o p o v [48])	
		n	$\bar{x}$	n	$\bar{x}$
Head length	men	340	188,28	595	187,36
	women	337	178,10	273	179,46
Head breadth	men	340	158,25	595	157,32
	women	337	151,09	273	151,57
Bizygomatical	men	340	144,97	595	141,93
diameter	women	337	137,39	273	133,80
Morphological	men	340	127,29	595	127,42
face height	women	337	116,95	273	118,76
Head index	men	340	84,17	595	84,03
	women	337	84,90	273	84,43
Morphological	men	340	87,85	595	89,60
face index	women	337	85,25	272	88,52

Fig. 11. Percentage distribution of face morphological index categories in men →
a — euryprosop; *b* — mesoprosop; *c* — leptoprosop; *1* — Vidin region; *2* — Mihaylovgrad region; *3* — Vratza region

Fig. 12. Percentage distribution of face morphological index categories in women
a — euryprosop; b — mesoprosop; *c* — leptoprosop; *1* — Vidin region; *2* — Mihaylovgrad region; *3* — Vratza region



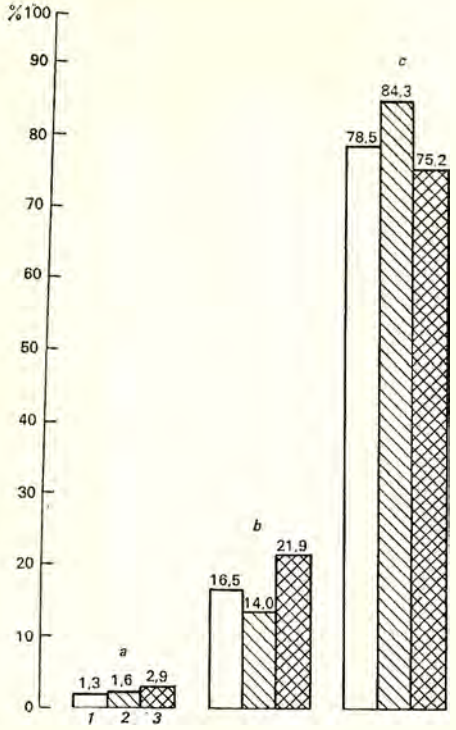


Fig. 9. Percentage distribution of head index categories in men
a — dolichocephal; *b* — mesocephal; *c* — brachycephal; *1* — Vidin region; *2* — Mihaylovgrad region; *3* — Vratza region

Fig. 10. Percentage distribution of head index categories in women
a — dolichocephal; *b* — mesocephal; c — brachycephal; *1* — Vidin region; *2* — Mihaylovgrad region; *3* — Vratza region

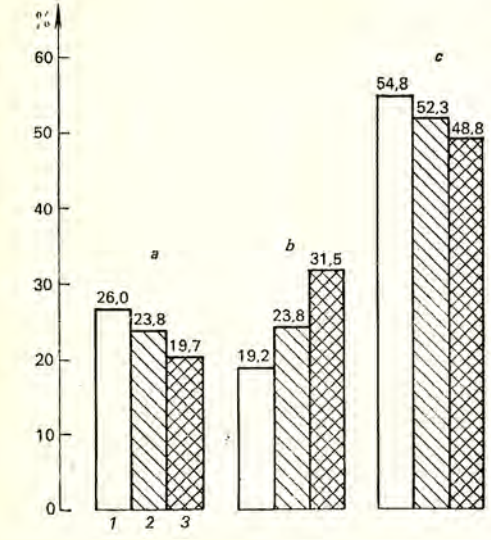


Fig. 11

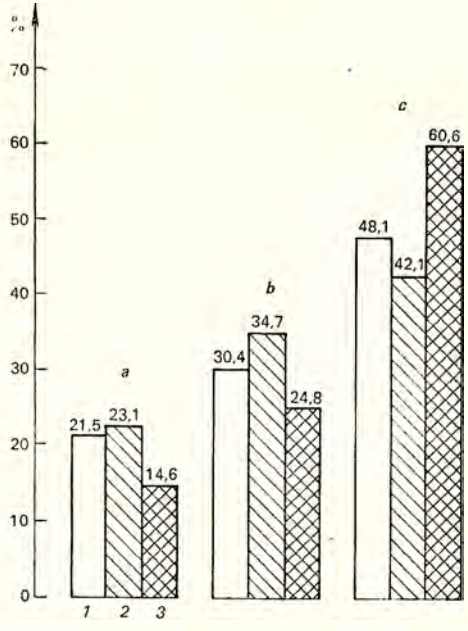
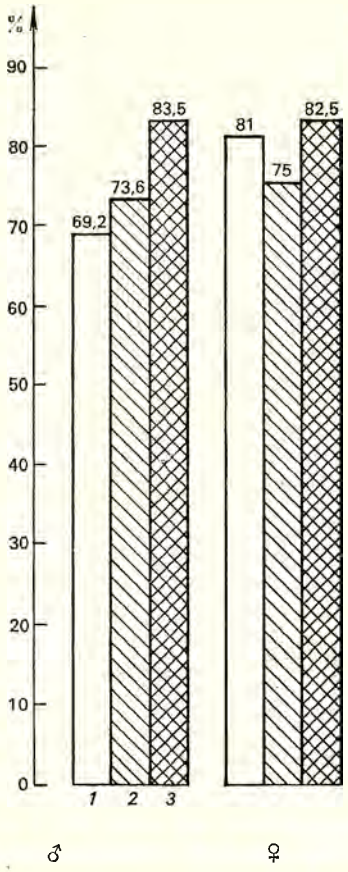


Fig. 12



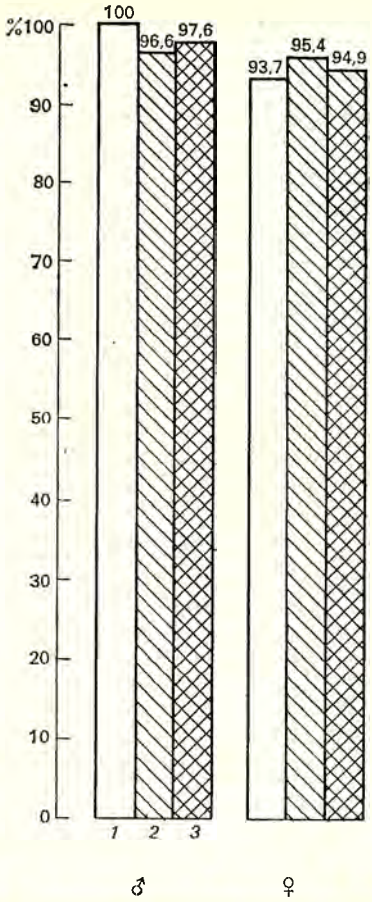


Fig. 13. Percentage distribution of fair skin
1 — Vidin region; *2* — Mihaylovgrad region; *3* — Vratza region
Fig. 14. Percentage distribution of dark hair (P—Y)
1 — Vidin region; *2* — Mihaylovgrad region; *3* — Vratza region

The investigated population of North-West Bulgaria is characterized with long and broad head, wide zygomatica and long face. The men from Vratza region have the lowest medium values of the head length, and the highest ones of the head breadth, the bizygomatic diameter, and the morphological face height. The women from Vratza region have the lowest medium values with regard to all the investigated cephalometric features except the morphological face height. Both sexes can be defined as brachycephalic after the middle value of the head index. The biggest percentage of brachycephalic men are in Vidin region (Fig. 9), and of brachycephalic women–in Mihaylovgrad region (Fig. 10). The middle value of the morphological face index for the men is at the upper line of mesoprosops, and for the women — of leptoprosops. The biggest percentage of leptoprosops for the men is in Vidin region (Fig. 11), and for the women — in Vratza region (Fig. 12). For both sexes of the investigated people in North-West Bulgaria predominate the white skin, especially in Vratza region (Fig. 13). The investigated population has much more high percentage of dark-haired people, particularly for the men in Vidin region, and for the women of the Mihaylovgrad region (Fig. 14). The mixed eyes predominate in the men of Vratza region (Fig. 15), and the dark eyes — in women especially in Mihaylovgrad region (Fig. 16).

