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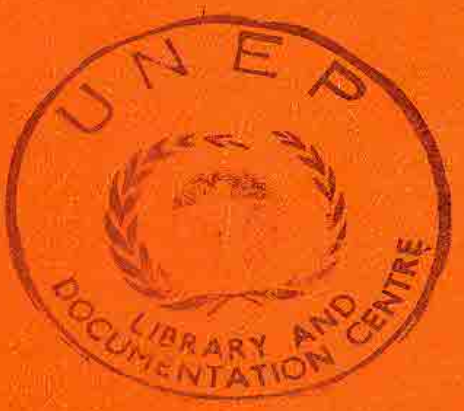
EXPERIMENTAL AFLATOXIC CANCEROGENESIS AND UNDERNUTRITION ULTRASTRUCTURAL AND PATHOMORPHOLOGICAL BASIS



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EXPERIMENTAL AFLATOXIC CANCEROGENESIS AND UNDERNUTRITION
ULTRASTRUCTURAL AND PATHOMORPHOLOGICAL BASIS

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Knowledge about aflatoxins has developed during an era of increasing avareness of the importance of natural as well as man-made chemicals as environmental contaminants (SHabad et al. 1976, Heathcote a. Hilbert 1978, Reiss 1971, Higginson 1963, Chaclin 1979). Discussing the role of geografical pathology in environmental cancerogenesis, Higginson (1972) points to the fact that approximately 80% of the tumors occurring in North America today are conditioned by out present environment. One of the factors of this environment although not the first, is aflatoxin.

The fact that some food-spoilage fungi are capable of producing mycotoxins has been recognised for a long time (Cardeillak a. Nair 1974, Rees 1966, Alpert et al. 1971, Ferrando a. N'Diaye 1979, Kleibel 1977, Jemmali et al. 1969). However, the importance of toxic mold metabolites as food contaminants, used to be evident principally to veterinarians, who frequently encountered outbreaks of poisoning of farm animals.

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The story of aflatoxin pathology begins almost as a fairy-tale. "Once upon a time", in particular 20 years ago,

there occurred the death of some 100,000 turkey poults in England. This at the beginning, designated as "X" disease, was also found to affect duclings, pheasants and other farm animals; involved liver necrosis, bileduct proliferation, haemorrhage, and in fish was characterised by the development of hepatomas (Sinhuber et al. 1976). Since then it was postulated that in all animals poisoned by aflatoxin, the liver was the primary, if not the only organ, specifically affected.

The histological changes varried from hepatic necrosis to liver cell dysplasia and the development of tumors (Wogan 1973, Butler 1966, Dao et al. 1970, Boshnacova 1979). Cuthbertson, Lausen and Pratt (1967) fed 1.8 ppm toxic groundnat meal to cynomolgus monkeys; the animals survived for 3 years. Cirrhosis and liver dystrophy were induced, but no tumors.

In the mean time afletoxin has been found in many foodstuffs including peanuts, soya beams, corn, rice, wheat, barley, cotton seed and others, when stored under conditions favourable for the growth of fungi, but Purchase (1967) was unable to show a relationship between the incidence of hepatic carcinomas and average temperature ot humidity in South Africa. On the other hand recent epidemiologica surveys in 3 climatically different areas of the Muranga district of Kenya showed an association between aflatoxin levels in foodstuffs and the incidence rate of liver tumors (Peers a. Linsell 1973). It was established that there was significant correlation between the calculated ingested daily dose and adult mail incidence of primary liver cancer in different

parts of Swaziland, Africa. Samples of foodstuffs other than the plate samples reflected the same correlation (Peers et al. 1976), which supports the hypothesis that aflatoxin investigation is a factor in the genesis of primary liver cancer in Africa.

Rensburg et al. (1974) reported that aflatoxin consumption by Africans living in the Inhambane district of Mozambique was as uniquely high as the incidence of liver cancer in this area. There was also mostly the same association between aflatoxin content of food and hepatoma frequency in different tribes in Uganda (Albert et al. 1971). The highest incidence - 15 cases per 100,000 per year was found in the Karamojong tribe, where aflatoxin contaminated 44% of the investigated food samples.

