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A PRELIMINARY STUDY ON EXPERIMENTAL HAEMOBLASTOSIS PRODUCED IN RATS BY TANNIC ACID*

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In previous experiments we had succeeded in producing liver cirrhosis [8], then liver tumours [9] in rats, by protracted parenteral administration of an aqueous solution of tannic acid. Considering that the rats treated in the way described developed tumours only in the liver, it had been assumed that tannic acid has a selective blastomogenic effect [10]. It has, however, been proved by protracted oral administration of tannic acid that the assumption is erroneous. The transformation of the liver structure was less profound after oral administration of tannic acid than that on parenteral treatment [7] and in none of the orally treated animals has a liver tumour developed. On the other hand, systematic histologic examination of the organs revealed unexpected changes in the haemopoietic and reticular apparatus.

Experiments

The data of two experimental series will be discussed. In one series a pharmaceutical tannic acid product (Acid. tannic U. S. P., Johnson and Sons Ltd., Hendon, London) was given orally while in the other an impure product (Valex) was administered subcutaneously to the rats for a long time.

In *the first experiment* 2 groups were treated. The first group consisted of 20 rats which received 4 doses of tannic acid weekly. 40 rats belonged to the 2nd group; they received 1 dose per week. Each dose, consisting of 4 ml of the aqueous tannic acid solution, was administered through a stomach tube before meals.

The *first group* was treated at the beginning with a 1 per cent solution; later the concentration was increased by 1 per cent, every second month, so that from the fifth month on until the end of the experiment the rats received a 5 per cent solution. The group consisted of an equal number of male and female young albino rats. At the beginning of the experiment their average weight amounted to 107 gm, on the 100th day to 210 gm, on the 200th day to 240 gm and on the 300th day to 238 gm. 17 rats survived 100 days, 11 rats 200 and 7 rats 390 days. These last seven animals were killed on the 391st day by destroying the medulla oblongata.

The *second group* consisted of 20 male and 20 female rats. In the first month they were given a 3 per cent, later a 5 per cent, solution of tannic acid through a stomach tube. The following changes occurred in their body weight during the experiment.

	Initial weight	100th day	200th day	300th day	400th day
Males	152 gm	215 gm	240 gm	269 gm	199 gm
Females	153 gm	184 gm	202 gm	211 gm	196 gm

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16 males and 16 females survived the 200th day, 14 males and 14 females the 300th day, and only 2 male and 2 female rats the 500th day of the experiment. Treatment was interrupted after 6 months for 5 weeks, then it was continued in the same way. The last, i. e. the 65th, treatment was performed on the 507th day.

In *the second experiment*, 40 male and 40 female white rats of an average age of 6 months were used. They were treated with a fresh aqueous solution of Valex subcutaneously every fourth day. The rats of longest survival were given of the Valex 11 times 50 mg per kg, 69 times 100 mg per kg, and 7 times 150 mg per kg. At the beginning the males weighed 144 gm on the average, females 142 gm. Treatment was generally well tolerated ; male animals seemed, however, to be more susceptible than females, considering that 31 females and only 13 males survived the 250th day, 12 females and only 4 males the 350th day, of the experiment.

In both experiments, white rats bred in our institute were used. The animals were fed with remnants of patients' meals and the diet was completed with curd at least twice weekly. Rats dead in the course of the experiment were as a rule immediately dissected. In a number of them intercurrent diseases (pneumonia, pulmonary abscess or purulent otitis) could be found. All organs were fixed in a 4 per cent aqueous solution of formaldehyde. The sections were stained with haematoxylin-eosin, according to van Gieson, Giemsa, Gram, with Gömöri's reticulum stain, and for iron.

Gross and Microscopic Changes

In the rats orally treated with tannic acid for a longer period (Experiment 1), only an enlarged liver and spleen were found in some animals, and it was noticed that the colour of the spleen was somewhat lighter than in other rats *(Fig. 1)*. Subsequent histology revealed grave lesions of the haemopoietic system in several cases.