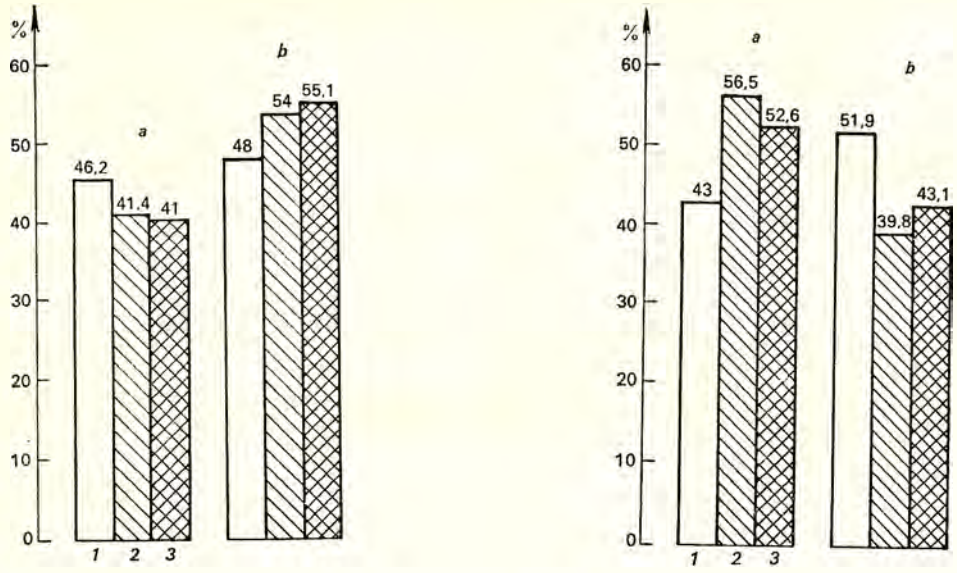


Fig. 15. Percentage distribution of dark (*a*) and mixed (*b*) eyes in men
1 — Vidin region; *2* — Mihaylovgrad region; *3* — Vratza region

Fig. 16. Percentage distribution of dark (*a*) and mixed (*b*) eyes in women
1 — Vidin region; *2* — Mihaylovgrad region; *3* — Vratza region

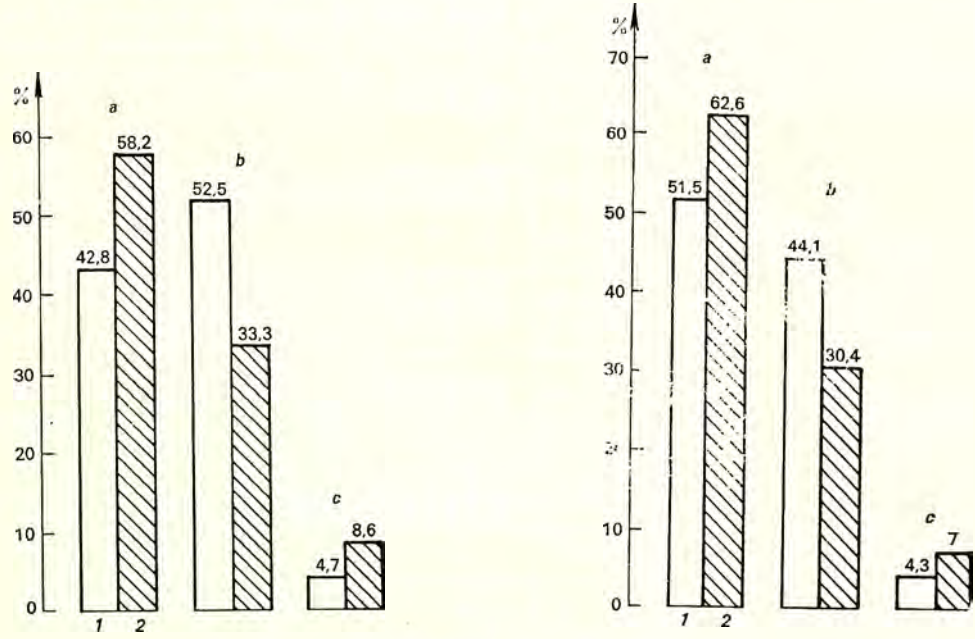


Fig. 17. Comparative data about eye's color in men
a — dark (1—6); *b* — mixed (7—12); *c* — blue (13—16);
1 — North-West Bulgaria; *2* — Vratza district (M. P o p o v, 1959)

Fig. 18. Comparative data about eye's color in women
a — dark (1—6); *b* — mixed (7—12); *c* — blue (13—16);
1 — North-West Bulgaria; *2* — Vratza district (M. P o p o v, 1959)

Table 11. Cephalometrical data of the investigated features in the different regions

Features	Sex	Vidin region			Mihaylovgrad			Vratza region			North-West Bulgaria		
		n	$\bar{x}$	*S*	*n*	$\bar{x}$	*S*	*n*	$\bar{x}$	*S*	*n*	$\bar{x}$	*S*
Head length	men	104	188,40	5,80	109	188,95	7,03	127	187,61	6,62	340	188,28	6,52
	women	79	178,64	6,24	121	178,09	5,68	137	177,80	6,23	337	178,10	6,04
Head breadth	men	104	158,19	5,91	109	157,83	6,00	127	158,65	5,99	340	158,25	6,00
	women	79	151,72	5,67	121	151,71	4,77	137	150,15	5,45	337	151,09	5,27
Bizygomatical diameter	men	104	144,89	5,11	109	144,44	6,20	127	145,49	6,10	340	144,97	5,85
	women	79	137,94	5,46	121	137,47	4,23	137	137,00	5,58	337	137,39	5,10
Morphological face height	men	104	127,32	6,29	109	126,74	7,16	127	127,73	6,86	340	127,29	6,79
	women	79	118,05	6,45	121	115,49	5,77	137	117,60	6,01	337	116,95	6,03
Head index	men	104	84,01	3,35	109	83,67	4,47	127	84,72	4,04	340	84,17	3,99
	women	79	85,08	3,72	121	85,18	3,42	137	84,54	3,87	337	84,90	3,68
Morphological face index	men	104	88,02	5,28	109	87,71	4,93	127	87,84	5,00	340	87,85	5,12
	women	79	85,77	5,52	121	84,24	4,94	137	85,84	4,79	337	85,25	5,04

The middle values of the analysed cephalometric features of the population in North-West Bulgaria don't show a big variations and are near to the same data of M. P o p o v [48]. The zygomatica wide, which middle value is much more high for the investigated groups, makes an exception (Table 11). In the scopic data, more good expressed in the eyes colour (Fig. 17 and 18), some variations are noticed.

The detailed analysis of the final results of the investigated cephalometric and cephaloscopic features included in the National anthropological programme will give the possibility to be cleared up the anthropological characteristics of the up-to-date population in Bulgaria taking into account the genetic factores, as well as the migration processes, and the territorial distribution of the features.

IV. Dermatoglyphics

The dermatoglyphic investigations can be used as a criteria for the norm, pathology, and the transitive status of the human organism, for being constructed an authoritative conception prognosticating different deseases, and looking for genetic markers in medicine and anthropology. The heredity of the dermatoglyphic features is very high. They can be used as genetic markers in the population and medical genetics. To be estimated this fact, however, it's necessary at first to be known the dermatoglyphic characterization of the healthy population.

The aim of this study is to be given a common dermatoglyphic characteristics of the population in North-West Bulgaria, as a part of the whole dermatoglyphic characteristics of the population in Bulgaria.

Finger and palm prints of 262 adult Bulgarians from both sexes (127 men and 135 women) at the age of 30-39 years are investigated. The material is taken from 18 settlements in Vidin, Mihaylovgrad and Vratza regions. Typographic ink is used to get the prints. The rotatory method is used for finger prints. Features are read after C u m m i n s, M i d l o [5] and P e n r o s e [17]. The

T a b l e 12. Frequency of the finger papillar patterns

Sex	Hand	A	R	U	W	Formula
Men	right	2,85	5,54	47,47	44,14	U>W>R>A
	left	4,25	3,31	57,64	34,80	U>W>A>R
	total	3,55	4,02	52,56	39,47	U>W>R>A
Women	right	4,00	2,37	63,11	30,52	U>W>A>R
	left	6,68	2,67	65,13	25,52	U>W>A>R
	total	5,34	2,52	64,12	28,02	U>W>A>R

T a b l e 13. Formulae of the types of the papillar patterns on fingers

Sex	Hand	Patterns			
		A	R	U	W
Men	right	II>III>I>IV=V	II>III=IV	V>III>I>IV>II	IV>I>II>III>V
	left	II>III>I>IV>V	II>III>I	V>III>I>IV>II	IV>II>I>III>V
Women	right	II>III>V>I	II	V>III>I>IV>II	IV>I>II>III=V
	left	II>III>I>V	II>I=III>V	V>III>IV>I>II	IV>II>I>III>V

Table 14. Distribution of the papillar patterns on fingers — separately

Finger of hand	Type of patterns (men)				
	A	R	U	W	Formulae
$Right_1$	2,36	0	45,67	51,97	W>U>A
$Right_2$	5,51	24,41	25,20	44,88	W>U>R>A
$Right_3$	4,80	1,60	60,00	33,60	U>W>A>R
$Right_4$	0,79	1,59	29,36	68,25	W>U>R>A
$Right_5$	0,79	0	77,16	22,05	U>W>A
$Left_1$	2,36	0,79	59,05	37,80	U>W>A>R
$Left_2$	11,02	13,39	31,50	44,09	W>U>R>A
$Left_3$	5,51	2,36	64,57	27,56	U>W>A>R
$Left_4$	1,57	0	50,40	48,03	U>W>A
$Left_5$	0,79	0	82,67	16,54	U>W>A

alternative statistical analysis is used. The T-criterion of Student $p<0,05$ is used for a bilateral and intersexual comparisons.

From the finger papillar patterns (Table 12), the ulnar loops (U) in men on both hands are found most often, followed by the whorl patterns (W). The frequency of arches (A) on the right hand are least, and the radial loops (R) on the left hand show the same tendencies. The formulae for the frequency of the total finger papillar patterns in men is U>W>R>A. In 8 cases (6,30%) there are whorls on all the fingers of both hands, and in 6 cases (4,72%) — ulnar loops. No men has arches on all ten fingers.

The results for the frequency of the papillar patterns in women are the same in commonly. In women most often are the ulnar loops followed by the whorls. At the third place in contrast to the men are the arches in the women on both hands, followed by the radial loops. The formulae of the papillar patterns on both hands is: U>W>A>R. Whorls and arches on all 10 fingers didn't found in women, and ulnar loops was found in 9 cases (6,67%).