Turning to Asia, the picture is a bit different. In Thailand 49% of peanut, 25% of corn and 11% of millet and dried chill pepper samples were found to contain significant amounts of aflatoxin B1. Comparing the data from three different parts of the country, there was a correlation between the amount of aflatoxin content in highest values in the food and the highest incidence of liver cell cancer (Shank Wogan, Gibson 1972, Shank, Bhamarapravati, Gordon and Wogan, 1972, Shank, Bourgeois, Keschamras and Chadawal 1972).

Data from China show also high incidence of liver cancer (Wellmann, Gerstmann 1979). At the end one should point also some review data according to which liver tumor prevalence shows a remarkably variable incidence in different parts of the world, accounting for 0.5% of all post mortems in Europe

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and as much as 7% some areas of Africa and Asia. Among the main ethiological factors, a high contamination grade with aflatoxins is underlined (Bretholz, 1979).

In addition to their importance as public health hazards, the aflatoxins are useful for investigation in experimental chemical carcinogenesis, as they are highly substituted coumarins. Aflatoxins are produced by only a few strains of Aspergillus parasiticus fungi, whose spores are widely distributed, especially in soil. When they occur as food contaminants, aflatoxin from the "G" serie is not so frequent but the absence of "B1" is almost impossible. As a matter of fact the mold can produce aflatoxins on virtually any food that will support growing, but in this case many other factors have also to be taken into account.

Before going to the morphology, some aspects concerning cancerogenicity and biology in general should also be mentioned, although in brief. Firstly we have to remember that notcompletely refractor species to acute or subacute aflatoxin poisoning is known. In domestic animals, symptoms of poisoning are produced by aflatoxin levels in the food of 10-100 mg per kg (ppm), or less. As regards lethal potency to experimental animals, the oral or parenteral LD50 values are generally in the range 5-15 mg/kg body weight for afl. B1 (aflatoxin B1), the main target organ being the liver (Butler, Barnes 1968, Cameron et al. 1976). Administration of the toxin to rates is quickly followed by pronounced inhibition of INA and RNA polymerases in liver; similar responses have been observed in human and animal cell cultures. Protein synthesis is also

impared, particularly under conditions where synthesis is strongly influenced by alterations in messenger RNA synthesis. It seems that polymerase inhibition is an indirect consequence of impared template activity of chromatin, due to toxin-chromatin interaction. Consequently, interaction between afl., or some of it derivatives, with RNA or another component of chromatin is view as the initiating event in the observed series of reactions.

Another line of evidence comes from the effect of afl. on the E.R. (endoplasmic reticulum) and thereby alter polysomal binding to the ergastoplasmic membranes. May be in this connection one can discuss also the next problem - <u>aflatoxic cancerogenesis and nutrition</u>.

Because of the possibility that exposure to aflatoxins could occur in human populations suffering from malnutrition, this problem has been investigated not only in human but also in experimental pathology (Wogan 1975, Krustev 1972, Galikov 1969, Bobrov 1976, Shabad et al. 1976).

The effect of dietary protein has been evaluated in different studies, but with some what contradiotory results. Madhaven et al. (1965) and Gopalan et al. (1972) found that ratesfed a low (5%) protein diet were sensetized to the toxic effects of afl., but developed liver tumors at a lower incidence than controls fed 20% protein ration. On the other hand Newberne et al. (1966), Wogan, Newberne (1967) found that diets containing 9% protein resulted in higher incidence of liver tumors in a shorter period of time than a diet containing 22% protein when both groups of rates were intubated with the

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same quantity of afl. B1 per animal.