In the liver of a rat which had been treated once a week and which succumbed on the 332nd day, an extremely intensive focal and diffuse myeloid infiltration was observed : a large number of myeloid cells was present not only in periportal foci and dilated sinusoids, but in all the vessels *(Fig. 2)*. The nucleus of the cells was of a varying shape, mostly roundish, sometimes of the form of a kidney or a horse-shoe. A minor part of the cells were polymorphonuclear leucocytes. Occasionally mitosis could be noted in the immature cells. The bands of liver cells have become thin, and in areas where the cellular infiltration was the thickest, they have completely disappeared *(Fig. 3)*.

The architecture of the enormously enlarged spleen was distorted and only a few small follicles could be found. The parenchyma was replaced by a tissue containing a great amount of cells, mostly roundish, with remarkably large nuclei, a fine chromatin meshwork, and large nucleoli. Mitosis occurred in many of these cells. The bone marrow was extremely rich in cells and normal bone marrow cells were mainly replaced by fairly large cells possessing a large nucleus and nucleolus. The number of mitoses in the bone marrow was so high that under high power magnification 5 or 6 or more mitotic figures were present in each visual field *(Fig. 4)*. The major part of the cells of the bone marrow and spleen were most immature, corresponding to the Ferrata cells, haemohistioblasts.

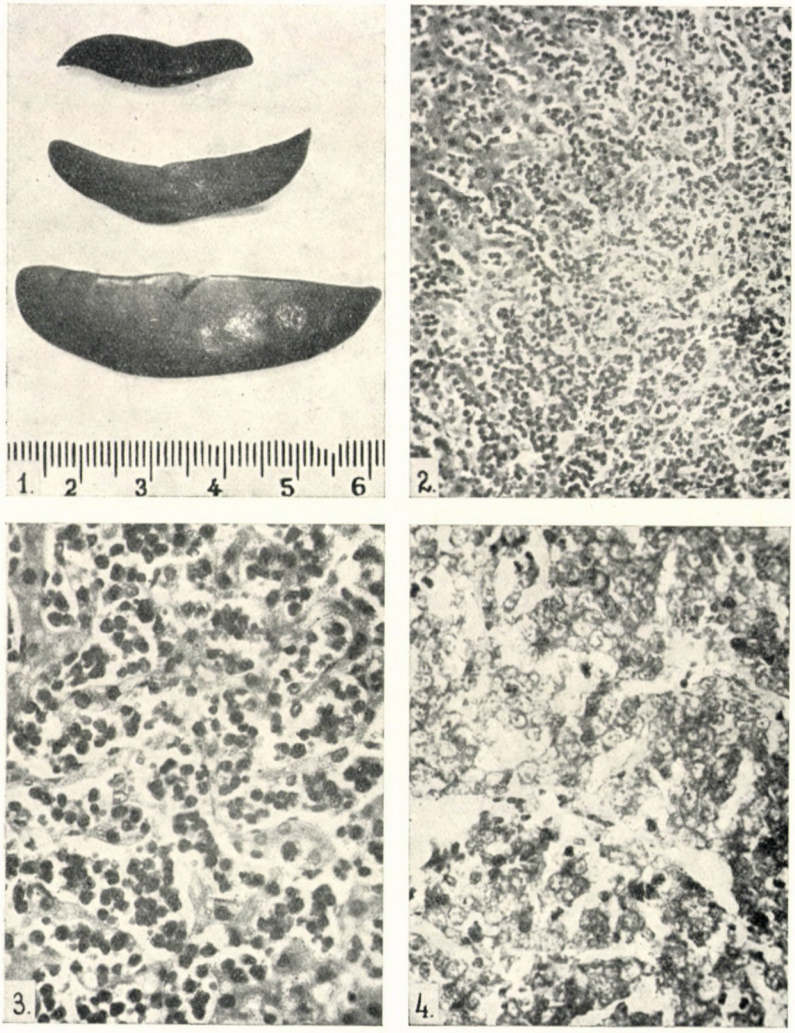

Fig. 1. Rat VI/9. Oral tannic acid. treatment 4 times weekly. Killed on 391st day. Weight 200 gm, weight of spleen 2,75 gm. Nearby : spleens of 2 untreated rats

Fig. 2. Rat OK16. Oral tannic acid treatment once weekly. Died on 332nd day. Liver, haem.-eo. stain, 200 ×

