ACTA MORPHOLOGICA

ACADEMIAE SCIENTIARUM HUNGARICAE

SUPPLEMENTUM XIII

AKADÉMIAI KIADÓ, BUDAPEST
1965

PROCEEDINGS OF THE ANNUAL MEETING OF HUNGARIAN PATHOLOGISTS AND ANATOMISTS

Kecskemét, 1964

PLENARY SESSIONS

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Current Problems of Experimental Teratogenesis

Any problem we make the object of inquiries may be interesting for either its practical or its theoretical aspects; most problems are so for both aspects together. Great was the practical significance of teratology right from the beginning, at a time when its scope of inquiry was still limited to the study of spontaneously developing abnormalities or malformations. When studying the genetic causes of such abnormalities of development, we have to give the physico-chemical approach priority to comparative morphological and taxological methods. The beginning of nuclear era a few decades ago and the discovery that many chemical substances are teratogenic, have only added to the practical importance of teratology; its theoretical significance, as viewed from the view of general biology, need not be specially emphasized.

In the approach of a given problem, there are always several phases. The first is usually the phase of selecting a suitable model allowing to study the influence which produces changes similar or at least analogous to those brought about by the natural phenomenon. Then follow analytic inquiries as to the manner in which these influences are inducing their effects. When trying to state general rules about a biological process, we have to determine the common element in the different pathogenic influences and from this point it may likely be that we are led on to finding means of prevention or even therapy.

In 1836, *G. S. Hilaire* furnished unquestionable evidence for the teratogenic effect of anoxia. In the period of one and a half century up to now, it has been discovered that many chemical substances and types of physical influences have a similar result. Few, however, have been investigated further than the first rough approach. In the present report I shall chiefly discuss those substances and physical influences which allow some insight into the mechanisms of their action, and to draw some conclusions of general validity which might help to find out the causes of the teratogenic effect of other substances the way of action is not known.

The teratogenic effect of *ionizing rays* was first observed at the beginning of this century by GILLMAN and BEATJEV [1] and has since then been

tested and confirmed by experiments on various species, including man. The pertaining tests fall into two groups: firstly radiotoxicological observations of the embryonal organism, discussed here so far as they are significant in revealing the mechanism of action; secondly experiments to test the teratogenicity of ionizing rays, with reference to the morphology, in some measure to the vital functions of the monster they bring about, and to the teratogenic mechanism. A general shortcoming of the relevant literature is that it fails to indicate whether air doses or absorbed doses have been used and at what degree of homogeneity the rays have been applied. Right, in my view, is to measure the absorbed dose by thermoluminescent methods.

The radiotoxicologic cardinal question sounds like this: what is the minimum total dose and the minimum dose rate that will kill the embryo within 20 hours (acute lethal dose, LD) and within 10 to 15 days (delayed lethal dose), respectively? Answers in the literature are fairly agreeing. KARNOFSKY [2], studying X-rayed chicken embryos 3, 4, 5 and 13 days of age, found the 20 hour LD50 to be 2000, 1800, 1450 and 750 r. This agrees on extrapolation with BOLAND's results [3] who found that the 6 hours LD50 for 3, 4 and 5-days chicken embryos is 946, 905 and 872 r, respectively. FULLY confirming KARNOFSKY's results were those of GOLDMAN et al. [4], while slightly lower values were obtained by GOFFI (Fig. 1). Whatever the results of these different experiments, they all testify to the fact that the very young embryo is resistant to acute lethal radiation and that the resistance gradually decreases in the course of embryonal life, until it settles at a certain level. The *delayed* lethal ray effect is not amenable to this test and, unlike the acute effect, it is independent of the dose rate. With Dixon's words [6] as the physiological life reaches more complicated stages, the embryo becomes more vulnerable. The simpler forms of life generally go with a higher degree of resistance to radiation.

RUSSEL's studies [7, 8, 9] have furnished the fullest comparative data for the teratogenic effect of ionizing rays. He found that the embryo's response to radiation depends primarily on its age. As to the kind of reaction, there are three stages during intrauterine development (Fig. 2): the preimplantation, the organogenetic and the foetal phase. Irradiation before implantation led mostly prenatal death, very rarely to abnormalities of development. When exposure occured during the organogenetic phase, then abnormalities were frequent and prenatal death was rare. Histology ascertained a breaking of the chromosomes, with formation of micronuclei, to which most of the effect can be ascribed. The sooner before implantation we expose the cells to radiation, the greater part of them will suffer lethal chromosome injury, leaving no time for an abnormality to develop. The comparative rarity of developmental abnormalities explains itself by the regulating capacity of the totipotent blastodermal cells in the period closely preceding implantation, a capacity

also confirmed by NICHOLAS and HALL [10] and by SEIDEL [11]. Abnormalities of development are very different in nature. Most susceptible to injury are the eyes and the central nervous system. According to WOOLAM et al. the radioprotective qualities of some chemical substances give us a means to reduce the frequency of malformations and a clue to establish an analogy between the mechanism of teratogenesis and that of the general radiation effect [12].

Our knowledge about these mechanisms has greatly increased in recent years. Different theories have been advanced for the cardinal problem to explain the apparent discrepancy between the comparative smallness of the imparted radiating energy and the severe degree of biological harm provoked by it. However, a real understanding of the mechanism of action has not been achieved neither by the adherents of the "hit theory", with DESSAUER [13], BLAU and ALTENBURGER [14], CROWTHER [16], ZUPPINGER and MEISSNER [15] among its main supporters, nor by those of the "water activation theory" who took the high water content of biological systems as the starting point of their reasoning and regarded the ionized water molecule as the primary product of irradiation. None of these theories was able to answer some crucial questions of radiation biology, e.g. why is it inside the cell nucleus, more precisely inside the chromosomes that the rays induce the gravest morphological changes; or, in other words, what makes the DNA to respond most sensitively to the action of ionizing rays? Further, in what manner arise the mutagenic and teratogenic radiation effects at the molecular level and how do we account for what we call the biological "oxygen effect", and so on. The closest approach to these problems can be gained at our present state of knowledge from the angle of *molecular biology*. An essential component of *nucleic acid* is made up of the nucleotide bases in which there occur conjugated double bond systems (Figs 3, 4, 6). There is a marked difference between the double-bond π electrons and the single-bondage σ electrons. The probable spatial arrangement of these can be visualized in the form of electron clouds in which chemical bonds arise at the points of overlap. With σ electrons the whole of the overlap is located in between the two atomic nuclei, symmetrically to the axis; with π electrons it is not so (Fig. 7).

Whenever a molecule includes a conjugated double bond system, the π orbits merge, and each π electron unites, — not with the atom that has helped to bring it about, — but with the conjugated double bond system that forms an integral whole with it. So we find within the molecule a delocalized π electron system, the existence of which in purine and pyrimidine bases was first established by PULLMANN and PULLMANN [17, 18] and later confirmed with much greater accuracy by LADIK's computations [19] (Fig. 8). Owing to the fact that the nucleotide bases in the DNA settle in parallel one above the other, overlappings also arise between the π electron clouds of the

corresponding C-C, C-N and N-N atoms. Since these overlappings, as LADIK's computations have shown [20], are non negligable, the delocalized electron system not only exists within the nucleotide base or base pairs joined together by H-bridges, but extends over the entire DNA molecule. This peculiar electron structure explains the experimentally ascertained semi-conductor property of DNA [21] and suggests answers for the fundamental problems of radiation biology.

It follows that the energy, at no matter on which point of the molecule we impart to the DNA, will always belong to the molecule as a whole and will not be limited to the place of contact or its close surroundings. The imparted energy sets the DNA molecule in a state of electric inducation by which the local electric fields of force, presumably always present inside the cell, are activated, releasing a process of polarization which may be responsible for the breaking of chromosomes (Fig. 10). Such breakings arise, according to BUTLER [22], at the contact points between DNA and nucleohiston, the weakest point in the nucleoprotein chain, where only a feeble electrostatic binding force keeps the links together. The probability, however, is very little that of all places just here should the rays touch the molecule. It follows that unless we take into account the delocalized π electron system which extends over the entire DNA molecule, and unless we allow for the possibility of an intermolecular energy transfer, we shall hardly be able to explain the frequent occurence of chromosome breaking and chromosome disjunction. But we might be able to account for it on considering that the process of polarization calls forth a rearrangement of the charges with the result that the electrostatic forces of attraction cease to work, moreover that forces of repulsion are likely to arise between identically charged particles. A change of the base sequence may even result from a not quite perfect chromosome recombination (Fig. 11). Much more — the idea suggests itself — could such a change ensue inside the synthetizing complementaries of the linkages, as a result of the rather frequent enol-keto-tautomerism within the nucleotide bases, once these are liberated from the immobilization by H-bridges (Figs 12, 13), on the consideration that the repelling power of identical charges which accumulate at the linkage ends in consequence of the polarization, makes the nucleic-acid double-helices to despiralize. Thus we can explain both the teratogenic and the mutagenic effects of rays.

Alkylating agents as it is known from the literature, also bear a specific influence on DNA (Fig. 13/a). Their mechanism of action is closely related to that of ionizing rays, as evidenced by the tumour inhibiting, tumour generating, teratogenic and mutagenic influences they exert in intensities depending upon the dose and its way of administration. Numerous reports have confirmed the teratogenic effect of alkylating agents. SALZBERGER [23] observed grave abnormalities of development, mainly in the limbs and in the bony structure

of chicken embryos, under the effect of nitrogen mustard. So did TAKAGAKI [24], JURAND [25] conducted chicken embryo tests with two nitrogen mustard derivatives and found the neural plate and somite cells severely damaged by both, with disorders of differentiation following in the organs. Testing other derivates in the mouse embryo [26], he found that the same two tissue types reacted most sensitively. The various tissues ranged in a descending order of vulnerability are the neural plate mesoderm and mesenchyma, entoderm, ectoderm. Led by the results of their animal experiments, FRIZ and NEY [27] called attention to the danger involved in the use of cytostatics during the early phase of pregnancy. MURPHY et al. [28] conducted comparative tests concerning the effect of 5 polyfunctional alkylating agents on rat and chicken embryos, and observed frequent developmental disorders and structural changes. ALSON [29] found the heart to show the earliest signs of abnormal development under the influence of alkylating agents. Disorders due to the effect of TEM were reported by WADDINGTON [30], JURAND [31, 32] and DANKELL and SCHUMANN [33]. TÖNDURY [46] holds the view that ribonucleic acid, while it is able to prevent virus-induced embryopathies, is ineffective against the effect of TEM.

Today we have come to realize that the biologic effects of radiomimetic alkylating agents somehow depend on the kind of their reaction with nucleophilic chemical groups (Figs 14, 15, 16). There are many different nucleophilic groups in the cell, but few of them enter into reactions *in vivo*, if the alkylating agents are used at pharmocological concentrations. It is therefore difficult to assess ALEXANDER's experimental findings [3] who used excessive doses with the result that all nucleophilic groups were alkylized. Under physiological conditions, as STACEY [35] stated for the proteins, we can only expect the carboxyl groups to alkylize to some appreciable extent and it may safely disregard to what extent the amino and SH groups do so. Testing nucleic acids, ELMORE et al. [36] were the first to show that mustard gas esterifies the primary phosphate groups of the DNA and RNA molecules. ALEXANDER [7] investigated many other alkalyting agents and arrived at the same conclusion. STACEY [35] studied alkylating agents for their influence on DNA and found the sole reaction they provoke is to make the phosphorus groups to esterify to a degree varying with the DNA concentration, and inversely varying with the degree of their own hydrolysis. Similar reactions are taking place in RNA. The concentration of the alkylating agents determines the kind of reaction they induce in DNP: they give rise to esterification only or to esterification accompanied by hydrolysis. Among the carboxyl groups that occur in DNP, the terminal polypeptide is the only one to alkylize. It follows that there is a great difference between the biological effects of the monofunctional and the polyfunctional alkylating agents (Fig. 17): the former give rise only to intramolecular, the latter are able to produce intramolecular as

well as intermolecular cross linkages. The intramolecular bondages bear a decisive influence on the spatial configuration of the molecule, while they leave molecular weight unchanged; the intermolecular bondages alter both molecular weight and spatial configuration, moreover, they prevent the DNA double helices to despiralize and the complementary linkage to synthesize — all this being of great consequence in producing the biological effect.

Functional disorders of the nucleic acids may also be made responsible for the teratogenic effects of *purine* and *pyrimidine*-type *antimetabolites*. THIERSCH [38] demonstrated the teratogenic property in 6-mercaptopurine; KARNOFSKY [39], DAGG [40] and TENCER [41] that of 5-fluoruracil and 5-fluororotacid; KARNOFSKY et al [42] of 5-fluoruracil, 5-fluorocytidine and 5-bromoxyuridine; NISHIMURA [43] and WADDINGTON [44] of 8-azaguanine.

Antimetabolite properties, according to LADIK et al. [45], are inherent in purine and pyrimidine derivates essentially differing in electron structure from the corresponding physiological derivates. Incorporating into the DNA, they disturb those electron interactions between the nucleotide bases arranged one above the other, which bear a decisive influence on the electron structure, and on the function of the DNA molecule. There is experimental evidence that the biological effect of the purine and pyrimidine antimetabolites is proportional to their amount in the DNA molecule.

In order to obtain a fuller notion about the effect of ionizing rays on the embryonal tissues, we must not leave certain ray-influencing factors out of consideration. Most important among these is the *gas-phase* in which the rays are made to work. In a large variety of biological systems the ionizing effect is enhanced by O_2. Anoxia provides a kind of distinct protection. The embryologic aspects of this problem have been discussed by HOPKINS and TUTTLE [47]. Their graphs (Figs. 18, 19) demonstrate that the effect of ionizing rays on blastodermal growth, developmental inhibition and acute death of the chicken embryo, widely differs if they have been applied in an O_2, a normal or an N_2 atmosphere. The same applies for the morphological changes produced by such rays. All this leads to the study of the most current domain of experimental teratology, that of the influence of O_2 upon normal and pathological development, with additional reference to its significant role in human pathology, about which my colleague HOLLAND will report in detail. What lends particular interest to the problem is the fact that O_2 concentrations both lower and higher than normal lead to pathological changes. DEGENHARDT [48] found that the developmental disorders induced by hypoxia in rabbits were phase-specific, the injury having been confined to those organ parts which had developed at a maximum rate during the hypoxic period. Similar results obtained by LANGMAN [49] allowed to draw certain conclusions in respect of human pathology. Abnormalities of development in the chicken embryo experiments of BÜCHNER et al [50] presented

themselves mostly in the brain, chorda and eye, less frequently in kidneys, limbs and in the intestinal tract. INGALLS and PHILBROOK [51] produced grave hypoxic developmental disorders in fish, with major frequency in the eye and brain, and very severe ones in mice [52]. The hypoxic effect was appreciably decreased by hypothermia. GRABOWSKI and PAAR [53] distinguished, according to the degree of hypoxia, three phases of the injury in chicken embryos of the same age, viz. latent injuries with no apparent abnormality at between 100 and 70 per cent of the normal atmospheric oxygen concentration; a second phase, characterized by frequent disorders of development at concentrations lower than 60 per cent of the atmospheric and the third as the lethal phase induced by excessive hypoxia. Oxygen deficiency was found capable of producing a wide range of alterations, with scarcely perceptible defects at one end of the scale and complete monstrosities at the other. Disorders of development within the second phase increased with the decrease of oxygen concentration, presenting themselves in even graver form in more and more embryos. The number of embryonal abnormalities grew as the exponential function of the numerical drop of oxygen concentration. It was also found that the embryos grew more sensitive to hypoxia as they advanced in age. The lethal phase of hypoxia, too, is characterized by an exponential relationship between the drop of oxygen concentration and number of death, and by the fact that older embryos react more sensitively to the hypoxic effect. Studying the interdependence between the embryo's age and its sensitivity to hypoxia, NELSEN [54] also arrived at the statement that the blastoderm, before formation of the primitive streak, tolerates oxygen deficiency much better than the embryo does at any later stage of development. With just a slight qualification, this statement stands unchallenged by the obvious theoretical concept that, under suboptimal oxygen conditions for those stage of development, the younger the embryo, the graver harm is done to it. *Grabowski*'s graphs clearly show that disorders due to low oxygen concentrations only develop in very young embryos; older ones usually die from it. In other words, the limit of the teratogenic oxygen concentration diminishes, to make way for a rise in the limiting lethal concentration, as the embryo develops. If we realize the difference in body volume and surface and the oxygen diffusion in the initial and the more advanced periods of embryonal life, we will also realize that the same degree of oxygen shortage which gives rise to a malformation in the early stage, leads to death at a later stage of development. RÜBSAAMEN [55] arrived at similar conclusions from his experiments.

The teratogenic effect of superatmospheric oxygen concentrations has been studied in greatest detail by NELSEN [56, 57]. His results can be summarized as follows. Chicken embryos from incipient incubation till the late head-process state were able to stand 3 atm overpressure (4 atm. total pressure) without perceptible reaction, but nearly 50 per cent of them developed disor-

ders at 5 atm. overpressure (6 atm. total pressure). At later stages, ranging from the head-process state till the 15—20 somite state, 3 atm. overpressure was tolerated well, 4 atm. overpressure (5 total) produced some kind of disorder, 6 atm. overpressure grave changes, in almost every embryo. The increased oxygen tension alone was responsible for all the effect, because nitrogen gas of the same pressure not only failed to produce it but was even found to provide a measure of protection. Also important from the theoretical point of view was the observation that oxygen tolerance improved with the decrease of temperature. The areas most sensitive to hyperoxia were the eye, the forebrain and the midbrain. Similar results were obtained by CRUZ and ROMANOFF [58], and RIDDLE [59].

The fact that both hypoxia and hyperoxia give rise to developmental anomalies, which vary in frequency and graveness not with the absolute O_2 concentration but with the degree of hypoxia and hyperoxia, makes it clear that the kind of reaction that works in the O_2 effect differs from anything we have known in classical chemistry. The phenomenon has been noted in a variety of biological systems and set the necessity to find out what particular property of O_2 may account for it. Szent-Györgyi [60] was the first to call attention to the special relationship between the electron structure of O_2 and its biological function. The oxygen molecule, he found, was one of the very few materials which exhibit paramagnetic properties in the basic state. In terms of electron structure this means that not all electrons have compensated spins, although they occur in even numbers in the O_2 molecule. According to Pauli's law of inhibition, it is impossible that two electrons of fully identical state should move at a time along one orbit; it may happen that two electrons of identical chief, subsidiary and magnetic quantums describe the same path, but then they will necessarily rotate in opposite directions: their spins will be contrary, their spin momentums compensated and the molecules they build up, free from paramagnetism. Not so in O_2 where the identical state and the parallel spin of two electrones which lend a magnetic momentum to the molecule even in the absence of an external electric field of force, give rise to paramagnetism. This accounts for the readiness of O_2 to enter reactions. The experiments by Szent-Györgyi were the first to point at the influence of this unusual electron structure upon intermolecular energy transfer. The energy intake of a molecule in the process in which the electron or electrons induce themselves to a higher energy level whereby their spins usually remain unchanged. Sometimes, however, induction also makes the spin to reverse, with the result that the electron, rotating now in the same direction as this former counterpart, cannot resume the basic state because its passage along the original orbit is barred, according to Pauli's law. This is what we call the electron's triplet state and its singlet-triplet transformation which bears momentous consequences firstly by lengthening the excitation period up to any-

thing less than one million times, secondly by making the molecule paramagnetic so that it behaves like a free radical with a high capacity to react. The significance of this change in biological energy transfer is obvious. The reverse process from the triplet to the basic state, termed triplet-singlet transformation is again associated with a change of the electron spin. Important from the angle of biology was the discovery that both the singlet-triplet and the triplet-singlet process permit of adjustment. Whenever a paramagnetic substance enters a system, causing electromagnetic derangement in the surroundings, it also changes the frequency of singlet-triplet or triplet-singlet transformation. It is interesting in this respect to note that O_2 and I_2 tend to produce opposite results: while O_2 makes the triplet unstable and favours triplet-singlet transformation, I_2 stabilizes the triplet state, enhances the reverse process from singlet to triplet. There is little to wonder that both substances — O_2 on its own and I_2 as a component of the thyroid hormones — play significant roles in bioenergetics and any defect in their supply gravely affects embryonal development. Worthy of note is the fact that in many biological systems the effect of NO is analogous to that of O_2; this is the more striking because the only feature that the two kinds of molecule share is the presence of electrons with non-compensated spin, making them both paramagnetic. Summing up what we know about normal intermolecular energy transfer, there is need for a definite O_2 concentration from which deviations to either side gravely affect the essential mechanism of vital processes and lead to malformations in the course of embryonal development. O_2 therefore plays a decisive role in bioenergetics not only as final electron acceptor but also as preserver of the submolecular structure which is the hinging point in the process of intermolecular and intramolecular energy transfer.

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The aim of my own investigations was to find a more exact evidence for the teratogenic nature of other than normal biologic energy transfers. The stain I found to serve my purpose best was Janusgreen-B (JgB), long known as a vital mitochondrial stain of the organelle of paramount importance in biological oxidation processes. In addition, as I had demonstrated in other biological systems, the dye causes grave changes by damaging the energy transfer of the substance.

In the first group of experiments [61] I have shown the teratogenic property of JgB, in the second I tried to clarify the mechanism of action. As to the first point, I succeeded in producing 27 different kinds of developmental abnormalities and found that the teratogenic effect on the chicken embryo of JgB was much stronger than that of scarlet red, safranine, malachite green or lithium chloride. The disorders were of widely different nature, ranging from mildest deformity to complete monstruosity. Their frequency and grav-

ity varied greatly with the point of time of application. All the 27 tabulated forms of abnormality presented themselves only when the stain had been made to enter the egg during the initial phase of incubation. Treatment after 24 hours of incubation induced only 16 types of deformity, after 48 hours only 7 and after 72 hours only 5 types.

To elucidate the *mechanism of action*, I studied the dye distribution and reduction of the dye in the chicken blastoderm and found that the dye did not spread over the whole layer, though it was free to do so, but was bound in fairly well circumscribed areas and reduced to a colourless leukobase. In embryos of the same age, these dye binding areas always presented the same shapes in the same locations. Their displacement therefore allows to infer that these areas are of a morphogenic character and most likely develop a long time before any morphogenic change becomes apparent. In the figures we can distinguish two types of blastoderm area: those to which the dye is firmly bound without undergoing reduction and those which reduce the dye. My own findings as also those of *Cooperstein* and *Lazarow*, *Showackre* and *Du Buy*, have shown that reduction of the dye has always to be interpreted as a sign of active dehydrogenase function; the findings also seem to prove that every visible morphologic change is preceded by two phases of submicroscopic alteration, namely the chemical synthesis of substances capable of binding JgB, followed by the development of dehydrogenase activity. The specific temporal character of malformations induced by JgB, considered together with the temporal appearance of stain binding areas, makes it understandable why certain kinds of developmental disorders only present themselves upon exposure between 0 and 24 hours of incubation, while others are likely to develop at a much later point of time.

Investigations into the teratogenic mechanism of action have raised two questions, viz. which is the cell component binding the dye, and what basic processes does the bond interfere with? To the first question there is no direct answer in the literature. There were experiments concerned with the dye reducing properties of the many different dehydrogenase types in isolated condition but they failed unequivocally to establish the particular type or types that would reduce JgB *in vivo*; much less has there been attempted to determine the site of bondage *in vivo*. Let us ignore the contradictions in the literature and put the question like this: Is it the mitochondrium alone that binds JgB *in vivo* and accounts for the vital staining mechanism, or is it the entire cell that does so? In the latter case what makes the mitochondrium only remain in the stained state, if not the presence of the citochrome system which counteracts the dye reduction process?

In collaboration with Dr. *Udvardy* we have succeeded in separating the particular lipoprotein complex that represents the sole dye binding component of the mitochondrium in a variety of biologic substances. I found material

to be identical with the structural protein *Green* et al had isolated from mitochondrium. In a second series of model experiments, using mouse amytal ascites cells, which are not liable to damage by the isolation process, we have shown *in vivo* that all the dye links itself to the mitochondrium, more particularly to its structural proteins.

To understand what essential processes the binding dye interferes with, let us briefly survey the function of the structural protein. About 50 per cent of the protein in the mitochondrium is characterized by the absence of enzyme activity and by a readiness to polymerize. The types of reaction fall into three groups, according as the structural protein molecules polymerize among themselves or with other mitochondrial proteins (mainly with the citochromes) or are bound to phospholipids (Fig. 21). Thus the structural proteins are functioning not only as coupling links between several components of the elementary particles responsible for electron transport, but also constitute an essential part of the mitochondrial membranes to which are linked both the elementary particles and the primary dehydrogenase complexes. Electron transport, along with energy transfer, is effected across the bridge that the structural proteins bound to lipids form between the enzymes of the respiratory chain. What happens when some foreign material, actually JgB, incorporate the structural protein molecule? The particular kind of molecule which includes a conjugated double bond system, i.e. a delocalized π-electron system and which — as I have demonstrated in other types of biological systems — exerts an influence on the energy transfer reactions, forms a pathologic shunt, obstructing electron passage and manifesting itself morphologically with a deformation. The strongest evidence for this action mechanism have been furnished by VERBRODY [62] and by DIANZANI and SCURO [63] who demonstrated that JgB acts as an uncoupling substance in respect of oxidative phosporylation.

At this point I have to refer once more to *Nelsen*'s experiment and to call attention to the fact that the same kind of prevention he demonstrated in the case of N_2 counteracting the teratogenic effect of O_2 was observed in connection with H, He, Ne, Ar and Xe, though there were wide differences in the degree of protection. The last mentioned gas, for instance, was 60 times more active than H_2. What mainly bears upon our present inqu'ries, is that this activity of the said gases vary in proportion to their lipid solubility. Since it is at the mitochondrium that energy transfer is at its strongest within the entire cell, furthermore considering the significant role that the mitochondrial lipids are playing in the process, there is little to wonder at the great effectiveness characterizing these gases of very close electron structure.

The O_2 molecule, as we have seen, exerts a decisive influence upon energy transfer. The question arises: does or does not this influence the teratogenic effect of JgB? Seaking for the answer, I treated chicken embryos with JgB

and subsequently divided the eggs for incubation into three groups. Those of group 1 were placed immediately in air: the eggs of group 2 were exposed to pure O_2 for 15 minutes and those of group 3 to N_2 for approximately 60 minutes, long enough to reduce the dye into the leukobase. After exposure all the eggs were incubated in air. The malformations observed in the three groups were widely different both in frequency of occurrence and degree of severity. In group 3 no deformity whatever had arisen, as was to be foreseen on base of the experience gained in connection with other biological systems, that reduction into the leukobase makes the dye ineffective. Preincubation in pure O_2 (group 2) produced abnormalities in nearly every specimen. Much fewer and far less severe were the malformations in the air-incubated eggs of group 1. A more detailed account of this question will be given by Dr. Erdélyi.

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An interference with the energy supply during development also explains the teratogenic effect of *insulin* [64, 65]. Some authors demonstrated that one single dose of insulin suffices to induce lasting hypoglycaemia in the embryo [66, 67, 68]. ZWILLING [68] found a relationship between the level of hypoglycaemia and the frequency and severity of developmental disorders. LANDAUER [69] was the first to claim that the teratogenic effect of insulin can be compensated with nicotinamide or α-ketoglutaric acid. The observation that insulin not only induces hypoglycaemia but also shows a certain functional relationship with the uncoupling substances of oxidative phosphorylation [99, 100, 101], led LANDAUER and CLARK [71] to studying some of these substances for their effects upon teratogenic insulin activity. The tests were performed most suitably with chlorpromazine which, as DAWKINS [98] has shown prevents the electron to pass from the reduced DPNH to the citochrome C, Landauer found that even low doses of chlorpromazine were intensively enhancing the teratogenic insulin effect. His experiments claim increased attention, considering the significance Szent-Györgyi attached to chlorpromazine in the process of energy transfer, owing to its peculiar electron structure.