Quite interesting also the changes in the ultrastructure of liver cell caused by afl., included into adequate rations containing 18 and 4.5% of protein respectively. In an acute experiment on rats, Pokrovsky et al. (1972) found that the toxic effects of afl. were intensified by protein deficiency. The normal protein content in food however lowered this toxic effect and simultaneously intensified the carcinogenic effect of afl., although to certain extent. Electron microscopy revealed preneoplastic changes in the hepathcytes at the early period much the same as those induced by other well-known chemical hepatocarcinogens. A few years earlier Madhaven, Rao a. Tulpule (1965), and Madhaven et al. (1965) found that high protein diet had preventive effect towards that of afl. on the liver of mankeys, while Smith et al. (1971) came to the same conclusion concerning broiler chicken. Still more this group points to the beneficial effect of high lipid content of the food intake.

Another aspect of the problem concerns the role of deficiencies of choline, methionine and other lipotrope factors. Rogers and Newberne (1969) found that marginal lipotrope deficiency protected rats against doses of afl.B1 that were lethal to 60-100% of rates on an adequate diet. On the other hand, when treated with a carcinogenic regimen, animals with a lipotrope deficiency developed liver tumors much earlier and at a higher frequency than did control animals. One can easily deduce that the mechanism respocible for this interactions are hardly to be claimed as understandable, even now.

When we came to the man, it could be stated that lesser degree of protein and other nutrient defioiencies, which are common amongst adults in the tropics, have an enhancing role in carcinogenesis as they do in some animals. Kwashiorcor also affects other organs in the body, notably the limphoreticular system; atrophy of the thymus and limph nodes, were found in untreated cases at autopsy (Mugerva, 1971). This would render apparently healthy adults more susceptib to infection with oncogenic viruses and generally, less able to withstand tumor genesis by any agent including aflatoxins.

The immunopathogenic effect of afl. is also well illustrated by the fact that afl. reduces the titres of the complement in guinea pigs (Thurstin et al. 1972). Some papers even postulate a new role for afl. in the production of hepatocellular carcinoma. Rather than acting as a primary carcinogen, as it seems to do in animals, it is suggested that afl. supresses cell-mediated immunity. This effect on the immune system would allow the hepatitis-B virus, highly endemic in certain populations, to maintain itself more easily in the liver, to produce more chronic infection and cirrhosis, and in the long term to lead to a higher incidence of hepatocellular carcinoma (Lutwick, 1979).

Several words concerning aflatoxin metabolism. Experiments with the 14-C-labelled toxin indicate that more than 90% of a single dose is excreted within 24 h by rats. Feaces represents the principal excretory route - 75% of the dose, with urine - additional 15-20%. Retained activity is present mainly in the liver (Cameron et al. 1976). This pattern of

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tissue distribution and excretion is generally similar in rodents, monkeys and other species given a single dose i. peritoneally or orally.

Hydroxylated derivatives of afl.B1 are formed through several routes. Ring hydroxylation at the 4 position producing M1 appears to be a common pathway. This derivative has been found in milk, tissue, urine of animals and people ingesting afl.B1. Ring hydroxylation of the carbon atom 8 to the carbonyl function of the cyclopentenone ring to form afl.Q1 was recently discovered in minkey liver preparations and represent the major in vitro conversion by human microsomes. However this pathway seems to be of only minor importance in rodents, still more, rodent liver microsomes convert the toxin to its reactive 2,3-oxide, which has been trapped as an RNA-adduct (Garner 1980). One should not forget also that some metabolites of afl.B1, retaining the vinyl-ether function, such as afl.M1, afl. 1 or aflatoxicol, could also be epoxidized and some of these epoxides might react with nucleic acids. These other adducts may be minor in amount (less than 10% of the total bound afl.B1), but may be extremely significant biologically.

It appears reasonable to speculate that the extraordinary carcinogenic potency of aflatoxins may be due to its related capacity to inflict multiple molecular results on target cells (Mainigi, Sorof 1977); to the changes in the metabolism of nuclear RNA and particularly to the diminution of the nuclear RNA-polymerase activity as well, as to those in the metabolism of RNA (Lafarge, Fraysinnet 1970, Friedman et al.

1970, Pokrovsky 1972).