The bilateral comparison shows that the whorls are with more high frequency on the right hands, than on the left ones in both sexes, but the difference is statistically significant in men ($p<0,05$). The ulnar loops in men are found more often on the left hand ($p<0,05$), while such a difference almost missed in women. Significant bilateral differences for the other two patterns were not found.

Comparing the frequency of the papillar patterns in both sexes, we can say that more frequent are the whorls in men, and the ulnar loops in women ($p<0,05$).

The data about the frequency of the finger papillar patterns follow the same regularity as the investigations from another authors about Bulgarian population samples [19, 45, 51].

Two kinds of formulae are worked out about the distribution of the papillar patterns on the fingers. The frequency of every type papillar patterns separately for the right and the left hand in both sexes are pointed at the first type of the formulae (Table 13). The arches and the radial loops are more frequent for the II-nd fingers, the ulnar loops — for the V-th fingers, and the whorls — for the IV-th fingers. The frequency of the different patterns on the separate fingers are pointed at the second type of the formulae (Table 14). Different variations can be seen between the separate fingers, as well as between the right and the left hands, and the men and women. The comparison between the finger formulae and the common formulae about the frequency of the papillar patterns in men shows that on the right hand (common formulae U>W>R>A) there is no similarity for the separate fingers, but for the left hand (common formulae U>W>A>R) the coincidence

Type of patterns (women)				
A	R	U	W	Formulae
0,74	0	63,70	35,56	U>W>A
12,59	11,85	40,74	34,81	U>W>A>R
5,18	0	79,26	15,56	U>W>A
0	0	48,89	51,11	W>U
1,48	0	82,96	15,56	U>W>A
5,93	1,48	61,48	31,11	U>W>A>R
17,04	9,63	38,52	34,81	U>W>A>R
8,89	1,48	73,33	16,30	U>W>A>R
0	0	63,70	36,30	U>W
1,49	0,75	88,80	8,96	U>W>A>R

missed only for the II-nd finger. The coincidence with common formulae in women (right hand, left hand U>W>A>R) exists for every finger of both hands with the exception of the IV-th finger of the right hand. The results pointed out in Table 13 and Table 14 show that the analysis of the finger patterns frequency must be done separately for each hand and each finger to be understood better the regularity of their distribution.

The mathematical expression of the finger papillar patterns' frequency are the indexes. That's why the indexes of Dankmeijer and Poll have more high values in women, and the Furuhata and the delta-index are higher in men (Table 15). This differences are due to the different frequences of the papillar patterns in both sexes. Analogus to this intersex differences are spoken about from another authors [45, 51]. The values of the Dankmeijer index are higher in the studies of T o r n j o v a - R a n d e l o v a [51] and K a r e v [45] (respectively — ♂ — 12,04, ♀ —

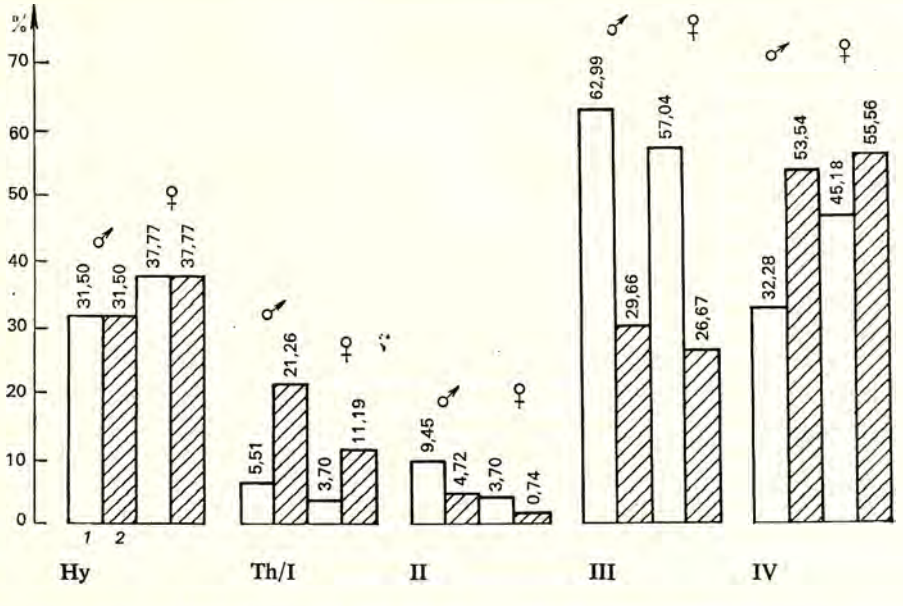


Fig. 19. Frequency of palm patterns
1 — right; *2* — left

Table 15. Index characterization of finger papillar patterns

Sex	Dankmeijer			Poll			Furuhata			Delta index		
	right	left	total	right	left	total	right	left	total	right	left	total
Men	6,46	12,21	8,99	5,38	6,97	6,24	83,28	57,10	69,27	14,13	13,06	13,59
Women	13,11	26,18	19,64	6,11	9,85	7,98	46,61	37,64	42,12	12,65	11,88	12,26

Table 16. Frequency of the type patterns on Hypothenar

Type	Men			Women		
	right hand	left hand	total	right hand	left hand	total
A^u	51,18	55,11	53,13	37,04	42,22	39,63
A^c	12,60	11,81	12,20	22,96	18,52	20,74
A^r	4,72	0,79	2,76	2,22	1,48	1,85
L^u	11,02	7,09	9,06	7,41	8,89	8,15
L^c	3,15	0,79	1,97	3,70	0,74	2,22
L^r	11,81	18,11	14,96	20,00	22,22	21,11
W+S	4,72	3,15	3,94	3,70	0,74	2,22
L^r/L^u	—	1,57	0,79	0,74	2,96	1,85
L^r/A^c	0,79	—	0,40	1,48	1,48	1,48
L^r/T	—	—	—	0,74	0,74	0,74
L^u/A^c	—	0,79	0,40	—	—	—
V	—	0,79	0,40	—	—	—

24,53; ♂ — 11,43, ♀ — 16,29), which is due to the minimum differences in the frequency of the arches and the whorls of the investigated groups.

The frequency of the palm patterns, the type of the Hypothenar patterns and the axial triradius from the palmoscopy are studied.

The frequency of the patterns on the six areas of the palm are analysed combining the Thenar and the I-st interdigital area (Fig. 19). Most patterns are found on the III-th interdigital area (IA) in men on the right hand. A little lower is the frequency of the patterns on the Hypothenar. Smallest are the patterns on Thenar / I-st IA. Most are the patterns on the IV-th IA on the left hand, at the second position are the Hypothenar patterns, followed from the patterns on the III-th IA. Least are the patterns on the left hand, on the II-nd IA in opposite to the right hand.

The comparison of the palm patterns' frequences on the right and the left hand shows that the patterns on the Th / I-st IA and IV-th IA are more frequent on the left hand, and the patterns on the II-nd IA and III-th IA prevailed in right ($p < 0,01$). The Hypothenar patterns are with equal frequency on both hands.

Most numerous are the patterns on the III-th IA in women on the right hand. Lower frequency have the patterns in IV-th IA, followed by the Hypothenar patterns. Comparatively scanty of patterns are the Th/I-st IA and the II-nd the IA. The highest frequency of the patterns is on the IV-th IA of the left hand. In contrast to the right hand, on the left one the patterns on the Hypothenar prevailed the patterns on the III-th IA. Most rearly are the patterns in the II-nd IA.

Bilateral differences are found in women too. Considerably often are the patterns in the III-th IA on the right hand, than those on the left one, and the patterns on the IV-th IA and Th/I-st IA on the left hand, than those on the right one ($p < 0,05$). Here the frequency of the Hypothenar patterns is equal for both hands as in men.

The intersexual comparison shows that the patterns on the Th/I-st IA on the left hands and those ones in the IV-th IA for the right hands have siqnificant statistical diff erences ($p < 0,05$).

It can be said, in general, that the bilateral differences prevailed to the intersexual ones in all palm area instead of the Hypothenar.

The Hypothenar (Hy) is the richest and the multiformed from all the palm areas (Table 16). From all the patterns, on the Hy most frequently in both hands and both sexes are the ulnar arches (A^u) and from the real patterns — the radial loops (L^r) followed by the ulnar opened loops (L^u). The comparison between the two hands didn't show significant differences ($p > 0,05$).

A particular attention deserves one of the very rear Hypothenar pattern, namely, the radial arch (A^r). G e i p e l [6] call it "classic arch pattern". G y e n i s [9] says that it isn't a separate pattern, but only a variation of the radial arch. Radial arch are found in both sexes frequently on the right hands in our investigation. By the literature [9] the frequency of this Hypothenar patterns for the European populations is 0,2-2,0%.

The analysis of the axial triradii coincided with those in the literature. In the healthy populations, in the nations all over the world, there is only one triradius — t (50-75%). The intermedial triradius (t′) is rear (10-20%), but more rear is the central triradius (t″), and the presence of two or three triradii. In our material, the most numerous is the carpal axial triradius (t) in both sexes and both hands (♂ — 62,99%, ♀ — 61,48%); on the second place with more lower frequency is the intermedial triradius (t′) (♂ — 17,72%, ♀ — 21,11%). The central triradius (t″) is very rear (♂ — 2,36%, ♀ — 4,07%). Statistical significant bilateral and intersexual differences are missing.