Among the various factors discussed at great length and described as necessary for the maintenance of a normal energy transfer, we failed so far to mention a very important one, *water*. It was again Szent-Györgyi who, setting out in his experiments from the dipole nature of the water molecule, gave the first description of the water structure taking shape within the cell, and demonstrated that, whenever a substance disarranges this structure, it also brings a heavy change in the physiological process of energy transfer. *Lithium* has been known for nearly one century as a substance of this kind, having a tendency to cause deformaities. RANZI [102] furnished the experimental evidence that lithium, by disorganizing the water structure, prevented protein and ribonucleic acid from denaturation by urea and claimed that there exists

a close relationship between this kind of inhibition and the development of abnormalities.

All these experiments testify that normal development can only take place within a highly organized structure, involving a considerable amount of energy, at a fully intact state of the biological energy transfer system: and inversely, energy transfer can never be perfect without a highly organized structure. Whenever, by any influence, one of the factors is put out of the normal state, it upsets the balance and gives rise to developmental disorders.

Numerous reports in the literature have been concerned with malformations due to *vitamin deficiencies*. Since vitamins, according to our present knowledge, are coenzymes associated with enzymes playing a significant role in cellular metabolism, it is not surprising that their absence bears decisively upon the normal course of development. The most instructive way to study the teratogenic effect of vitamin deficiency is by the antivitamin technique, ACKERMANN and TAYLOR [70], administered 3-acetylpyridine to induce nicotinamide deficiency and observed grave developmental disorders to arise, an effect whose specificity was borne out by the competitive antagonism between 3-acetylpyridine and nicotinic acid. LANDAUER and CLARK [71] caused abnormalities with another nicotinamide analogue, 6-aminonicotinamide. With 2,4 dimethyl-3hydroxy-5-hydroxymethyl pyridine, CRAVENS and SNELL [72] produced a state of vitamin-B_6 deficiency, involving a grave damage to embryonal development. The effect was the more intensive, the younger the embryo was at the time of exposure, but became of course compensated under vitamin-B_6 supplies. Methoxy-pyridoxine and desoxypyridoxine proved in chicken embryo tests to be potent vitamin-B_6 antagonists [73]. There is plenty of evidence that folic acid is essential to normal embryonal development [74, 75] and that its absence may be the source of many different and grave deformities, especially in the osseous system. Using folic acid antagonists KARNOFSKY et al. [77] induced developmental disorders, while SNELL and CRAVENS [78] succeeded in counteracting the teratogenic effect with thymidine or thymidine and hypoxanthine combined with desoxyribose. Similar results were achieved by NABER et al. [76], *Biotin* shortage may be another cause of grave abnormalites [79, 80], GIROUD et al. [81, 82] reported malformations due to the lack of pantothenic acid. WARKANY attributes significance in human pathology to the teratogenic effect of hypo- and hypervitaminosis A.

Numerous reports [84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94] on the development of deformities in humans and animals after treatment with *thalidomide*, the sedative also known as Contergan, called attention to the consequences of vitamin-deficiency states and the effect of antivitamins. LECK and MILLER [95], studying the action mechanism, noted that there is much resemblance in clinical symptoms between the effect of thalidomide upon humans and that of riboflavin deficiency upon rats. This experience induced MITVEDT [96]

to study the thalidomide effect on riboflavin-consuming bacteria; he found that the thalidomide changed the growth curve of bacteria most distinctly whenever riboflavin was the growth-limiting factor, but left it practically unalterated whenever it was nicotinamide or biotin. Fig. 22 shows the structures of thalidomide and riboflavin, both containing the -CO-NH-CO- group. Since the -NH group is one of the sites where the coenzyme is bound to the apoenzyme, substitution of riboflavin with thalidomide is apt to produce grave changes in the enzyme function. WARKANY's experiments [97] also reveal why the limbs, especially the osseous structures, are the most frequent locations of thalidomide deformities. He found that the function of riboflavin was mainly in the process of differentiation which obviously requires more energy than any other; so the lack of riboflavin counteracts mesenchymal differentiation during the process of desmoid bone formation.

Here ends my report in which I only touched upon a few current problems of teratogenesis, as I had indicated in the title. Many teratogenic substances have not even been mentioned, except by some causal reference in the tables. The aspect under which I selected for discussion a few agents out of the multitude, was the inquiry into their general action mechanism. Whatever our interpretation of these mechanisms, the question is open to discussion and needs to be argued about. What I wish to emphasize in conclusion is the need for a complex view of the problem, in which structure and function cease to constitute separate concepts and form parts of a higher unity, which is the essence of the natural phenomenon.

RELATOR

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Molecular-Biological Aspects of Heredity

Scientific discoveries of the last decade, by disclosing the chemistry and the molecular pattern of biological structures, have enabled us to find the connection between these structures and the fundamental, terminal factors of the functions. It has become clear that specific molecular structures must be regarded as the ultimate carriers of all biological functions. Recognition of this principle has given rise to molecular biology.

It is exactly in respect of one of the most obscure biological processes that molecular biology has achieved the most striking results, viz. in respect of the much studied and extremely intricate process of hereditary transmission, in the course of which living organism transmit some of their properties to their descendants. Hereditary transmission — "biological memory" — preserves certain racial properties, individual characters and anatomical and physiological characteristics, so that it ensures the continuity of the species and, by

way of individual variations as also through changes occurring during life, is at the same time a significant factor in the evolution of the species.

When cytology reached the molecular level or, in other words, when submiscroscopy revealed some morphological and biochemical structures governing specific cellular functions, molecular biology has alloved a deeper insight into the mechanism of hereditary transmission. Modern genetics, called also molecular genetics, has, thus, developed into a separate branch of molecular biology.

All inherited morphological properties of the living organism, all its inherited behavioural manifestations can, according to the principles of molecular biology, be traced to those processes which, occurring on the molecular level, have formed the biological structures and the morphological features of the given organism in the course of ontogenesis, biological structures able to perform the given normal or pathological functions.

Expressed in a simpler form, the essence of hereditary transmission is the inherited capacity of the descendant to develop its structures and evolve its functions in the likeness of its progenitor.

What is inherited are not the properties themselves but, so to say, the program of the progenitor's properties, the disposition to develop these properties. If and to what extent the "program" is then realized depends, apart from the existence of an adequate organism, on a multitude of favourable and unfavourable environmental factors. Modern genetics avails itself of the concepts and the theory of cybernetics: hereditary transmission requires genetic information (program), a system suitable for the reading and the execution of the information, further adequate conditions of life, i.e. the existence of exogeneous factors which are in the service of the executive system. Again, genetic information cannot be received and preserved unless there exists a biochemical structure which — by utilizing a few simple "sings" and combining them in all possible ways — is capable of storing (encoding) all the informations concerning the hereditary properties. It follows that (using once more the terminology of cybernetics) the executive system must be able to "read" the code, to institute the processes prescribed by the information, and it must, at the same time, posses a certain elasticity so as to adapt itself to incessant changes in the relationship between living organisms and their environment. It is this elasticity which ensures the organism's adaptability, and it is this elasticity which ensures the non-hereditary phenomenon of variability.

The vital functions of the organism, development and growth in the course of ontogenesis, are due to metabolic processes. These inherited specific and individual processes are governed in the intricate biochemical protoplasmic structures by an organized system of enzymatic activities. The synthesis of enzyme proteins i.e. the executive system constitutes, therefore, at least part of the process of heredity.

The synthesis of proteins is known to depend on the specific structure and the activity of ribonucleic acids (RNA). The base triplets of the RNA molecules determine the order in which the amino acids are linked on the surface of the ribosomes, the submicroscopic cell organelles which contain likewise RNA. One type of the RNA molecules carries, therefore, information regarding the sequence of amino acids in the course of protein synthesis. RNA belongs to the executive system since it is not capable of reduplication: its synthesis occurs on the surface of the nuclear deoxyribonucleic acid (DNA) molecules. The information concerning protein synthesis is, thus, primarily laid down in the DNA molecules of the cell nucleus, in the sequence of the nucleotid base pairs. DNA is the carrier and transmitter of genetic information, and its base pairs provide the code for the specific synthesis of RNA. The base sequence of the RNA so produced provides the code for the synthesis of proteins, and — finally — the spatial and temporal organization of the so produced specific enzymes is responsible for the normal or pathological metabolic processes, on which life is based. Metabolism ensures, at the same time, the identical reproduction of DNA, the synthesis of RNA and proteins.

The scarcity of available space forbids a detailed analysis of these processes, the biochemical aspects of which have been widely explored. The processes under review are — partly or in their essential outlines — well-known, and we can content ourselves with touching upon the principal data.

The model constructed by WATSON and CRICK may be regarded as a true structural respresentation of the DNA molecule. As regards biochemical composition and the laws respecting base pairs, this molecular structure satisfies all requirements concerning systems carrying genetic codes. The double spiral and its arrangements represents a system which, though biochemically relatively stable, allows the possibility of identical reproduction. The biochemical structure of the bases, their tautomeric forms, or their chemically somewhat modified types attracting other pairs, make "spontaneous" or induced mutations possible. The genetic code is therefore, molecularly and biochemically relatively stable and conservative, but metabolic phenomena and exogeneous factors may change the code, and the activity of the executive system will undergo a corresponding change.

The structure of the RNA molecule is similar to that of the DNA molecule with the difference that the spiral is simple. This explains in theory the synthesis of RNA on the surface of the nuclear DNA molecules, but the real mechanism of the process is still obscure. The biochemistry of the process, in the course of which the genetic code is transmitted to the RNA which provides the direct code for protein synthesis, has been more or less explored. We know that the production of proteins requires several types of RNA, viz. the transfer RNA, the RNA of the ribosomes, and the messenger RNA which brings information from the DNA concerning the amino acid sequence. However, there

are only hypotheses as to the interrelations of these different types of RNA, the molecular arrangement of the cell structure, briefly, those factors, which ensures the precise execution of the program. Let us expatiate upon just one of the many phenomena in this connection, namely, the problem how the long spiral transfer RNA, with its triplets, is able to determine the incorporation of an amino acid into the polypeptide chain. The transfer RNA is, in fact, likewise a double spiral, but it is the result of the duplication of one and the same elongated molecule, whereas the DNA results from the linkage of two molecular chains. The triplets at the loop-like end of the molecule join the complementaries of the messenger RNA, and become interlinked to constitute the proteins of the sequentially arranged amino acids. A recent theory (RISENBROUGH et al., GRIERER, GROS, WATSON) attributes the linkage of the amino acids, occurring in conformity with the information conveyed by the messenger RNA, to the activity of whole ribosome groups and not to that of a single ribosome.

It is, to a considerable extent, due to the study of bacterial and viral genetics that our present biochemical knowledge concerning the interpretation of the genetic code and the synthesis of enzyme proteins is no longer based on mere theory. Many detail problems have been solved, and the significance of the term "gene" has undergone a fundamental change. An increasing number of detail is being explored and interpreted regarding the action-mechanism of the genes, i.e. the process of heredity, a process which leads to promising results in the recognition and also in the control of hereditary diseases. Hereditary diseases mean, essentially, false or deficient genetic information. According to the old terminology, pathologic genotype means false informations, and phenotype means its execution, its manifestation. Molecular genetics offer an unequivocal and clear interpretation of phenocopies and open, at the same time, certain possibilities of controlling hereditary diseases. Life processes will take a normal course if the execution of a false genetic program is inhibited in the executive system, or if we succeed in introducing into that system a factor from the environment for the production of which the system has no information, finally, if we suppress those environmental factors which — owing to false information — the executive system would fail to utilize or would utilize in an undesirable manner. The phenotype is intact, although the information is wrong, the individual is a phenotypical copy of the intact organism.

Molecular genetics have two biochemical problems to solve, viz. [1] protein synthesis i.e. carrying out the genetic program by "reading" the genetic code; [2] the identical reproduction of the systems which preserve and transmit the genetic program. It is with these processes that the problem of individual variations and adaptability, and that of mutation, i.e. a change in the genetic program, are connected. It has to be determined to which particular points of the cell structures these biochemical processes are bound.

Morphologists imbued with the teachings of classical cytology and accustomed to the dimensions of microscopic magnification must be highly interested in finding out how submicroscopic molecular structures compare with the morphological units of classic cytology as also with the cell structures revealed by the electron microscope.

As regards the enzymes which accomplish the cell's organized metabolic processes, it should be noted that in principle, the protein structures of the cell are also capable of enzymatic activity. It has been found by means of the electron microscope that these important protein structures usually form double membrane systems, and that they contain lipid-protein complexes. The cell membrane, the endoplasmic reticulum, the mitochondria and the double nuclear membrane belong to this group. It is known that protein synthesis takes place in the endoplasmic reticulum and the robisomes, and that oxidative metabolism occurs in the mitochondria, while most processes of decomposition occur in the lysosomes. It is likewise known that the pyroninophilic areas of the cytoplasm abound in RNA, and that pyroninophilia indicates protein synthesis. There are, thus, no serious difficulties in respect of the executive apparatus.

The principal agent of heredity is the nucleus, and even the classical theory of genes supposed these hypothetical units to be situated in the chromosomes of the nucleus. That the chief component of the chromosomes is DNA and that the nucleoli contain RNA, has long been known, and it is, therefore, here that we must first look for the apparatus of information.

The regularity of chromosomal phenomena, as observed in cell division, has long seemed to substantiate the assumption that the nucleus or rather the chromosomes therein are the centres of hereditary transmission. All this does not, however, explain the essential nature of the process of heredity. When the lessons of molecular biology enabled the investigators to reach the very roots of genetic processes, they were faced with apparently unconquerable difficulties. These arose from the differences between the data of molecular genetics with their Ångstrom order of magnitude and the data of microscopic morphology with their micron order of magnitude.

The question which arose when Watson and Crick constructed the model of the DNA molecule was how macromolecule which has only a thickness of 18 to 20 Å but a length of several cm or even considerably more, produces chromosomes with a thickness of $1/10\mu$ and a length of several microns. To bring the dimensions of the intrachromosomal axoneme, those of the chromomere and those of the chromatid in relation to molecular dimensions constituted a special problem. Why is it that the chromosomes of nuclei are usually invisible during the interphase, how should we interprete the reduplication of chromosomes, how can we understand the identical reduplication of the intrachromosomal DNA molecules? How shall we explain the differentiation

of cells and tissues in the course of ontogenesis, or, in other words, how do their genetic potentials become restricted? How is it possible that information is transmitted adequately, precisely and in a differentiated form or in the chromosome, a very coarse structure in the molecular sense?

Before going into details, we have to admit that it is as yet impossible to give unequivocal and reliable answers to these questions. Electron microscopy — in combination with cytochemical and autoradiographic investigations — has nevertheless elucidated numerous details and allowed the elaboration of a few useful working hypotheses.

Let us examine, by way of example, a genetically determined regular ontogenetic process and let us see whether there is some morphological and biochemical sign to show that the system of genetic information is functioning, that the realization of the program is in progress.

Observation of the salivary glands of insects has shown that, in the course of the larval, pupal and imaginal stages, the giant chromosomes invariably develop ring-shaped tumefactions, "puffs", at variable points and variable times. Since these swellings forecast the different phases of ontogeny it was concluded that developmental changes (metamorphosis) are released by certain chromosomal areas becoming active. Another example is provided by the insect *Acritopus lucidus*. Rings at different points of the homologous giant chromosomes in the three lobes of its salivary gland indicate that three different kinds of saliva are being produced in the three lobes.

It can be seen at the locus of such rings (puffs) that the chromatids of the chromosome are loosened and arched forward.

The synthesis of uracil, a component of RNA, can be followed by means of tritium labelled uridine. Autograms reveal a strong activity in the puff, indicating that the genetic information of DNA is being transmitted there, i.e., that RNA is being synthesized. This closes the circuit. By way of RNA synthesis, the DNA sends the information to the cytoplasm at a given point of the chromosome at a given time, and the characteristic glandular secretion signals the execution of the command performed through enzymatic activity.

However, this is one of the least difficult among the numberless problems. A much harder nut to crack is the still unelucidated question regarding the relation between the microscopic structure and the molecular arrangement of chromosomes; a still more obscure problem is the reduplication of DNA within this coarse microscopic structure, further the question as to how the chromosomes themselves, the paired chromatids, are evolved and reduplicated.

Let us, as a reminder, recall the schema of the chromosome based on microscopic observations. Such pictures show the spiral axonemes, the chromatids, chromomeres, etc. Photomicrographs in textbooks present the delicate double spiral structure of the chromosomes. The discovery of this structure

explained the disappearance of the chromosomes in the interphase, and offered certain clues to their preexistence.

It is now evident that the chromosomes of the nucleus do not cease to exist in the interphase but become invisible because, uncoiled, they are elongated filaments of a thickness that is below the resolving power of the light microscope. Forming multiple coils at the time of cell division, the chromatids become bulky structures and present the classical microscopic picture of the chromosome. This seems to be a step towards the solution of our problem, since the DNA molecule, too, is a double spiral, yet, the matter is not as simple as it would seem, for the thickness of the DNA spiral amounts to 18—20 Å and that of the chromosomes to several tenths of a micron so that the disproportion between the two magnitudes is too great.

The so-called "lamp-brush" chromosomes that have long been observed in some ova seemed to offer a clue to the molecular arrangement of the chromosomes. They led TAYLOR to formulate the theory that the DNA double spirals attach themselves to both sides of a central double axial fiber (protein?) in the chromosome. The division of this axial fiber and the uncoiling of the double spiral result in a doubling of the chromatid, while the entire process of reduplication can start anew at the same time. This theory was, however, disproved by the discovery that the axis of the "lampbrush" chromosomes consists of DNA (instead of protein), and that the brush-like projections are in fact loops of DNA and RNA.

COLE's ingenious hypothesis seems to be substantiated by morphological considerations. He thinks that the spiral DNA and the likewise helical histones strongly hydrated proteins, are linked by ionic bonds. As a result, the protein is partly dehydrated, and the DNA-protein complex assumes a spiral shape. Similar mechanisms may produce further tertiary, quaternary, etc., spiralizations which become then visible as the spiral structure of the chromatids (chromosomes). COLE explains by a likewise ingenious biochemical argumentation the uncoiling of the DNA molecule, its reduplication, as also the transmission of the code in the course of RNA synthesis. COLE's highly interesting theory has the snag that all these processes of coiling, uncoiling, etc. involve considerable mechanical activity which may amount to 100 rev./sec. at the chromosomal reduplication in mammals.

Other investigators, e.g. FREEZE (1958) and KELLENBERGER (1960) also constructed similar models, but all are open to criticism on account of the unknown nature of the connections between the DNA chains. It has been found that, uncoiled, the chromosomes of the phage T4 at least 50 μ long, and that the length of certain chromosomes reaches lengths of and more than a mm. Taking the molecular weight of DNA (of a magnitude of a hundred millions) into account, it must be presumed that not only a single filament of DNA runs along the chromosome, and that there exist several segments, i.e. DNA

chains, which are somehow interlinked according to some law; from this it would follow that distortions of the information, i.e. mutations, are caused by disturbances occuring in these "subunits" owing to reduplications, environmental influences, ruptures, etc.

The process of replication (or the essentially identical process of RNA synthesis), occurring in a given DNA chain evidently does not require a rearrangement, uncoiling, i.e. the genetic activity of the entire system of a length of several mm or cm. This explains the distribution of the complete genetic information over several loci, to wit, the fact that only parts of the genetic command are executed at a time in the course of differentiation. Certain parts of the information are utilized in certain phases of ontogenesis, while other parts bound to spatially and temporally predetermined cells — are utilized at other stages of the individual's development. Investigators who assume the intrachromosomal existence of more than doubly spiralled DNA molecules look upon the process of uncoiling as the source of activation. This theory seems to be substantiated by the fact that no chromosomes can be seen during interphase, i.e. at a time of cellular activity, and that the chromatids of the "puffs" become loose and blurred in giant chromosomes. It is, however, difficult to bring the halving and reduplication of the chromatids and chromosomes in harmony with the reduplication of DNA COLE's theory and its limitations have already been mentioned, and we want to refer to TAYLOR's pertinent hypothesis which, while likewise limited in certain respects, has been borne out by electron-microscopic observations.

Relying on the model constructed by FREEZE, TAYLOR accepts that the chromosomes contain two doubly helical DNA chains which are interlinked in regular segments. Considering that the molecular weight of the segments amounts to several millions, it is difficult to determine the nature of the linkage, so that only conjectures are possible. Bonds are, according to TAYLOR, formed between the 5'phosphate groups of the nucleotides at the end of the nucleotide chains and between 3'OH groups contained in the carbohydrate of the complementary chain. These helical chains are bilaterally stabilized in a ladder-like fashion by H-bonds which process results in the formation of chromatids. As a first step towards reduplication, the 3'bonds are ruptured owing to enzymatic activity, the double spiral of DNA is uncoiled and immediately thereafter reduplicated, and a new 3'bond, then a 5'bond is formed. The chromatid, now a double one, then opens like a slide fastener, and, finally, the ladder-like structure is restored by means of the H-bonds lacking on one side.

How the synthesis of RNA takes place in this model or in other models remains to be elucidated. It is likewise difficult to understand why RNA, when formed, does not serve as the template of DNA, although all precursors and all necessary premises are given. As regards RNA synthesis, it may take place on the double spirals of DNA, but it is likewise possible that the DNA

chain opens at special points, while the synthesis of RNA occurs at points and times different from the reduplication of DNA.

This preliminary report had the purpose to adduce but a few examples in order to prove that molecular biology is capable of giving at least an outline of the extremely intricate and ramified process of heredity which still contains a great number of unsolved details.

RELATOR

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Pathology of Chromosomal Aberrations

The existence of human chromosomal aberrations and of pathological conditions based on such alterations has long been suspected. Yet, it was not until 1958 that a method suitable for studying the human chromosomal sets had been developed.

The essence of the method modified several times since its first description is that cells from bone marrow or peripheral blood, or specimens of cutis or fascia are explanted for periods varying from 24 hours to several weeks, depending on the object to be grown. There are two important features ensuring the success of the procedure, viz. enrichment of the phase of division, the metaphase, "this being the period suitable for study and the induction of swelling of the cells arrested in mitosis, whereby the individual chromosomes separate from one another". The former is attained by treatment with colchicine, the latter by the use of hypertonic solutions. Then a specimen is dropped out, dried and stained; in such preparations readily analysable chromosomal patterns are visible by immersion microscopy. It is particularly interesting for the pathologist that bone marrow cultures can be prepared and studied even one ore two days after death. Our own preparations, too, have been obtained from cadavers.

Although it can demonstrate only rough morphological or quantitative changes of the chromosomes, this relatively simple method represented an important step in human cytogenetics, in spite of the fact that such rough changes seldom occur in human pathology, because they would produce such gross quantitative and at the same time qualitative changes of the genetical material (including functional alterations), which are usually incompatible with foetal growth and life.

Before beginning to discuss the aberrations, it is necessary to deal with the normal morphology of the chromosomes. The chromosomes are analysed in detail in microphotograms, studying the chromosomes of 30 to 70 dividing

cells in each case. The microphotogram is suitable for determining the chromosome count, for qualitative analysis and mainly for making a karyotype showing the suitably arranged and coupled pictures of the individual chromosomes cut out of the microphotogram. (Fig. 1.) As we are examining dividing cells in the metaphase, the chromosomes are visible as X or V shaped structures, i.e. in doubled form, joined at one point, the centromere. At this point insert the pulling filaments of the spindle, that pull apart the chromatide, one half of the chromosome, into two new daughter cells.

In general the chromosomes are composed of two symmetrical halves, the two chromatides, which are distinguished partly on grounds of their size and partly on the basis of the position of the centromere. When the centromere is in the centre, we speak of metacentric chromosome, if it is near the tip, we speak about an acrocentric one. An intermediate type is a submetacentric chromosome. The two parts separated by the centromere are called the arms of the chromosome. Their length and relative size are the most important pointers in the identification of the chromosomes, apart from their size which in humans varies from 7 to 1.4 micre. There are satellites on the acrocentric chromosomes, appearing as short, lightly stained process over the short arm.

The normal human cell contains 46 chromosomes, 44 somatic and 2 sexual. The 44 somatic chromosomes are composed of 22 homologous pairs, whereas the sex-chromosomes constitute in the female an identical pair of two X and in the male they are represented by the different X and Y ones (Fig. 2.). In the normal case 90 to 95 per cent of the cells show the regular 46 piece set, in the rest, depending on the technique, we find more or less.

On the basis of an international agreement the human chromosomes are designated with numerals 1 to 22 according to size and grouped morphologically, showing the sex-chromosomes separately. It is also customary to designate the single groups by letters from A to G. Within the groups distinction of the single pairs is uncertain.

In cytogenetics the chromosomal aberrations are divided into two large groups: divergence in the number of chromosomes and changes in their structure.

Numerical differences

If the total chromosomal set is doubled or multipled, we speak about polyploidy. If only single chromosomes are affected, the following terms are used. Under normal conditions the chromosomes occur in pairs, there are two of each; this is called disomy. Monosomy means that of the pair one chromosome only is present, trisomy means that there are three, tetrasomy if there are four of one kind, etc. In such cases it is designated which chromosome is so affected (e.g. 21 trisomy).

In man, of the above possibilities polyploidy occurs in tumor cells; only one case without tumour has been reported. Trisomy has been observed in several pathologic conditions. Tetrasomy or an even greater number of one kind of chromosome is known to occur exclusively with the sex-chromosomes. On the basis of theoretical considerations no possibility seems to exist for monosomy to occur with the autosome chromosomes, as since the absence of one single chromosome has most serious consequences, as either its genetic material is indispensable, or the effect of a recessive lethal gene may become manifest.

The mentioned changes in the number of chromosomes arise as a result of defective cell division; this is called non-disjunction. The essence of it is that the chromosomes duplicating during division and separating into two chromatides do not separate and divide equally into two daughter cells. As a result, one of the two new cells will have less, the other more, chromosomes. In the adult such defective divisions are without significance, because they may occur only locally, or in special tissue.

The situation is entirely different when non-disjunction takes place during the meiotic division of sexual cells. The mechanism of this is represented in a diagram (Fig. 3). At the beginning of its maturation the ovum possesses 46 chromosomes; for the sake of simplicity these are represented by 6 chromosomes (6 dots), corresponding to 3 homologous pairs. While preparing for the first meiotic division, in the prophase the homologous chromosomes (pairs) lie side-by side, each is split into two chromatides; this is indicated by the four-dot tetrades. Under normal conditions during the first meiotic division the chromosomal pairs separate, one into one, the other into the other daughter cell, and so two diploid secondary ovocytes arise. But if one pair fails to split, both members of the pair will enter the same cell, and as a result one of the secondary ovocytes will have 48, the other 44 chromosomes. When the 48-chromosome ovocyte undergoes the second meiotic division, every chromosome separates into two chromatides, i.e. two haploid cells of 24 chromosomes arise. The fertilization of this gamete by a normal haploid spermatozoon will result in a zygote with 47 chromosomes i.e. a trisomic one. In the further development this will pass over the pathological, 47-chromosome set to every one of its daughter cells.

If the ovocyte having 44 chromosomes continues to mature, after the second meiosis an ovocyte possessing 22 chromosomes arises, which, when fertilized by a 23-chromosome normal spermatozoon will yield a zygote with 45 chromosomes, i.e. a monosomic one. This process of non-disjunction, with exactly the same result, may take place during the second meiotic division.

Non-disjunction may occur also in the course of spermiogenesis, and in exactly the same way zygotes with monosomy or trisomy may arise, if such a pathological spermatozoon is involved in fertilization. Experience obtained

in Drosophila and to some extent in man indicates that it is mainly the maturing ove of older females that have a tendency to non-disjunction.