What are the pathohistological and histocemical findings due to aflatoxins? In an experiment carried by Kalengayi and Desmet (1975) male rats were treated by gastric intubation with a single dose of afl.B1, lethal to 50% of animals (7.20 mg/kg). An early peiportal liver cell necrosis was seen, that was fully developed in rats examined at thirs and sixth days. This necrosis subsequently regressed and disappeared in livers of rates killed from 11 to 32 days and later, but many large polyploid liver cells (megalocytes) were seen. A depletion of glycogen and marked decrease of cytoplasmic RNA occurred early in the periportal region and subsequently extended to the whole liver lobule. There was also fatty infiltration.

During the period of periportal necrosis, acid phosphatase activity increased in the same area with the appearance of typical acid phosphatase tich cytosomes. Except for the acid nucleases, which were practically unchanged all over enzymes-ATP-ase, glucose-6-phosphatase, glucose-6-phosphatase dehydrogenase and succinate dehydrogenase decreased or disappeared in the periportal area; but alcaline phosphatase showed a striking increase in the centrolobular area. When parenhymal necrosis was subsiding, the enzyme activities progressively returned to the normal state. However the morphology of canaliculy still remained strongly altered up to 6 months as revealed by alcaline phosphatase. When periportal necrosis was maximal - at 3 days, the biliary proliferation was still discrete, but at the 6th and 11th day extensive biliary proliferation with ductular differentiation was to

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be seen. On the 6th day there were also some clusters of small and basophilic regeneration liver cells along the areas of necrosis and biliary proliferation. Well developed regenerative foci occurred only from the 11th to the 32nd day, but were almost absent at the 3rd month. This regenerative foci showed a variable content in glycogen and RNA and were characteristically enzyme deficient, which reflects the immaturity of regenerating hepatocytes.

Much the same were the results of Pokrovsky, Meshkov and Kravchenko (1972) who studied the histological, histochemical and biochemical-enzymologic changes of the liver of rats exposed to the acute action of a mixture of aflatoxin B and G. Even on the 12th after the gastric intubation of afl., there were some necrotic foci in the liver lobules. Around the 24th, periportal micronecrose were formed which grew to zonal necrosis with infiltration by neutrophilic leucocytes and monocytes. On the 72nd hour, in the periphery of the necrotic foci appeared more hystocytes and fibroblasts; on the 96th - some oval cells emerged and on the 7th day the picture was dominated by the proliferation of bile ducts. As hould be expected there was the drop in the glycogen and RNA content of the gepatocytes at the early periods, as well as to correlation between the biochemical and morphological changes. Progress in necrobiosis of the liver parenhyma was accompanied by a considerable activation of the acid hydrolases. Rise of the activity of lysosomal enzyme was noted already during the first hours following the administration of afl., and as a rule preceded the destructive alterations of the li-

ver.

Kalengayi and Desmet (1975) carried also some chronic experiments. They treated by gastic intubation male rats with 50 micrograms of afl.B1, twice weekly for 4 weeks, and there - after with 75 micrograms twice a week for the subsequent 10w. The livers of the animals were investigated histologically and histochemically along the above mentioned parameters on the 3, 5, 8, 15, 25, 35, 44, 51, 81 and 86 w after the beginning of treatment. The results showed that the livers of the afl.B1-intoxicated animals killed before 15w had no significant changes. Many "megalocytes" were always noted from 44w onward. From the 15w onward, multiple small foci of liver cell hyperplasia occurred; these foci progressively increased in number and size and became more and more distinct and com - pressed the adjacent parenchyma, thus corresponding to real microscopic nodules ranging from 2-5 mm in diameter. Three morphological types of such areas could be distinguished: foci and nodules type 1, composed of small or large clear vacuoleted liver cells showing some pyonotic nuclei; type II - with small hyperbasophilic hepatocytes, sometimes with mitoses; abd type III - comprising large eosinophilic or pale liver cells in which mitotic figures and acifophilic inclusions could be seen.