Fig. 3. Rat OK16. Liver, haem.-eo. stain. 400 ×

Fig. 4. Rat OK16. Bone marrow, numerous mitoses. Giemsa stain. 600 ×

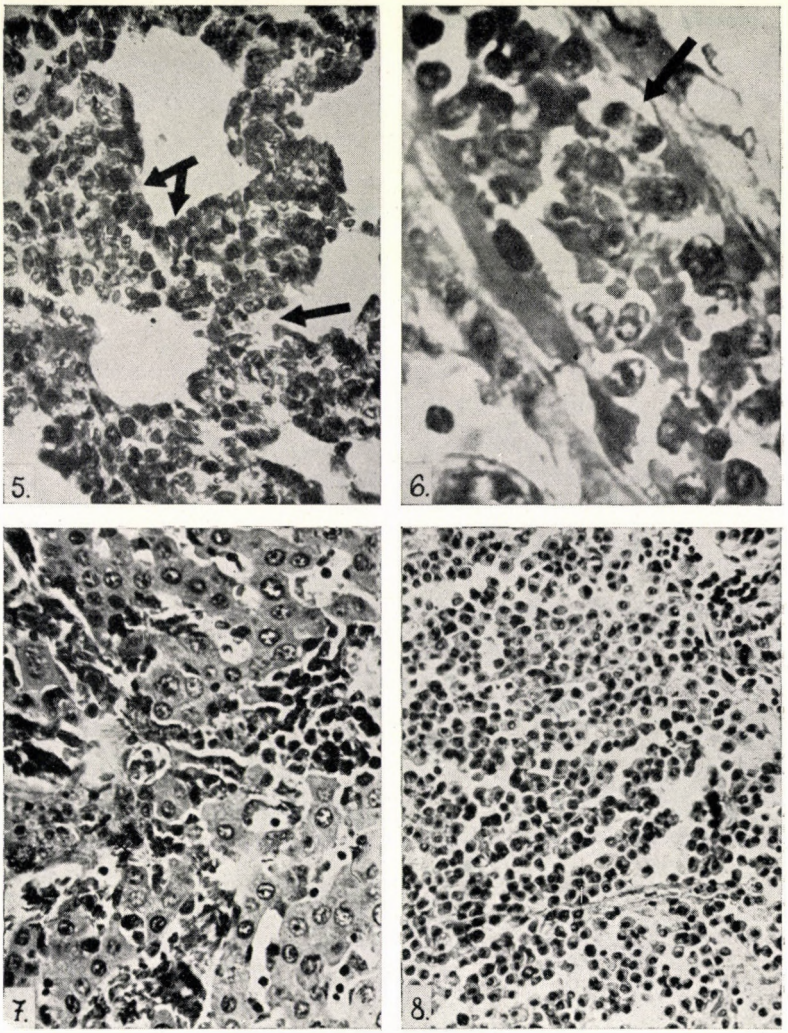

Fig. 5. Rat OK16. Lung, 4 mitoses signed with arrows. Haem.-eo. stain. 600 ×
Fig. 6. Rat OK16. Cerebral vessel, mitosis signed with arrow. Haem.-eo. stain. 1200 ×
Fig. 7. Rat Valex 10/III. Died on 179th day. Liver, haem.-eo. stain. 400 ×
Fig. 8. Rat Valex 7/VI. Died on 217th day. Lymph node. Haem.-eo. stain. 200 ×

In the vessels of all visceral organs large numbers of mainly immature elements of the granulocyte series could be observed. In addition, a mild degree of myeloid infiltration was present in the kidneys, adrenals and ovaries. The histological appearance of the lungs and brain was also impressive. In the lungs, the alveolar walls were thickened, with considerable narrowing of the lumen, in consequence of the enormous distension of the capillaries full of myeloid cells. Mitosis was very frequent *(Fig. 5)*. The cerebral vessels were also dilated and contained many immature myeloid cells with a few mitoses *(Fig. 6)*.

In the organs of another rat which had been treated once weekly an essentially identical histological picture was observed. This animal had died on the 429th day of the experiment. In one of the rats treated four times a week the same changes occurred. This animal had been killed on the 391st day of the experiment.

Apart from the 3 rats mentioned, in both the first and second groups a mild or moderate degree of focal and diffuse myeloid infiltration frequently occurred in the liver *(Fig. 7)*. In the spleen the follicles were smaller than usual and foci of myeloid tissue, accompanied by reticular hyperplasia of a varying degree, could often be found, sometimes with giant cells of the megakaryocyte type. The bone marrow was hypercellular in all rats and beginning from the 100th day of treatment it displayed an intense leukoblastic reaction. The nucleus of the myeloblasts, containing a large, roundish nucleolus, was remarkably large and light.