Non-disjunction may occur also at a third point of time, notably during one of the early divisons, or even during the first division, of the zygote. In the latter case the first two blastomeres will have 47 and 45 chromosomes, respectively. The one with monosomy will perish and the pathological one will give rise to an individual with trisomy. When non-disjunction takes place at a later stage, but still during the early divisions of the ovum, the trisomy will be restricted to certain areas of the organism, while in the rest disomy is to be found, provided that the simultaneously arising monosomic blastomere perishes. This phenomenon i.e. the existence of cell populations possessing different chromosome sets in certain tissues of one organism, is called mosaicism. Mosaicism of the somatic chromosomes is a rarity and has little significance. With the sex chromosomes the situation is different in that following non-disjunction the monosomic cell is also viable. Thus, when the ovum divides for the first time, non-disjunction of some sex chromosome will result in a 45/47-mosaicism. If it is some later, second or third, division of the ovum that is pathological, triple mosaicism will result, i.e. one normal and two pathological chromosomal sets will be present in the same organism. These mosaicisms are of great importance as the cells with abnormal chromosomal sets may give rise to abnormal growth.

Structural changes of chromosomes

Whereas the numerical changes described above are easy to recognize by the present methods, the structural changes may be of such orders of magnitude that they escape detection. For example, the loss of a chromosomal segment 0,5 μ in size may be of great functional significance, but remain undetectable by our present procedures. We shall therefore deal exclusively with the structural changes detectable by the methods now available, the clinical significance of which is known.

In this respect the most important changes is the one termed translocation. This means that a segment broken down from a chromosome, or even a complete chromosome, becomes attached to another. If this does not involve a loss of substantial genetic material, the individual built up from such cells will be of a pathological genotype, but a normal phenotype.

Translocations occur mostly in the course of meiotic division, and therefore the arising situation is not simple. Fig. 4. illustrates the consequences of a translocation which occured during the maturation of an ovum. The tips of a bigger black and a smaller white chromosome pair are translocated. In this case four kinds of mature ovocytes may arise: a normal one, a genetically balanced one, in which there is translocation without loss of genetic material, and two pathological ones. In one of the latter there is a great excess beside

a small deficiency of genetic material, in the other there are a slight excess and a great loss. If one of such ovocytes is fertilized, zygotes of the following phenotypes may result: a normal one: one with normal phenotype and a genotype with pathological translocation; a non-viable one with a lack of genetic material approximating monosomy: and, finally, a pathological genotype with an insignificant deficiency of genetic material, but with an excess of genetic material approaching trisomy. If, as we shall see later, this excess of genetic material originates from chromosome 21, the individual will suffer from Downs disease. I should like to call attention to the zygote which has a normal phenotype, because it is its gametes that transfer the pathological condition to the offsprings. This is the way the familial cases of Down's disease develop.

Diseases based on chromosomal abberation

Before dealing with the clinical conditions due to, or secondary to chromosomal aberrations, it should be pointed out that the disorders connected with abnormalities of the sex chromosomes occur much more often than those connected with the somatic ones. Down's disease is the only exception to that.

In connection with the sex-chromosome aberrations it should be mentioned that the diagnosis of such pathological conditions and in general the recognition of the disorders of sexual development have been greatly facilitated by the chromatin test. According to the present view, sex chromatin is a heterochromatic part of one of the X chromosomes in a functionally inactive condition; it stains well even in the nucleus in the interphase, and does not loosen up. It follows that an inactive X chromosome can be present exclusively in the female. The sex chromatin test truly reflects the polysomy of the X chromosome, because in such cases two or more sex chromatin corpuscles can be found in the cells.

In the following the most often encountered and best known conditions based on chromosomal aberrations will be discussed.

1. *Klinefelter's syndrome.* The variants will not be dealt with, owing to lack of space. The commonest and most important symptoms are: eunuchoidism gynaecomastia, small testicles, mental defect, sterility. Morphologically there is a dysgenesis of the testicular tubules. The patients are males by phenotype, but they have chromatin positive nuclear sex and correspondingly have pathological sex chromosome sets. In typical cases the chromosome count is 47, there is sex chromosome triosomy with a sex chromosomal set of XXY. Less often XXXY, XXXXY and XXYY sets, and in addition various forms of mosaicism may occur. The sex chromatin test usually reflects the presence of the 3 or 4 X chromosomes. Trisomy is due to non-disjunction taking place either during meiosis or at the earliest phase of foetal development.

2. *Turner's syndrome.* There is one case to every 3000 births. Clinical symptoms: small stature, sexual infantilism, ovarian dysgenesis, pterygium, cubitus valgus, usually, but not always, sterility. In spite of the female phenotype nuclear sex is chromatin negative. Correspondingly, the chromosomal set is abnormal, inasmuch as there is a 45-chromosome set, i.e. monosomy with one X chromosome. The chromosomal pattern is designated XO. The variations of the syndrome will not be discussed here, it is only mentioned that there are also cases displaying mosaicism with two or three kinds of cell population. The cause of monosomy is non-disjunction, which probably takes place during spermatogenesis, as a result of which a spermatozoon possessing no sex chromosome arises and this fertilizes the ovum. The defective sex chromosomal set is unable to govern gonadal development, but the rest of the genital system develops in the female direction. The usual negativity of the sex chromosome test and the fertility found in some cases may be explained by mosaicism.

3. *Female with multiple X.* Earlier, in analogy with entomological observations such individuals were called superfemales. This terms is misleading, as these patients are usually and paradoxically less feminine. Most of the patients are mentally backward and have borne children. There may be 2 or 3 sex chromatin corpuscles in one cell, depending on the presence of 3 or 4 chromosomes. The triple-X, i.e. the X trisomy, occurs more often than the tetra-X, or tetrasomy.

4. *Down's disease.* The symptoms of the condition are wellknown. There is one case to every 600 to 700 births. This is the only frequent disease based on somatic chromosomal aberration. The chromosomal count is 47, there is trisomy of one of the smallest chromosomes, chromosome 21. As a result of cytogenetical investigations, the cases of Down's disease can now be divided into sub-groups.

a) Regular Down's disease. This is the commonest form. Most of the patients are delivered by aged mothers. The incidence in an average population is 0.16 per cent, but if mothers under 25 years of age are included, the incidence is much lower. In the case of mothers over 40 years of age it may be above 1 per cent. This too lends support to the genetical finding revealed by other studies that non-disjunction during meiosis occurs especially among elderly females. On this basis it appears that this type of Down's disease may be ascribed to non-disjunction of maternal origin.

b) Translocational Down's disease. Clinically, the disease is typical of Down's disease, but the chromosomal count is normal, 46. On the other hand, one chromosome is irregular in shape, and at the same time a big or a small acrocentric is lacking. The chromosome of irregular shape arises as a result of translocation, either a big and a small, or two small acrocentric ones adhere to each other. Thus, there is also a latent trisomy on chromosome 21. The

supernumerary chromosome 21 is translocated to some member of the 13—15 group or of the 21—22 group. Unlike the regular Down's disease, this form develops in the offsprings of young mothers.

c) Familial Down's disease. Many cases occur in one family, the age of the mother plays no role. The patients belong individually to the regular or translocational group. The hereditary background is that the translocation that had taken place in the course of gametogenesis of some parent or more distant predecessor may lead to the arisal of a gamete possessing a genetically balanced translocation, from which a zygote with a normal phenotype develops after fertilization. In the course of gametogenesis of this zygote, gametes inducing Down's disease, i.e. gametes with latent (translocated) trisomy may also arise. In this way is inherited the translocation on which the disease is based. In such cases, too, the latent translocation has been successfully demonstrated in some of the clinically normal parents.

Finally, there are cases of incomplete Down's disease, with only a few pregnant symptoms; eventually even the most typical signs may be absent. For example, there may be no mental retardation and therefore the clinical diagnosis is often questionable. In such cases mosaicism has been detected, 21 trisomic and normal cell populations i.e. chromosome counts of 46 and 47 have been found. For example, in the blood cultures both cell populations occur with the same frequency, whereas in skin biopsy specimens a normal cell population can only be demonstrated.

The universally accepted rule is that to exclude or prove mosaicism various tissues should be examined simultaneously, taking specimens possibly from both halves of the body.

5. *Other autosomal trisomys* are uncommon, and it is hardly likely that new types will be discovered, because it is vital that the genetical substance content of the somatic chromosomes be balanced.

a) *Trisomy 17—18, or E-trisomy.* The two questionable chromosomes cannot always be distinguished. Seven cases have been reported. The typical symptoms are mental retardation, small mandible and mouth, low-lying, deformed ears, pterygium, flexion deformities of the fingers, short, thick toes. The patient usually dies in the first extrauterine life.

b) *Trisomy 13—15, or D-trisomy.* The three questionable chromosome pairs cannot always be distinguished. Four cases are known. The symptoms are mental retardation, grave eye defects, hyperconvex fingernails, split palate and split lips, haemangiomas, irregular palmar skin folds, interventricular septal defect. These patients liable to die within a few months after birth.

6. *Chronic myeloid leucaemia.* In some patients an irregular so-called Philadelphia chromosome, can be found. The condition does not belong to the true chromosomal diseases, as the change does not involve every cell of the

individual, but is restricted to the tumour cells. One member of the chromosome pair 21 has an irregular shape, ascribed to a partial loss of chromosomal substance.

7. *Hermaphroditism.* In the true hermaphrodites, i.e. the patients possessing both kind of gonad, there ought to be theoretically a malefemale chromosomal mosaicism. A few cases have been found. In the various forms of pseudohermaphroditism there are no chromosomal changes. The chromosomal sex is the same as the gonadal sex. It would be outside the scope of this report to deal with the hitherto demonstrated other genetical changes.

As we have seen, during the six years of the advance of human cytogenetics, many valuable observations have been made, disclosing in various diseases the underlying chromosomal aberrations. It would be interesting to know the causes responsible for the discussed disorders of cell division, notably nondisjunction and translocation. One factor is the age of the mother. The various radioactive effects, too, undoubtedly play a role. In this connection I wish to quote the evidence reported in 1961 by GARDNER. He claimed that if every man living on earth had received the officially tolerated dose of irradiation, that which for example the radiologists get, the number of the so-called spontaneous mutations would be doubled. At any rate, we can agree with the opinion that in view of the extremely complex process of mitotic cell division involving systematic grouping and regrouping, then re-separation of the genetical material, it is not asthonishing that errors occur in the process. What is surprising is that they occur so infrequently.

RELATOR

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Aetiology of Mental Deficiency

Every functional or morphological change affecting tissues, organs, organ systems or the organism as a whole, outside the range of variation for the given species, that develops while the development-determining factors are active, should be classified as a developmental disorder. An analysis of this definition will show that developmental defects may be of genetic origin, but may also arise as a result of environmental changes.

The two factors, the genetic and the peristatic often act in combination on the developing individual. The definition makes it clear that the concept of developmental defect extends to functional changes as well. In defining the concept, we must take into consideration the principles of molecular biology; this is clearly reflected by the term inborn error of metabolism.

These conditions usually arise as a result of recessive homozygote allele-gene configuration, showing no morphological characteristics at birth, and manifesting themselves later, in infancy or childhood. In addition to the errors of intermediate metabolism, the disturbances may affect various other functions, including the development of mental activity, which, on the other hand, may also arise as one of the symptoms of a metabolic error.

The sensory and motor activitates as well as the neurohormonal regulations develop through well-defined morphological and functional stages until the individual has become capable of acting purposefully, thinking logically and of adapting himself to the environment. Essentially, what we deal with is a development of the intellect as a functional unit, showing characteristic stages in the course of life.

If in some stage something interferes with development and as a result the mental capacities of the individual will not meet the average standards for its age, making it impossible for the person to fit into society or to lead an independent life later, we speak about mental backwardness.

It must be stressed that however severe the mental backwardness, oligophrenia (idiocy, imbecility, debility), it is merely one symptom of a given pathological condition, the morphological basis of which is determined by irreversible changes in the cerebral cortex. For this reason we should speak about conditions associated with mental backwardness and not merely about oligophrenia, which term does give much information about the defect but as far-reaching consequences as regards the fate of the individual in society.

Like all other developmental disorders, the conditions associated with mental deficiency are classified according to genesis into four groups,

1. Gametopathies
2. Chiemopathies
3. Neogonopathies
4. Postnatal injuries

Ad 1. Gametopathies, in PACHE's terminology are the developmental disorders of preconceptional origin.

Conditions associated with mental defects of gametopathic nature may be divided into two groups.

I. Idiotypic gametopathies
II. Heterotypic gametopathies
    1. recessive hymozygote combination
    2. blastophoria
        a) point mutation
        b) karyopathy
            i. aberration in chromosomal count
            ii. structural chromosomal anomalies.

Idiotypic gametopathies are inherited dominantly. The best method for detecting them appears to be to construct a detailed family tree and to analyse it in detail.

The characters of the individual suffering from heterotypic gametopathy differ from those of the parents. The oligophrenic conditions based on recessive inheritance are the enzymopathies, as also the proportionate from of osteogenic microcephaly and other mesodermal changes (for example APERT's disease).

The enzymopathies accompanied by mental defects are

Disorders of carbohydrate metabolism

1. Galactosaemia
2. Sucrosuria
3. Glycogen thesaurosis
4. Gargoylism
5. Spontaneous hypoglycaemia

Disorders of lipid metabolism

1. Progressive leucoencephalopathy
   a) type Schilder
   b) type Krabbe
   c) type Scholz
   d) type Greenfield
   e) Metachromatic
   f) Baló's disease
   g) Pelizaeus-Merzbacher's syndrome
2. Progressive polylipoidosis
   a) amaurotic Idiocy
      i. Norman-Wood' disease
      ii. Tay-Sachs' disease
      iii. Bielschowsky's disease
      iv. Spielmayer-Vogt' disease
      v. Kuf's syndrome
   b) Niemann-Pick'disease
   c) Gaucher's disease
   d) Triglyceridaemia

Disorders of protein metabolism

1. Phenylketonuria
2. Succinoargininuria
3. Cystothionuria
4. Maple syrup disease
5. Hartnup's disease
6. Cystinuria
7. Oasthouse-uria
8. Glycinuria
9. Pseudo-hypertrophic muscula dystrophy
10. Histidinuria
11. GABA-uria
12. Hyperbilirubinaemia without isoimmunization
13. T-pigmenturia
14. Thyrosinosis

Disorders of calcium metabolism

1. Infantile hypercalcaemia
2. Pseudohypoparathyroidism
3. Epilepsy due to cerebral calcification

Other metabolic disorders

1. Wilson's disease
2. Congenital renal tubular acidosis
3. Lowe's syndrome
4. Microcephaly (sex-bound)

5. Pyridoxine-dependence
6. Hypsarrhythmia
7. Joseph's syndrome
8. Idiopathic cretinism

The above conditions are based on molecular changes of the genetic code, i.e. on the alteration of the structural protein matter of the gene locus (cistron). Thus according to the theory of "one gene-one enzyme" the above conditions are due to damage to the genes, inducing defective protein synthesis or enzyme synthesis on grounds of an *ab ovo* defective transposition of the messenger and transfer RNA. Thus a hereditary enzyme block or enzyme deficiency will arise, leading to an accumulation of intermediate metabolic products damaging the tissues, first of all the nervous system, and producing a progressive mental disturbance with readily demonstrable neurological symptoms.

Almost 10 per cent of the mentally defective persons suffer from enzymopathies.

Blastophthoria means a damage to germ cells caused by peristatic factors; it occupies a prominent position in the aetiology of conditions associated with oligophrenia.

Blastophthoria may be induced by infectious diseases (mainly parotitis and hepatitis), industrial poisoning, brain commotion, peculiar psychic conditions, disorders of nutrition, but first of all by ionizing radiations. The best known blastophthoric condition is karyopathy.

The olygophrenic conditions with abnormal karyotype are as follows

I. Chromosome count anomalics

Clinical pattern	Chr. complement	Chr. count
Down's syndrome	21 trisomy	47
Klinefelter's syndrome	XXYY	48
Turner's syndrome	XO	45
Klinefelter + Down	XXY 21 trisomy	48
Superfemale	XXX	47
? (oligophrenia)	6 trisomy (?)	47
facial anomaly	22 trisomy (?)	47
oligophrenia	XXXX	48

According to the March 1963 UN commitee report on the effects of atomic radiations, background radiation on Earth is steadily increasing. There is a linearity between dose and effect, but there is no threshold. Therefore it cannot be postulated categorically that there is such a small dose which has or may have no effect. As shown by geophysical measurements, there is a

II. Anomalies of chromosomal structure

Clinical pattern	Chromosoma pattern	Chromosoma count
Polydyspondylia	22 ~ (13—15)	45
Familial oligophrenia with speech defect	22 ~ (13—15)	45
Down's syndroma	21 ~ 22	46
Down's syndrome	21 ~ 21, or 19 trisomy and 21 monosomy	46
Hypertonic oligophrenia	(1—2) ~ (6—12)	46
Complex fr.	16 ~ 21, or 21 trisomy and 16 monosomy	46
Pseudohermaphroditism	21 ~ Y	46
Turner's syndrome	thickened X	46
Marfan's syndrome.....	thickened satellits	46

meteorological phenomenon amply discussed in the report, notably that of the relative stability of air masses situated along the meridians. This is responsible for the fact that the radioactive fallouts of war-time and experimental atomic explosions reach constant concentrations corresponding to the meridians. This is indicated by the 90 Sr concentrations in milk found in 9 countries, as well as by the increase in the 90 Sr content of soil measured at different meridians. These are merely two selected data from among a great number of similar ones. From the point of view of genetic population, 14 C with its half-life of 5 760 years is particularly dangerous.

According to HAHN and HEVESY, DNA synthesis is highly radio-sensitive. WOLFF has pointed out that ionizing radiation may cause breaks in the chromosomes, followed either by restitution, or by an adherence of the broken off parts to other areas. These aberrations may cause an uneven distribution of chromosomes at cell division. Such breaks are produced mainly by the incorporation of 14 C, 3 H, but may be induced also by UV radiations, it must be emphasized that a cause-effect relationship exists between ionizing radiation and the incidence of chromosomal aberrations, despite the fact that in an Editorial of the British Medical Journal (*1*, 1960, 1944) it has been pointed out that the sudden increase in the incidence of leucaemia in Great Britain had nothing to do with the increase in background radiation.

According to the UN report, the frequency of chromosomal aberrations is 1 to 100 births. One-fitfth of the patients suffer from Down's syndrome, 1/5 from Klinefelter's syndrome. Down's syndrome is not always diagnosed in newborn age. According to ØSTER, in 21 per cent of the cases the obstetricians fail to report it.

In Hungary we devote great attention to children with Down's syndrome reaching school age. According to a survey made in 1960, there are certain

areas in the country (for example county Somogy) where hardly any of such children born in the period 1945—1955 had reached school age. In the same period the largest number of mongoloids were born (excluding Budapest) in county Zala. Another survey will be made in 1965, to facilitate comparison with the above data.

The incidence of gametopathic conditions are remarkably high in the 1962 WHO statistics of the children

1 per cent had chromosomal aberration

1 per cent had sex-bound or dominantly inherited disturbances

1.5 per cent suffered from mental, or severe constitutional hereditary disease

2.5 per cent had morphologically well-defined developmental anomalies.

Total : 6 per cent.

Thus, almost every 17th child suffered from gametopathy.

The information concerning the frequency of gametopathies is supplemented by the 40-year data on the genetic and peristatic causes of death published by CARTER:

Year	Cause of death, per cent			
	Peristatic	Unknown	Partly genetic	Genetic
1914	68	15.5	14.5	2
1954	14.5	48.0	25.5	12

The *chiemopathies*, disturbances acquired during intrauterine life, are often associated with gametopathic conditions. There are symptoms which may be of both gametopathic and chiemopathic origin. Such changes are for example microcephaly, spina bifida, microphthalmia, palatoschisis, degeneration of the organ of Corti, congenital cataract, etc.

In an effort to determine the origin of teratogenetic time-table for use at the Pregnancy Polyclinic (Head: Dr. D. Margitay—Becht). Whenever there is reason to suspect sense disturbance, the expecting mother is kept under observation from the first or second month of pregnancy the data of conception (some mothers can tell it for day or even hour), and the factors that may cause harm are recorded on the time-table. After delivery the newborn is subjected to detailed examination, and at the age of a few months its mental development is appraised by the Bühler—Hetzer test. Thereby we may obtain early information as to eventual defects in mental development.

The chiemopathies fall into 3 groups.

a) The zygopathies arise in the first fortnight after conception and usually lead to early termination of the pregnancy. Zygopathic factors are hypoxia, chemical agents (Li, drugs, quinine, sulphonamides) and ionizing radiation.

The zygopathies have no significance in giving rise to mental defect, except when segmentation is of karyopathic character.

b) The embryopathies develop from the gastrula till the haematotrophic stage, i.e. till the development of the placenta (2nd week to 12th week).

During that period the factors affecting the embryo cause necrosis and degeneration of tissues, usually by intra- or extraembryonic haemorrhages.

The quality of the anomaly is chronospecific, even when the noxa is definitely organspecific.

The causes of embryopathy are:

1. *Hypoxia* (Braun, Gere and Szekeres, Ingalls)
2. *Microbiological effects* (viruses: Gregg, Töndury)
3. *Chemical factors* (Thalidomide /Speirs/, barbiturates /Potter/, sulpha drugs /Csabay/).
4. *Metabolic diseases* (diabetes mellitus) (Büchner, Kelemen, Worm)
5. *Ionizing radiation* (Ottweiler, Weiss).

Development of the neural tube and the 3-vesicle stage of the brain fall to the third and fourth weeks of pregnancy. During the 5th and 6th weeks develops the 5-vesicle stage, while stratification of the pallium begins at 6 weeks and is well under way by the end of the embryopathic stage. It is beyond doubt that the embryopathic conditions are accompanied in almost every case by severe and irreversible changes.

Diagnosis requires a very thorough examination of the patient, because the embryopathic changes detectable in other organs supply reliable information to the time of teratological termination. Special attention should be devoted to pregnancies of nurses, nursery employees and teachers who are often exposed to viral infection. Viral embryopathies are now thought to occur less frequently as it was supposed earlier. In 400 cases of our material some injurious factor (e.g. carbon monoxide intoxication, barbiturate poisoning, viral diseases such as German measles, hepatitis, parotitis, postvaccination syndrome) had certainly demonstrated occured during embryogenesis yet the newborn showed no pathological change whatever. On the other hand, in some cases German measles in the environment, oligosymptomatic herpes, or megalerythema produced classic embryopathic conditions.

c) Foetopathies are acquired during morphogenesis, in the period from the 12th week till birth.

This period is characterized by haematotrophic nutrition, by a certain degree of cellular and humoral protection of the foetus, with the appearance of the reticuloendothelial system and the beginning of reactivity to different factors: Thus, the foetus responds to such noxae not only with degeneration, but also with an inflammatory process. In general the resulting abnormality is non-specific. *Wohlwill* found pathological changes in the placenta in 42 per

cent of spontaneous abortions at 3 to 6 months. This must stimulate the investigators to carry out studies of the placenta in every case of late spontaneous abortion.

The causes of foetopathy may be divided into three groups:

1. Microbiological causes
2. Immunbiological causes
3. Metabolic disorders.

1. From the point of view of infection the foetus is endangered from two directions.

a) The allantoic fluid may be infected as a result of parietal chorioamnionitis or early rupture of the membranes, preceded by some non-sterile manipulation. The infected allantoic fluid is partly aspirated, partly swallowed by the foetus, leading to infection through the respiratory or digestive tract.

b) The chorionic villi may be infected: the pathogenic agent enters the foetal circulation and gives rise to haematogenic generalization.

Thus, the microbiological factors exert their foetopathic action by affecting the membranes (parietal chorioamnionitis, placentitis) in the first place: such factors are the viruses of cytomegaly, chickenpox, poliomyelitis, influenza, smallpox; further tuberculosis, syphilis, listeriosis, toxoplasmosis, and Entamoeba histolytica.

2. To the immunbiological factors belong the main blood group and RH incompatibilities which affect 0.3 per cent of the foetuses, with the spread of early exchange transfusions they have lost much of their significance.

3. The effect on the foetus of maternal metabolic diseases have been studied extensively, yet foetopathies must still be investigated. — Evidence is still scarce relative to the foetuses of mother suffering from endocrinological diseases other than diabetes mellitus. The foetopathic relations of diabetes are fairly well-known. *White* found developmental disorders in 12 per cent of the children of diabetic mothers. These disorders were of embryopathic nature. The spasticastatic-hyperkinetic syndrome associated with mental defects, described by MAYER, is considered to be a diabetic foetopathy.

The neogonopathies

Perinatal factors effect mainly the brain of the newborn during progressive cephalisation, which is a process peculiar to man, that in turn may have a decisive influence on the further development of the nervous system, including intellectual development.

The neogonopathies may be divided into three groups.

1. Hypoxia or ischaemia.
   a) Protracted delivery: disorders of rotation, posture, prolapse of the cord, uterine inertia, tetanus-trismus uteri, developmental disorders of the uterus, narrow pelvis.
   b) Haemorrhage during delivery: placenta previa, vasa previa, premature detachment of the placenta, utero-placentar apoplexy, foetomaternal shunt.
2. Trauma
   a) Narrow pelvis
   b) Obstetric hyperactivity (expression of the foetus, forceps)
3. Premature delivery

Neogonopathic nervous changes are usually characterized by asphyxia. The methods of resuscitation applied in such cases may also harm the nervous system. The foetus tolerates hypoxia well, but the rewarning of the newborn increases the rate of enzymatic processes and thereby the oxygen demand of tissues, is not a fortunate intervention. Slow, progressive warming up would cause less damage to the nervous system.

Micro-lesions of the nervous system are recognized only later. It is advisable to examine the newborns from the first month on with the Bühler—Hetzer test; in this way defects may be detected at an early period.

It is my intention to omit the infectious diseases of the newborn; because in possession of our modern weapons these must not be allowed to develop.

Postnatal affections

Meningoencephalitis and encephalosis are undoubtedly the most important in this group. The syndromes consequential to bacterial, viral and allergic meningoencephalitis will not dealt with here. Encephalosis may give rise to a condition just as severe as that produced by encephalitis. Every loss of consciousness may produce serious and irreversible changes in the central nervous system, irrespective of it having been caused by bacterial toxin, carbon monoxide, alcohol, vitamin B_1 deficiency, liver disease, grave anaemia or ketosis.

It has to be mentioned that encephalitis or encephalosis usually develop on grounds of previous nervous affections (intrauterine lesions, damage sustained during delivery).

Ionizing radiation is a factor that must not be ignored when discussing postnatal disturbances. We may mention in brief 21 cases, in which erroneous calculation has led to exposure of the child's head infected with Microsporon to fourfold X-ray doses to induce epilation. The radiation injury caused oligophrenia which is still in progress.

The aim may be formulated briefly: teratological prophylaxis. This is part of the fight begun by Pasteur, Semmelweis, Koch, Ehrlich, Fleming and many others and carried on by the unknown labourers of medicine day by day. By the recognition of the cause-effect relationship of the phenomena and by the elimination of the causes we may save many lives, probably more, as HEISER has put it, than what many a general has been allowed to sacrifice.

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Teratogenic Aspects of the Principal Morphogenetic Phases in Early Chick Embryos

Teratogenic investigations have been made by means of Janus green B, N-methyl-phenazonemetasulphate, lithium chloride and rhodanates. Recording of the morphogenetic process and a new method of enzyme detection have made it possible to extend analysis to the early phases of development.

Treatment of the chick blastoderm with diluted Janus green B in the early phase of morphogenesis causes the appearance of mitochondrial succinic dehydrogenase positive areas, which reflect the actual stage of development. This means that, although there is abundant dye for the entire embrionic disk, only the presumptive areas corresponding to the age of the embryo are capable of binding the dye. The dye is decomposed under the influence of dehydrogenase, a phenomenon indicative of the presence of mitochondrial activvity. Higher concentrations of Janus green B give rise to the production of living monsters wich are phase specific in accordance with the detected presumptive areas.