The histochemical pattern of cytoplasmic RNA, glycogen and fat in these hyperplastic areas was quite variable from one type to another and even within the same area. However, all 3 types of foci and nodules exhibited a marked bur variable decrease of even complete loss of enzyme activities, but

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occasional liver cells with enzyme activity could be seen in these lesions. Some histochemical-histological discrepancy was observed in different sacrifice periods. Even when liver tumors were developing, hyperplastic and enzyme deficient areas could still be seen in the nontumoral parenchyma, although they became less numerous.

From 44 w onward, liver cell carcinoma occurred. They were multicentric, variable in size, and solid or cystic. Of all the rats that were completely dosed, 69% developed malignant hepatomas. Histologically the predominant type (69.56%) was the pure, well-differentiated liver cell carcinoma; other types were heterogeneous or mixed, and they disclosed areas of well-differentiated liver cell carcinoma alternating with either less - differentiated or cholangiomateous areas, or both. Metastases were noted in the lungs and/or mesenteric nodes in 4 animals. They all showed histological features of pure well-differentiated liver cell carcinoma. RNA, glycogen and fat content in the tumors were variable. All the liver cell carcinomas showed absence or decrease of enzyme activities as observed in the hyperplastic areas, except for glucose-6-phosphate dehydrogenase and alkaline phosphatase, which sometimes showed special features.

In some other experiments feeding of male Fisher rats with a diet containing 4-5 ppm of afl.B1 for 6 wm followed by return to control diet, results in 100% incidence of hepatocarcinoma (Judah et al. 1977). During the first 3 w histologically there was an actually toxic response with death of a large proportion of the hepatocytes and depressed nucleic

acid synthesis in the rat liver. It is interesting that in the above mentioned experiments there was no underlying of the familiar for large part of the literature abbundance of "oval cells" on the 3-4 w of afl. intoxications (Butler, Barnes 1963, Beal, Butler 1978). These cells are known not only in connections with afl., but with many other toxic and carcinogenic factors, including DAB also (Goldfarb 1973, Farber 1963, Buicorez and Pintchuck 1976, Farber 1956). The role of "oval cells" in neoplasia and the problems surrounding their histogenesis can hardly be claimed to be solved. As far as hepatomas are themselves concerned, even a single or several injections of afl.B1, can produce it in newborn rats around the 52nd week of age (Vesselinovitch et al. 1972). Data from experiments with other animals - mice, guinea pigs, ducklings and others, follow similar histological and histochemical pattern of liver changes, with minor differences. For example in guinea-pigs, the effect of afl., was characterized by the development mainly of liver cirrhosis (Gedek, Hofmann 1970); necrosis and fatty degeneration of the liver in ducks (Richer et al. 1964, Richer et al. 1969); multinucleated giant cells and portal fibrosis in marmosets (Svoboda and Lin 1971).

As far as the connections between human pathology and afl. are to be discussed, except cancer only cirrhosis of the liver has been until now incriminated (Peers, Linsell 1976, Jadgiri et al. 1970). In fact afl. is supposed to play also a role in the etiology of reyess syndrom in humans and in experimental animals, where among others fatty degeneration is to be found (Olson et al. 1971, Bourgeois et al. 1971).

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Ultrastructural changes due to afl. One of the most often mentioned changes that affect the nucleus consists of redistribution and quantitative differences in its fibrilar and granular components (Grundmann, Kirsten 1977, Higginson, Svoboda 1975, Pokrovsky et al. 1972, Edwards et al. 1971). In fact the micro- and macrosegregation of the nucleoli of the hepatocyte is common to most of the carcinogenic factors of the liver - DEN, DMN, DAB, TAA as well as by hepatocarcinoma in humans (Svoboda a. Higginson 1968, Reddy et al. 1976). In all of these cases, by rounding off of the granular and fibrilar zones, the separation of nucleolar components becomes more pronounced, and this can be one of the first effects of acute single afl. exposure (Bauer, Tulusan, Müller 1972). With the progressive separation of nucleolar components, the granular elements become markedly coarser while the cytoplasm shows loss of density. The nucleolar zoning and segregation is a characteristic alteration produced by substances that bind with DNA and interfere with its template activity in DNA directed RNA synthesis. It is interesting that nucleolar segregation has been described in other cells - lymphocytes, leyding cells, in the aging human tests by prostate gland cancer, and in nuclei of human epidermis cells in precancerous disease (Valkov 1975, Yasuzumi et al. 1975). In these cases the occurrence of nuclear bodies is preceded by the separation of the amorphous protein component from other components in the nucleous. The nuclear bodies originating from such a protein microsegregation of the nucleolus. This occurred at the second week and persisted during treatment with