In the rats treated *parenterally with a solution of Valex* (Experiment 2) neither cirrhosis nor tumours could be produced in the liver, probably because in this experimental series only about half the amount of tannic acid was administered as in those [8, 9, 10, 12] in which cirrhosis and tumours had been induced. Nevertheless, a histological examination of the haemopoietic and reticular systems revealed remarkable changes also in these animals.

The spleen and lymph nodes were moderately increased in size. In some cases the cervical lymph nodes were bean-sized, their longest diameter attaining one centimeter. Microscopically the most characteristic change consisted in a varying degree of hyperplasia of reticuloendothelial elements.

The architecture of the lymph nodes showed a gradual transformation. First the disintegrating lymphocytes are replaced by plasma cells. The endothelial cells of the dilated sinuses are swollen, partly desquamated. Later, the normal cell population becomes replaced in an increasing degree by plasma cells, histiocytes, polyblasts and endothelial cells, from among which one or the other type frequently predominates. The cortex becomes thin and in animals treated for more than six months it either appears in the form of a marginal layer containing no follicles or it has completely disappeared. The diffuse reticular hyperplasia soon results in obliteration of the sinuses whereby normal architecture becomes distorted or fully unrecognizable.

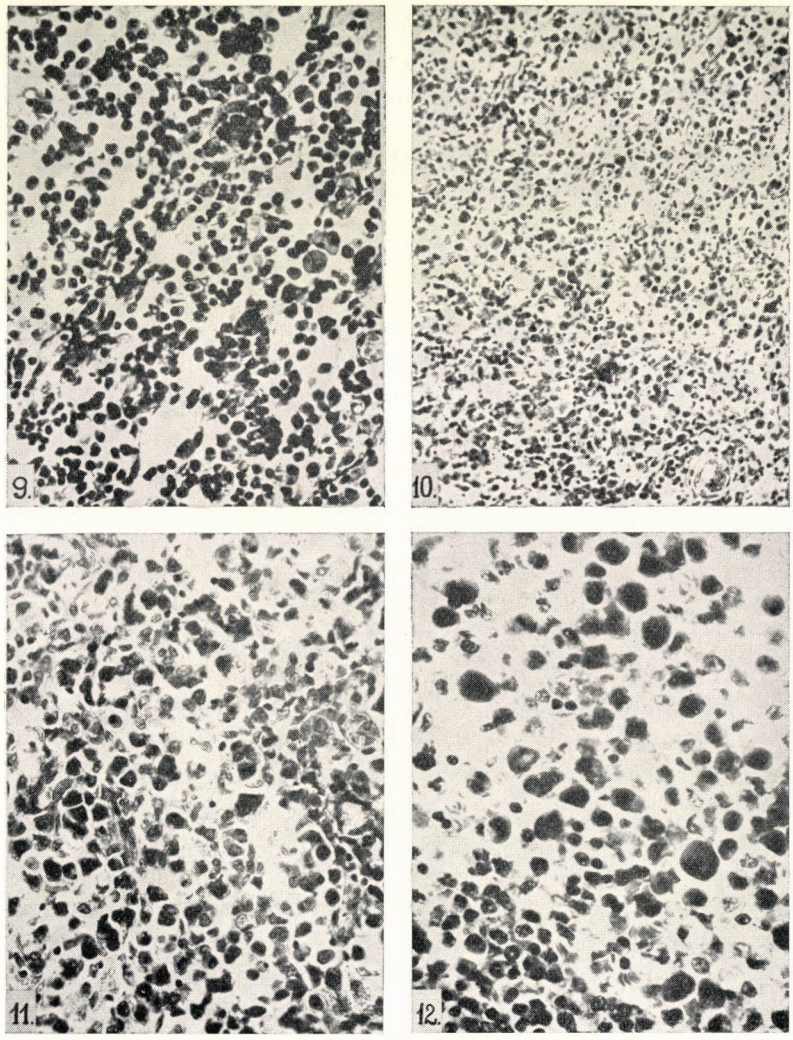

Fig. 9. Rat Valex 4/VIII. Died on 240th day. Lymph node, 400 ×
Fig. 10. Rat Valex 4/VI. Died on 229th day. Lymph node. Haem.-eo. stain. 200 ×
Fig. 11. Same as Fig. 10. 400 ×
Fig. 12. Rat Valex 4/VI. Bone marrow, haem.-eo. stain. 600 ×