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Morphogenetic Characteristics of Young Chick Embryos in Teratological Experiments

Chick-embryos are widely used in teratogenic model experiments. Most investigators have been concerned with the late phenogenesis, the final result, of experimentally induced developmental anomalies. The study of early phenogenesis, of the initial minute alterations, is important for the elucidation of the teratogenic mechanisms, but such studies require a statistical evaluation of the specimen employed in the model experiment. The stages, as described by HAMBURGER and HAMILTON, are usually accepted in experiments on chick-embryos. These stages are not quite satisfactory in the early phase of growth (long intervals between the stages, small number of quantitative morphogenetic data), concerning only the embryo's body, without its different annex formations (blastoderm, area vasculosa, area pellucida).

With a view to overcoming these limitations and to elaborate a quantitative method serial photographs of 1200 Rhode-Island-chicken eggs were taken between the 16th and the 56th hour of incubation. The photos were made at one-hour intervals *in vivo* and after brief

fixing with acetic acid. An original method of serial photography with contrast making China-ink injections into the subgerminal cavity, has been elaborated.

Measurements of the embryos and the embryonic appendages were made on the eggs and on the pictures.

The appearance of 13 morphological formations were worked out statistically and represented graphically. Further 12 morphological correlations between the main formations have been established. Thus, a complex, statistically evaluable picture of the basic morphogenetic characteristics in the early chick embryo was obtained, allowing a more exact teratological testing.

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Morphological and Histochemical Studies of Human Nidation Disturbances

Abrasion products fixed in the living state and living teratoid structures observed in the course of ectopic pregnancies of patients suffering from diseases of the uterine cavity and other chronic disorders were examined under the light- and the phase-contrast-microscope, and also histochemically as to aldolase, acid and alkaline phosphatase, succinic dehydrogenase and mucopolysaccharides.

A further object of the investigations was the differentiation of decidual from trophoblastic elements which cannot be distinguished reliably by the usual procedures.

Parallel investigations were made in normal (control) material.

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Seven-Day Old Human Ovum

In abrasion material sent in for the suspicion of chrionepithelioma a freshly embedded ovum has been found. The age of the ovum, on the basis of Hertig's microphotographs, was set at 7—7 1/2 days.

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Incidence of Developmental Anomalies

Among 43 348 corpses dissected in 10 hospitals in the period 1953 to 1963, developmental anomalies were found in 2016 (4.1 per cent). Analysis of the annual figures revealed the number of individuals in whom developmental disorders are found *post mortem* to fluctuate in 3-year cycles. The relatively highest number of such cases occurred in the hospitals of Esztergom and Tatabánya, the lowest in those of Szombathely.

Overall differences in respect of sex were insignificant (males, 54.1 per cent, females, 45.9 per cent). On the other hand, sex differences in respect of particular organs (except genital disorders which do not lend themselves to this kind of evaluation) were notable. The incidence of neural disorders was 15.2 per cent,
of respiratory disorders 17.4 per cent,
of sensory disorders 23.6 per cent higher in females than in males, whereas the incidence of disorders of the digestive tract was 20.4 per cent,
of the muscles 30.6 per cent,
of the urinary tract 46.2 per cent higher in males than in females.
The anomalies of the skeletal, circulatory and endocrine systems and other non-classified disorders showed no significant sex-conditioned differences.

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Lethal Developmental Anomalies

The records of a 10-year material from January 1, 1954, to December 31, 1963 have been surveyed.

The rate of perinatal mortality was 4.8 per cent. Of the 721 foetuses who died at or shortly after delivery in 92 (12.7 per cent) death was due to some developmental anomaly.

The number of developmental anomalies totalled 157 in the 92 necropsied cases. Anomalies of the nervous system represented 57.4 per cent; those of the secretory organs 20.1; those of the circulatory apparatus, 12.3; and those of the digestive tract, 10.2 per cent.

As regards occupation, 43 per cent of the cases occured in families engaged in agriculture, 31 per cent in those employed in industry, and 26 per cent in families with an intellectual profession. The high percentage in the agricultural category is worthy of note. The quota of pregnants who had received prenatal care amounted to 94.3 per cent. Of the monsters, 47 per cent were females and 53 per cent were males.

Developmental anomaly caused intrauterine death in 34.7 per cent of the cases. Death ensued in the first postnatal hour in 19.4 per cent, on the first day of life in 20.3 per cent, and only 15.5 per cent survived the first day. More than 50 per cent of the material consisted of prematures, 44.0 per cent weighed less than 2000 g, 28.0 per cent between 2000 and 3000 g. 15.0 per cent between 3000 and 4000 g and 4.2 per cent more than 4000 g.

In the mother's history febrile episodes during the first 3 month of pregnancy were mentioned in 27.4 per cent of the cases.

Seventy per cent of the mother were primiparae. No mother younger than 16 years gave birth to monsters, and only in a single case out of a total of 92 was the mother of a monster olden than 40 years.

Measures for the inhibition of developmental anomalies should concentrate on the fight against alcoholism, on adequate nutrition, and on the prevention of fevers during the first trimester of pregnancy. All such measures depend on the improvement of routine prenatal care.

S. Ferenczy, J. Kovács

(Hospital of Zala County Council, Zalaegerszeg)

Teratogenic Phenomena in a 10 Years Necropsy Material

The number of congenital malformations among the 3204 cases autopsied in 10 years totalled 317, i.e. 9.89 per cent. Developmental anomalies were more frequent in and around Zalaegerszeg, the county seat, as also in the south-western region of the county. The frequency was especially high in gypsy colonies, where about 10 per cent of the registered anomalies had originated.

About 50 per cent of the malformations occurred in the age group 21 to 30 years which may be due to that the percentage of births is highest in this category. Another peak appeared at 40-years of age, a fact in agreement with the phenomenon that malformation is more frequent in the offspring of middle-aged mothers. Approximately 50 per cent of the offspring with malformations were children of primiparae and 20 per cent those of secundiparae; the incidence decreases after the third pregnancy, although deformed children were born also from the 10th to 13th pregnancy.

A case has been recorded in which after 3 abortions a mother with congenital dislocation of the hip was delivered of a stillborn baby with three malformations. In another case, two successive children were born, with bizarre embryopathies. Cardiac anomalies were found in 3 children *post mortem*, and in a fourth child clinically, all in one and the same gypsy family. A chondrodystrophic mother gave birth to twins, both of whom had likewise chondrodystrophy. These anomalies appear to have been inherited.

The records contain 3 cases of congenital malformation in which the pregnant mothers had been active in spraying DDT and had then had a transitory indisposition. Although the two events need not necessarily stand in causal connection, teratogenic possibilities of this kind should be taken into consideration.

Several records show cystitis of the mother treated with antibiotics, sulphonamide and phenazopyridine, often in a haphazard manner and without medical supervision. It would seem that it is not so much the often uncertain cystitis but rather the polypragmatic and sometimes superfluous medication which should be regarded as the pathogenic factor in such cases.

Influenza was frequently mentioned in the history. It should be borne in mind that this term is usually applied by mothers to all febrile affections of the respiratory tract. Some of the cases were those of true influenza, but there were also a number of other viral diseases (including Coxsackie) referred to as "summer influenza". Although influenza is held to be harmless teratogenetically, the percentage of babies born with malformations was higher after influenza epidemics, as proved by a duplication of such births in the years 1958 and 1961.

Back in 1959 — reference has been made of Coxsackie infections observed in the second half of the year 1958, emphasizing the possibility of their teratogenic effect. The incidence of congenital malformations rose to 29 per cent in the first 4 months of 1959, and in the mothers' history episodes of Coxsackie-infection was mentioned. The observed cases were mostly embryopathies in contrast to the last quarter of 1958 which displayed a majority of foetopathic cases. The incidence of hydramnion rose at the same time, and that of abortions increased almost sixfold in the last quarter of 1958, and threefold at the beginning of 1959.

The pregnancy histories contain also abdominal operations, further different forms of toxicosis.

L. Galambos, Magda Scholz

(Department of Pathology, Uzsoki Street Hospital, Budapest)

Perinatal Mortality with Especial Regard to Developmental Anomalies

With a view to ascertaining the mortality rate for babies below the age of 12 months, the necropsy records from the period 1954 to 1964 have been surveyed. The number of foetuses, newborns and infants who had died between the 28th week of pregnancy and the end of the first year of extrauterine life totalled 470 (322 = 68.51 per cent during the first, 148 = 51.49 per cent during the second half of the 10-year period).

The causes of death were (1) obstetrical factors; (2) aspiration and its complications; (3) premature delivery; (4) extrauterine infections; (5) intracranial haemorrhage; (6) congenital defects; (7) other developmental anomalies; (8) RH incompatibility; (9) intrauterine death of unknown aetiology. Extrauterine infection was the most frequent cause of death (23,47 per cent), premature birth was the next in frequency (15,39 per cent), while the 3rd and 4th place were occupied by intracranial haemorrhage and congenital defects (14,26 per cent, each); the fifth in frequency were other developmental anomalies (9,57 per cent). Infantile mortality should, therefore, be fought by paying especial attention to the said factors.

A comparison of the data of the two 5-year periods revealed the following. The number of obstetrical complications decreased, that of developmental anomalies (except heart defects)

decreased considerably, and also the death rate for premature delivery showed a significant decrease. The incidence of death caused by aspiration and intrauterine deaths of unknown aetiology remained essentially unchanged. The number of lethal extrauterine infections and RH-incompatibilities showed a considerable, that of intracranial haemorrhages and congenital heart defects moderate relative increase.

Summing up, the rate of mortality fell considerably during the last decade, since 68,51 per cent of the deaths occurred during the first five years. Developmental anomalies were responsible for about a fourth of the deaths, although cases in which some other definite cause of death (e.g. intracranial haemorrhage) could be ascertained were not classified under this head. The incidence of congenital heart defects increased while that of other developmental anomalies decreased significantly. That both the absolute and relative number of obstetrical disorders, aspiration and its complications decreased must have, among others, been due to the systematic prenatal care and a change in obstetric indications. The latter factor is not yet manifest in the numerical change of intracranial haemorrhages. Data for prematures can be evaluated from a regional point of view only, since either the premature delivery took place in the obstetrical ward or the living premature baby was transferred to a special institution. The regional rate of extrauterine infections displays a moderately upward tendency. This together with other similar findings, shows that the satisfactory development of systematic postnatal care rends to prolong the survival of constitutionally impaired infants.

G. Garay, Gy. Szederkényi

(Department of Radiology and Pathology, County Council Hospital, Kecskemét)

Incidence of Lethal Developmental Defects and Number of X-Ray Examinations

The infantile death rate of developmental defects in the period 1956 to 1962 was compared with the number of X-Ray examinations performed in the same area during the same period. The number of X-Ray examinations and the incidence of developmental abnormities causing death in infancy were found to exhibit a parallel increase (by 20 per cent and 40 per cent, respectively). A causal relationship is presumed to exist between the two figures.

E. Czeizel

(Department of Pathological Physiology, National Institute of Public Health, Budapest)

Teratogenic Effect of Ionizing Radiation

Ionizing radiation can be advantageously applied for teratological investigations. Time and dose of radiation affecting the embryo can be defined precisely, the placental barrier is eliminated, the radiation affecting the tissues diffusely make an investigation of elective changes possible, etc.

In the present studies pregnant rats were irradiated with 300 to 500 r. Irradiation on the 1st day caused the incidence of pregnancy and the number of a live healthy foetuses to decrease. Irradiation on the 10th day of pregnancy decreased the number and weight of foetuses per mother animal and caused mainly craniocerebral developmental anomalies in part of the animals. Irradiation on the 16th day did not induce any visible abnormity. The direct effect of radiation is responsible for the injuries caused by ionizing rays. If high doses are given in the early period of pregnancy the mother's radiation injury also plays a part in the foetal damage. In this indirect effect, radiotoxins seem to play a subordinate role.

D. Tanka, Maria Keller

(National Institute for Rheumatism and Balneology, Budapest)

Acute Radiation Injury and Enzyme Activity

Changes of enzyme activity occurring under the effect of ionizing radiation seem to play a significant role in the development of late radiation injury, genetic injury and tumour formation.

According to the present histochemical and biochemical findings, the activity of one and the same enzyme decreases in some organs and increases in others. This might be due to differences in radiation sensitivity of the enzyme molecule's protein component; thus the problem of the so-called izoenzyme also arises.

B. Bugyi, J. Kunstadt

(Department of Radiology Jan Šafarik University, Košice;
Polyclinic of the Ganz Mávag Works, Budapest)

The First Case of Intrauterine Encephalopathy Caused by Irradiation

Hard ionizing rays are well-known to cause genetic changes. Radiogenic embryopathies usually result in the birth of nonviable foetuses, so that cases of such embryopathies with a record of long survival are extremely rare. A case of this kind has recently been detected in a man now 60 years of age, the son of a radiologist. This is the first radiogen embryopathy of the world-literature.

The father had conducted radiological investigations at the end of the last century, when the noxious effect of X-rays was unknown. He studied the development of the vertebral column by means of X-rays in prematurely born died babies and aborted foetuses and used his own pregnant wife as control of whom he took serial radiographs.The wife was then delivered of a son who had perinatal encephalopathy and was imbecile. The child exhibited no somatic anomalies with the exception of anisocoria. Imbecility must have been due to radiogenic encephalopathy since the three previous children of the same parents have exceptionally high mental faculties. Although parts of the original X-ray apparatus are still extant, there are no records to reveal the number of radiographs taken or the phases of pregnancy in which the pictures were made, so that it is possible to determine the quantity of irradiation to which the pregnant woman was exposed. The radiologist father had never referred to the probable origin of the intrauterine encephalopathy and it was only by piecing together information obtained from the family, the environment, from clinical data, from a knowledge of the father's practice and scientific achievements that this unique case could be demonstrated.

L. Szentendrei, Emma Kurusa, Gy. Szederkényi

(Department of Paediatrics and Pathology, County Council Hospital, Kecskemét)

Complex Developmental Malformations in a Child after X-Ray Examination during Pregnancy

A retarded female child born with clubfoot and treated for chronic pyelonephritis died with renal failure. Autopsy revealed hydrocephalus due to agenesis of the aqueduct and an ectopic kidney. History revealed that the mother had been submitted to gastric X-ray examinations in the 2nd and 3rd weeks of pregnancy. Analyzing the amount of radiation received by the mother and comparing this with the amount giving rise to similar malformations, it has been concluded that the defects observed in the child had probably been caused by the Y-rays.

Ilona Banga, D. Szabó

(First Institute of Pathological Anatomy and Experimental Cancer Research, University Medical School, Budapest)

Interconnexion between the Submicroscopic Structure and the Digestibility of Collagen

A close interrelation has recently been discovered to exist between the configuration and the digestibility of globular proteins. Methods for investigating the configuration of globular proteins are, however, less satisfactory than those applied for fibrillary or scleroproteins. Submicroscopic inspection of the longitudinal fibrillar arrangement in the molecules of the last named proteins is a great help in studying configurational changes. The question to be elucidated is whether changes in configuration are invariably accompanied by increased digestibility. Collagenous fibres are instructive in this respect, since they display a molecular birefringence the changes of which indicate changes occuring in the configuration or spatial reticulation. It has been possible to induce by chemical means (sulphatation, carbamide treatment) submicroscopic phenomena which gave rise to configurational changes. The fact that they were not always accompanied by increased digestibility of the collagen proves that not all but only some special kinds of configurational changes increase the digestibility of proteins, whereas certain other configurational changes may have the opposite effect.

I. Oláh, Sz. Virágh

(Institute of Histology and Embryology, University Medical School, Budapest)

Differentiation of Embryonic Skeletal Musculature

The transformation of mesenchymatous cells into myoblast has been studied with light- and electron-microscope in the somites and myotomes of rat embryos. Myoblasts are single or grouped cells with large nucleus, several nucleoli many, mostly free ribosomes, some glycogen and scattered fine filaments. The endoplasmic reticulum of myoblasts is undeveloped which helps to distinguish them from the neighbouring fibroblasts. It seems that primitive muscle fibres arise from the fusion of myoblasts (formation of syncytium). The adjacent myoblasts are interconnected at certain points by junctional complex like structures of the cell membranes. The tightly packed myoblasts are enveloped by a common basal membrane. The coherent cell membranes disintegrate into vesicles when the Syncytium is formed. The growth of differentiated sarcoplasm is preceded by the accumulation of undifferentiated glycogen-rich plasm in the primitive muscle fibres and in the so-called muscle tubes. The major part of the abundant glycogen disappears during the differentiation of the myofibrils. Development of the myofibrils have been discussed.

M. Palkovits

(Institute of Experimental Medical Research, Hungarian Academy of Sciences, Budapest)

Nuclear Volume and Cellular Activity

The correlation between changes in nuclear size and cellular metabolism constitutes a fundamental problem of karyometry. In other words, the problem is whether justified to infer from the changes of nuclear volume to cellular activity.

After the administration of substances of known action to animals, changes in the size of nuclei have been studied in the thyroid, liver, uterus, suprarenal gland, pancreas and in different parts of the diencephalon. Nuclear volume was found to increase when the examined organ was more active, and to decrease when the activity of the organ was inhibited. Excessive doses raised the nuclear volume threefold or reduced it to a third.

The action of the test substances was observed in acute experiments, i.e. measured at intervals of 10 minutes after their administration. Changes in nuclear volume took place even in these short periods, and there exist karyometric methods to quantitate such minute changes. Measurements made at different intervals make it possible to follow the action of the test substances chronologically.

A new procedure has been devised for the measurement and comparison of abnormally shaped (elongated, flat) nuclei; a two-dimensional diagram has been plotted for the representation of the results. The diagram expresses changes in nuclear size and shape, and makes it moreover possible to distinguish between different cell-types and to measure their respective activities.

Sz. Virágh

(Institute of Histology and Embryology, University Medical School, Budapest)

The Fine Structure of Cell Interconnection in Various Embryonic Tissues

Electron microscopic studies have been made of tissues of rat embryos and of human placenta. The surface of the plasma membrane of dispersed embryonic cells is covered by an indistinct, presumably proteinic substance which seems to constitute a loose connection between the cells associating to form tissues. A more solid attachment is formed by the invaginations and the junctional complexes — such as the desmosomes, zonula adhaerens, zonula occludens (FARQUHAR and PALAD, 1963) — of adjacent cell membranes in epithelial and mesenchymatous tissues. At the point of zonula adhaerens and desmosomes a homogeneous material condenses gradually on the inner and outer side of the cell membrane. Fine filaments (tonofilaments) attach on the condensed cytoplasm in this region. All the three junctional complexes mentioned above exist between the cardiac mesenchymatous cells and the myoblasts. The intercalated discs seem to develop on the basis of zonula adhaerens-type junctional complex. In the cardiac myoblasts the myofilaments attach secondarily on the cell membrane. The length and number of the intercalated discs progress paralelly with the cytoplasmic differentiation of the myoblasts. Certain ultrastructural problems of the basal membrane have been discussed.

T. Donáth

(Institute of Anatomy, University Medical School, Budapest)

Connexions between the Intensity of Fluorescence, Temperature and Exposures to Ultraviolet Light in Epithelial Cells

Using his own method for the semi-quantitative estimation of fluorescence, the author studied the effect of different temperatures as also that of ultraviolet light on the intensity of fluorescence in model-experiments and also in epithelial cells treated with different concentrations of acridine orange.

The intensity of fluorescence was found to increase considerably between 18° and 30° C, a phenomenon presumably due to a dissociation of the non-fluorescent molecules caused by the increased temperature.

The durability of fluorescence was found to depend on the nature of the binding between the molecules of the cells and those of the fluorescent dye. If the bond is physical, fluorescence is soon extinguished, while it is stable if the bond is chemical. The decrease in the intensity of fluorescence — due to continuous ultraviolet excitation — depends, thus, on the nature of the connexion between the dye and the chemical structure of the cell.

A. Ábrahám

(Institute of General Zoology and Biology, University, Szeged)

Contributions to the Knowledge of the Nerve Supply of Human Glomus Caroticum

Studies by means of the modified Bielschowsky method of the carotid body of human subjects of different ages have led to the following conclusions.

(1) In contradiction to current usage it is not possible to separate and classify the nerve plexuses which surpass all other organs of a similar structure and function as regards form and abundance.

(2) Age and pathologic conditions induce conspicuous changes in the shape and arrangement of the plexuses, in the shape of the fibres running in them as also in the relation of the terminal fibres to the receptor cells.

(3) Shape and structure of the intraglomerular plexus show a certain constancy, although the circular or ellipsoid form occasionally becomes elongated which leads, by an unilateral juncture with the periglomerular plexus, to longitudinal and sometimes irregularly shaped terminal systems.

(4) The nerve fibres constituting the plexuses are usually moderately thick, smooth-edged or delicately varicose; with advancing age or under pathological conditions there appear thick axons, broad, sometimes very long and occasionally sharply fibrillated varices which are interconnected by extremely thin homogenous filamentous portions.

(5) It is only in the whole of the glomerulus that the intraglomerular fibres form dense spirals; the terminal connexion with the cells is not the pericellular spiral as has recently been supposed by certain authors on the strength of light- and electron-microscopic investigations, but the comparatively large and conspicuous end-disk situated on or between the receptor cells.

I. Törk, J. Kiss, B. Aros

(Department of Histology and Embryology, University Medical School, Budapest)

Autoradiographic Examination of the Neurosecretory System of Invertebrates

Since labelled amino acids are incorporated by the neurosecretory cells of vertebrates, the protein metabolism of the neurosecretory system can conveniently be studied by means of autoradiography.

The present experiments were designed to observe the neurosecretory system of invertebrates (Lumbricus sp.) by means of the same method. Sexually mature animals were injected with a dose of 10 μC/gm thioaminoacid labelled with ^{35}S. The animals were killed 1, 6, 14, and 24 hours after the administration of the isotope.

It took 6 hours for perceptible amounts of the labelled amino acid to appear in the nervous system. Activity was most vigorous in the neurosecretory cells of the cerebral and the subpharyngeal ganglia. Incorporation reached a peak at 14 hours.

The experiments showed that there is an intensive protein metabolism in the neurosecretory cells. Their morphological appearance suggests a correlation between the radioactivity of the neurosecretory cells and their Gomori-positivity.

B. Aros, Ingeborg Teichmann, B. Vigh

(Department of Histology and Embryology, University Medical School, Budapest)

Histochemical Investigations of the Neurosecretory System of Invertebrates

Histochemical investigations made in numerous species of higher vertebrates show that neurosecretion is of protein nature. In our present examination we have studied the neurosecretory system of invertebrates with histochemical methods (Lumbricus sp.). The following stainings and reactions were used: PAS reaction with and without digestion; astra blue; toluidine blue; trypaflavine; tetrazonium reaction; eriochrome black T; methylgreen pyronine; Feulgen reaction; demonstration of protein bound SH- and SS-groups with the DDD-method; demonstration of tyrosine and tryptophane; Sudan black B.

The results of our histochemical reactions suggest that the neurosecretory material has protein character.

Ingeborg Teichmann, B. Vigh, B. Aros, Sára Koritsánszky

(Department of Histology and Embryology, University Medical School, Budapest)

Histochemical Investigation of the Periventricular Gomori-Positive Glial Cells in the Rat's Hypothalamus

In earlier experiments a correlation has been shown to exist between the periventricular Gomori-positive glial cells and the Gomori-positive ependymosecretion. Investigations have now been made to elucidate the histochemical nature of the Gomori-positive granules of the glial cells.

The stainings and reactions applied were the PAS reaction with and without digestion; astra blue; toluidine blue; trypaflavine; tetrazonium reaction; eriochrome black T; ninhydrin-Schiff reaction; demonstration of protein bound SH- and SS-groups with the DDD-method; demonstration of tyrosine and tryptophane; paraldehyde fuchsin with and without preceding digestion with trypsine; methylgreen-pyronine; Feulgen's reaction, Sudan black B.

All these reactions showed the material of the glial cells to consist — like ependymosecretory granules — of a muco- or glycoprotein. This is an additional fact to prove the correlation existing between the Gomori-positive substance of the ependyma and those of the glia cells.

T. Wenger, B. Vigh, B. Aros

(Department of Histology and Embryology, Medical University, Budapest)

Mitotic Activity in the Hypothalamus of Adult Rats

Colchicine is known to stop mitosis in the metaphasis. Therefore, its administration makes it possible to investigate whether, at a given time, there occur cell divisions in a given organ.

The mitotic activity of the periventricular layer has been examined in the hypothalamic region of adult rats (body weight 150 to 200 gm). This activity was found to be very intensive; the number of mitoses reached the peak 4 hours after the administration of colchicine. The number of mitoses, as observed in the hypothalamus, was compared with that observed in the Lieberkühn crypts of the same animals.

It is suggested that cell divisions in the hypendyma of the third ventricle provide for substituting the cells of the periventricular layer.

P. Kása, B. Csillik, F. Joó

(Institute of Anatomy, University Medical School, Szeged)

Histochemical Investigations in the Deafferented Cerebellum

The location of acetylcholine-esterase, pseudocholine-esterase, succinodehydrogenase and cytochromoxidase activity has been studied in the archicerebellar and neocerebellar cortex of rats both under physiological conditions and after deafferentation. Literature contains contradictory data regarding the localization of these enzymes. Experiments with acetylthiocholine at different pH values and incubation times have shown that, under normal conditions, acetylcholine-esterase is limited to the mossy fibres of the granular layer, while the molecular and the Purkinje layer are inactive. Deafferentation reduced acetylcholine-esterase activity but did not entirely stop it presumably due to the survival of the cholinergic Golgi axon endings. The appearance of acetylcholine-esterase activity in the Purkinje cells was probably due to the mobilization of an enzyme that had become latent in the course of ontogenesis.

Pseudocholine-esterase is normally restricted to the capillary walls while succinodehydrogenase and cytochromoxydase are active in the molecular layer, in the cytoplasm of the Purkinje cells and in the mossy fibres alike. Deafferentation caused no significant change in this respect.

It is suggested that the observed changes are partly the signs of degenerative processes and partly the result of dysfunction. It would follow that neurotomy in the central apparatus affects almost exclusively the activity of enzymes specific for synaptic function.

I. Törő, I. Oláh

(Institute of Histology and Embryology, University Medical School, Budapest)

Thymic Epithelium and Thymic Corpuscles in Tissue Culture

Electron microscopic observations were made on 6 to 12-day old tissue cultures obtained from newborn guinea-pigs.

The epithelial cells were found to be flattened; sometimes two or three layers were superposed, one on the top of the other. The marginal cytoplasm of the epithelial cells frequently had a more compact structure ("ektoplasm"). The cells were rich in free ribosomes. Some contained dilated ergastoplasmic cisternae filled with moderately electron-dense substance. The epithelial cells contained numerous tonofibrils.

Hassall's corpuscles were of two types. In one type, electron-microscopy revealed signs of secretory activity of the epithelial cells. Such cells were characterized by dilated ergastoplasmic cisternae containing moderately electron-dense substance, further by a well-developed Golgi apparatus. Signs of degeneration were visible towards the interior of Hassall's corpuscles. The other type of the corpuscles showed signs of cornification. Cells in this type contained numerous bundles of tonofibrils and islets of delicate granular substance which obscured the cell organelles. The epithelial cells are interconnected by well-developed desmosomes in both types of Hassall's corpuscles.

F. Hajós, K. Strazinczky, B. Mess

(Institute of Anatomy, University Medical School, Pécs)

Thyroid Secretion and Follicle Formation in the Chicken Embryo

Previous electronmicroscopic studies of chicken embryos have shown that thyreotropic hormone (TSH) caused changes in the quantity and quality of the ergastoplasm while development of the Golgi apparatus seemed to be independent of TSH. Now the development of

follicles has been studied. They were found to be dependent upon TSH since no follicles developed in decapitated embryos, whereas after pituitary transplantation their development reached a nearly normal degree. Two kinds of mechanisms of follicle development were observed, an intercellular and an intracellular one. The latter mechanism is discussed in detail, with special regard to the vesicles observable around the follicles and increasing on TSH administration.