afl., but reversed from 4-6w after withdrawal. Macronegregation was not present (Svoboda a. Reddy 1975). The RER was with slight dislatation and detachment of ribosomes from its membranes. It is claimed that afl.B1 may act directly at the steroid-dependent ribosome binding sites of the ER membranes (Williams et al. 1975). This is with the conclusion of Garner, Wright (1973 and 1980), Apffel (1978) and others, that aflB1 may not be a direct carcinogen, but has to be metabolized to an active metabolite which then binds to DNA, protein and some polynucleotides, the bindings being microsome-dependent. Confirmation of this point may be in the changes of the smooth ER which is very well expressed. Among the other changes of the liver cell organelles are mentioned the following: marked elongation of some of the mitochondria, patchy loss of glycogen in scattered foci of enlarged vacuolated cells, some fat dropplets and occasionally dilated bille canaliculi.

Tumor cells in the liver, due to afl. have few small mitochondria, moderate numbers of microbodies, and short dilated segments of ER. Nucleoli were usually abnormal in their configuration and showed some degree of segregation of constituents. In contrast to the nonneoplastic liver, there were extensive collections of interchromatin granules. In one of the few communications about ultrastructure of tumor cells induced by afl.B1 in other organs, namely kidney, these cells preserved some characteristics consistent with renal origin, but there was a striking loss of apical orientation of brush borders and a lack of any orientation of mitochondria.

The observations are compatible with a tubular origin for these renal carcinomas.

Some main pathomorphological and ultrastructural data from the experiments carried in our laboratory. The first series of our experiments were concerned with the effect of a single dose afl.B1. Male rats with body weight of 100 g each were derived as following: Igr, on standard pellot food and water ad libitum; IIgr. - the same food and single i. periton, inj. of 0.5 ml of 1.2 propylenglycol; IIIgr. - a single i. periton. inj. of 6 mg afl.B1 in the above mentioned vehicle, and IVgr - where the afl. was 10 mg of the afl., 98% was comprised of B1, and the other 2% - a mixture of B2, G1 and G2. Four hours after this treatment the rats were discapitated. Liver tissue was processed according to the classical histological techniques, for transmission electron microscopy, and for evaluating the acid phosphatase (after Gomory-Holt) activity in the lysosomes of the liver cells (Krustev 1972a).

The morphological picture in the first two groups did not deviate much from the known one in the normal rat, except for some fatty droplets in the hepatocytes. In the IIIgr. the u rastructure of the liver cell was characterised by segregation of the granular and fibrilar components of the nucleolus (fig. 1), cluming of geterochromatin at the periphery of the nucleus, some deformities in the nuclear membrane, dilated perinuclear space and lipid droplets in some of the nuclei (fig. 2).

The RER around the nucleus was less often to be seen, there was degranulation of its ribosomes, well expressed SER,

although with some vacuolisation. The complex of Golgy is presented by its vesicles, in any of which there were fine lipid droplets. From the other organelles of particular interest were the lysosomes, which were abundant not only in the hepatocytes, but also in the Kupfer cells. Among them were primary forms but also autophagolysosomes and citolysosomes with marked acid phosphatase activity, sometimes with membrane defects (fig. 3). The IVth and last group didn't appear to be much different from the third one, but the changes in the fine structure affected greater number of the hepatocytes. The conclusion being, that there was no differenses between our data and those in the literature, with one exception. It is the morphological and cittochemical proof of the effect of afl. on the structure and function (acid phase activity) on liver cell lisosomes.