In the cervical lymph nodes plasmacellular hyperplasia is the most frequent phenomenon *(Figs. 8, 9)*. The plasma cells filling the reticular meshwork are, however, not uniform, their nucleus being of a varying size, and mitosis is not infrequent either. Though the disappearance of the architecture and the atypical character of the cells makes the picture often appear as a neoplastic transformation, criteria of malignant proliferation are generally missing. There was one exception, a rat which had died on the 229th day of treatment, and which exhibited a cervical lymph node composed of a highly cellular tissue infiltrating even the capsule. The tissue consisted of unusually large, mononuclear, atypical cells with numerous mitoses *(Figs. 10, 11)*. In the marrow of the femur of this rat, groups of highly atypical cells, considerably larger in size than the haemopoietic ones, were present. The nuclei of these often oval shaped cells were mostly excentric and mitoses were seen among them in an unusually high number *(Fig. 12)*.

The structure of the spleen underwent profound changes in every animal. The follicles soon decreased in size and initially the pulp was extremely hyperaemic. Later the hyperaemia ceased and proliferation of reticulum cells blurring the normal architecture became more and more conspicuous. The swollen reticulum cells, some of which display mitosis, surround the wasted Malpighian corpuscles like a ring. Beside scattered groups of plasma cells and histiocytes containing a brownish-red pigment, focal myeloid metaplasia with some giant cells of the megakaryocytic type are not infrequently observed. The giant cells sometimes resemble those of the Reed-Sternberg type and the whole picture is similar to that of Hodgkin's disease *(Fig. 13)*.

After the third month of treatment the bone marrow becomes hypercellular and it is chiefly the immature elements of the myeloid series that seem to have increased in number. The large round myeloblasts contain unusually large nucleoli ; mitoses are frequent *(Fig. 14)*. Myelosclerosis or gelatinous transformation of the bone marrow was not observed in any of the cases. In the liver, mild focal or diffuse myeloid metaplasia was fairly frequent.

Large histiocytes, often appearing in groups, were observed in the spleen and the lymph nodes. The nucleus of these cells is small, excentric, and their greatly swollen cytoplasm is filled with a granular foreign substance *(Fig. 15)*. These serve obviously for the purpose of storage and contain presumably tannin or tannic complexes. In the spleen and lymph nodes, intensive erythrophagocytosis *(Fig. 9)* occurred frequently and deposited granular iron pigment could be observed.

The morphological appearance of the *neuro-endocrine system* was as follows. The adrenals, ovaries, the thyroid, parathyroid and pituitary glands were larger than those of the untreated controls. In the adrenal cortex chiefly the fasciculate zone was widened, containing little or no lipid, whilst the glomerular zone was rich in lipids. The ovaries contained a great number of large yellow