The dermatoglyphic characteristics is a part of the anthropological characterization. The analysis of the dermatoglyphic investigated persons for the country — total 2571 (1221 men, 1350 women) will help the presentation of a common dermatoglyphic characteristic of the population in Bulgaria. The importance of the dermatoglyphical status has two basic aspects — on the one hand, in anthropological and genetic-population aspect, and on the other hand, they can be used as a basis for a comparison and an interpretation of the results from different clinical deseases.

V. Odontology

Morphological specifities of teeth and dentition in the human are part of the genetically exactly determined features. The odontological characteristics represents an important section of the overall anthropological characteristics of the Bulgarian population.

338 men and 337 women at an age of 30-39 years from 31 foci of 19 settlements have been examined odontoscopically in the Mihaylovgrad region.

The programme of the odontological examination includes a total of 26 features. A part of them are genetically predetermined and another one are related to the evolution of the facial cranial portion in the human.

In the present study is analyzed the first group of odontological features which includes: type of bite, colour of teeth, shape of the tooth arch (upper and lower), shape of the upper central incisors, spade-shapedness of the upper central and lateral incisors and presence and degree of expression of Tuberculum Carabelli (Table 17, Figs. 20, 21 and 22).

The psalydont bite is found in 89,50% in women and 82,00% in men; tooth colour in men is darker (No 25 and 27) than it is in women (No 21 and 23); the shapes of the maxillar and mandibular tooth arches display an identical per cent distribution; the spade-shapedness of the upper central incisor is found in 28,50% of the males and in 17,60% of the females, stronger pronounced in the masculine

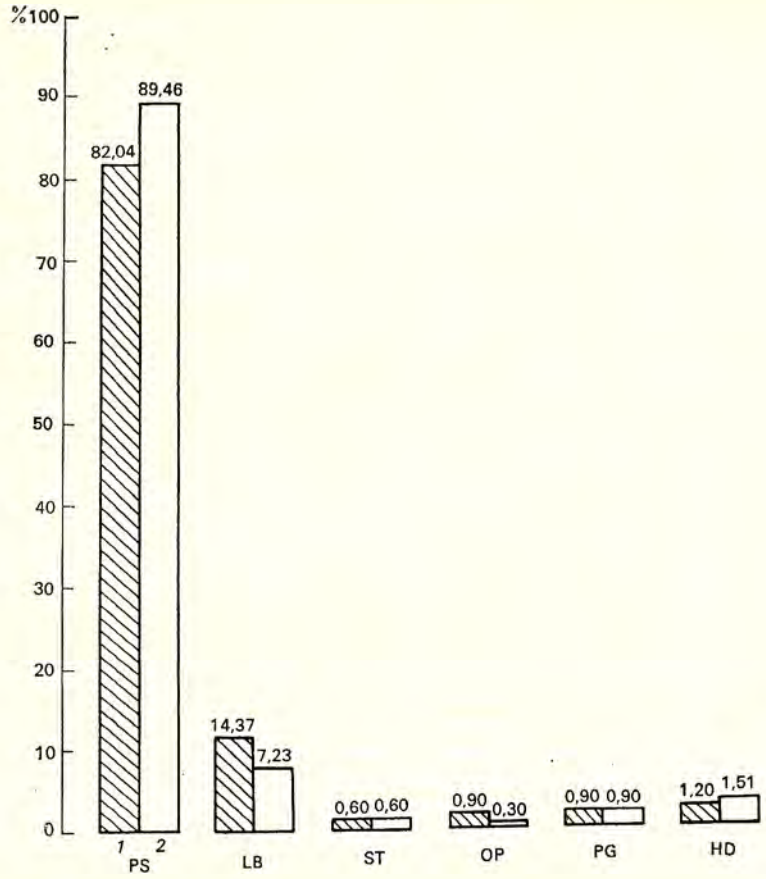


Fig. 20. Percentage distribution of bite type in men (*1*) and women (*2*)

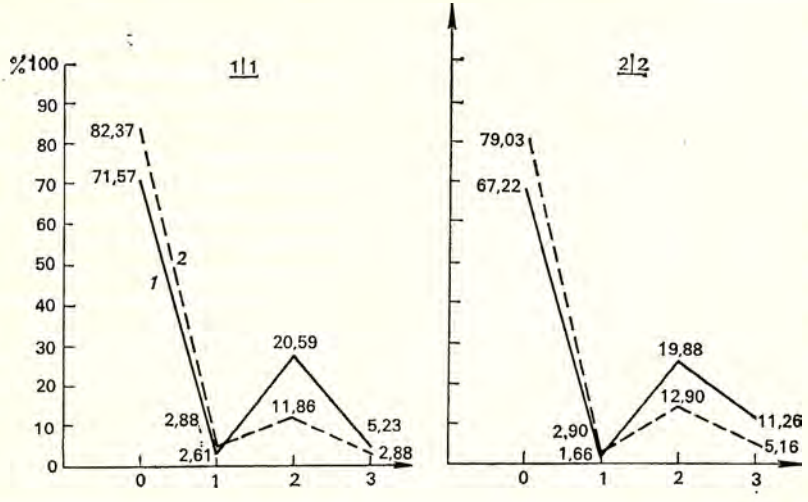


Fig. 21. Percentage distribution of shovel shape in the upper incisores
1 — men; *2* — women

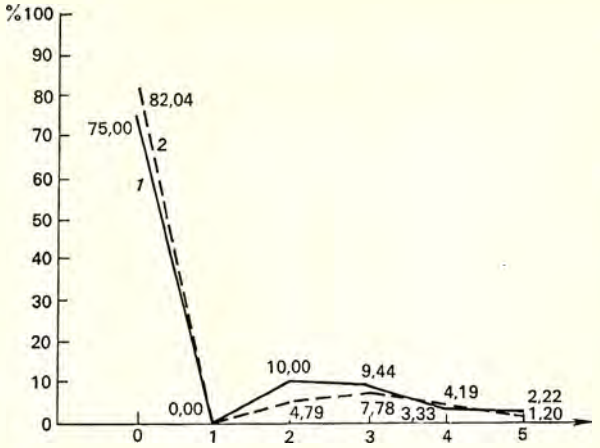


Fig. 22. Percentage distribution of Tuberculum Carabelli in the first upper molares
1 — men; *2* — women

Table 17. Frequency of teeth shade and forms of processus alveolaris maxillae and mandibulare and forms of the upper central incisores

Features		Men		Women	
		n	%	*n*	%
Colour	05	1	0,29	2	0,60
Shade by Duracryl	07	1	0,29	—	—
SPOFA, Dental Praha	19	—	—	1	0,29
	21	57	16,91	120	35,61
	23	81	24,03	135	40,06
	25	92	27,30	65	19,29
	27	71	21,07	13	3,86
	39	1	0,29	—	—
	41	10	2,97	1	0,29
	43	14	4,15	—	—
	45	10	2,97	—	—
Form of arcus alveolaris maxillae	1	42	12,61	39	11,89
	2	237	71,17	239	72,86
	3	26	7,81	29	8,84
	4	28	8,41	21	6,40
Form of arcus alveolaris mandibulae	1	9	2,96	8	2,42
	2	248	74,03	230	72,42
	3	51	15,22	59	17,88
	4	27	8,06	24	7,28
Form of the upper central incisores	1	161	50,95	171	53,94
	2	94	29,75	70	22,08
	3	61	19,30	76	23,97

sex; the spade-shapedness of the lateral incisor repeats the same tendency — 32,80% for men and 20,90% for women; the Tuberculum Carabelli is reared in 25,00% of the men and 17,90% in women.

It is quite clear judging from the data presented that the permanent dentition in women is more regularly arranged than it is in men. The spade-shapedness in the males significantly exceeds the frequency (by 11 per cent) and the degree of expres-

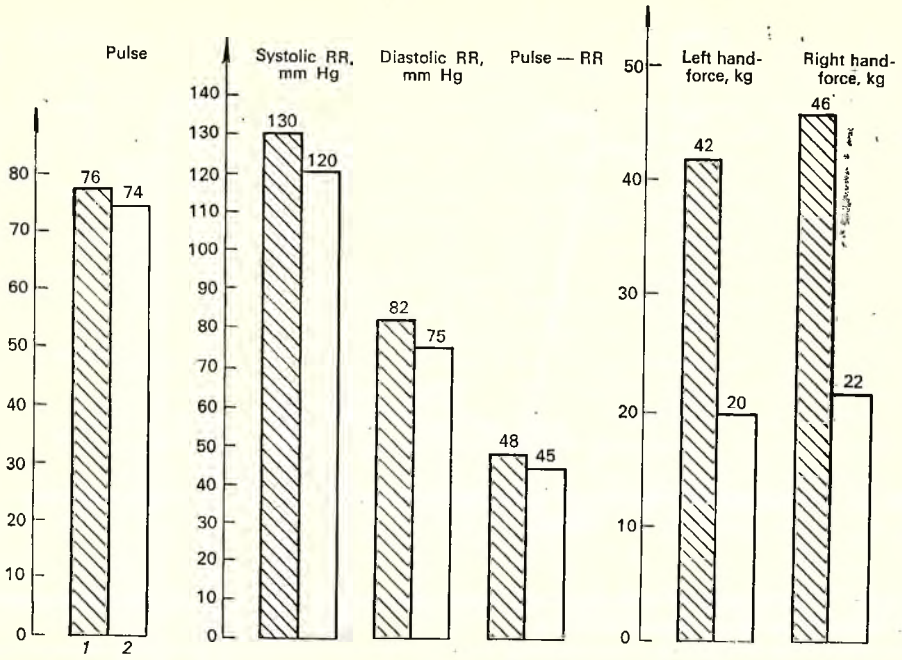


Fig. 23. Middle values of physiometrical features in men (*1*) and women (*2*)

sion by which it is found in women. The presence of the Tuberculum Carabelli repeats the same tendency, i. e. it is more frequently (by 7 per cent) registered in the males from Mihaylovgrad region.