The second experiment was performed on the same strain of rats, according to the above mentioned, sex, weight, etc. The animals were divided in the following groups: Igr. - water and standard pellet food ad libitum; IIgr. - same food, but also 10 microgramms of afl. in propylenglycol injected i. peritoneally 5 times weekly; III^{I}gr. - with only 20% of the standard pellet food of the rats in I and IIgr; IVgr. - the same 20% of the food that received the controls (Igr.) but treated with 5 i. periton. inj. of 10 microgramms afl.B1; Vgr. - food and water ad libitum and 5 times weekly i. periton, inj. with propylenglycol. Every group consisted of 10 animals. The experiment continued for 8 w. Methods used were the same as those performed in the acute experiment.

The results of these experiment in summary are as follows: Gr.I (controls), and that treated with propylenglycol (Gr.V), did not show any particular changes in the morphology or ultrastructure of the liver. In gr.2 (afl.B1 - for 8w), although the liver tissue was generally preserved, there was marked polymorphism of the nuclei (some in mitosis), with expressed degenerative changes in the parechimal cells; microgranulomas formed by lymphoid mononuclear and leucocytic cells, and portal inflamation with the same cellular characteristics (fig. 4). In group III - undernutrition with only 20% of the food of controls, the most important were the signs of atrophy in the liver (fig. 5) with dilated sinunoides, thiner liver cell plates, lipofuscin pigment and so on. Most affected were the animals in group IV - the same undernutrition as in group III, but also treatment with afl.B1. In fact, here the type of lesion was the same as in the group on sole afl., but the changes were more expressed. Instead of mainly vacuolar and lipid, there was acidophilic degeneration in the hepatocytes, with forming even of Councillman type bodies, ballooning of the liver cells etc. However in neither of the groups were cirrhosis or tumors to be found.

So we come to our last experiment which has the same parameters as those of the groups from the previous one, except for the period of treatment - 7 months and the percentage of food intake in groups 3 and 4, where the food was half, 50% of that given to the controls. The methods of investigations were the same in all experiments.

What were the results? In gr.1 - controls - the only si-

gnificant change was accumulation of fat droplets in the liver cells. Quite different was the picture in gr.II - normal food intake and 1400 microgramms of afl.,B1 i. peritoneally. Here, of 10 animals one died of bronchoneumonia in the course of the treatment of the 9 left, when decapitated on the 28 week, 3 were with tumors in the right abdominal region (fig. 6). Histologically the tumors were identified as leyomiosarcomas, fibrosarcoma and the highly undifferentiated mesenchymoma (fig. 7.8.). In the liver the lesions were exemplified by vacuolar, lipid and to a lesser extent acidophilic degeneration, with minor inflammatory and vascular changes, but with not signs of cirrhosis or cancer.

Electron microscopically in this group the signs of microsegregation of nucleolus could still be revealed, the chromatin was clumped around the nuclear membrane. The normal pattern of RER was distributed,wbut in different locations of the liver cell it formed stacks of cysterns. The ER in general was vacoolised and hypertrophied (fig. 9). Mitochondria were deformed, their crystae disorganized, with the general swelling of the organelle (fig. 10). The complex of Golgy was dilated, while the microbodies were in higher number than in controls. Lysosomes were more often than usually located all over the hepatocyte or Kupffer cell, and these is valid for all of their representatives - primary ones, or autophagolysosomes, cytolysosomes and residual bodies (fig. 11).

Acid P-ase was generally well expressed throughout the matrix, often in connection with the membrane, but with the locuses of the organelle without any activity. Glycogen was

seen in its usual rosette-forming pattern, while lipid droplets were mainly in the SER region or among mitochondria.