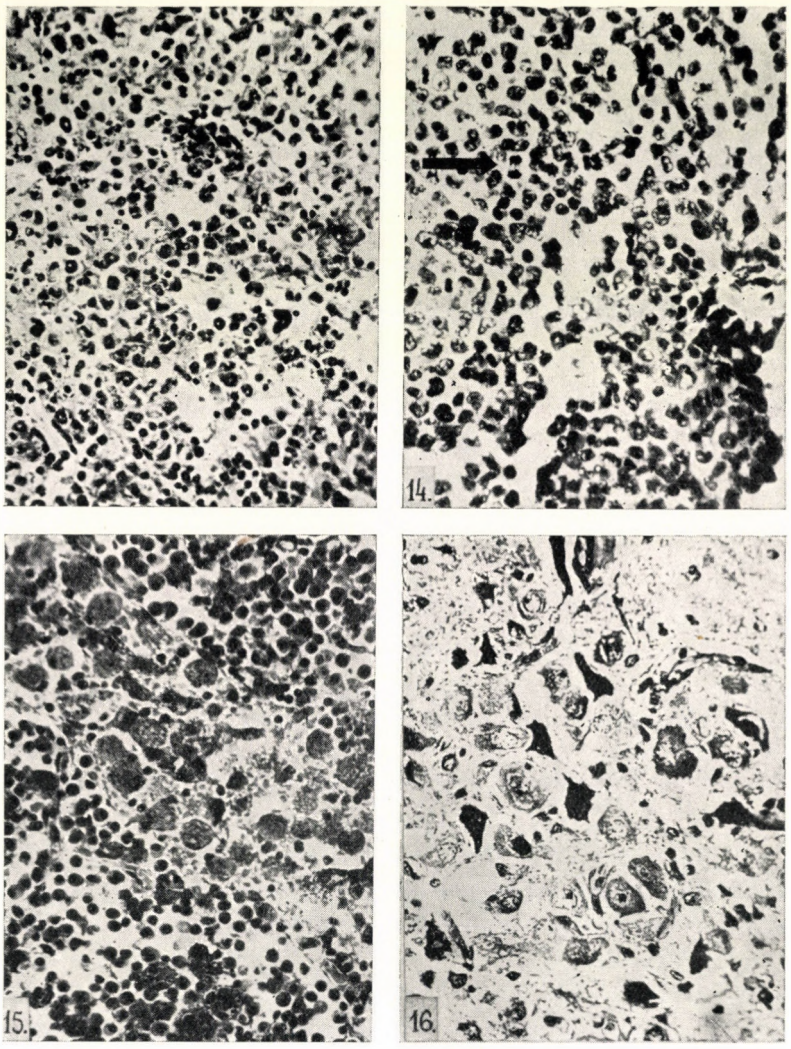

Fig. 13. Rat Valex 4/VIII. Died on 240th day. Spleen. Changes similar to Hodgkin's disease. Haem.-eo. stain. 350 ×

Fig. 14. Rat Valex 6/VIII. Died on 214th day. Bone marrow, leukoblastic reaction, several mitoses. Haem-eo. stain. 600 ×

Fig. 15. Rat Valex 1/VIII. Died on 486th day. Lymph node. 400 ×

Fig. 16. Rat Valex 5/II. Died on 313th day. Greatly enlarged, partly degenerated ganglion cells. Haem.-eo. stain. 400 ×

bodies. The enlarged parathyroids were rich in cells. In the thyroid the lining cells were often high, the colloid was either missing or it stained quite pale. In the anterior lobe of the hypophysis the number of basophile cells was significantly increased at the expense of the chromophobe ones and their nucleolus was also enlarged. The basophilia of the cytoplasm was increased in some cases while in others degranulation occurred; hyaline areas were also present sometimes.

Considerable changes were visible in the nuclei of the anterior hypothalamus; the large ganglion cells of the supraoptic and paraventricular nuclei were significantly enlarged, so their cytoplasm, as the nucleus and nucleolus. Apart from the hypertrophy of the large ganglionic cells, degenerative phenomena also occurred, in some cases together with slight haemorrhages *(Fig. 16)*.

Discussion

The chief problem arising in connection with the changes observed refers to the very *nature* of the lesions. In three of the 29 rats surviving after the 180th day of oral tannic acid treatment, the histological picture was suggestive of myelosis. No evidence has, however, been brought forward as to the existence of a real leukaemic myelosis, since no blood counts were performed and no transplantation was undertaken. Particular attention will be paid to these points in our experiments now in progress.

Although the presence of a real myelosis has not been fully evidenced, it is thought that the microscopical picture of the organs forms a sufficient basis for assuming its diagnosis. This is supported first by the presence both in the extremely hypercellular bone marrow and in the spleen of a large number of entirely immature cells corresponding to the least mature cells of the haemopoietic system, the Ferrata cells, haemohistioblasts. Another remarkable phenomenon was the very frequent occurrence of mitosis in the numerous immature haemopoietic cells in the vessels of the different organs. The disappearance or distortion of the splenic architecture, observed in all of the three animals is, in *Block* and *Jacobson*'s opinion (1950), an important finding in human leukosis, in contrast with myeloid metaplasia.