It is noteworthy that in contrast to what is known in the literature [37, 40] sexual dimorphism applied to the spade-shapedness in the upper first molars is strongly expressed. Final assessment of this fact is, however, only possible after the wholesome processing of the data characterizing the entire country.

VI. Physiometric features

Human organism is a self-regulatory system aimed at maintaining physiological homeostasis in it. That is why, it is impossible to work out a through objective anthropological characterization of the population without including the basic physiometric features: pulse frequency, systolic and diastolic blood pressure and as a derivative — the pulse blood pressure, vital capacity of the lungs, strength of left and right hand.

677 individuals were investigated (341 men and 336 women) at an age of 30-39 years from 21 foci in 19 settlements of the Mihaylovgrad region. Data about the district, as a whole, have been processed statistically, as well as about the three regions of Vratza, Mihaylovgrad and Vidin, plus the total of the three big towns (over 50 000 inhabitants), plus the eight small towns (under 50 000 inhabitants) and the eight villages.

Upon comparison of data concerning men and women as a whole for the entire Mihaylovgrad district (Figs. 23 and 24) it was established that there exist significant differences in the strength of the left and right hands and vital capacity between the sexes in favour of the male one. A tendency towards higher values of both systolic and diastolic blood pressures is also observed in the men.

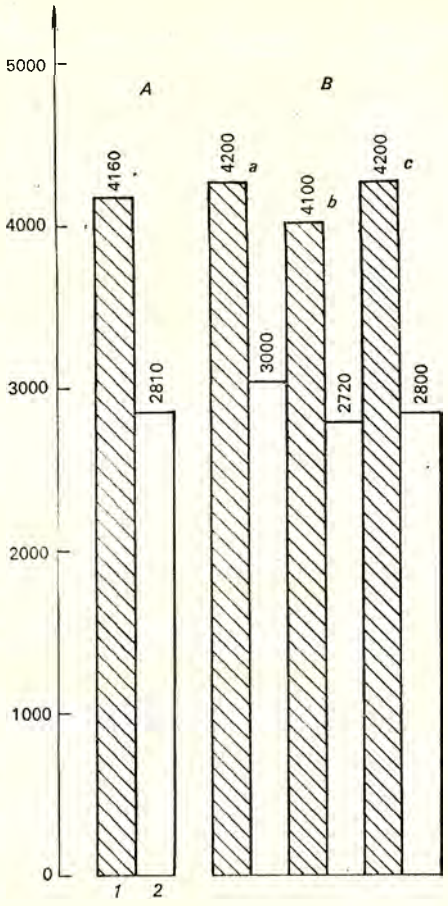


Fig. 24. Middle values of vital capacity in men (*1*) and women (*2*)
A — Mihaylovgrad region; *B* — three types of settlements; *a* — big towns (over 50 000); *b* — small town (under 50 000); *c* — villages

Aiming at determination of the influence of urbanization and geographic location of the given settlement the results valid for the three different types of settlements were compared (Fig. 25). Statistically significant differences in the pulse frequency, the diastolic blood pressure, and vital capacity of the lungs are not established. The systolic blood pressure reveals a tendency to higher values in the men from the smaller towns and villages. The strength of the left and right hands is of greater values in males from the villages which is logically explained by their greater physical activity. There are no differences whatever in the women of the different types of settlements.

The results obtained give grounds for the conclusion that excepting for the strength of hands there is no influence traced due to the urbanization and geographical location of the settlement on the physiometric features studied in the population of the Mihaylovgrad district. Even after the common analysis of the physiometrical features from whole Bulgaria it will be possible to be searched their connections with the individual's constitution type and the influence of the geographical, socio-economical, professional and other factors.

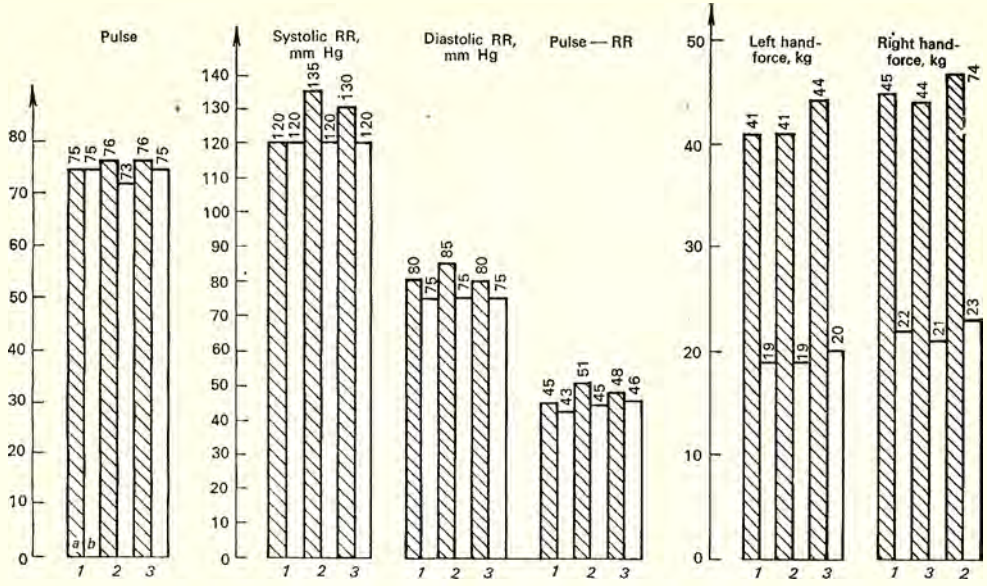


Fig. 25. Middle values of physiometrical features in three types settlements
1 — big towns (over 50 000); *2* — small towns (under 50 000); *3* — villages; *a* —men; *b* — women

VII. Epochal changes in the basic craniometrical features

In the study of the epochal changes in the configuration of the human skull major attention has been allotted to the breadth-length cranial index, which reflects the process of brachycephalization. To that end, numerous hypotheses have been

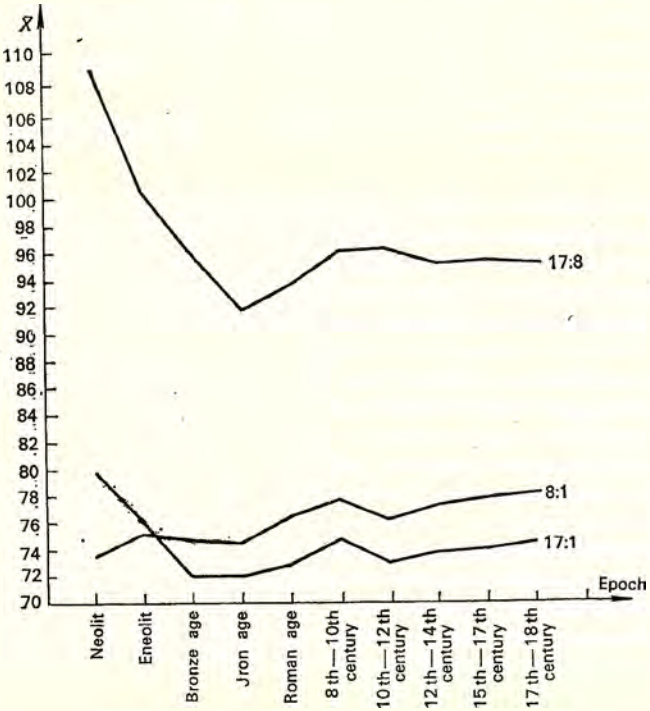


Fig. 26. Epochal changes in basic cranial indexes

Table 18. Objects studies

Epoch	Series	Authors of study
Neolith	Dewetashka peshtera, Karanovo Malak Preslavetz, Vaksevo	P. Boev [1] S. Cholakov
Eneolith	Rousse, Liljak, Kubrat, Varna, Targovishte, Omurtag	P. Boev [1] J. Jordanov [10] S. Sholakov, P. Boev [4] S. Cholakov, J. Jordanov
Bronze age	Belogradetz, Plachidol, Nova Zagora, G. Detelina, M. Detelina	J. Jordanov, B. Dimitrova [41 S. Cholakov
Iron age	Devetashka peshtera, Dolno Sachrane, Kalojanovo, Sborjanovo	P. Boev [1] S. Cholakov, J. Jordanov [53]
Roman age	Abritus, Varna, Plovdiv, Augusta Trayana	N. Kondova [46] P. Boev, N. Kondova, S. Cholakov [28]
8th-10th century	Ablanitza, Balgarevo, Kragulevo, Durankulak, Tabachka, Rasgrad	P. Boev, N. Kondova, S. Cholakov[25]; P. Boev, N. Kondova, S. Cholakov [2]; J. Jordanov, S. Cholakov [42]; I. Iordanov [39]; N. Kondova, S. Cholakov [47]
10th-12th century	Lovech, Tuchovishte, Kovatchevo, Karanovo, Kalugerovo, Odartzi	P. Boev [1] P. Boev, N. Kondova, S. Cholakov, P. Boev, S. Cholakov, N. Kondova, S. Cholakov, N. Kondova
12th-14th century	Lukovit, Kasanlik, Tatul, Urvitch, Poljanitza, Pernik, Kavarna, Kabile	P. Boev [1] P. Boev, N. Kondova, S. Cholakov [27] P. Boev, N. Kondova, S. Cholakov [29] S. Cholakov, N. Kondova, P. Boev [54]
15th-17th century	Nedelkovo, Kavarna, Kaliakra	P. Boev, N. Kondova, S. Cholakov [26, 29]
17th-18th century	Gradishte, Ilijantzi	S. Cholakov [52]