M. Ertl

(Institute of Histology and Embryology, University Medical School, Budapest)

Blood Volume and Juxtaglomerular Apparatus

According to recent observations, the juxtaglomerular apparatus has a certain role in the regulation of fluid spaces. The present experiments were designed to ascertain whether changes in circulating blood volume affected the granulation of the juxtaglomerular apparatus.

White mice of about 20 g body weight were bled through the caudal vein or by cardiac puncture. Each animal was carefully weighed, its blood volume as well as the volume of withdrawn blood was carefully measured. Then the animals were sacrified at different intervals. They were kept on a dry laboratory diet from the day preceding the withdrawal of blood until death. A number of animals from which blood had been taken received liquids at different times prior to being killed.

The kidneys of the sacrificed animals were fixed in formalin, embedded in paraffin, section 3 to 4 μ thick were made and stained with Mallory's haematoxylin.

The degree of granulation of the juxtaglomerular apparatus was found to change in conformity to the volume of withdrawn blood. Quantitative changes in granulation were illustrated graphically by means of Hartroft's index.

Gy. Ungváry, J. Faller

(Institute of Anatomy and Institute of Surgical Anatomy, University Medical School, Budapest)

Segmentation of the Liver

Livers of necropsied adult human subjects have been investigated by the corrosion method, X-ray, and filling with diffusible dyes. On the basis of both the hepatic venous system and the portobiliary apparatus, three lobes were distinguished. The right, middle and left lobes of the portobiliary system do not correspond to the similarly termed lobes of the hepatic venous system. Simultaneous filling of the two systems reveals four interlobar fissures, namely the right portobiliary fissure, right hepatic-vein fissure, left portobiliary fissure and left hepatic-vein fissure. Each fissure separating the lobes lodges a main trunk of the opposite system. As regards shape, there are livers of the regular and intermediate type in which size and position of the lobes and fissures are characteristically different. Differences in respect of minute intralobular details are still more pronounced so that it is practically impossible to distinguish intralobular segments. The frequent occurrence of the so-called magistral arch in livers of the irregular type makes a segmental division still more difficult.

Cs. Hadházy, K. Benkő, P. B. Ary

(Institute of Anatomy, Histology and Embryology and Central Electronmicroscopic Laboratory, University Medical School, Debrecen)

Electron-Microscopic Investigation of "Neodifferentiational" Chondrogenesis

The knee joints of 10 dogs were surgically treated by Krompecher's method, and the regenerating articular surface of the distal part of the femur was then examined under the electron microscope after 33 and 70 days. At these points of time in the granulation tissue

there occurs a process of chondrogenesis which starts in the fibroblasts and results in the formation of young chondrocytes. The submicroscopic structure of the resting and the active fibroblasts, that of the prechondroblasts, chondroblasts and of young chondrocytes is described. The examined (so-called neodifferentiational) chondrogenesis differs in some respects from the other types of cartilage formation. The main change occurs in the endoplasmic reticulum, whereas the role of Golgi's apparatus is more subordinate. Mucopolysaccharides secreted in the course of cartilage formation presumably originate in the hyaloplasm, and a considerable quantity of them passes into the endoplasmic reticulum. The ribosomes of the latter seem to be responsible for the production of non-collagenous proteins and the collagen precursor. The endoplasmic reticulum is supposed to contain a mixture of these three substances. They may be excreted in different ways; the major part probably passes through the pores of the cell membranes of the endoplasmic reticulum. Polymerization of the precursor of the fibres seems to take place on the outer surface of the cell membrane. The transitory swelling of mitochondria is suggested to be induced by anoxia, a phenomenon the metabolic aspects of which are pointed out. Small, empty vesicles form a chain-like structure between the cell membrane and the nucleus; these may be regarded as the morphological manifestations of micropinocytosis.

J. Vajda, Teréz Tömböl

(Institute of Anatomy, University Medical School, Budapest)

Lymphatics in the Small Intestine and the Intestinal Villi

The existence of lymphatics in the intestinal villi is a controversial problem. Experiments in dogs and cats have been made to clear the question. Vehement contraction of the intestinal mucosal muscle layer were observed on opening the abdominal cavity, so that it was impossible to inject the lymph vessels retrogradely under even approximately normal conditions. After the administration of antispasmodics, however, the central lymphatics filled satisfactorily and became well visible. A coherent plexus of lymph vessels was found at the base of the villi, in front of the mucosal muscle layer.

The factors maintaining lymph flow in the intestinal wall and the mesentery (dilatations at the intersections with vessels, arrangement of valves, etc.) have been investigated in a further series of experiments. The important role of the mucosal muscle layer in absorption should be taken into account.

I. Palkovich, E. Czeizel

(Second Department of Obstetrics, János Hospital, Budapest)

Lymph Vessels of the Oviduct

The surgically removed oviducts of patients 20 to 60 years of age have been studied. One oviduct was removed in the usual way while the other after clamping the efferent veins and lymph vessels for 30 minutes. As a result of the obstruction, lymph collections appeared in the mucosa of the tunica propria, concentric lymph vessels in the muscular layer and wide longitudinally running lymph vessel trunks provided with valves in the subserosa. Considering its abundant lymph vessel network the oviduct might influence physiological and pathological ovarial function.

H. Csermely

(MÁV Hospital and Policlinic. Department of Pathology, Budapest)

Vascular Developmental Defects of the Brain

Exogenous developmental disorders of the brain (proencephaly, hydranencephaly, microgyria) where the changes could be located to certain vascular supply areas have been studied. No pathological change was found in the afferent arteries, in other words organic

vascular disease had no aetiological role. This suggests that the malformations had been caused by a failure of the arterial supply to certain, usually symmetrical, cerebral areas during pregnancy. Various factors are responsible for the circulatory failure, and part of them is still unknown.

The resulting malformation depends on the intensity and duration of the circulatory failure and on the degree of immaturity of the developing brain.

A. Nagy, Zs. Szabó

(Department of Pathology, County Council Hospital, Tatabánya)

Circulatory System and Anencephaly

Developmental anomalies of the heart and major vessels have been studied in 235 anencephalic monsters and the observed incidence of cardiac defects was compared to their incidence in a similar group displaying other congenital malformations. The myocardium and the walls of the major vessels of 50 anencephalic monsters as well as the structure of the small and middle size arteries in various organs were examined histologically. It has been found that elastic fibres in the walls of small and middle size vessels have been missing but on other aries they have had hypertrophy.

At the same time around the capillaries micro-haemorrhages have been found nearly in every organ.

The pericardium, the position of the heart, the major vessels, the venous and arterial orifices, atrial and ventricular septa as the various other developmental disorders were subjected to gross examination.

A. Réffy, Zs. Szabó

(Department of Pathology, County Council Hospital, Tatabánya)

Anencephaly and Endocrine Glands

Changes in the endocrine organs have been studied post mortem in 235 cases of anencephaly.

Absence of the pituitary was observed in a single case only; in the other cases all the three lobes were present but the intermediary lobe was invariably poor in cells or empty. The stalk was often lacking an observation in agreement with literary data.

The adrenal glands (both the cortex and the medulla) were hypoplastic in all cases in several cases one of the adrenals was missing.

In contradiction to literary data, the thymus had double the normal size; the number of Hassall's corpuscles was higher, but their size smaller than normal. Occasionally, degenerated giant thymic corpuscles were found.

The thyroid was hypoplastic in two cases and normal in the others.

About 69 per cent of the examined cases were females; the gonads showed no changes.

The pancreas was normal in every case.

Klára Németh

(First Institute of Pathological Anatomy and Experimental Cancer Research, University Medical School, Budapest)

About the Malformations of the Central Nervous System with a Report of an Unusual Case

In the statistics concerned with developmental disorders the congenital defects of the central nervous system occupy an important place. According to ANDERSON, they constitute 49.5 per cent of the congenital defects incompatible with life.

In the 5-year material of the Institute, developmental defects of the central nervous system could be demostrated in 39 cases. This represents 1.9 per cent of the total number of necropsies, and 8.3 per cent of those who had died before the age of 1 year.

In the above mentioned material an interesting case has been observed. In a male infant aged 6 weeks a thickening 2 cm long was found in the thoracic spinal cord. No neurological symptoms had been described clinical. The homogeneous, greyish-white, dense intumescence proved to be a segment of small intestine of normal structure. As a result of the pressure exerted by the intestinal segment the spinal cord showed diastematomyelia. In the area of the intumescence the well closed spinal canal was bulging, but did not communicate either with the body surface or with the body cavities. The change may be ascribed to entoderma dysplasia, which could have developed up to the 20th day of foetal life. It is namely up to that time that the neuroenteric canal provides for a communication between the entoderm and the caudal end of the neural tube, from which the cord develops. In the present case no trace could be detected of the neuroenteric canal.

J. Veres

(First Institute of Pathological Anatomy and Experimental Cancer Research, University Medical School, Budapest)

A Case of Complete Cerebellar Agenesis

Complete agenesis of the cerebellum is one of the rarest developmental defects of the central nervous system. Even incomplete agenesis is a rarity. In the case to be described other malformations were present beside complete cerebellar agenesis. The infant had lived 18 days, and at autopsy the following changes were found beside the signs of KLIPPEL—FEIL'S syndrome. The cerebral hemispheres were well developed, but the corpus callosum was absent and the left lateral ventricular system was hypoplasic. Behind the medulla oblongata parts of the cerebrum were herniated in the foramen occipitale magnum, these proved to be parts of the dentate gyrus and the hippocampus lying as a coat on the posterior aspect of the medulla oblongata. Detailed examination failed to reveal even traces of the cerebellum. There were no cerebellar tentorium, pons, peduncles and the cerebellar arms were also absent, as in the cases of hypoplasia. The spinal cord was replaced by medullovasculous area. Histologically, at the base of the brain and near the surface of the medulla oblongata, gliomesodermal nodes were visible. The cerebellar agenesis might have been due directly to a herniation taking place early in foetal life, when under the effect of the compression the cerebellar anlage had perished. As a possible etiological factor, the mother had suffered for a few days during the second month of pregnancy, from a now not precisely identificable exanthematous disease resembling German measles.

L. Király, Gy. Gorácz

(Department of Pathology, P. Heim Children's Hospital, Budapest)

Classification of Connatal Heart Defects

The incidence of connatal heart defects has been studied in the 10-year necropsy material of the hospital. In early infancy (under 6 months of age) clinical diagnosis is difficult the procedures (e.g. angiocardiography) facilitating the correct recognition of the conditions being unfeasible. On the other hand, it is for this age group in the first place that the heart defect difficult to classify are characteristic. Those which can be classified readily and diagnosed easily during life generally occur after 6 months of age, owing partly to the fact that most infants with unusual heart defects die in the first 6 months of life.

Gy. Gorácz, L. Király

(Department of Pathology, P. Heim Children's Hospital, Budapest)

Myocardial Changes in Connatal Heart Defect

In the hospital's necropsy material cases of connatal heart defect have been studied in which, owing to right to left shunt, mixed blood was flowing in the coronary blood vessels. It has been found that the anoxid damage to the heart muscle is characterised in the first place by a pathological water uptake and necrosis extending to single fibres. The myocardial changes developing in connexion with connatal heart disease have a decisive role in the fatal outcome.

R. Jankovics, J. Simon, I. Kő

(Department of Pathology, Semmelweis Hospital, Budapest

Teratogenic Effect of Drugs Taken during Pregnancy

Several years data for perinatal mortality in Pest county have been analysed with special reference to drugs taken by the mother during pregnancy and to the changes they may have produced in the foetus. Special attention was focussed on foetal damage caused by barbiturates, these being the drugs most mothers stated to have taken.

In animal experiments the effect on the foetus of phenolbarbital administered during pregnancy has been studied. The results obtained indicate that in toxic doses phenobarbital exerts a teratogenic action; low doses influenced growth of the foetus.

In a comparative study it has been found that even if administered in toxic doses glutethimide is less noxious for the foetus than phenolbarbital.

J. Temes, G. Tóth

(Department of Pathology, László Hospital, Budapest)

Hepatitis and Pregnancy

The course of pregnancies complicated with infection hepatitis observed in the period 1957—64 has been studied with special reference to the result (abortion, premature or normal delivery), and the fate of the babies. Autopsy and histological findings of such cases were analysed in detail.

For comparison pregnancies complicated by non-infectious jaundice were studied, with special attention devoted to the serum bilirubin level, liver function and other factors involved.

P. Kertai, M. Sajgó, E. Czeizel

(National Institute of Public Health, Budapest)

Endotoxin-Foetopathy

Typhoid endotoxin, injected intraperitoneally in doses of 0,08 ml/100 g into pregnant rats before nidation and during organogenesis, did not harm the zygote or the embryo. Similar treatment in the last third of pregnancy killed the foetuses within 24 hours. With a view to studying the mechanisms at play necrosed and macerated foetuses and placentas were subjected to histological study and placental O_2-consumption and lactic-acid synthesis, as also the DNA and RNA-contents of the placentas and the foetal livers were assayed.

J. Molnár, L. Nagy

(Institute of Urology and Second Institute of Pathology, University Medical School, Budapest)

Teratospermia

In almost every case of pathospermatism there are a few teratoid sperms to be found. Sometimes they are present in astonishingly high numbers. These cases are termed teratospermia. The teratoid changes occur in the first place in the head of the spermatozoon, but they may be detected in the middle piece and tail as well. The morphology is variable. As to the fertilizing ability of such spermatozoa, it seems certain that only few of them contact the ovum, because their advance, incordinated owing to the deformed shape, is interfered with and also because they move at a speed much slower than the usual 3 mm/min. If amphimixis takes place, the offspring will probably show developmental defects. There is no decisive proof that this would happen in man, but the results of animal experiments confirm this view. In the genesis of teratoid sperma radiation effects, too, may play a role; such cases have occured in our material as well. It is likely that the spermatogenetic cells are affected in the first place and thus give rise to teratoid sperms. — A direct action on the spermatozoa is less significant. Finally a few pertaining observations (teratospermia in certain diseases, etc.) are described.

J. Bazsó, L. Karmazsin, K. Gelei

(Clinic of Obstetrics and Gynaecology, and Clinic of Pediatrics, University Medical School, Debrecen, and Municipal Neurologic Station for School-children, Debrecen)

Physical and Mental Development in Childhood Following Intrauterine Growth Retardation

Beside the reduction of the perinatal and infantile mortality rates, one of the main tasks of modern obstetrics and pediatrics is to recognize and combat all those prenatal factors, which may produce permanent physical or mental damages of the surviving infants.

In spite of the precariousness of gestational estimates and signs determining the prenatal age of newborns, it is well known, that children are born a birth weight of less than 2500 g at or near full-term. From 1950 to 1962 the incidence of such newborns was 1.13 per cent in the single-birth material of the Obstetrical Clinic of Debrecen.

Studies of the physical and mental development of 103 children, born with intrauterine growth retardation and now aged from 2 to 14 years, indicate that the growth disturbance suffered during intrauterine life continues unsatisfactorily after birth and leads to temporary or permanent stunted growth and (or mental deficiency in very many cases among 103 children were physically under the average: 50.4%, and I. Q. under 90 : 33.9%).

An analysis of the complications of pregnancy or associated with pregnancy and *the dysfunction of the placenta* observed in many cases suggests that a variety of factors may give rise to intrauterine growth retardation.

The cause of the difference between the development of infants born at term with low birth-weight and the subsequent childhood development of truly premature children may well be a permanent brain damage caused by the prolonged deprivation in utero.

K. Méhes

(Institute of Pathology, University Medical School, Pécs)

Practical Value of Sex Chromatin Examination

While sex chromatin examination is a simple and useful method of genetic sex determination, the sources of error inherent in the procedure should not be disregarded.

The distribution of and changes occuring in the sex chromatins of the epithelial and other somatic cells afford reliable information only if several preparations, stained with differ-

ent methods, yield identical results. Among the nuclear projections of neutrophils only the so-called drumsticks (forms A) should be regarded as sex-specific.

The possibilities and limitations of sexchromatin determination could be well observed during the examination of 104 individuals whose clinical symptoms pointed to intersexuality. Surgical and laboratory results, further the sexchromatin findings revealed intersexuality in 31 cases. It was the sex chromatin examination which turned attention to the genetic anomaly in 11 cases; it confirmed the diagnosis in 18 cases, while it caused momentary confusion by misleading results in 2 cases.

Complete chromosome analyses are only suitable for the exact elucidation of developmental genital anomalies. The present practical value of sex-chromation examinations consists in their being simple, easily realizable and often revealing the existence of certain chromosomal anomalies.

B. Flerkó, P. Petrusz, C. Muha

(Institute of Anatomy, University Medical School, Pécs)

The Significance of Androgens in the Sexual Differentiation of the Hypothalamus

There is ample experimental evidence to prove that the hypothalamus is exclusively responsible for the male-type, i.e. continuous, and for the female-type, i.e. cyclic production of the gonadotrophic hormones, and at the hypophysial level is no difference in this respect between male and female.

According to the data in the literature and our own investigations, however, at birth the hypothalamus is capable of maintaining cyclic production of gonadotrophic hormones in both sexes, but it loses this ability in the male a few days after birth, first of all in response to the androgens produced by the testicles. In the present experiments the mechanism of this effect of the androgens has been subjected to a detailed study.

The results suggest that, at least in part, the androgens give rise to sexual differentiation by desensitizing in the anterior hypothalamus the nervous elements sensitive to sexual steroids, the intactness of which is indispensable for the maintenance of cyclic gonadal function.

In the second group of experiments it has been investigated why the androgens present during intrauterine life fail to exert their effect on the hypothalamus before birth. The results indicate that this is made impossible not by an underdevelopment of the hypothalamus, but by the sexual hormonal milieu of the maternal organism.

A. Traub, Sz. Virágh, F. Szontágh

(Clinic of Obstetrics and Gynaecology, and Institute of Pathology, University Medical School, Szeged, and Institute of Histology and Embryology, University Medical School, Budapest)

The Electron Microscopic Structure of the Human Placenta during the First Trimester of Pregnancy

Chorionic villi obtained at interruption of normal pregnancies of young and healthy women have been subjected to electron microscopic study. The main results may be outlined as follows.

a) The syncytiotrophoblasts form a true syncytium. On their surface large numbers of microvilli can ge found (like on these intestinal and renal epithelial cells actively involved in fluid transport). Some of the vesicles visible in the cytoplasm originate from pinocytosis, others may be part on the endoplasmic reticulum. Of the corpuscles in the cytoplasm some may represent hormones. The syncytium and Langhans's cells are connected by desmosomes.

b) The cytotrophoblasts (cells of Langhans), mononuclear cells with a light cytoplasm, are attached to the thick basement membrane of the chronic epithelium, and emit microvilli toward the interstitial interspaces. Granules believed to be those of steroid hormones (Terzakis) occur in smaller numbers in this kind of cell.

c) Among the stromal cells fibroblasts, cells of Hofbauer, mesenchymal cells and plasma cells, which occur rarely, may be distinguished.

d) The blood vessels of the villi show certain differences from the vessels of other organs.

M. Scholz

(Department of Pathology, Municipal Uzsoki Hospital, Budapest)

The Myocardium of Infants Died with Congenital Heart Defect

It has been shown that in early age congenital heart defects are accompanied by grave alterations in the histological structure of the coronary arteries. The degree of the lesions does not depend on the type of defect as rather on the age of the child, i.e. the duration of the cardiac deformity. Myocardial lesions consequent upon coronary damage have been surveyed in the necropsy records from the period 1954 to 1964. The material contained 67 cases of congenital heart disease, defect of atrial septum (2 cases); defect of ventricular septum (14 cases); cor biatriatum monoventriculare (2 cases); cor monoatriatum biventriculare (1 case); cor biloculare (1 case); tetralogy of Fallot (2 cases); corrected transposition (2 cases); common arterial trunk (3 cases); congenital pulmonary stenosis (1 case); adult type stenosis of aortici sthmus (10 cases); juvenile type stenosis of aortic isthmus (5 cases); patent foramen ovale and patent ductus arteriosus (21 cases); congenital fibroelastosis (3 cases). The age of the subjects varied from stillborn to children of 4 years.

Sections made from different points of the heart musculature were stained with haematoxylin-eosin, Van Gieson's stain, Masson's trichrome, Heidenhain's iron haematoxylin and Gomori's silver impregnation; carbohydrate components were demonstrated with Best's carmine and Schiff's periodic acid leukofuchsin, nucleoproteins by means of Feulgen's method, fats and lipoids with Scharlach R- and Lorrain Smith's Nile blue; in some cases the "Nadi"-oxidase reaction was also performed.

Routine procedures revealed parenchymatous, vacuolar and hydropic degeneration of the heartmuscle fibres. Striated fibres disappear or become scarce in the myocardium at an early stage. Proliferation of connective tissue was not observed. The amount of glycogen and polysaccharides was considerably diminished. The presence of neutral fats and lipoids substances was especially conspicuous in the myocardium of infants surviving for some length of time. Acid and alkaline phosphatase activity of the heart muscle fibres was considerably reduced. Compared with that of normal children of equal age, the results of Feulgen's reaction showed no significant discrepancy.

The myocardium of infants and children suffering from congenital heart defects displays grave degeneration without other symptoms of anoxia (e.g. accummulation of connective tissues).

L. Liszkay, F. Kneiszl

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Intrauterine Vessel Changes in Premature Infants

In infants died with hyalin-membrane, productive pulmonary vessel changes of intrauterine origin have been found. Therefore, 30 premature infants were studied for vessel changes; such findings were made in about two-thirds of the material. The vessel changes had begun or developed during foetal life.

Julia Szentner, J. Juhász, A. Bajtai

(First Institute of Pathological Anatomy and Experimental Cancer Research, University Medical School, Budapest)

Connective Tissue and Vascular Changes in Osteogenesis Imperfecta Congenita

According to the present view, imperfect osteogenesis is a hereditary systemic connective tissue disease presenting three forms: congenital, infantile and late. Opinions vary as to the incidence of the congenital form, FREDA in 1961 collected 106 cases from his own material and from reports in the literature. According to certain data, the congenital disease is rarely inherited.

Four cases of congenital imperfect osteogenesis have been studied. In none of these instances was there a similar change in the parents, or even in the family.

The changes were found mainly in the bones. Although osteoblasts occured at sites in considerable numbers, they were spindle-shaped, deviating from the normal. As a result, there was a marked disturbance in the genesis of the bony trabecules. Microcysts were found to have replaced the trabecules, which were absorbed in many parts; in other parts, areas of angiomatosis were visible. Attention is drawn to the changes of the periosteal blood vessels, which show an increase of elastic fibres, intimal proliferation and narrowing of the lumen. Connective tissue changes occuring in imperfect osteogenesis and constituting part of the mesenchymal developmental defect, are described.

K. Viszlóy, Gy. Kasza

(Department of Pathology, Municipal Hospital, Ózd)

Polarization-Optical Study of Imperfect Osteogenesis

Tissues taken from individuals suffering from imperfect osteogenesis, a congenital abnormality, contain collagen as does the normal tissue, with the difference that the formation of polysaccharide ground substance necessary for the collagenous structure is increased. This is supposed to lead to the development of fibres of abnormal submicroscopic structure. Fibres of this kind desintegrate spontaneously under certain endogenous or exogenous influences. This would explain why high polysaccharide contents and polysaccharide orientation were observed in certain areas (sinciput), while the original polysaccharide content of the fibres was just perceptible in other areas (ribs, femur) where the rapid disintegration made it impossible to demonstrate their fibrillar orientation.

T. Vizkelety

(Clinic of Orthopaedics, University Medical School, Budapest)

Hereditary Properties of Congenital Dysostoses

Preformation and formation of bone proceed in various ways and with various intensity during intrauterine life and after birth. The inherited pathological gene state may give rise, to a systemic mesenchymal lesion and on this basis to a generalized disturbance of ossification but may also act, as a predisposing factor, on grounds of which the disease will manifest itself differently in response to the influence of extrinsic factors. An example for this are the localized forms of enchondral dysostosis.

The disease may show characteristic sex variations. In the case of an enchondral dysostosis appearing in similar form in the members of one family, a similar gene damage may be suspected. When the symptoms are different, extraneous factors may be at play. The time factor is involved in the sense of Murk Jansen's law, and influenced the form of appearance and the character of the condition. A case is demonstrated which at the age of 12 years had presented a severe ossification disturbance in the bones of the hand while no sign of malformation was visible at the age of 18 years.

The course of inheritance is often irregular. Even most extensive and detailed familial studies have failed to demonstrate a regular course of inheritance in every case.

G. Miklós, J. Marton

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Foetal Adrenocortical Megalocytosis, with Special Reference to its Relationship to Certain Developmental Defects

Systematic examinations revealed an increase in the number of megalocytes in the foetal adrenal cortex in 5 out of 80 unselected foetuses and newborns. In three cases the adrenocortical megalocytosis was marked, in two cases it was slight. The cells varied from 50 to 120 μ in diameter, had hyperchromic nuclei, the nucleus to cytoplasm relation was normal, the cytoplasm was eosinophilic, finely granulated or vacuolized, and contained sometimes inclusion-like globules. Polynuclear cells were rarely observed. The megalocytes were found around the abundant sinusoid vascular network in the foetal zone, accompanied by many regressive, necrotic cells. No megalocytes were found in the outer zone of the adrenal cortex and the medulla. The adrenals exhibited no gross change; they were not enlarged. In the cases exhibiting severe megalocytosis the diagnoses revealed at necropsy were:

1. Tetralogy of Fallot and polydactylia; 2. Intracranial haemorrhage; 3. Icterus gravis, in the slight forms they were, 4. transposition of the great vessels with ventricular septal defect; 5. common arterial trunk, ventricular septal defect, renal dystopia; hyospadias undescended testicles, and external genital organs of feminine character. Of the three cases with disorders of development 2 were boys, the third, showed a predominance of masculine character; the remaining two were girls.

There is scarce evidence in the literature as to the occurence of megalocytes in late foetal- or newborn age. The data relative to the terminology, origin, frequency and evaluation of the megalocytes are divergent. HETT in 1925 was the first to mention these mononuclear giant cells of the adrenals, which in the course of normal development are present during the second month of foetal life and disappear during the 4th and 5th months. Their presence in the late foetal period or in newborns is indicative of a pathological condition (hyperplasia, tumour). CRAIG and LANDING consider them to be anaplastic cells resembling cells of the adrenocortical cancer responsible for Cushing's syndrome in adults, though there are no mitotic forms among them. As opposed to this BEATTY and HAWES correlate the cells in question with involution of the foetal cortex. POTTER described them as cases of adrenal cytomegaly, but did not consider the change to be identical with the cytomegalic inclusion disease due to a viral infection of the salivary gland, though he could find inclusion bodies of the so-called Cowdry B type. We, too, suggest the term "*megalocytosis*", to underline the differences from cytomegaly. The incidence of such cells in newborns has been estimated from 1 to 6.5 per cent by different authors. Most authors ascribe the appearance of such cells in late foetal life to toxic intrauterine factors, but Rh incompatibility and asphyxia may also play a role. It is particularly remarkable how often they occur together with developmental defects. It has been proved that the megalocytes would have hormonal activity. There are few, and mostly contradictory, data as to their eventual correlation with the adrenogenital syndrome.

In the present material in 3 cases such cells were found together with developmental defects in other organs in 3 cases. In one case they were associated with icterus gravis due to Rh incompatibility, and it was in one case only that their presence was not accompanied by developmental disorders. The frequent occurence in combination with the latter suggests a common teratogenetic effect. The cell-deformity change corresponds to a hyperplasia of embryonic cells. The possibility of a malignant tumour arises at the most in the *in situ* form described by MALLORY, because the nucleus cytoplasm relation is normal and there are no mitotic forms to be found. However, megalocytes may play a role in the development and growth of adrenocortical cancers in infancy and childhood.

S. Ferenczy

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Complete Duplications of the Large Intestine

In man, duplications of the gastro-intestinal tract and especially those of the large intestine are rare developmental disorders. Such malformations are much better known in veterinary pathology (JÁRMAI) they have been observed by JOST and KOCH, as well as HOLZBAUER in birds and cattle.