The rats belonging to gr.III - 50% underfed for 2 and 7 month were with histological signs of atrophy and degeneration of the parenchimal cells. El.microscopically on the 7th month, the nuclei were with polymorphism, the perinuclear space was dilated, but the nucleus was without substantial changes. The quantity of the RER was diminished; there were signs of degranulation and disorganisation of its structures (fig. 12). Diminution affected also the elements of SER and miochondria and Golgi complex was moderately dilated; microbodies were not changed, while most of the lysosomes were of the autophagolysosomal type, with the usual acid P-ase reaction.

The IV group - undernutrition and afl.B1 - was comprised of 5 rats. The other five died in the course of treatment due to intracurrent infection. Of the five that were left one died with tumor in the right part of the abdomen. Histologically the picture was the same as that in the tumor of the rats treated sollely with afl.B1 - leyomyo- and fibrosarcoma. However this time the large bowel was also affected (fig.13). When we came to the liver, this was the only case with the metastase from the sarcoma. It also of interest that this same liver showed tendency towards cirrhosis in the region around the metastase.

Ultrastructurally in this animal there was polymorphism of the nucleous (fig. 14), the usual picture of segregation and even capping of the nucleous of the hepatocytes, diminu-

tion, degranulation and disorganisation of the ER, marked changes in the mitochondria (fig. 15), increased number of different lysosomes (fig. 16) with high acid P-ase activity (fig. 17). In the other 4 rats of this group, no tumors were to be found. However the histology and ultrastructure of their livers did not differ dignificantly from the picture of the nontumorous part of the liver in the sarcomatous rat.

So at the end of out investigations we can conclude firstly that afl. is not so much a hepatocarcinogenic agent, but has a more general and powerful blastomogenic effect; secondly, that means of application, tissue homeostasis and may be other, unknown factors are of greater importance than usually assesed; and third - effect of afl. leads at the end to a more malignant pattern of the tumor process.

Many and different points concerning the aflatoxin B1 - induced sarcomas could be rased. Not smaller number are those of nutritional origin; even the morphology and ultrastructure, including the role of lysosomes, bare some difference when compared our data with that of the literature. So it seems at the end that our knowledge about aflatoxic apathology and morphology raises more questions, or at least some new ones, without resolving allthose that still exist.

Maybe this makes problems surrounding aflatoxins and mycotoxicoses in general so fascinating for biology and pathology.

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TEXT OF FIGURES

Fig. 1. - Segregation of nucleolus in the hepatocyte. Magn. x 20,000

Fig. 2. - Lipid droplet in the nucleus of the hepatocyte. Magn. x 8,000

Fig. 3. - Well expressed activity of acid phosphatase in the autophagplysosome. Magn. x 50,000

Fig. 4. - Degenerative changes of the liver parenchyma with inflammation of the tissue. Hematoxilin, eosin staining. Magn. 10x10

Fig. 5. - Atrophic changes of the liver tissue. Hem. eosin. Magn. 10x10

Fig. 6. - Tumor in a rat treated 7 months with Afl.B1

Fig. 7. - Leiomyosarcoma in part of the tumors. Hem. eosin. Magn. 10x10

Fig. 8. - "Mesenchinoma" in part of the tumors. Hem. eosin. Magn. 10x10

Fig. 9. - Vacuolisation of the RER in the hepatocyte. Magn. x 20,000

Fig. 10. - Disorganisation of oristae mitochondriales in the hepatocytes. Magn. x 19,500

Fig. 11. - Two autophagolysosomes in the hepatocyte. Magn. x 26,000

Fig. 12. - Disorganisation of the RER in the hepatocyte. Magn. x 16,000

Fig. 13. - Leomyosarcoma of the large intestine. Hem-

eosin. Magn. 10x10

Fig. 14. - Deformities in the nuoleus of the hepatocyte. Magn. x 18,000

Fig. 15. - One of the mitochondria is with a marked disorganisation of its crista. Magn. x 24,000

Fig. 16. - Increase in the number of the different types of the lysosomes in the hepatocyte. Magn. x 18,000

Fig. 17. - Marked acid phosphatase activity of the lysosomes in the liver cells. Magn. x 3,000

Зак.3517 ПИК ВИНИТИ