Table 19. Epochal changes in basic cranial measures and indices

Epoch	Measures					Indices			
	1	8	17	45	48	8:1	17:1	17:8	48:45
Neolith	186,7	137,0	151,0	138,0	70,7	73,4	79,9	108,8	54,4
Eneolith	185,2	139,9	140,9	129,6	69,2	75,2	75,8	100,2	53,3
Bronze age	190,4	142,5	136,7	136,8	72,5	74,8	71,9	95,8	53,0
Iron age	189,7	141,0	134,6	128,0	70,8	74,6	72,0	91,6	55,9
Roman age	186,0	143,2	133,2	134,2	69,2	76,5	73,1	93,7	51,5
8th-10th century	183,4	142,0	135,0	133,0	71,5	77,7	74,7	96,2	53,9
10th-12th century	186,1	142,1	136,1	133,2	71,5	76,2	72,9	96,3	53,4
12th-14th century	185,2	143,0	136,3	133,5	70,4	77,3	73,8	95,2	52,5
15th-17th century	183,4	142,6	136,3	133,8	70,4	77,8	74,1	95,5	52,6
17th-18th century	181,9	142,3	135,7	131,3	68,9	78,3	74,6	95,4	52,6

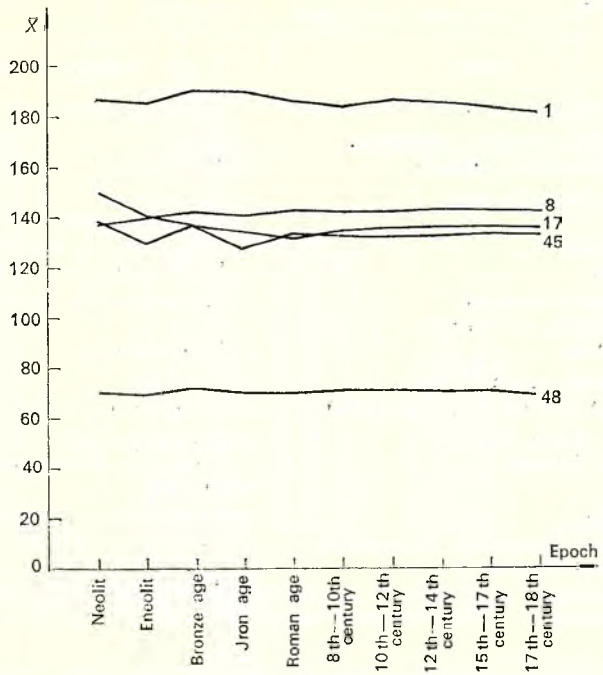


Fig. 27. Epochal changes in basic face measurements

proposed and the influence of a number of factors, bearing on evolution, selection, isolation, migration, some climatic and geographic conditions, etc., has been traced. The changes in the anthropological characteristics of skulls found in the Bulgarian lands reflecting the influence of those factors, along with the peculiarities, specific of this country, can be traced from the neolith to the beginning of the 20th century [47].

The object of this study are 48 craniological series from archaeological excavations of necropolises, dated from the early neolith to the end of the 18th century (Table 18). The comparative analysis is based exclusively on data concerning 980 male skulls. The 19th century period is represented by bone material preserved in monasteries [33], and the skulls from the Sofia military graveyard supply data about the early decades of the 20th century [43].

When the values of the cranial index are considered, its gradual increase becomes evident (Fig. 26). Starting at 73,4 in the neolith, it reaches 77,8 by the end of the 17th century and 78,4 in the 18th and 19th centures. Up to the end of the Iron Age dolichocrany prevails.

The early neolith crania are long, of medium breadth, and markedly high (Table 19). In Central Europe at that time the first manifestations of brachycephalization are observed, related to the emergence of the alpine racial type and the brachycranial cromagnoids. Protomediterranean racial types prevail among the neolith crania in the Bulgaria lands [1].

During the eneolith in these lands there is clear evidence of gracilization and brachycephalization, although dolicho-mesocranic, hipsicranic and acrocranic types still prevail. In the eneolithic population from Rousse alpine racial types was observed and emergence of dinarization among the population [1]. It must be emphasized that the eneolith witnesses the initial rise of brachycephalization

Table 20. Territorial variations of cranial index during the Roman period

Series	Measures					
	1	8	17	45	48	8:1
Plovdiv	181,8	145,0	133,5	131,0	66,4	79,7
Abritus	186,0	144,6	134,1	132,0	66,3	77,5
Varna	188,2	142,6	134,7	139,5	72,0	76,4
Augusta Trayana	186,7	141,9	134,5	134,4	70,5	76,0
Total (n=102)	186,0	143,2	133,2	134,2	69,2	76,5

Table 21. Territorial variations of cranial index during the Early Middle Ages (8th-10th century)

Series	Measures					
	1	8	17	45	48	8:1
Ablanitza	179,2	144,7	127,6	124,8	64,3	80,8
Rasgrad	185,5	144,3	138,7	137,0	75,2	77,9
Tabachka	183,6	142,0	132,8	132,0	71,1	77,7
Durankulak	182,4	140,9	137,3	133,2	72,3	77,6
Bulgarevo	183,6	141,0	135,3	135,7	64,7	76,9
Kragulevo	186,8	140,7	135,9	133,6	71,2	75,3
Total (n=85)	183,4	142,0	135,0	133,0	71,5	77,7

whose peak is clearly observable on the diagram (Fig. 26). In the Bulgarian lands this can be attributed to the diversity of dolichomesocranic, intermediate variants marking the transition from protomediterranean to gracile mediterranean racial types [1, 4, 10]. In the Bronze Age the skulls remain dolichocranic, with a certain increase of both their length- and width measures [41]. The cranial length reaches its maximum (Fig. 27).

The Iron Age is likewise repsesented by dolichocranic skulls, though lower and a little wider, with considerably narrower faces [1, 53].

The basic cranial measures substantially alter their characteristics during the Roman period, when the cranial and bizygomatic breadth reach their maximum, while the height from the basion and the upper-face height reach the minimum. Distinct territorial difference are also characteristic of the period. Although all the studied series are mesocranic, substantial differences in their structure are observed. In Augusta Trayana (Southern Bulgaria) [28], for instance, the predominant number of crania are long and very long, 40 per cent of them being dolichocranic, while among the Abritus and Varna populations (Northern Bulgaria) [46] dolichocranic and brachycranic skulls are evenly represented. Great variability, with different combinations of width- and height measures, is also observed in the facial part (Table 20). These peculiarities are probably related to the more intensive migration processes in the Northern Roman province — Mizia, while in Trakia it was the representatives of the authochthonic population and immigrants mainly from the Mediterranean regions that prevailed.

The second distinct peak in the curve, representing the cranial index changes, is reached during the Early Middle Ages. Its higher values are connected mainly with the cranial length decrease (Fig. 26, 27). At the same time, an increase in the upperface height is in evidence, along with other specific changes in the facial part. Mesobrachycranic skulls appear, with mongoloid traces in the chapes of the orbita, the nasal bones and the face profile. The territorial variations become even more conspicuous. One population from South-Western Bulgaria (Ablanitza)

Table 22. Territorial variations of cranial index during the Middle Ages

Series	Measures					
	1	8	17	45	48	8:1
			10th-12-th century			
Kalugerovo	180,8	141,8	134,0	132,8	70,5	77,7
Odartzi	185,6	143,4	134,3	133,8	71,4	77,2
Karanovo	185,8	140,1	135,6	131,6	69,6	75,5
Lovech	188,0	144,2	137,0	130,8	74,0	76,7
Tuchovischte	198,3	143,1	138,3	135,4	73,3	76,1
Total (n=69)	186,3	142,1	136,1	133,2	71,5	76,2
			12th-14th century			
Poljanitza	180,8	142,8	136,8	—	—	79,8
Pernik	183,8	145,4	135,9	135,9	70,2	78,9
Urvitch	187,2	145,1	133,2	135,0	69,5	77,8
Kabile	184,6	142,7	136,0	134,1	70,8	77,4
Tatul	187,8	147,7	139,5	135,7	70,5	77,0
Kavarna	183,0	140,8	137,8	131,6	72,0	76,6
Lukovit	186,4	140,7	139,4	129,1	66,4	75,5
Kasanlik	189,6	140,4	137,0	129,0	69,6	75,2
Total (n=163)	185,2	143,0	136,3	133,5	70,4	77,3
			15th-17th century			
Kaliakra	183,7	144,0	134,7	134,8	71,0	78,1
Kavarna	182,7	141,7	136,5	133,3	70,6	77,7
Nedelkovo	187,1	145,7	136,9	135,3	68,2	77,9
Total (n=134)	183,4	142,6	136,3	133,8	70,4	77,8

[25] is particularly prominent, with strongly brachycranic, narrow-faced skulls of small height. The opposite deviations are observed in the north-eastern part of Bulgaria. The crania from Durankulak [42]; Tabachka [39]; Kragulevo [24] and Bulgarevo [2] are longer, higher, with bizygomatic width and upper-face height and obvious mongoloid traces (Table 21). These measurements prove that the process of brachycephalization in the Bulgarian lands from the 8th to the 10th century was characterized by considerable regional variations.