Partial duplications occur more frequently. Complete duplications have been described in the literature in a total of 28 cases, viz. SUPPIGER 1876, LESBRE 1878, PICCOLI 1892, BLANK 1896, GROHÉ 1900, VOLPE 1903, BAR 1909, BALLANCE 1930, ASAI 1936, OMBRÉDANNE 1936, GRAY 1940, MONTAU and DENNY 1945, WEBER and DIXON 1946, BRUNSCHWIG—DARGEON—RUSSEL 1948, ALARD—ROSS—HOPKIRKE 1949, AITKEN 1950, FISCHER 1950, DIAZ 1951, ZWALENBERG 1952, NEVILLE 1953, RAVITSCH 1953, FELLMANN 1955, VALZER—BURRICH—JENKINSON 1956, BERNSTEIN 1957, JUDY 1957, COOK—ZINGER—FRENK 1960, DAN 1960 and OECONOMOPOULOS—DARGEON—RUSSEL 1962.

Of these 28 cases only 7 were examined post mortem, the rest were diagnosed at operation or radiologically. It is clear from the above reports that the duplication is always associated with other developmental defects, mostly of the rectum and the urogenital system, there was one single instance where the duplication occured alone.

The following two cases have been observed.

1. In a stillborn male infant, 48 cm long, with a head circumference of 33 cm, the large intestine was completely duplicated, the duplication extended to every layer; the rectum was absent. Bilateraly polycistic kidneys, hydroureters and urethral atresia were also present.

2. A premature female infant, 34 cm long who had lived 5 hours at necropsy showed duplication of the large intestine a defect of the abdominal wall and fissure of the urinary bladder. Atresia ani, dorsal rachischisis, bilateral hydronephrosis and hydroureter, as well as an urachal cyst were also present.

Thus, in our cases a variety of malformations accompanied the duplication of the large intestine, involving in both cases the rectum, kidneys and ureters.

No data relative to the genetics of the disorder could be found. In Case 2 the mother, a primipara 22 years old, in the earliest phase of her pregnancy had been spraying DDT and subsequently had been sick for 2 days. Similar contacts with DDT have been observed by the author in connection with other developmental defects.

J. Kovács

(Department of Pathology, Hospital of the Zala County Council, Zalaegerszeg)

Congenital Agenesis of the Kidney

Congenital agenesis of the kidney is a rare developmental defect, especially on both sides. According to BELL, DAVIDSON and ROSS, as well as BELL and NATION about 230 cases have been described. BELL found the condition in 21 cases among 59.000, NATION in 3 out of 27.000 necropsies. Bilateral agenesis of the kidney is often associated with other developmental defects. Some of the patients are stillborn.

Of the 317 developmental disorders found during 10 years, 7 were cases of renal agenesis including 2 bilateral ones. Both babies were born alive. One of them lived 5, the other 25 minutes. Of the 5 cases of unilateral agenesis in 2 the solitary kidney showed congenital hydronephrosis and hydroureter. In both cases the direct cause of death was uraemia.

Unilateral agenesis of the kidney could be demonstrated in two males, one aged 35, the other 49 years, and in a female aged 72 years. None of them showed any other developmental defect.

The infant with bilateral agenesis, who had lived 5 minutes exhibited bilateral clawfoot and bilateral sternocleidoid agenesis. In the other case hypoplasia of the urinary bladder and an urachus cyst were found.

Two cases were transitory between unilateral and bilateral agenesis. In one of them the right kidney was absent, and in addition to a hypoplasia left kidney, Ellis—van Creveld's syndrome and a ventricular septal defect were found. In the other case (an infant aged four months) the left kidney and adrenal were absent, the right ureter was stenosed, the right kidney showed hydronephrosis, the Botall's duct was patent and the foramen ovale was also patent.

Zs. Csapó, Judit Lantos, B. Zolnay, J. Ormos

(Institute of Pathology, University Medical School, and Institute for Public Health, Szeged)

Experimental Pyelonephritis

Various methods have been applied for inducing pyelonephritis in rats by the intravenous administration of *Escherichia coli* originating from infected human urinary tracts. Injecting a bacterial suspension in itself was not sufficient for inducing changes in the rats. If, however, the unilateral ureter was ligated for 24 or 48 hours after administration of the bacterium suspension, in about 36 per cent of the animals acute apostematous pyelonephritis developed on the same side, to turn later into chronic scarred pyelonephritis. Chronic ligation of the ureter led to hydronephrosis, and in 65 per cent to pyonephrosis. It was also attempted to induce pyelonephritis by producing symmetrical renal cortical necrosis by the hormonal method described by Kovács and Dávid before administering the bacterial suspension; this method has failed and it was established by renal bacterial counts that the mentioned hormonal treatment did not influence the bacterial contents of the kidneys significantly. Ligation of one ureter for 48 hours increased the renal bacterial count already during the obstruction; after releasing the clamp the bacterial count still remained higher than in the other kidney.

J. Ormos

(Institute of Morbid Anatomy and Pathohistology, University Medical School, Szeged)

Electron Microscopic Studies of Renal Cortical Necrosis

An electron microscopical study has been made of symmetrical renal cortical necrosis induced hormonally by the method of Kovács and Dávid. The renal structures are affected in various degrees. The most severe changes are found in the proximal and distal convoluted tubules, but even there relatively intact tubular epithelial areas alternate with gravely damaged parts; significant differences can be found between adjacent cells, too. The cell body shows a more marked "oedematous" swelling than what occurs normally. The mitochondria dissociate and disintegrate, or become stunted. The brush border and the basal labyrinth gradually disappear. Many lipid-rich cytosomes appear, containing sometimes membraneous elements (lysosomes). The residues of the cell body constituents leave in part through the basement membrane toward the interstitium, while some are ejected into the lumen. After it has undergone necrosis the cell body is osmiophilic, displays a spotted structure, then, becoming gradually more and more homogeneous, enters the lumen and forms a cylinder. 48 hours later among the necrosed ones very light, undifferentiated cells occur: these might correspond to regenerated epithelium. Alongside the variable cytoplasmic changes the nuclei seldom show alterations, such as hyperchromatosis of the nuclear membrane. The tubular changes preponderate over the glomerular ones, which arise at a later time. It is first of all the podocytes that are damaged, showing for example a merging of the pedicle processes.

E. Szalay, I. Vargha

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Amyloid Glomerulonephrosis

Although amyloidosis (including that of the kidney) is in most cases diagnosed only *post mortem* (in 50 per cent in the present authors' material), a careful scrutiny of the clinical and morphological findings is necessary for a better understanding of the etiology, pathogenesis and the effect of new potent therapeutic agents.

12 cases (eight per cent) of the autopsies performed during 6 years on subjects who had died of renal disease revealed amyloidosis of the kidney. The ratio between primary and secondary amyloidoses (3 : 9) and the four tuberculous cases among the secondary amyloidoses do not show the potent action of new antibiotics and new antituberculous drugs described in recent literature. The interval between the first manifestation of the renal lesion and its fatal termination (due to uraemia in 50 per cent of the examined cases) was notably short. Cortisone treatment was found to be decidedly noxious.

It is emphasized that gross diagnosis is insufficient, that amyloidosis is often associated with other more patently manifest renal diseases (which may represent the primary lesion, e.g. pyelonephritis), and that the staining reactions are extremely variable. Increased attention to the disease would reveal the existence of many more cases.

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Vital Staining of Juxtaglomerular Granulated Cells

After administering intravenously or intraperitoneally a 1 per cent solution in physiological saline of different dyes (azine, oxazine, thiazine, azo, diazo, triphenylmethane, xanthene) to white mice, squash preparations of their kidneys were examined under the light microscope and in some cases for fluorescence. The results have confirmed the neutral red effect described by Japanese authors. We succeeded in staining the granules of the juxtaglomerular cells also with brilliant cresyl-blue, Nile-blue sulphate and acridine orange. The probable mechanism of staining has been discussed.

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Quantitative Examination of Glomerular Changes Following Experimental Renal Infarction

In experiments performed on dogs, guinea pigs and albino rats renal infarctions were induced by ligating and dividing the anterior or posterior (sometimes the polar) branch of the unilateral renal artery. After having made laboratory examinations during the infarctions, the animals were sacrificed at different intervals. Both kidneys were worked up histologically or injected with polyvinyl chloride. On the histological sections volumetric changes of the ligated and undamaged renal glomeruli were recorded and compared to those of the other renal structures by means of Palkovits—Csapó's nuclear variation table. Glomerular counts in the corrosion preparations were made according to Zolnai's method. After the infarction had developed, results obtained for the ligated and the undamaged kidney of the test animals were compared with those for the kidneys of control animals, taking the time-factor into consideration.

A. Bajtai, Klára Németh

(First Institute of Pathological Anatomy and Experimental Cancer Research, University Medical School, Budapest

Histological Changes After Cardiac Surgery and Resuscitation

Cardiac operations (interventions with extracorporeal circulation, ventriculotomy, commissurotomy, resuscitation), may give rise to different myocardial changes, which may decisively influence the success of the surgical intervention. The significance of such changes has been analysed on the evidence of 50 post-mortem findings.

Lesions observed in the heart are of three kinds.

The first group includes those very conspicuous infarctionlike necroses on both sides of the ventriculotomy which measure 3 to 4 mm in width and may extend over the entire thickness of the ventricular wall. Lesions of this nature are equivalent to infarctions both clinically and anatomically.

To the second category belongs the focal myocytolysis which, occurring independently of the ventriculotomy in the surroundings of the operation area, manifests itself with a swelling and homogeneous staining of the muscle cells, weakening of nuclear staining, and a well-preserved sarcolemma. Diffuse necrosis with leukocytic infiltration and necrobiosis also occurs. The latter form of the lesion is characterized by oedematous swelling of the cells, enlargement of the nuclei, loosening and marked staining of the chromatin.

Haemorrhages constitute the third category. They may be subendocardial, subepicardial and also intramural suffusions. The intramural ones, by compressing the branches of the coronary artery, lead to myocardial necrosis. Haemorrhages in the ventricular septum promote post-operative complications by damaging the A-V bundle.

Gy. Nagy, L. Csanádi

(Department of Pathological Anatomy, Bugát Pál Hospital, Gyöngyös)

Aetiology of Non-Rheumatic Myocarditis

The autopsy material of the hospital in the period 1960 to 1963 contained 11 cases of acute and one case of chronic non-rheumatic myocarditis, out of a total of 1579. Five of the acute cases were myocarditis of the Fiedler type in infants or children. There is an increasing number of clinicians and virologists who hold that Fiedler's myocarditis is of viral origin. This theory has been substantiated by the present morphological findings. Four of the cases of Fiedler type myocarditis occured within 5 months, so that one is justified in speaking of a moderate epidemic. One case appeared after smallpox vaccination which may have constituted a pathogenic factor. Virological investigations will probably help in elucidating the aetiology of Fiedler's myocarditis.

B. Veress, T. Kerényi, I. Hüttner, H. Jellinek

(Second Institute of Pathology, University Medical School, Budapest)

Submicroscopic Study of the Phases of Muscle Necrosis

Mallory's phosphotungstic acid haematoxylin has proved to be the stain of choice for visualizing muscle necroses. It was possible to distinguish three phases in the necrosis of muscle fibres, (a) an initial phase in which the dye was intensively bound; (b) a second phase in which a granulated disintegration of muscle fibres is visible; (c) a third phase in which the dye failed to be bound. Polarisation, phase contrast and fluorescence microscop revealed that in the first phase the optical properties of the necrosed muscle fibres were considerably different from those of normal muscles, whereas a structural desorientation could be seen in the later phases. Transitory forms were also observed. The intensity of the damaging factor influences the picture and determines the morphological changes.

P. Röhlich, I. Törő

(Institute of Histology and Embriology, University Medical School, Budapest)

Early Phase of Phagocytosis in the Mesenteric Lymph Node

The phagocytosis of chyle particles has been examined in the reticular cells of the marginal sinus of mesenteric lymph nodes. Adsorption of particles on the surface of the cell membrane may be regarded as the first phase of phagocytosis. A moderately electron-

dense layer, about 500 Å in thickness, separates the particles from the osmophilic portion of the cell membrane. The cell membrane then forms a fold into the cytoplasm so that the moderately electron-dense layer and the particles attached to it gain access into the fold. The invaginated part becomes detached from the cell membrane and changes into a round phagocytotic vacuole. The moderately electron-dense layer remains on its inner surface for a long time. A delicate striation perpendicular to the surface was also observed; it consists of fine filamentous structures measuring about 20 Å in thickness.

T. Kerényi, L. Haranghy, I. Hüttner, B. Veress

(Second Institute of Pathology, University Medical School, Budapest)

"Senile Pigmentation" in Young and Old Age

The accumulation of "senile" pigments at selected points of the central nervous system has been studied in human subjects and in rat experiments. The morphological and histological properties and intracellular arrangement of the pigments have been studied and electron-microscopic examinations were made to elucidate their fine structure and origin.

L. Barla Szabó

(Department of Pathology, Uzsoki Street Hospital, Budapest)

Development of Cutaneous Pigmentation in Man and in Mammals

It has been observed by ZIMMERMANN and BECKER JR. that in certain cephalic regions of negro embryos melanocytes filled with native melanin, revealed in unstained sections by their brown colour, have been found as early as in the 3rd month of foetal growth. The authors quoted do not discuss the fate of these melanocytes rich in native melanin in the course of further development, but they mention that they did not find any native brown melanin in the epidermal basal cells of "dopa stained" of negro newborns. In the present studies similar melanocytes rich in melanin have been found in the outer acoustic meatus and nasal skin of white human embryos aged 3 to 5 months. The finding is interesting because

1. ample quantities of similar native melanin-containing melanocytes, developed in the course of intrauterine life, occur in Caucasian and coloured adults only in the hair follicles;
2. pigmentation due to melanocytes of the same type can be found in most mammals in these areas not covered by hair, so on the head, in the first place,
3. the native melanin-bearing melanocytes observed in the outer acoustic meatus of Caucasian embryos were at a high level, i.e. about to be ejected.

To interpret this finding, the pigment-producing system of mammals and man is compared from the point of view of morphology and function. According to REYNOLDS the epidermal melanocytes of mammals are

1. pigmental melanocytes, capable of melanin production;
2. pigmented or active melanocytes, containing visible melanin;
3. non pigmented melanocytes, which do not contain and cannot be induced to form melanin.

The pigmented epidermal melanocytes capable of producing melanin can be activated by chemicals, but according to QUEVEDO JR. and ISHERWOOD only in the case of growing hair, suggesting that in mammals epidermal pigmentation is correlated with pigmentation of the hair.

In Caucasian and coloured human races melanocytes showing such a constant and ample pigment content as displayed by the active melanocytes in the non-furred mammalian skin occur exclusively in the hair follicles. The epidermal melanocytes of humans are of the so-called "clear cell" type of MASSON; their activity especially in the Caucasian race depends upon ultraviolet radiation.

As far as the developmental relations are concerned, in the mammalian embryo the pigmented active melanocytes develop fully in the epidermis and hair follicles during intra-

uterine life. In the present studies it has been found that the pigmentation observed in Caucasian human embryos in the 3rd to 5th months of embryonal life and described also by ZIMMERMANN and BECKER JR, is temporary, as indicated also by the "high level" position of pigmented melanocytes in the outer acoustic meatus, and disappears after the 5th month of pregnancy. From then on till foetal maturation no native brown melanin can be demonstrated in the epidermal melanocytes, and the complete epidermal pigmentation reacting to ultraviolet radiation only develops during the first months of extrauterine life. Therefore, brown melanin granules can be observed in the epidermis of Caucasian and coloured races only after birth. Before birth the epidermal granules impregnable with silver consist of premelanin.

In primates (the anthropoid apes) the mammalian pigment composed of active pigmentes melanocytes and developed during intrauterine life is marked in the skin of the face and the ear, including the inner ear, i.e. the sites where the hairs disappear with the advance of development. On this basis the similar pigmentation occurring in the ear and nasal skin of the human embryo seems to be a vestige indicative of man's relationship with the primates.

Gy. Földes, T. Holló

(Postgraduate Medical School, Budapest)

Generalized Herpes Simplex

Herpes simplex, an acute vesicular eruption affecting the skin, the eye and the mucous membranes of the external orifices of the body, has long been known as a viral disease. In 1919, GOODPASTURE succeeded in inducing it in the embryo. LIPSCHÜTZ subsequently demonstrated the presence of eosinophile inclusion of herpes simplex. It was long believed that the virus did not affect the internal organs. HASS described in 1935 post-mortem findings made in a premature of 14 days who displayed necroses surrounded by a haemorrhagic zone in the liver and the suprarenal gland. HASS observed the eosinophile inclusion bodies of LIPSCHÜTZ in the intact cells around the necrosed areas, and diagnosed, on the evidence of this observation, the case as one of herpes simplex.

Since then, herpes virus infections have often been diagnosed on the strength of pathologic changes alone. Several authors (e.g. DEBRÉ, LELONG, ZUELZER) succeeded in demonstrating the virus in addition to the characteristic histological changes. Cases with fatal termination have been described in infants only.

The case of a premature female infant of 10 days is reported whose autopsy revealed the above-described lesions. The mother of the baby had had a herpetic infection in the second half of pregnancy.

P. Bianchini

(Modena)

Some Aspects of the Clearing Factor Problem

Two groups of experiments have been performed to study two different aspects of the CF problem. The first one is the exhaustion of the CF producing or repeated injections of small doses of heparin (250 μg/kg) (rat) cause a decrease of blood. CF activity in exhaustion is almost complete after four injections of heparin but the CF releasing system can be completely restored after 40 minutes.

The second one is the inhibition of the CF system. Some inhibitors of CF both in vitro and in vivo (Triton WR 1335, salmine sulphate, supernatant of India Ink) cause an increase of blood triglycerides. This inhibition does not seem complete despite the fact that no activity is shown by the Ediol test. In fact the injection of heparin in CF blocked rats (on Ediol test) is effective in reducing plasmatriglycerides.

Two hypotheses are offered. The existence of two different enzymatic systems, both activated by heparin, is supposed. It is however possible that injection of heparin causes a release of CF inactivated substrate-complexes from blood vessel walls and that new enzymes take place on the cellular surface.

G. Lusztig

(Section of Pathology, County Council Hospital, Kecskemét)

Blood Coagulation Factors and Atherosclerosis

Some factors have been studied which are capable of influencing coagulation, the heparin houshold, and the development of atherosclerosis. The investigations were extended to the role of macromolecular substances, histamine, old age, alimentary lipaemia, further to interconnection between certain hormonal factors and endogenous heparin, as also to the effect of chemical thyroidectomy, thyroid hormone and thyrotrophin on certain factors of blood clotting.

(1) High molecular dextran liberates heparin or a heparin-like substance and produces, at the same time, a histaminic effect.

(2) The fact that the hyperheparinaemia appearing on dextran treatment does not affect coagulation is presumably due to the concomitant increase in the blood histamin level.

(3) The blood heparin level is inversely related to the amount of total lipids. Rise in the lipide level of the blood is preceded by a drop of the heparin level in cases of alimentary hyperlipaemia, and vice versa.

(4) The blood heparin level in aged femals falls below the earlier level in 75 per cent of the cases. This phenomenon is somewhat less frequent in males.

(5) The amount of endogenous heparin is increased threefold in certain infectious diseases such as hepatitis, measles and scarlet fever.

(6) The amount of endogenous heparin diminishes in cases of hypothyroidism and invariably increases in those of hyperthyroidism.

(7) Treatment with thyroid extract does not influence the amount of endogenous heparin in rabbits, while the level of heparin falls, the clearing effect is antagonized, and coagulopathy develops after chemical thyroidectomy. Treatment with thyroid extract eliminates the coagulation disturbances.

(8) Thyrotrophin does not seem to affect the heparin level and the examined blood coagulation factors.

The investigations were designed to show that most factors involved in the pathogenesis of atherosclerosis are at the same time capable of influencing one or the other factor of blood coagulation.

L. Józsa

(Department of Pathology, County Council Hospital, Kecskemét)

Effect of Thyrotrophin and Thyroid Hormone on the Composition of the Aortic Wall

Biochemical and histochemical investigations have been carried out to study the effect of the pituitary-thyroid system on the acid mucopoly-saccharide content in the aortic wall of animals. Normal animals, further animals treated with thyrotrophin or thyroid hormone, as also thyroidectomized animals were used. In addition to the acid mucopolysaccharide contents, the proportion and amount of chondroitin sulphate A + C, chondroitin sulphate B, hyaluronic acid and other fractions, further the amount of glycoprotein were determined.

It was found that in the aortic wall the acid mucopolysaccharide content increased by 79.9 per cent after the administration of thyrotrophin and by 117.8 per cent after chemical thyroidectomy, whereas it decreased, on the average, by 43.3 per cent following treatment with thyroid hormone. Treatment with thyrotrophin and thiamiazole reduced the fraction of chondroitin sulphate B and elevated the hyaluronic acid contents. Thyrotrophin caused a considerable rise in the amount of chondroitin sulphate A + C while chemical thyroidectomy increased it but moderately. The other fractions (heparin, heparitin sulphate) were reduced by treatment with thyrotrophin and thyroid hormone, and notably increased by chemical thyroidectomy.

The significance of biochemical changes and their role in the diseases of vessel walls have been discussed and the results of animal experiments compared with observations made on the aorta of human subjects suffering from thyroid deficiency.

H. Jellinek, I. Hüttner, T. Kerényi, B. Veress, Éva Konyár, Klára Szentágothai

(Second Institute of Pathology, University Medical School, Budapest)

Fibrinoid Necrosis of Small Blood Vessels

In various experiments it has been observed that the smooth muscle cells play a decisive role in the developments of fibrinoid necrosis of the vascular wall. The early stages of smooth muscle cell necrosis are readily demonstrated by different methods. Necrosis of smooth muscle cells develops on painting with acid, in hypertensive states, in response to nor-adrenaline treatment. Imbibition with plasma, which together with muscle necrosis constitutes the "fibrinoid substance", may vary in intensity. In small vessels not possessing an elastic membrane, imbibition with plasma and muscle necrosis develop simultaneously. The internal elastic membrane reduces the rate and extent of imbibition. In fibrinoid necrosis appears in dependance on the severity of the lesion. Muscle necrosis, plasma imbibition and the precipitation of fibrin can readily be distinguished from one another by staining methods, polarization optical analysis, fluorescence microscopic examination, and phase contrast microscopy.

Anna Kádár, Éva Konyár, H. Jellinek, F. Lajosi

(Second Institute of Pathology, University Medical School, Budapest)

Fibrinous Vessel-Wall Necrosis Induced by Horse Serum

Periarteriitis nodosa has been induced in rabbits by means of horse serum according to RICH et al., and the affected minor vessels of the muscular type have been studied.

Vascular changes were observed in various parenchymatous organs, lungs, heart, kidneys and the intestinal submucosal vessels. Various staining methods, and polarization optical methods were applied to follow the successive phases of the vascular changes from their earliest plasmatic manifestations to the final fibrinoid necrosis.

The vascular changes observed in these experiments were identical with those induced by hypertension experimental or noradrenaline treatment.

L. Józsa, Mária Perneczky, Gy. Szederkényi, G. Lusztig

(Department of Pathology, County Council Hospital, Kecskemét)

Role of the Pituitary-Thyroid System in the Regulation of Serumal Mucopolysaccharides

Investigations into the effect of chemical thyroidectomy, thyrotrophin and thyroid hormone on the mucopolysaccharides in the blood of rabbits have led to the following conclusions.

(1) Thyrotrophin and chemical thyroidectomy induce a significant rise in the blood level of glycoprotein, mucoprotein, hexosamine, sialic acid and total mucopolysaccharides. The effect takes about 14 days to develop.

(2) The said changes are less pronounced after the administration of thyroid hormone.

(3) The effects of chemical thyroidectomy are antagonized by thyroid hormone.

It is assumed, that the thyroid is just an intermediary factor in the regulation of the blood mucopolysaccharide level and thyrotrophin is thought to be the dominant factor in this respect.

L. Nagy, Klára Szemenyei

(Second Institute of Pathology, University Medical School, Budapest)

Vascular Changes in Rats Painted with HCl and Fed with Cholesterol

The abdominal aorta and the femoral artery of rats were painted with concentrated hydrochloric acid, and the animals were then fed with cholesterol. The appearance and incorporation of cholesterol in the impaired vessel walls, as observed by means of different staining procedures and seen under the polarization microscope is discussed and a survey of the pertinent literature is offered.

I. Hüttner, Éva Konyár, B. Veress, T. Kerényi, A. Tóth, H. Jellinek

(Second Institute of Pathology, University Medical School, Budapest)

Enzymatic and Histochemical Observations in Rats with Experimental Hypertension

Earlier investigations of the present authors have shown that non-specific esterase and alkali phosphatase reactions provide the best information about lesions of the vessel walls.

The present experiments were designed to study, mainly by means of the said enzymatic reactions, the damaged minor vessels of the muscular type in rats with hypertension.

Non-specific esterase activity was found to be vigorous before, and cease after, the necrosis of muscle cells. A similar phenomenon was observed in the first phase of the necrosis of striated and heart muscles. The non-specific esterase reaction is thus suitable for the study of new cells involved in the process of regeneration, and also for a determination of the necrobiotic condition of the cells. The adventitial alkali phosphatase network around the media of the small vessels showed increased activity in experimental hypertension.

J. Balogh

(Department of Pathology, Balassa Hospital, Szekszárd)

Histology of the Coronary Vessels after Transitory Experimental Neurogenic Hypertension

Moderate hypertension has been induced in albino rats by unpleasant stimuli and the changes occurring in the coronary arteries, of the animals have been studied at different intervals after ending the stimulation. The observed morphological and histochemical changes and those occurring in the vessel walls, smooth muscles and elastic fibres, as also the interrelations of the phenomena are discussed. The correlation between the vascular changes, the degree and duration of hypertension and the changes provoked by it is analysed.

K. Farkas, M. Keller, D. Tanka

(State Institute of Rheumatology-Balneology, Budapest)

Enzyme Histochemical Changes on Exposure to Ultrasound

Rats were exposed to various doses of ultrasound to determine the effect of such treatment on enzymic activity. Sections of liver, kidney, spleen and skin have been studied.

From among the hydrolases the most characteristic change was shown by aminopeptidase, the activity of which was strongly reduced in the organs, especially in the spleen.

Redoxases were found to respond most sensitively to ultrasound; even low doses caused excessive reductions in activity.

J. Plank

(Prešov, ČSSR)

The Morphology of Congenital Atelectasis

The fetal atelectasis with cleft-like alveoli is generally known as the orthologic picture of the non-aerated lung. But this is only the structure of retracted lungs which has his origin at autopsy.

The lungs of newborn infants were fixed *in situ* in order to eliminate the post-mortem by autopsy caused retraction of the lungs. It was thus possible to study the true picture of the lung newborn infants, one that is in many regards different from the generally known retracted lungs.

Alveoli in the non-aerated newborn lung are similar to crumpled sacs but always distended. Their lumen contains a Hale-positive fluid. Numerous pad-like protrusions of the alveolar walls are a characteristic sign of non-aerated alveoli.

The aerated lung of newborn show globular expanded alveoli and smaller portions of cleft-like alveoli. In the unvetilated paravertebral parts the alveoli present the same picture as that seen in the lungs of stillborn babies. Atelectatic portions with cleft-like alveoli in the aerated part of the lungs should be regarded as a compensation for the expanded alveoli.

L. Wallacher, J. Balogh

(Department of Pathology, Balassa Hospital, Szekszárd)

Histology of the Carotid Body in Chronic Pulmonary Hypertension

The carotid bodies were examined histologically in 50 subjects dead with chronic *cor pulmonale* due to pulmonary parenchymal degeneration.

In all the cases right heart failure was revealed as the cause of death.

A significant increase was observed in the number of dark cells in the carotid bodies together with signs of hypoxia due to cardio-pulmonary damage.