In the subsequent period (10th-12th century), the process of brachycephalization slows down (Table 22). The cranial index decreases, while the cranial length is the greatest as compared to the whole medieval period.

From the 12th-14th century on the basic cranial measures and indices preserve a constant tendency in their changes. The cranial length progressively decreases, along with a parallel, but less marked reduction of width. Although all the series studied are mesocranial, there are considerable inter-group difference, which makes it possible to trace the territorial variations in the brachycephalization process. The comparison between the two large groups of Pernik [27] and Kabile [54], representative of Southwestern and Southeastern Bulgaria, reveals that the small variation in the cranial index values covers substantial structural differences (Table 22). The Pernik population in fact is mesobrachycranial (36 per cent brachycrany), with obvious mongoloid traces in the facial part. In the Kabile series which is mesocranial as well, brachycranial forms are almost completely lacking. The skulls from that region are narrower, relatively longer and more gracile. That, along with the prevalent mediterranean features, makes the differences between the two

Table 23. Changes of cranial index in children

Cranial index	Infans I		Infans II	
	12th-14th century	15th-17th century	12th-14th century	15th-17th century
Dolichocran	—	2,61	—	5,00
Mesocran	34,78	28,70	53,85	40,00
Brachycran	56,52	47,83	30,77	43,33
Hyperbrachycran	8,70	20,87	15,38	11,67

populations even more obvious. They reflect the later migration processes, related to the kuman and pecheneg invasion in the 11th century.

In the Late Middle Ages Kaliakra, Kavarna [29], Nedelkovo [26] the intergroups differences are less clear-cut. The cranial length continues to decrease and the index is slightly increased (Table 22).

The 18th century is represented from the populations in Gradishte and Ilienzi [52] which were characterized with a rise in the value of the cranial index, in return for a decrease of the cranial length (Table 19).

The 19th century is represented by data from Vatev's research, in monastic bone-vault supply evidence concerning three regions — Mizia, Trakia and Southwestern Bulgaria [34]. The cranial index changes are most significant in Mizia (79,1) and least so in Trakia, where also the relatively longest speciment (77,6), with correspondingly the least cranial width, have been found.

In the beginning of the 20th century, in the crania from the Sofia military graveyard, the length is once again strongly reduced. In ten of the regions studied it varies from 174,6 mm to 178,3 mm, and in the Rila-Pirin region alone it is 180,1 mm. The percentage of brachycranial forms is significantly increased as well.

These regularities observed in the process of brachycephalization are also corroborated by the evidence concerning the cranial index changes in children (Table 23). Among the 12th-14th century populations in Infans I, for instance, 65,2 %of the skull are brachy- and hyperbrachycranial, while in the next age group (7-14 years) the cranial index drops to mesocranial in 54 % of the cases. In the late medieval population this ratio does not change until Juvenis-group, when brachycrany drops below 50 %.

Among the factors affecting the changes in the basic cranial measures those that rank first are indubitably migration process and metissation. However, other factors such as isolation, epidemics, the different protein balance, jodid and iron deficit, etc., shoud not be underestimated. It is selection that is considered to be the main factor in the process of brachycephalization in Central Europe during the period between the 8th and the 18th centures [18]. Epidemic diseases and the different constitutionally conditioned resistance to them rank first. There is historical and archaelogical evidence of a severe epidemic (probably plague) that struck the population of the roman city Augusta Trayana at the end of the 5th century.

With the Ablanitsa population the influence of the unintentional artificial skull deformation should be noted.

When compared to other European countries, the brachycephalization process in the Bulgarian lands is characterized by a significant retardation after its upward development during the Early Middle Ages, although some local fluctuations are observed. Conversely, in neighbouring Romania and Jugoslavia, the cranial index increase accelerates after the 16th century [16, 12]. Even steeper is the rise of the index change in Poland and Czechoslovakia [13, 8].

The results of this study suggest the following conclusions: the process of brachycephalization in the Bulgarian lands is comparatively slow, with considerable regional and diachronic variations; as a major emerges the reduction of skull length in human evolution; at the end of the Roman period and during the Middle Ages a significant influence was exerted by migration process and the various selection factors.

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Short communication

Effects of oxytocin on progesterone secretion by hen granuloza cells

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Oxytocin (OT) dose-dependently increased progesterone secretion in granulosa cells (GCs) of preovulatory follicles F_2 and F_3 in laying hens. The effects of OT 1, 5 and 10 mIU were weakest at 24 h and strongest at 72 h after cultivatin of granulosa cells. LH stimulated the OT-induced progesterone secretion while FSH inhibited the OT effect. It is concluded that OT is an intraovarian regulator of steroidogenesis in the preovulatory follicles of laying hens.

Key words: oxytocin, progesterone secretion, hens preovulatory follicles, granulosa cells, gonadotropins.

The nonapeptide oxytocin (OT) originally detected in the magnocellular neurons of the hypothalamus has recently been found in human ovaries and in the ovaries of many mammalian species [3, 4, 8, 9]. In the ewe and in the cow the plasma levels of OT are low during the follicular phase and increase during the luteal phase. These fluctuations are due to changes in the ovarian OT [8]. Chandrasekher and Fortune [1] and Voss and Fortune [6] have reported OT stimulation of progesterone secretion in bovine GCs isolated from preovulatory follicles. This stimulant effect of OT is blocked by the oxytocin antagonist. The authors suggest that OT is an intraovarian regulator of steroidogenesis in mammalian preovulatory follicles. We failed to find data about the synthesis of OT- or OT-like peptides in the ovaries of birds and about the OT effect on steroidogenesis in granulosa cells. This stimilated us to study the effect of OT on isolated GCs from hen preovulatory follicles, as well as the possible modulating action of gonadotropins on OT-induced progesterone secretion.

Material and methods

Hens were killed by cervical dislocation and the F_2 and F_3 large preovulatory follicles were removed and immediately placed in ice-cold 0,9% saline. GCs were collected and dispersed with 0,3% Collagenase (type 2) diluted in sterile DMEM (Flow Labs) as described by Tilly and Jonson [5]. Cell number and via-

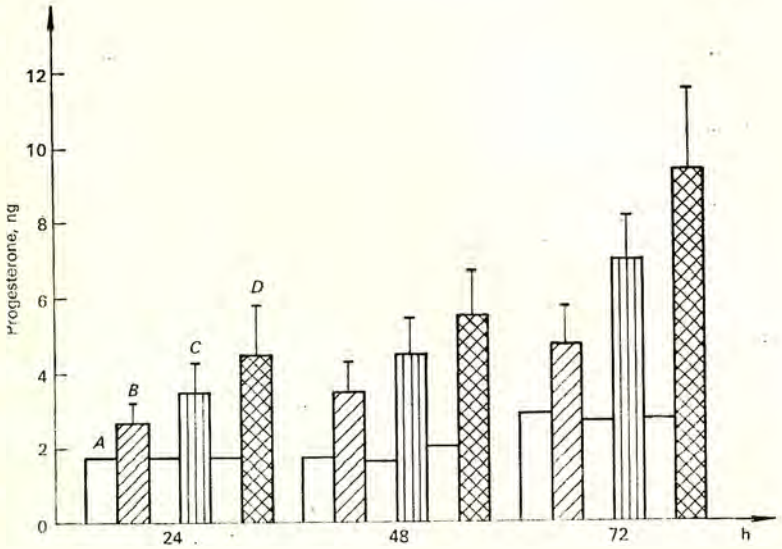

Fig. 1. Secretion of progesterone (nanograms per 400 000 cells ± SEM; n=9 cultures) by granulosa cells in the presence of oxytocin during 24, 48, and 72 h after cultivation
A — control; *B* — oxytocin 1 mIU; *C* — oxytocin 5 mIU; *D* — oxytocin 10mIU

bility were estimated by a hemacytometer and viability was evaluated using the trypan blue exclusion technique. In all cases viability was greater than 90%. Finally the cell suspension placed in sterile DMEM incubation medium supplemented with 5% fetal calf serum (FCS) (Difco Labs), 50 IU/ml penicillin, 50 µg/ml streptomycin, 2,5 µg fungisone to give a final concentration of 400 000 viable cells per 1 ml medium aerated with 5% CO_2 and 95% O_2 at 39°C.

OT (Richter) was added to the cultures in increasing doses to acheive final concentration of 10 m_1 U, GCs were incubated in the above described medium with the addition of OT in the presence or in the absence of gonadotropins — 300 ng/ml LH (Boehringer mannheim) or 250 ng/ml FSH (Boehringer mannheim).

Progesterone assay: At the end of cultivation (96 h) the medium was centrifuged and the supernatants were stored at — 20°C until progesterone assay. The concentration of progesterone in the medium from cultured GCs was determined by the method of K a n c h e v et al. [2] using rabbit antiserum (RD/4.10) at a dilution of 1:10 000. The antiserum was prepared against progesterone — 11-succinyl-BSA. The sensitivity of the method was 10 pg per tube.

Results and discussion

Oxytocin (OT) dose dependently increased the progesterone production in cultured GCs from F_2 and F_3 large preovulatory follicles of the ovum sequence. The 24h-treatment of GCs with 10 mIU OT led to a twofold increase of progesterone secretion as compared to the controls, while the 72h-treatment caused a fourfold increase of this secretion (Fig. 1). These results are in agreement with the data of other authors [1, 6] concerning the effect of OT on the progesterone secretion in GCs bovine preovulatory follicles.

Gonadotropins exerted a different effect on the OT-induced progesterone secretion in GCs from F_2 and F_3 large preovulatory follicles: LH increased the

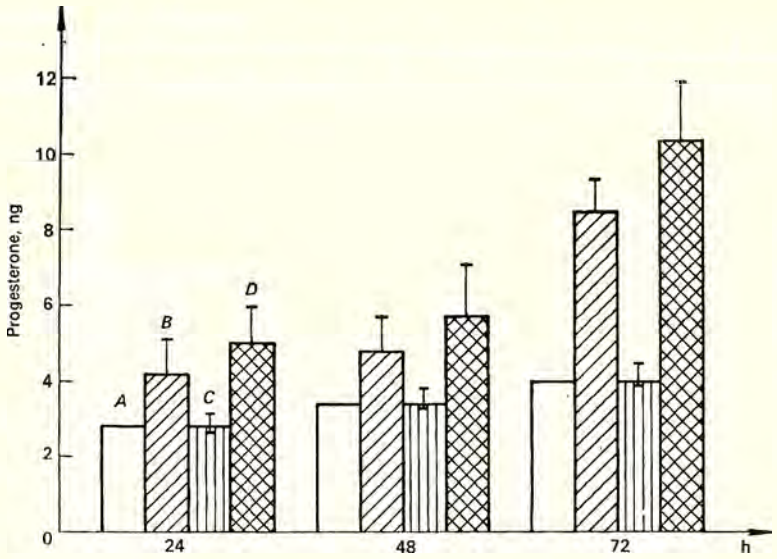

Fig. 2. Secretion of progesterone (nanograms per 400 000 cells ± SEM; n=9 cultures) by granulosa cells cultured with oxytocin in the presence of LH or FSH
A — control; *B* — oxytocin 10 mIU; C — oxytocin+FSH; *D* — oxytocin+LH

effect of 10 mIU OT on the progesterone secretion, while FSH decreased it. Chandrasekher and Fortune [1] and Voss and Fortune [6] also found an increase of progesterone secretion induced by OT in the presence of LH and a decrease of the OT effect in the presence of FSH.

The present results strongly suggest not only a paracrine but also an autocrine role of OT in birds. Like in the mammalian preovulatory follicles, in the preovulatory follicles of birds OT performs as an intraovarian regulator of steroidogenesis.

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Polymorphism of human mitochondrial glutamate-oxaloacetate transaminase (m-GOT)—a new allele

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The aim of the present study is to investigate the mitochondrial form of glutamate-oxaloacetate transaminase (m-GOT) in human liver and placental homogenates by the method of horisontal starch gel electrophoresis. The theoretical importance and actuality of this kind of investigations for population genetics are related to the origin of different human populations. By this way it is also possible to characterise more precisely their gene pool. The practical application of results obtained for the purposes of forensic medicine is recommended.

Key words: population genetics, mitochondrial form of enzyme glutamate-oxaloacetate transaminase, liver and placental homogenates, electrophoresis, gene frequencies.

Glutamate-oxaloacetate transaminase (EC 2.6.1.1.) occurs in two distinct subcellular forms: cytoplasmic (s-GOT) and mitochondrial (m-GOT). The both forms of the enzyme are known to show genetic polymorphism independently. In this report we describe for the first time a new genetic variation of m-GOT in the Bulgarian population.

Materials and methods

110 liver extracts from healthy Bulgarian people died of accidents, as well as 80 placental extracts were investigated. All materials were homogenizated and supernatants obtained were kept at —20°C. Horizontal starch gel electrophoresis was carried out with tris-EDTA-boric acid buffer, pH 8,4 for 18 hrs at —4°C, 20 μA, 150 V. The isoenzyme profile was investigated by the formasan method of Dikov and Lolova (1974), in our modification (I a n e v a, H a d j i o l o f f, 1979).

Results and discussion

Each form of the isoenzyme of m-GOT is detected as a sharp single band, as we show on Fig. 1*A* — *1*. m-GOT activity is observed as triple bands in two placental samples and in three samples from the liver (Fig. 1*A—2*; *B—2*). We designated the present variant type as m-GOT 2-1, triple banded heterozygote of m-Got[1] and

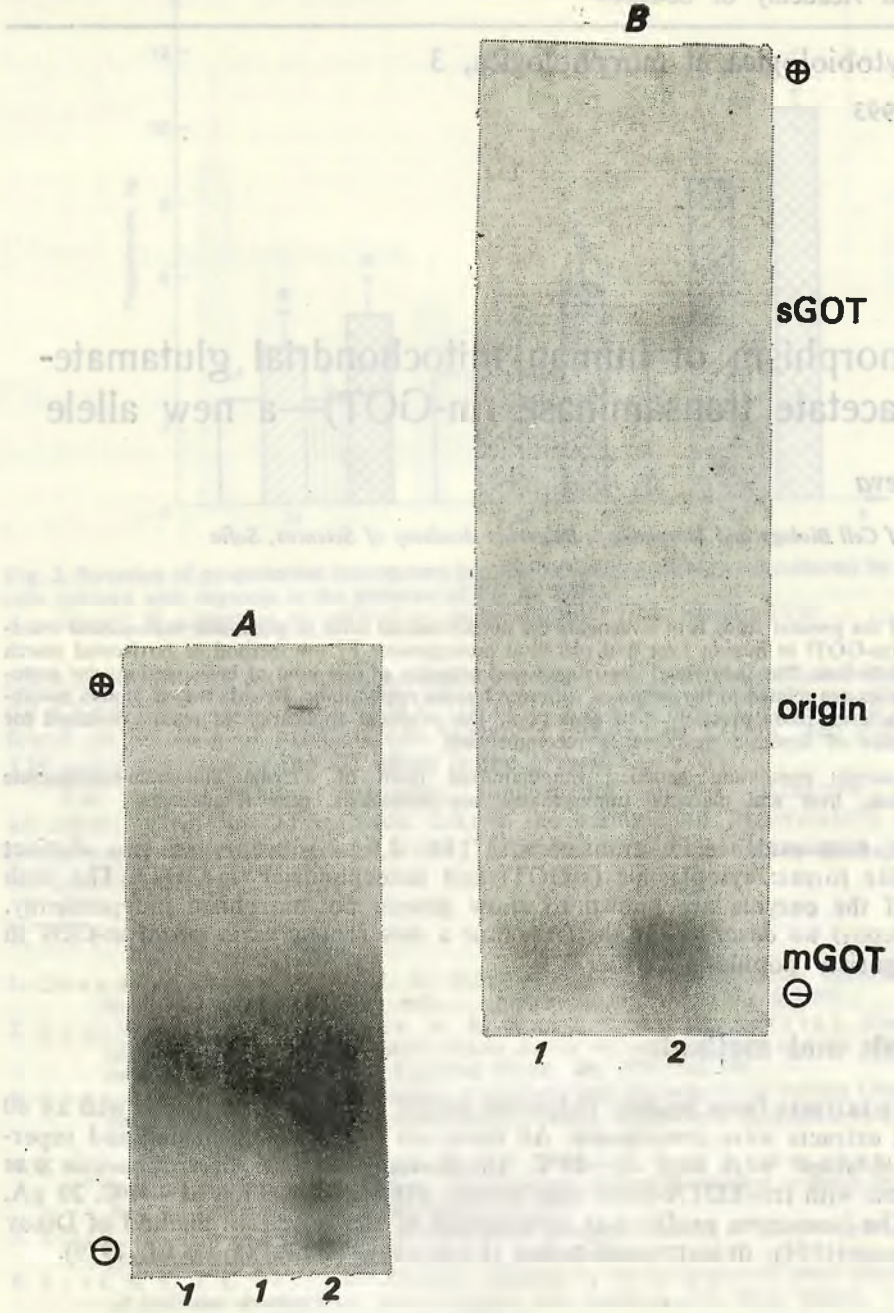

Fig. 1. GOT phenotypes of placental samples (*A*) and of liver homogenates (*B*) on the starch gel

m-Got^2 alleles. The frequency of this variant is 3 out of 110 liver samples, and the estimated allele frequency for the m-Got^2 in the observed Bulgarian population is 0,0136 (m-Got^2=0,0136). The data of the frequencies of the m-GOT in various populations are presented in Table 1.

Table 1. Frequency of the alleles of m-GOT

Population	m-Got[1]	m-Got[2]	m-Got[3]	Author
Europeans	0,983	0,017	0,000	Hackel et al. [2]
Negrous	0,961	0,000	0,040	
Indians	1,000	0,000	0,000	
Germans	0,993	0,007	0,000	Ananthakrishnan et al. [1]
Chinese	0,9947	0,0053	0,000	Teng et al. [4]
Malaysians	0,9946	0,0054	0,000	
Japanese	0,9957	0,0043	0,000	Toyomasu et al. [5]
Bulgarians	0,9986	0,0136	0,000	Ianeva, 1992

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