L. Haranghy, Anna Kádár, Éva Konyár, Z. Kováts, M. Siska

(Second Institute of Anatomical Pathology, University Medical School, Budapest)

Transfer of Deals Tumour

Experiments with the transmission of Deals tumour have been reported at Perugia in 1961. Further 330 animals were inoculated through 45 passages from 1961 to the end of 1963; the rate of susceptibility rose to 81.2 per cent which means that not more than two of ten animals inoculated with the tumour were refractory; in contrast to the previous findings, now there were series in which the tumours has taken in every animal. Incrase in the susceptibility went hand in hand with certain histological changes. Of a total of 122 tumours 53 were sarcomas with small, and likewise 53 with large round cells; some other form of tumour was found in 5 cases among which only a single one proved to be fibrosarcoma. These figures show that the character of the tumours tends to become uniform in the course of years, that their overwhelming majority is of the indifferent type, and that the percentage of the more benign fibrosarcomas has dropped to 0.82 per cent. The tendency to form metastases has increased, and the rate of cardiac deposits was especially high (26 out of 83 cases). The tumours were subjected to chemical analysis; the contents of dry substance and ash, and in this the amount of sodium, potassium, calcium, inorganic salts, total N, and the amino acids contents were determined and compared to the analysis of Guérin's tumour. Except in respect of Ca, the chemical results showed no significant deviation from those obtained in the previous years.

Whereas in 1960 the calcium content was 400 mg per 100 g dry substance, it was 160 mg in 1962 and 80.2 mg in 1963, a statistically significant difference. The spleen of the tumorous animals contained 2.83 mg per 100 g of Ca against 5.43 mg per 100 g in the corresponding organ of controls of equal weight. The reticulum was found to have increased in the spleen of 94 out of 124 animals and around the follicles a homogeneous substance was observed in the spleen of 16 animals; this substance proved to be negative for amyloid and was weakly polarizing.

T. Tiboldi, Sz. Virágh, M. Julesz, K. Kovács, M. Hódi

(First Institute of Medicine, and Institute of Pathology, University Medical School, Szeged)

Oestrogen-Induced Adenohypophysial Tumour

Homologous adenohypophysial tissue was transplanted to the anterior chamber of white male rats. The animals were then treated with oestrogen for 6 months which induced neoplastic growth in the majority of both original pituitary and the grafts, a phenomenon to prove that pituitary tumours may develop even if the direct hypothalamic connexions have been interrupted. Tumours tissue excised from the tumour of the anterior chamber and transferred to the eye of other animals, continued to grow during oestrogen treatment. The tumour whether obtained from the "in situ" hypophysis or the graft was an adenoma consisting mostly of degranulated chromophobe cells. The pyroninophilia pointed to an increase of the RNA contents of the tumour cells. In the tumour cells the electron microscope revealed granules of variable amount and maturity with sizes exceeding 300 mμ. Only a few cells were devoid of secretory granules. The highly developed ergastoplasm and the fact that the extensive Golgi's apparatus containing even unripe secretory granules, points to a process of active protein synthesis in the cells.

G. Kendrey, Klára Németh

(First Institute of Pathological Anatomy and Experimental Cancer Research, University Medical School, Budapest)

Cytological Changes in Thioacetamide Carcinogenesis

In recent years, much attention has been paid to research concerning the mode of action of carcinogenic agents. For this purpose experiments are suitable, in which the changes can be followed up dynamically from the early phase till the development of cancer.

On this basis experiments have been performed with thioacetamide (TAA), a hepatocarcinogenic agent. Male rates maintained on a constant diet were given 50 mg/kg of TAA intraperitoneally daily during 6 months. The animals were sacrified successively, and the observations were continued until cholangiocellular cancer had developed.

In the earliest phase of carcinogenesis the glycogen disappears from the pericentral liver cells, at the same time the granular basophilia of the cytoplasm is replaced by diffuse basophilia. The liver cells, liver cell nuclei and especially the nucleoili, increase considerably in size in the centre of the lobule. As carcinogenesis progresses, these changes expand gradually and during the 4th to 6th weeks of the periphery of the lobules. Nuclear and cytoplasmic inclusions are seen in the chronic phase of treatment (4 to 6 months).

The cytological changes indicate that TAA interferes in the first place with hepatic nucleic acid and carbohydrate metabolism and there is reason to believe that this effect plays a role in the carcinogenesis. In this respect TAA occupies a peculiar position among the hepatocarcinogenic agents.

G. Honti, Gy. Putnoky

(County Polyclinic, Kecskemét, and Postgradual Medical School, Budapest)

Imidazole Ring and Carcinogenesis

Cancerous tissues contain no or hardly any histamine (FELDBERG, LOESER, ROSENTHAL). HATEM has demonstrated that several carcinogenic substances form complex compounds with histamine, and that carcinogenesis starts only after the elimination of histamine from the tissues. Achlorhydria, refractory to histamine and independent of the location of the tumour, has often been observed in cases of malignant cachexia. Inoculation of a suspension of tumour cells failed to take and neoplasmic growth was inhibited if, together with the inoculation, histamine was administered to the mice. The time of survival was longer in the animals so treated. Inhibition of neoplastic growth was observed also in mice treated with antihistamines. The essential difference between the effect of histamine and the examined antihistamines is that the life of the animals is prolonged by the former and curtailed by the latter. The virulence of tumour-cell suspensions pretreated with histamine increases, whereas isotonic chlorpyramine inhibits the growth of tumour cell *in vitro*. Tolazoline was found to be a less potent inhibitor.

The fact that both histamine and antihistamines seemed to produce a similar effect, drew attention to the similarity of their chemical structure. It is known that, for instance, the antazoline molecule contains an imidazole ring which, in several antihistamines is substituted by a dimethylamino group. The amine, as in histamine, is linked to it by the ethylene group. Supposing that histamine, the antihistamines and the carcinogens (assuming an indirect action in this connection) have the same point of attack, the problem arises as to the location of this point. The experiments of HUSZÁK and DOMOKOS have shown antihistamines to be incorporated by cytochrome B.

Z. Vass, Z. Erdei, I. Tapasztó

(Department of Ophtalmology, University Medical School, Szeged)

Myoepithelial Elements in Visceral-Precancerosis

The behaviour of the myoepithelical cells in human biopsy material has been studied. Proliferation of myoepithelial elements occurs most frequently in cystic mastopathy, in its forms consisting mainly or exclusively of myoepithelial-proliferation. Cases have been found in which myoepithelial-proliferation had turned malignant. In the present cases myoepithelial malignization was associated with malignant degeneration of the mammary glandular epithelium, thus a mixed tumour (myoepithelioma + cancer of the breast) had arisen. Myoepitheliomas of the skin, as well as malignant cystic mastopathy have also been studied.

Edit Beregi, R. Jankovics, Z. Brasch

(Department of Pathology, Semmelweis Hospital, Budapest)

Connection between the Histological Picture and Prognosis of Malignant Thyroid Tumours

In the period 1943—1963, 128 malignant thyroid tumours occurred among 14,122 strumectomies performed in the Semmelweis Hospital. The tumours could be divided into eight groups according to their biological behaviour: I. Slight malignancy: 1. malignant adenoma, 2. malignant papilloma; II. Medium malignancy: 3. follicular cancer, 4. solid cancer: III. High degree malignancy: 5. planocellular cancer, 6. anaplastic cancer, 7. sarcomatous haemangioendothelioma and 8. sarcoma. From circulars sent to 128 patients it was established that where the cause of death was a tumour, the histological finding was mostly sarcoma or

anaplastic carcinoma. The longest survival occurred with malignant adenoma, malignant papilloma and follicular cancer. From the point of view of survival, the patients' age was decisive, the prognosis of cases under the age of 40 proved to be more favourable than of older ones.

Erzsébet Zombai, G. Kelényi

(Institute of Pathology, University Medical School, Pécs)

Biological Properties of the Moloney Leukaemia

The Moloney leukaemia, a virus induced lymphoid tumour pathogenic to mice, rats and hamsters, has been transferred with both cells and cell-free material method; the number of inoculated animals was 524, and about a half of this material is at present suitable for evaluation. The inoculated animals, their haemopoietic tissues in particular, were examined under both light and electron microscope. Albino rats and mice of the Institute's closed colony were found suitable for the transfer of tumour with cellular material as also with cell-free ultracentrifugate. Most of the animals died of thymoma, and only a few showed signs of a generalized disease. Isolated thymomas were diagnosed radiologically. The virus was demonstrated in the mogakaryocytes of the bone marrow and the spleen, further extracellularly in the tumorous infiltrations of thymus and spleen. Genesis and development of the tumour have been discussed.

Gizella Karácsony, P. Lantos

(Institute of Pathology, University Medical School, Szeged)

Serum and Tissue Lipids in Rabbits with Brown-Pearce Carcinoma

Brown-Pearce carcinoma was transplanted into the testicles of rabbits kept on a normal diet. Serial blood samples showed a significant increase in the serum phospholipide level. A direct connection between progress of the tumour and increase of the phospholipide level could be established. The phenomenon does not occur in hyperacute cases and in those where the tumour undergoes early necrosis. Histochemical reactions revealed phospholipids in the periphery of the tumour tissue. Oilred-0 staining showed fat droplets in peripheral living tumour tissue as well as in central necrotized parts.

O. Szücs

(Department of Pathology, MÁV Hospital and Polyclinic, Budapest)

Histochemistry of the Mucopolysaccharides of Mixed Parotid Tumours

Mixed parotid tumours display metachromasic connective tissue mucus and cartilaginous tissue. Sulphated mucopolysaccharides could be demonstrated by the rhodizonate and acriflavine sulphate reactions in cartilage, but the connective tissue mucus was negative, thus its metachromasia was not due to the presence of sulphate groups. To elucidate the problem the metachromasia was studied after blocking the sulphate radical with barium salt. The results obtained by this modified technique have confirmed the evidence obtained by the rhodizonate and acriflavine methods.

A. Nagy, Sarolta Petrás

(Department of Pathology, County Council Hospital, Szolnok)

Carcinomatous Myocarditis

Literature contains numerous reports on cardiac metastases of tumours, mainly clinical observations of cases diagnosed *in vivo*. The fact has usually been disregarded that the metastases elicit a response of the heart muscle and thus clinical signs and ECG changes arise.

A review of the material discussed in earlier communications has led to the conclusion that myocardial metastases are surrounded by lymphocytic infiltration which represents the inflammatory reaction elicited by the neoplastic growth. This may manifest itself with clinical symptoms. When the clinician diagnoses a cardiac metastasis *in vivo*, it is on the evidence of the clinical symptoms of myocarditis that its presence is inferred to.

S. Keresztury

(Section of Pathology, Semmelweis Hospital, Miskolc)

Plasmacytoma of the Uterine Portion

The uterus of a 60 year old patient was removed on account of a toumor thought to be a carcinoma in the first clinical stage. Histology revealed a plasmacytoma. Only one report has been found to deal with extramedullary plasmacytoma of a similar localization. The clinical manifestations and morphological changes are described in detail.

Z. Zámbó

(First Institute of Pathological Anatomy and Experimental Cancer Research, University Medical Schoool, Budapest)

Extracranial Metastasis of Intracranial Tumour (Meningioma)

There are few reports on metastasizing intracranial tumours. GLASAUER (1963) could collect in the literature only 35 authentic cases of this kind.

In a female 59 years of age necropsy revealed a meningioma in the left parietal region which had given rise to a histologically verified metastasis in the left lung. A study of the reports on the problem of metastasizing meningeal tumours revealed the following.

(1) Intracranial intervention was performed in 33 of the 35 reported cases.

(2) As regards histological structure, mainly tumours belonging to the category of sarcomatous meningiomas (according to Cushing's classification) produced metastases.

(3) The most frequent site of metastases were the lungs; metastases in the cervical and mediastinal lymph glands, further in the liver were less frequent.

A. Haraszti, B. Ringelhann

(Department of Pathology, County Council Hospital, Eger)

Serum Glutamic-Oxalacetic Transaminase (SGOT) in Rats with Liver Injury Induced by Carbon Tetracloride

The correlation between hepatic morphology and the SGOT level has been studied in rats poisoned with carbon tetrachloride. SGOT began to rise 3 hours after the injection, at a time when the liver showed no sign yet of necrosis, and it was still elevated after a week, at a time when necrosis was no longer observable. Parenchymal necrosis alone cannot be responsi-

ble for the rise in SGOT level. The increased passage of GOT into blood is presumably facilitated by the increased permeability due to the histotoxic hypoxia caused carbon tetrachloride, an assumption corroborated by the appearance of vacuoles filled with droplets of protein in the liver cells and of hydropic cells.

Aranka László, K. Streitmann, I. Bartók, D. Bara

(Institute of Pathology, University Medical School, Szeged)

Effect of Experimental Liver Lesion on the Antidiuretic Centres

In liver disease the antidiuretic activity of body fluids is increased and this is one of the causes of water retention. The increase of the ADH titre may be correlated with a decrease of inactivation in the liver, or with an increase in the activity of the centres inhibiting diuresis. The latter problem has been subjected to investigation. The histology and the biological activity of the antidiuretic centres of albino rats have been studied 1—4 and 12 days after subtotal hepatectomy.

At 24 hours following sham operation (laparotomy, lifting of the liver) neurohypophyseal antidiuretic activity was lower than in the controls; at the same time, a depletion of neurosecretion could be demonstrated histochemically. After subtotal hepatectomy no temporary ADH mobilization occurred and histochemically a storage of neurosecretion was demonstrable in the neurohypophysis. As compared with the controls, the antidiuretic activity of the anterior hypothalamus decreased in the sham-operated animals during the first 48 hours, whereas in that period and at 12 days following operation subtotal hepatectomy produced no appreciable changes (decrease or increase of concentration).

The results indicate that for the increased antidiuretic activity of the body fluids in the early phase of liver disease not a hyperactivity of the hormone-producing centres, but other factors, first of all a decrease of hepatic inactivation are responsible. The experiments do not rule out the possibility that in chronic and progressive liver disease the antidiuretic centres may also become activated.

Anna Tószegi, I. Török, I. Bartók

(Institute of Pathology, University Medical School, Szeged)

Changes in the Regeneration of the Cirrhotic Liver in Response to Humoral Effects

Liver cirrhosis was induced in rats with CCl_4, afterwards subtotal hepatectomy was performed. Following the operation, one group of the animals was treated with serum from normal, subtotally hepatectomized rats, and a second group was treated with serum from normal, non-operated rats; a third group served as control. At 48 and 72 hours, as well as 10 days following the operation identical numbers of animals were sacrificed from every group and the regenerating livers were studied.

The serum of partially hepatectomized rats was found to promote the regeneration of the cirrhotic liver to a considerable degree. 1. The extent of regeneration was about twice as great as in the controls, 2. the serum produced a prolonged stimulating effect on mitosis; whereas that of the controls had no such effect, 3. it prevented the development of regressive changes otherwise observable in regenerating cirrhotic liver; 4. the RNA content of the cytoplasm increased for a long period and to a much greater extent than in the controls, 5. it considerably influenced the changes in succinic dehydrogenase and cytochrome oxidase activities, making them similar to those changes observed in the course of the regeneration of intact liver; the changes in alkaline phosphatase activity were not influenced by the serum.

The serum from normal, non-operated animals neither stimulated nor inhibited the regeneration of the cirrhotic liver and did not exert any influence in the activities of the oxidative enzymes.

I. Török, I. Bartók, Sz. Virágh

(Institute of Pathology, University Medical School, Szeged and
Institute of Histology and Embryology, University Medical School, Budapest)

Changes in the Subcellular Structure during Regeneration of the Liver

Livers of partially hepatectomized rats killed at 24, 48, 72, 96 hours, 10 and 20 days, respectively, after operation were studied electronmicroscopically.

At 24 hours following operation the principal changes are a disintegration of the subcellular structure, first of all of the endoplasmic reticulum and the mitochondria, an accumulation of free ribosomes and fat and a marked glycogen depletion. Subsequently, and most markedly between 48 and 96 hours, the structure shows a rapid differentiation: the mitochondria regain their usual shape, and meanwhile the accumulated microbodies turn into mitochondria. In some cells the preexistent endoplasmic reticulum is reorganized, in others a neogenesis of the endoplasmic reticulum occurs. The organized ergastoplasm will be formed out of the earlier developed smooth-surfaced endoplasmic reticulum in close topographical correlation with the glycogen gradually increasing again and with the mitochondria. A rapid mobilization of fat is observable between 48 and 96 hours.

The changes of the biliary canalicules (marked distension, intracellular interaction with a decrease in number or swelling of the microvilli), as well as the hypertrophy of the Golgi apparatus in the early phase of regeneration may be ascribed to an excessive functional overstrain caused by the excretion of bile and other substances in the considerably diminished liver parenchyma. At 20 days structural restitution is complete.

I. Bartók, Sz. Virágh

(Institute of Pathology, University Medical School, Szeged and
Institute of Histology and Embryology, University Medical School, Budapest)

Submicroscopic Examination of the Regeneration of the Cirrhotic Liver

The regeneration of the liver of albino rats rendered cirrhotic by treatment with CCl_4 was studied at 24, 48, 72, 96, hours, 10 and 30 days following subtotal hepatectomy.

The subcellular changes differed significantly from those seen in the course of regeneration of the normal liver. The structural disintegration observable in the early phase of regeneration is protracted; the dilatation of the endoplasmic reticulum might reach extreme severity and an increasing swelling of the mitochondria can be observed. In 48 hours to 10 days necroses appear, pointing to the irreversibility of some changes. During the first four days regressive phenomena are in the foreground, the new formation of the endoplasmic reticulum becomes marked only after 96 hours. The glycogen content of the liver cells increases slowly, fat mobilization is slow. Even by the 20th day regeneration is not complete: in some areas the structure is normal, in others changes noted in the early phase of normal liver regeneration are visible, in still others milder changes indicative of damage to the mitochondria and endoplasmic reticulum are still present. The changes observed may be explained by assuming that the damaged liver adapts itself with difficulty to the overstrain caused by the loss of a substantial part of its parenchyma, and some of the surviving liver cells are not capable of reproducing the normal structure. This explains why it is only later that the regenerative phenomena are more marked and as a result the process of regeneration is more protracted than in the normal liver.

L. Szentendrei, M. Perneczky

(Department of Paediatrics and Pathology, County Council Hospital, Kecskemét)

Extensive, Bilateral Bronchiectasis and Secondary Chronic Cor Pulmonale due to Chronic Intrauterine Arsenic Poisoning

A boy aged 11 years was treated for bronchiectasis and incompensated valvular heart disease, which on autopsy proved to be a functional tricuspid insufficiency, caused by an extreme hypertrophy and dilatation of the right ventricle. The latter condition could not

be influenced and the patient died 4 weeks after admission. During pregnancy the mother and the rest of the family had been drinking water regularly contaminated with a pest-destroying spray containing arsenic. The family suffered a more or less severe intoxication, the father died of an arsenic cirrhosis seven years later. Ever since infancy, the patient often suffered from pneumonia, his body was affected with arsenic melanosis, and hyperkeratosis was observed on his palms and soles. At necropsy the considerable bronchiectasis and cutaneous changes are ascribed to the intrauterine arsenic poisoning.

Anna Horváth-Kovács

(Institute of Pathology, University Medical School, Pécs)

Early Morphological Manifestations of Experimental Silicosis

The first reaction to experimental silicosis has been studied in the rat lung 2 and 96 hours after dust inhalation. Granules containing quartz were found to have been phagocyted by leukocytes intrabronchially, in the alveoli and in the peribronchial connective tissues. Numerous disintegrated leukocytes were seen around the dust granules. This phenomenon was not observed after the inhalation of amorphous silica-gel or medicinal charcoal, and is thought to be indicative of the toxicity of quartz crystals.

Gy. Kup, F. Brenner

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Aorta Angusta

Aorta angusta as a technical term and as an anatomical entity has been known since 1761 and was mentioned by MORGAGNI. Its essence was defined by VIRCHOW who also described the clinical and pathological pattern of the condition.

Aorta angusta is undoubtedly a rare cardiovascular anomaly; HENKE and LUBARSCH described it as a pathological entity and constitutional deformity closely correlated with the so-called degenerative type.

KAUFMANN'S observations and investigations of many decades were confirmed by SUTOR, SCHEEL, KANI, JAFFÉ and STERNBERG, but all of these authors have failed to recognize the entity under consideration.

ASCHOFF then formulated the final definition "Hypoplastic changes of the aorta and other arteries have been described. Their significance is doubtful. This applies to the aorta in the first place, and to the aorta angusta established by Virchow. This entity cannot be substantiated anatomically and is therefore no longer tenable".

It is only natural that, after such antecedents, the concept of aorta angusta was erased from all important textbooks.

Far from being non-existent, aorta angusta — though a rare condition, — is a real and very much existing disorder, and has been repeatedly observed even under conditions for which the present authors have coined the term "aorta angusta familiaris".

Of the six children of a family, four have died of aorta angusta (cor bovinum familiare) during the last 9 years, and the diagnosis was verified *post mortem* in two of these cases.

Congenital aortic anomalies are of three kinds:

(1) Aortic coarctation (stenosis of the isthmus)

(2) Aortic hypoplasia

(3) Aorta angusta.

In cases of aorta angusta, the heart and the valves are normal at birth, the aorta in hypoplastic. The disease has two phases:

(1) Latent phase. The heart grows in size, and cor bovinum develops gradually and imperceptibly.

(2) Second phase. The picture is dominated by a gross enlargement of the heart which leads to death usually between 15 and 18 years of age.

The aorta is both narrower and shorter than normal. Its lumen may shrink to one third of its normal width. The heart weighed about 1000 g in both cases examined post-mortem.

The above-described cases make it clear that aorta angusta is an existing condition notwithstanding the fact that many pathologists have not come across such a deformity during their whole career. The now reported familiar occurrence of the condition seems to be unique.

Aorta angusta is different from aortic hypoplasia, and the two anomalies can well be distinguished as no cor bovinum develops in cases of aortic hypoplasia.

Since the familiar occurrence of aorta angusta has been verified, the existence of the entity should be recognized in both clinical practice and pathology.

Gy. Sétáló

(Institute of Anatomy, University Medical School, Pécs)

On the Mechanism of Hypothalamic Adiposity

After inducing obesity in male rats by electrolytic destruction of the anteromedian portion of the hypothalamus, their endocrine organs have been examined. Nuclear hypertrophy was found in the zona fasciculata and in the cells of the Langerhans islets, while thyroid activity appeared to have diminished. The starving level of blood sugar was the same in the experimental and the control animals. Dextrose tolerance was reduced in obese animals.

It follows that — beside hyperphagia, reduced spontaneous activity and diminished thyroid function — changes in the function of the Langerhans islets might play a decisive role in the mechanism of hypothalamic adiposity.

G. Krausz

(Institute of Pathology, University Medical School, Debrecen)

Lethal Gastric Haemorrhage from a Submucosal Artery of Abnormal Calibre

Haematemesis and melaena occured in a 47-year, old female patient, although she had not mentioned episodes of ulceration or hyperacidity in the hystory. — Post-mortem examination revealed a superficial acute ulcer covered with thrombus on the posterior wall of the stomach. A large, (2.2 $\times$ 0.8 mm) arroded artery was found in the centre of the ulcer. The vessel, showed the phenomenon of calibre persistence which occurs when the arterial branch supplying the submucous plexus passes into the submucosa without any essential reduction of its calibre. Both this anomaly and the partial defect of the gastric musculature beneath the abnormal vessel should be regarded as developmental anomalies. Aberrant vessels create a local disposition for peptic ulceration. The presence of calibre-persistent vessels in the gastric wall is of a pathological significance since even its superficial ulceration may be accompanied by fatal haemorrhage.

J. Laczkó, G. Ley

(Section of Pathology, Semmelweis Hospital, Miskolc)

Duodenal Ulcer in Infants and Children

Duodenal ulcer has been revealed at necropsy in 4 infants and young children. In 3 cases the cause of death was haemorrhage from the ulcer. The pertinent clinical data, the gross and micoscopic postmortem findings are described, and the special features of the lesion as observed in infants and children are discussed. It is emphasized that the possibility of duodenal ulcer in infants and children has to be taken into consideration. Attention is drawn to the increasing number of such cases.

L. Megyeri, Gy. Szeleczky

(First Institute of Surgery, University Medical School, Debrecen)

Histomorphology of Oddi's Sphincter in Biopsy and Necropsy Specimens

Since the introduction in recent years of new techniques, the morphology of the region of Oddi's sphincter is gaining significance in surgery. Still there are hardly any recent studies on this subjects.

At operations on the biliary ducts biopsy specimens were taken from the pathologically altered sphincter area in 32 cases. As a control a similar number of Oddi's sphincter area specimens were taken at necropsy of cases who had not suffered from diseases of the biliary ducts, liver or pancreas. — The results, and the significance of the area in question in modern surgery has been discussed.

B. Rohonyi, Mária A. Mázló

(Institute of Pathology, University Medical School, Pécs)

Preparation of Semi-Thin Histological Sections

It seemed necessary to study the technique of histological sections' preparation, in the thickness of 0.5 to 2.0 μ that, would constitute a transition between sections required for electron microscopy and, those suitable for light microscopy.

Sections of the mentioned thickness ("semi-thin sections") are sometimes particularly suitable for affording some insight into the finer histopathological changes.

Although there exist several methods for the preparation of sections ranging in thickness from 0.5 to 2.0 μ, the widespread use of electron microscopic methods favours the use of preparations fixed in osmium tetroxide, embedded in synthetic resin and sectioned by ultramicrotome.

The technique of preparing semi-thin histological sections for the purposes of light microscopy is discussed. Such sections have been prepared from various specimens of renal disorders, tumours and from other materials too.

FORENSIC MEDICINE RELATOR

S. Ökrös

(Institute of Forensic Medicine, University Medical School, Budapest)

Thrombosis of the Syphons of the Internal Carotid and the Vertebral Arteries

The brain has the richest blood supply of all organs. Two central and two lateral arterial connexions, communicating by way of the aortic arch, are situated between the two subclavian-vertebral and carotid branches; they supply, connected with Willis' circle, the brain with blood even if a branch of the vertebral or carotid artery is completely occluded. Obstruction of these vessels may sometimes occur in the hidden segments the so-called syphons. A new method for the post-mortem isolation and convenient examination of the lower and upper syphons of the carotid and vertebral arteries has been worked out. Eleven cases of the carotid syphons and 2 cases of those of the vertebral artery are demonstrated.

I. Gy. Fazekas, Erzsébet Virágos-Kiss

(Institute of Forensic Medicine, University Medical School, Szeged)

The Free and Total Histamine Content of the Constriction Mark

BARSUM and GADDUM, further CODE determined by a modified method, which they elaborated for blood, the free and total histamine content of the constriction mark of the skin of the neck by hanged persons and by post-mortem hanging of human corpses at different intervals after death. The total histamine content of the constriction mark and of the intact skin of the neck of the same corpse was identical by both groupes. The free histamine content *of the vital constriction mark* was in mean value the 90% of the total histamine content, and the free histamine content of the intact skin of the neck in the same corpse was only 24%. *The post-mortem constriction mark* and the free histamine content of the skin of the neck of the same corpse does not differed from one another and it was in mean value 34%. The free histamine surplus between the vital restriction mark and the intact skin of the neck may be taken into consideration as a new vital reaction, and by the help of which one can make a distinction between the vital and postmortal hanging.

Erzsébet Virágos-Kiss

(Institute of Forensic Medicine, University Medical School, Debrecen)

Unusual Mechanisms of Suffocation in Children

Two 18-month-old children died from suffocation during their stay in hospital. One child, left unattended for a short time, had its neck stuck between the protecting bars of the bed. The other child, likewise left unattended for a short time, crawled out of the bed, and the vestlike linen bridle, serving for safe attachment, slipped up and compressed the neck. The importance of utmost care in the supervision of young children is emphasized.

B. Rex-Kiss

(Institute of Forensic Medicine, University Medical School, Budapest)

Correct Transfusion-Serological Nomenclature in Medico-Legal Practice

The task of forensic pathologists in connection with fatally blood transfusions requires the correct use of serological terms. A survey of post mortem records, expert opinions and pertinent reports shows that numerous misinterpretations of serological and immunological terms occur in connection with transfusions. To ensure a correct interpretation of the most frequent terms, the different blood-group systems, their interrelations, some of the related genetic concepts, as also their role in transfusions are discussed, with special attention to the usual expressions and designations (subgroups, factors, etc.). Also, a few fundamental concepts of immunology (immunization, sensitization, etc.), further the terms group-identity, incompatibility, imperilled and not imperilled recipient, dangerous, and universal donor, the differences between in-vitro and in-vivo reactions, and finally the precise meaning of the terms cross-matching, homospecific and heterospecific pregnancy, etc. are analysed.

The medico-legal importance of a familiarity with immunological concepts connected with blood transfusions is emphasized.

I. Gábor, A. Potondi

(Institute of Forensic Medicine, University Medical School, Budapest)

Topography and Morphology of Basicranial Aneurysms

Morphological features (size, shape, etc.) of basicranial aneurysms are described on the evidence of many hundred observed cases and those reported in international literature. It is especially the site of the examined aneurysms which shows peculiar features; their analysis is of clinico-pathological importance and facilitates the diagnosis.

Gy. Rózsa, E. Somogyi, P. Sótonyi, J. Irányi, B. Orovecz, Klára Irányi

(Institute of Forensic Medicine, University Medical School, Institute of Rheumatology and Balneology; Hospital of the First Aid Service and Department of Psychiatry, University Medical School, Budapest)

Histochemical and Fluorescence-Optical Investigation of Dermal Lesions Caused by Eelectric Current

Patches in the skin of persons killed by electric current have been investigated histochemically 2 to 12 hours after death. Burstone's dinitrofluorobenzene and the β-naphtol methods were employed for the demonstrations of the reactive protein groups, eriochrome black for that of basic proteins, Pearse's method for that of disulphide and sulphhydryl groups, and the Yasama-Ichikava ninhydrin reaction for the demonstration of α-amino acids. Acid mucopolysaccharides were identified by means of azure A, toluidine blue, thionine, further with Hale and Eskelund's method and the periodic acid diamine reaction recently elaborated by Münch and Ernst. Nucleic acids were determined with Narson's gallocyanin chrome alum, Taft's methylgreen pyronine and Danielli's naphtolic acid hydrazine. For fluorescence analysis the sections were stained with adequately diluted solutions of acridine orange, choriphosphine, flavophosphine, thioflavine S, geranine and vitolin-yellow. A range of veronal-acetate buffers extending from pH 2.52 to pH 9.18 served as controlled milieu.

It was found that pigmentation had decreased in or disappeared from the examined spots, and that these areas gave a stronger reaction with alkaline dyes. Fluorescence microscopy revealed the same damage as was demonstrable by means of the routine staining procedures. Swelling of the collagenous fibres with consequent decrease of staining, homogenization of the gravely damaged skin displaying homogenous diffuse staining, and the discrepancy between slightly lesioned and uninjured areas are regarded as significant. Lesions caused by electric currents are divided in three categories according to the degree of damage.

E. Somogyi, Gy. Rózsa, P. Sótonyi, J. Irányi, B. Orovecz, Klára Irányi

(Institute of Forensic Medicine, University Medical School, Institute of Rheumatology and Balneology; Hospital of the First Aid Service and Department of Psychiatry, University Medical School, Budapest)

Polarization Microscopy of Dermal Lesions Caused by Electric Current

Patches in the skin of persons killed by electric current have been studied under the polarization microscope 2 to 12 hours after death. The frozen sections were stained or treated with the Canada, aniline, phenol, anisotropic ethacridine, Romhányi's methylene blue and toluidine blue anisotropic precipitation procedures. Amylacetate was substituted for terpinol in the aniline reaction, and 1 : 2 and 1 : 4 aqueous solutions of the 0.1 M ethacridine stock solution were used. Precipitation in the case of methylene blue was performed with 2 per cent potassium bichromate and in the case of toluidine blue with 2 per cent potassium ferricyanide. Staining was performed with 1 per cent solutions in Michaelis buffers ranging from pH 2.9 to pH 8.9.

The connective-tissue fibres were digested at 37° C by means of 0.25 to 5.0 mg/ml of elastase dissolved in 0.1 M borate-phosphate buffer of pH 4.5, 6.5 and 7.5.

Aniline, which extinguishes the birefringence of the collagenous fibres of intact skin, revealed anisotropy of the fibres in the affected spots. 50 per cent phenol-Canada reinforced the double refraction of collagenous fibres in the intact skin but failed to do so at the affected spots, while ethacridine increased the anisotropy of fibres in the damaged area and failed to do so in the uninjured skin.

Elastase, applied in a concentration of 4 mg/ml, almost completely digested the damaged fibres in 3 hours at 37° C. No digestion of the damaged fibres by elastase was obtained if the acid radicals had been bound by 24-hour methylation with 0.1 N hydrochloric acid dissolved in methanol at 60° C.

Exposed to monochromatic red light, intact collagenous fibres showed positive dichroism and positive birefringence, while electrified fibres revealed a negative dichroism and negative birefringence.

L. Buris

(Institute of Forensic Medicine, University Medical School, Debrecen)

Histochemical Analysis of Injuries

The area of the cervical groove caused by the rope on hanged persons has been examined in cases of intravital and posmortal injuries. It was observed previously that after an intravital injury the epithelium, the loose subepithelial connective tissue and the muscles showed increased pyroninophilia. This was interpreted as indicative of ribonucleic acid accumulation in the traumatized area, an assumption confirmed by fluorescence microscopy. The present investigations by means of ribonuclease digestion, and examination of the ultraviolet absorption and the isoelectric point of the injured area have also corroborated the assumption that RNA accumulates in the injured area and have revealed an accumulation of basic proteins.

V. Földes

(Central Police Laboratory, Budapest)

Signs of Fresh Vital Injury in Striated Muscles

Signs of fresh vital injuries are highly significant in criminology. The detection of such signs is of special importance if the time between the vital injury and the moment of death amounts to a few minutes only. Pathological changes in striated muscle tissue caused by fresh vital injuries are demonstrated as observed by the author in both animal experiments and human practice.

E. Maárová, S. Koudela, J. Lukáči

(Institute of Forensic Medicine, P. J. Šafarik University Medical School, Košice, Czechoslovakia)

Some Biochemical and Morphological Changes in Heart Muscle in Lethal Injuries

Myocardial sodium and potassium contents have been analysed in cases where death had ensued at the very spot of the accident within a few minutes. The potassium content was found to have considerably decreased and sodium content to have increased. The difference was more pronounced in the right heart. Simultaneously with the biochemical analyses, the myocardium was subjected to morphological examination, to ascertain the correlation between the fragmentation of heart-muscle fibres (a phenomenon observed in every case) and the K-Na balance, further the connection between the said fragmentation and the observed disturbance of myocardial water balance.

B. Rengei

(Institute of Forensic Medicine, Medical University School, Szeged)

Identification of Blood by Means of Thin-Layer Chromatography

Fresh spots suspicious of blood were treated with physiological saline, old ones with 1 to 5 n KOH and then neutralized with HCl. If necessary, the solution was concentrated with the paper-chromatographic procedure of Kirk and Schaidt.

Silica gel-C was used according to Stahl's method for the preparation of plates. These measured 20 cm by 10 cm and were dried in air for one hour, then at 105° C for 30 minutes. Various dilutions of blood samples were then run parallel to the test substance in a solvent composed of 99 parts of methanol and 1 part of glacial acetic acid. After running for 1—2 hours, during which the solvent migrated 15 to 18 cm, the plate was removed from the tank and kept for 5 minutes at 100° C in order to inactivate the eventually present peroxidase of vegetable origin. The plates were then treated with a freshly prepared 1 per cent alcoholic solution of benzidine and, after a few minutes, with a 3 per cent solution of hydrogen hyperoxide, Positive result was indicated by a blue colour betweeu R_F 0.55 and 0.65.

The method under consideration is quick and highly sensitive. Even 0.005 ml of a 1 : 10 000 dilution of blood gives a clearly positive reaction. Dilution should not be less than 8 to 10 000-fold since higher concentrations yield diffuse spots. Besides, the method renders specific the otherwise non-specific benzidine reaction, eliminates the disturbing action of vegetable peroxidase, and identification is made reliable by the parallel running of control blood.

Gy. Szuchovszky

(Institute of Forensic Medicine, University Medical School, Budapest)

Legal Aspects of Death during Surgery

Cases falling under the concept of unexpected death are not precisely defined by the existing legal code. Practice in this respect relies on common law and decisions as to what should be regarded as death due to unknown causes depend more or less on individual judgement. Since similar cases are qualified differently by the different official organs, practice in this respect is not uniform.

Examining the problem on the evidence of Hungarian and foreign statutory provisions as also on that of the existing (and statistically analysed) practice, cases of death during surgery have been investigated, as *mors in tabula* and tries to determine whether they satisfy the criteria of unexpected death. It is claimed that death during surgery has to be regarded as a special case of death which requires the institution of particular official proceedings as also autopsy to be performed by an authorized medical officer. It is imperative that future legal regulations, circumscribing the category of special cases of death, should include a precise definition of death during surgery. Only adequate legal regulation will lead to uniform practice and settle the problem of medical responsibility in a satisfactory manner.

L. Takácsy

(Institute of Forensic Medicine, University Medical School, Budapest)

Medico-Meteorological Aspects of Sudden and Unexpected Cardiac Death during Surgery

Recent investigations have thrown fresh light upon the meteorological factors involved in the occurrence of sudden cardiac death during surgery.

It has been found in 12 cases that meteorological phenomena occurring before or on the day of operation may so affect the heart and the circulation as to make the patient susceptible to sudden death during the surgical intervention. In nine cases there was a frontal passage or even several frontal passages on the fatal day, whereas air masses known to be of biological influence were present on the days without frontal passage.

It is suggested that surgeons should test the meteorological sensitivity of the patients and set the day of the intervention accordingly. If, in a given case, the intervention is vitally urgent, the patient's meteorological sensitivity should preliminarily reduced by adequate sedatives.

L. Nagy, M. Szabó

(Institute of Forensic Medicine, University Medical School, Debrecen)

Sudden Death during Trichloroethylene Anaesthesia

Cases of sudden death under trichloroethylene anaesthesia during gynaecological interventions are described.

Post-mortem examination revealed in all cases accumulation of adipose tissue in the right ventricle of the heart, an underdevelopped circulatory system, persistent thymus and small adrenals. After discussing the chemical properties of a tricholorethylene a theory is advanced for the explanation of the observed fatal episodes. The indications and counterindications of trichloroethylene anaesthesia should precisely be determined.

D. Horváth

(Pharmacy of University Medical School, Pécs)

Significance of the History for the Correct Induction of Anaesthesia

The spread of numerous up-to-date methods of anaesthesia has raised the necessity to subject the sources of error inherent in, and the complications following, the existing anaesthesiological methods to a medico-legal analysis. The increasing number of drugs employed for anaesthetic purposes lends a growing importance to the eventual complications.

There are many different drugs to be administered to induce and maintain anaesthesia as well as in preoperative and postoperative treatment. The most suitable technique and drug have to be selected by the anaesthetist: when making his choice he should not merely consider the desired immediate effect but regard the patient as a complex physiological unit as well. The very first task is carefully to record a reliable and detailed history; this is so important that one is tempted to suggest that a special anaesthesiological history should be taken.

The medical expert cannot obtain a clear picture of the hazards of anaesthesia unless he takes extreme possibilities into account. This is only feasible if the drugs in use have been carefully examined and analysed.

The connexion between the data in the history and the substances selected for anaesthetic purposes is stressed. A knowledge of such connexions will make it possible to prevent and recognize anaesthetical complications.

L. Harsányi, V. Földes

(Institute of Forensic Medicine, Medical University School, Budapest)

Medico-Legal Aspects of Unexpected Death Following Surgical Intervention

Unexpected death in the postoperative period may be independent of the preceding intervention being due to some disease or other factor in the patient's organism. Another part of the fatalities belongs to the category of surgical complications and represents operative hazards. Still another part may be due to a violation of professional rules. A few typical cases are presented to show the different forms of postoperative fatalities and their medico-legal aspects.

E. Somogyi, J. Irányi, Gy. Berentey, L. Tolnay

(Institute of Forensic Medicine, University Medical School, Budapest)

Surgical Treatment of Craniocerebral Injuries, Analysed on the Evidence of Autopsy Material

A total of 197 cases of craniocerebral injury have been studied, of which 14 had been lethal. Polytraumatized persons who had sustained some mechanical injury suffered, among other lesions, also from non-penetrating cranial injury. The study involved the determination

of the nature of injury, the surgical and neurological aspects of the fatal cases, and the post-mortem findings. There were discrepancies between the clinical and the necropsy findings, the reason of such discrepancies was examined. The question whether and how far surgical procedures had been helpful in establishing the correct diagnosis, has been studied and an attempt was made to point out those surgical interventions which may lead to a further improvement in diagnostics and surgery.

Comparison of the clinical diagnoses and the post-mortem findings has led to the conclusion that the identification of traumatic lesions is difficult because of the indistinctness of the focal symptoms in the nervous system. Unilateral mydriasis is not always a reliable clue. Symptoms of already existing diseases such as previous cerebral haemorrhage, pneumonia, etc., hide or distort the picture of the actual injury and make correct diagnoses difficult.

The clinical and the pathological findings agreed in 13 cases; *post mortem* examination proved moreover the correctness of the therapeutical plain in these cases. Tracheotomy, angiography and timely diagnostic probing contributed to the achievement of these results. The making of 3 diagnostic drill holes on both sides is suggested if the traumatic condition is rapidly progressing. It is thus possible to stop epidural and subdural haemorrhage in comatose patients who display uncertain neurological symptoms and a general rise of cerebral tension. In judging such cases from a medico-legal angle, possible diagnostic errors should be viewed in the light of the existing difficulties.

The prognosis of craniocerebral injuries has considerably improved owing to the development of neurologic diagnostic, neurosurgical and anaesthesiological procedures, the improvement in the treatment of states of shock and the prevention of secondary complications. A further progress may result from a co-operation of the traumatologist, neurologist and the medico-legal expert.

Leontin Jegesi, L. Tolnay

(Institute of Forensic Medicine, University Medical School, Budapest)

Spinal Injuries Sustained in Traffic Accidents

Spinal injuries due to traffic accidents have been examined in order to study the mechanism of the damage and to ascertain the connection between the nature of the injuries and the phases of the accident. Muscle injuries were likewise taken into account.

Characteristic overextensive spinal injuries sustained in collisions are not essentially different from those occurring when a person has a fall and dashes against the pavement. The muscle injuries are, however, more serious in the latter case. Injuries of the thoracic vertebrae or those at the boundary of the thoracic and lumbar vertebrae arise almost exclusively at the impact of the body on the road.

Characteristic fractures of the spinous processes occur in the phase of being run over by a vehicle, especially if the chest is crushed. This facilitates diagnosis in the case of young persons with elastic thorax even if no rib is broken.

The degree of muscle injuries depends on muscular strength and, in particular, on the age of the patient which should always be taken into consideration.

L. Szodoray, J. Nagy

(Institute of Dermatology and Institute of Forensic Medicine,
University Medical School, Debrecen)

Thrombotic Thrombocytopenic Purpura

A case of thrombotic thrombocytopenic purpura, Moschcowitz's syndrome, a disease rare in Europe, is described.

Biopsy has made it possible to establish the correct diagnosis *in vivo*. The medico-legal aspects of the case are discussed.

J. Lukáči

(Institute of Forensic Medicine P. J. Šafarik University Medical School, Košice, Czechoslovakia)

Demonstration of Group Substances in Biological Material

Serological analysis of biological material is frequently needed in medico-legal practice. Promising results have been reached in their field by the application of ultrasound for manipulations of the biological material and especially for the demonstration of group properties.

The present study had the purpose to determine group substances in stains of seminal fluid found on the female underwear on cases of rape or rape and murder. Identification of group properties in human cerebral tissue is of importance in cases of traffic accidents, since fragments of crushed brain tissue are often found on parts of vehicle responsible for the fatal injury.

Gy. Farkas

(Institute of Forensic Medicine, University Medical School, Pécs)

Comparative Blood-Alcohol Tests

It is well-known that Widmark's officially adopted microidometric method is not specific for ethanol, its results being modified by a number of non-alcoholic reducing agents the level of which may reach 0.1 per cent in the blood of corpses. It has been attempted to make the alcohol test more specific by introducing the fermentative antidiuretic — hormone (ADH) procedure. Results of the two tests performed in parallel in blood samples from 122 corpses and in 218 subjects were compared. The ADH method yielded values 0.03 to 0.05 per cent and 0.01 to 0.02 lower for cadavers and living persons, respectively in two cases of alcoholic intoxication Widmark's test yielded values of 0.366 and 0.254 per cent, respectively, while the ADH tests were negative. All this in agreement with literary data, warrants the conclusion that Widmarks's test, if applied alone, offers false results, especially for cadaveric blood. The ADH test is not the only means of improvement; gas chromatography ought to be utilized, since the latter method shows not only the difference between the respective levels of the reducing agents of alcoholic and non-alcoholic origin but also indicates the nature of the reducing substance responsible for the difference. The ADH-test is eminently suitable for the control of results obtained by means of gas chromatography.

S. Koudela, J. Lukáči

(Institute of Forensic Medicine, P. J. Šafarik University Medical School, Košice, Czechoslovakia)

Gas Chromatographic Analysis of Chlorinated Hydrocarbons

After a brief review of the fundamental principles of gas chromatography, its medico-legal utilization for the demonstration of chlorinated hydrocarbons is discussed. Gas chromatography makes it possible to separate and distinguish the various derivates in cases of poisoning, an important achievement in consideration of the different toxicity of the chlorinated hydrocarbons.

D. Horváth, A. Nádor, I. Barth

(Pharmacy of Medical University, Pécs)

Certain Organizational and Practical Problems Regarding Toxicological Information

Contact with — mainly organic — poisonous chemical substances is now an everyday occurrence. The number of the various toxic substances used in all walks of life has so increased

that their knowledge cannot be expected either from the clinician and the general practitioner or the organs of security.

First aid in cases of poisoning is usually hampered by the toxic agent, being unknown. Although the symptoms usually admit of certain inferences regarding the pharmacological nature of the poison, they are as a rule too vague for a precise guidance of the medical aid.

Led by such considerations, toxicological centre has been organized at the University Medical School of Pécs, under the name "Service of Toxicological Information".

The agents most commonly causing poisoning are drugs, household substances, and plant protectives. The service has therefore been organized to provide information regarding these substances in the first line.

A few cases are reported to illustrate the above-discussed points.

Klára Zsigmond, J. Nagy

(Institute of Forensic Medicine, Medical University School, Debrecen)

Determination of the Alcohol Contents in the Expired Air. Use of the "Alcohol-Sound"

The so-called alcohol sound has been used in 50 000 cases, and an account is given of the experiences. Experiments have been made at different blood alcohol concentrations and it has been shown that it is possible reliably to distinguish different degrees of alcoholic intoxication. The result of the alcohol-sound test offers a sufficient basis for the institution of criminal or other proceedings in the given case.

Gy. Czirbusz, L. Póka

(First Institute of Surgery, Medical University School, Pécs)

Chronic Intravenous Alcohol and Sugar Therapy

The effect of large doses of alcohol and sugar infusion has been studied. In agreement with literary data, the oxidation of alcohol in the organism was found to be enhanced by the addition of sugar. Up to date 1200 litres of a solution containing 5 per cent alcohol and 15 per cent sugar have been administrated. The beneficial results and harmlessness of such treatment are emphasized on the evidence of toxicological analyses.

Gy. Dallos

(Hungarian Army Medical Corps, Budapest)

Microchemical Identification of Trace Elements Around Shot Wounds

In cases of short-distance shots, antimony, barium and lead are precipitated around the bullet's entrance. Identification of these trace elements may help in determining the direction and distance of the shot. A microscopic method of assay is described which is simpler and has a wider scope than the current analytical procedures.

P. Guth, J. Székely, A. Major

(Institute of Forensic Medicine and Obstetrical Clinic, University Medical School, Pécs)

Immunological Diagnosis of Pregnancy: Its Significance in Legal Medicine

Recently interrupted pregnancy can reliable be determined on the basis of certain signs. The Ascheim-Zondek and Galli-Mainini tests are often used for ascertaining the existence or non-existence of pregnancy. However, these tests are not routinely employed in medico-legal practice.

A new preparation, Pregnosticon, has been tested in order to study whether the immunological test is more sensitive than the biological tests, and how long the sensitivity persists after the termination of pregnancy. Twenty women who had had a normal pregnancy and a full-term delivery, further 10 women whose pregnancy had been interrupted, were chosen for the test; the immunological and the Galli-Mainini test were performed simultaneously. Urines excerted in the morning were sampled.

The sensitivity of the Pregnosticon test was found to exceed that of the Galli-Mainini test: 500 I.U. per litre of urine were necessary in the first and 1000 I.U. in the second case for obtaining a positive result. After normal delivery, the Galli-Mainini test remained positive for 1 to 4, the Pregnosticon test for 3 to 7 days; the corresponding values after interrupted pregnancy were 4 to 9 and 6 to 12 days. The urines of 15 non-pregnant women served as controls. Pregnosticon did not cause agglutination inhibition in any of the controls.

The present results as also literary data make it clear that the immunological pregnancy tests are more sensitive than biological testes, whether urine or serum is used for the determination of gonadotrophin. Being highly sensitive, more lasting, specific and simple, immunological pregnancy tests are preferable for the determination of pregnancy, artificial abortion and miscarriage.

M. Fehér

(Institute of Forensic Medicine, University Medical School, Budapest)

Results of Anthropological-Genetical Investigations Made in Course of 15 Years

Anthropological-genetical investigations made in 4238 cases (mainly in connection with affiliation suits) are reported.

The paternity tests showed the following results:

	Total %	After the examination of	
		one male %	several males %
Almost impossible	3.2	2.0	6.8
Highly improbable	7.9	5.4	15.5
Improbable	27.0	24.4	34.8
Possible	5.0	4.9	5.0
Probable	32.2	37.0	18.0
Highly probable	19.0	20.7	14.3
Almost certain	5.6	5.6	5.6
Of the utilizable expert opinions Negative proofs	40.1	33.3	60.1
Positive proofs	59.9	66.6	39.9

Irrespective of these examinations, blood tests were made in 94 per cent of the suits. In 7 cases (0.2 per cent) the two kinds of tests were in agreement. In 5 of these cases, the children were so young that careful examination failed to yield reliable results. There was a case in which the alleged mother had delivered a child in her home lived uninterruptedly

with her child; repeated blood typing disproved the woman's maternity (mother M-type, child -N-type), whereas detailed genetic investigations proved both maternity and paternity. In contrast in a father case anthropological and genetical tests proved the father, a paternity beyond doubt, although blood typing had yielded an opposite result (The Court confirmed the paternity).

M. Szabó

(Institute of Forensic Medicine, University Medical School, Debrecen)

Buoyancy of Submerged Pulmonary Tissue

The buoyancy of various samples of submerged specimens of intact and diseased lungs has been studied by means of a specially designed instrument.

The method allows a numerical expression of the different properties of the lung tissue and of the volume of air contained in the pulmonary tissue of newborn infants.

L. Harsányi, Gy. Szuchovszky

(Institute of Forensic Medicine, University Medical School, Budapest)

Responsibility of the Nursing Personnel

Medico-legal experts are often required to name the person responsible for some noxious sequelae arising in connection with medical treatment. As sometimes judgement has to be given against nurses, theatre sisters, etc. It is necessary to analyse the question as to when and how far such persons can be taken to account because of mistakes or omitted actions. The law is governed by the principle of subjective responsibility, and it is discussed how far this principle is realized by the existing medical by-laws, hospital regulations and other statutes. It is concluded that the responsibility has to be borne by the physician if the personnel acts under his supervision, whereas responsibility rests with the nurse as soon as he (or she) performs some independent action (even if commissioned to do so). The scope of responsibility devolving upon persons acting as medical auxiliaries is precisely defined in the existing statutory provisions; this scope is limited, but — within these limits — criminal responsibility is subjective and absolute.

N. Kapusz

(Institute of Forensic Medicine, University Medical School, Debrecen)

Cases of Post-Partum Death

Cases of death following delivery are analysed.

Among the factors responsible for the fatal outcome the followings were present in all examined cases: considerable haemorrhage into the broad uterine ligaments, clinically latent uterine injuries sustained during delivery and, finally, constitutional factors.

The reported cases are discussed in detail and practical conclusions are drawn.

I. Berta, I. Szabó

(Institute of Gynecology, Institute of Radiology and Institute of Forensic Medicine, University Medical School, Debrecen)

Medico-Legal Evaluation of Hysterosalpingography

The results of 1200 hysterosalpingographic examinations are described. Complications connected with the examinations, especially the sterile inflammations are discussed, and the usefulness of contrast media in water and oil is analysed.

The diagnostic value of the patency of the tubes is examined from the angle of forensic medicine, and it is emphasized that only salpingograms revealing patent fallopian tubes are reliable, whereas great precaution is advisable in connexion with radiographs showing occlusion.

Gy. Kiss, A. Steczik

(Department of Pathology, County Council Hospital, Kecskemét)

Fatal Cases of Septic Abortion

The material of a large administrative area has been surveyed in order to ascertain changes in the frequency of different kinds of abortion and the incidence of fatal cases.

The number of birth, after a transitory increase, began to diminish in 1956 and amounts at present to about two thirds of the original value. Together with this decrease there began a striking in that of abortions, so that — compared to the initial number — the latter has risen fourfold. Of 78,089 abortions, 51006/65 per cent were artificial. According to statistical data and careful computations, of the 27,083 abortions which occurred outside the hospital about 75 per cent were artificial.

Among the abortions with fatal termination death was due to septicaemia in 43 cases, and about 60 per cent of the fatal cases were presumably criminal abortions.

In 22 cases (51 per cent) of these, post-mortem examination revealed genital complications (perforating injury, exudate, adnexitis, abscesses). Changes in the genitals were minimal in 8 cases (18 per cent), and only the microscopic study of the endometrium showed the gate of infection. The picture was dominated by purulent peritonitis in 4 cases (9.3 per cent) (septicaemia was associated with adrenal haemorrhage in 2 cases (4.7 per cent). Death was due to tetanus in 5 cases (11.6 per cent) and to gas gangrene in one case. The pathogenic organism was demonstrated in the latter case. Since the roots of various plants are still applied by the ignorant layer of the population in rural districts, the incidence of such cases might have been expected to be higher. Four additional cases in which septicaemia was accompanied by haemoglobinuria and renal injury belong likewise to this group, so that the incidence of fatal infections due to soil bacteria totalled 23.2 per cent.

Extramarital pregnancy was interrupted by means of an unknown tool in one case. The patient developed double pneumonia after the abortion. She had been suffering from chronic heart disease and heart failure when entering the hospital. The case was regarded as one of infection because the development of septicaemia was in our opinion prevented only by the patient's death.

It is noteworthy that the major part of the fatal cases belonged to the culturally and socially lowest layer of the population.

I. Gábor, L. Danka

(Institute of Forensic Medicine, University Medical School, Budapest)

Isolated Pancreatic Rupture

A man received in the course of a tavern brawl a single powerful kick in the abdomen. The injury was recognized in time and the patient was operated upon. Laparatomy revealed a rupture of the body of the pancreas as the only internal damage. This kind of injury rarely reported in the literature. The injury was sutured but the patient died a week later.

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FORENSIC MEDICINE

Printed in Hungary

A kiadásért felel az Akadémiai Kiadó igazgatója

Műszaki szerkesztő: Farkas Sándor

A kézirat nyomdába érkezett: 1965. V. 4. — Terjedelem: 8 (A/5) ív

65.60738 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György

23,—Ft

Index: 26.017