As to the character of the changes of the lymphoreticular system, it is rather difficult to form a definite opinion. On the basis of the often excessive hyperplasia of various cells pertaining to, or originating from, the reticular system, as observed in the lymph nodes all over the body and in the spleen, it seems justified to speak, at least in some of the cases, of a diffuse reticulosis. According to *Haranghy*, the diagnosis of such patterns may be very difficult since, considering the capacity of reticuloendothelial elements ready to proliferate, the limit of simple hyperplastic processes cannot be stated with certainty.

The several times observed disappearance of the architecture of the lymph nodes, the various degree of differentiation of the proliferating cells, the nuclear polymorphism and the frequent mitoses, all suggest that the proliferation of the reticular system has, at least in some of the animals surviving the 180th day of the experiment, passed the limit of simple reactive hyperplasia and turned into proliferation of a neoplastic nature.

According to the literature, spontaneous leukosis is very rare in white rats. *Oberling*, *Guérin* and *Guérin* (1939), found leukaemia in only 9 from among 6000 rats. All the nine were old animals, most of them beyond 29 months of age. *Arai* (1940) found spontaneous myeloid leukaemia in one out of 500 rats of different strains.

Most of the efforts to produce experimental leukaemia in rats have failed. Several investigators *(Bernard, Storti* and *Storti)*, using *Engelbreth—Holm's* method [17], injected carcinogenic substances into the bone marrow. The procedure was successful e. g. in chicken, but failed in rats. *Shay et al.* (1952) administered methylcholanthrene through a gastric tube to 39 Wistar rats in order to induce cancer of the stomach. Though they failed in producing cancer in this way, myelosis was developed in 3 rats and lymphoid leukaemia in 6.

In contrast with rats, in mice it is relatively easy to induce leukosis by means of chemical carcinogens. In this respect noteworthy are the experiments of *Morozenskaya* (1949) who produced leukosis in white mice by subcutaneously administering dimethylaminoazobenzene in oil, a compound which had been considered a substance selectively producing liver cancer and having, in this respect, the same effect as tannic acid.

According to the literature, tumours of the lymph nodes are also rare in rats. *Ratcliffe* (1940) observed in Wistar rats 273 spontaneous tumours out of which only 2 were mediastinal lymphoblastoma and 2 lymphosarcoma. *Farris* and *Yeakel* (1944) dissected 1000 Wistar rats and observed reticulum cell sarcoma in 9.

Concerning reticulosis and lymphatic tumours, *Gillman* and *Gillman* (1952) have obtained essentially the same results as the ones under discussion by administering Grübler's trypan blue subcutaneously. The changes appearing in the lymph nodes between the 150th and 300th day of the experiment were considered on the basis of the predominant cell type as plasmocytoma, histiocytoma, endothelioma, paramonocytoma or haemohistiocytoma. *Willheim* and *Ivy* (1953) have attempted to produce cancer of the stomach by administering different dyes by mouth. Instead of a cancer, malignant lymphoblastoma has been developed in the abdominal lymph nodes of 7 out of 33 rats.

As to the present investigations, there is no cause why to regard the findings as spontaneous changes. The experiments were made in rats coming from a strain which has been bred by us for more than 6 years and during that time several thousands of animals were dissected, many of them 2 to 3 years old,

without finding either leukaemia, or a lymphatic tumour in any of them. It seems therefore warranted to assume that the treatment performed is in causative relationship with the changes observed in both the haemopoietic and the reticular systems.

Though further work is needed for solving the problem of pathogenesis, it is thought that the changes observed were due to stimulation of the active mesenchyma.

Summary

(i.) In three of 29 white rats surviving after the 180th day of *oral* administration of a tannic acid solution, morphological changes indicative of myeloid leukosis have been found.

(ii.) Diffuse hyperplasia of the haemopoietic and reticular systems has been induced in white rats by protracted subcutaneous treatment with a commercial tannic acid product. The reticulum cell hyperplasia in the lymph nodes displayed sometimes a neoplastic character.

(iii). The morphological changes observed in the endocrine glands and in the nuclei of the anterior hypothalamus suggest that in prolonged tannic acid treatment the neuroendocrine regulation becomes changed.

(iv.) The hyperplasia induced in the haemopoietic and reticular systems by prolonged oral or parenteral tannic acid treatment is attributed to a stimulation of the active mesenchyma.

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ПРЕДВАРИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ЭКСПЕРИМЕНТАЛЬНОГО ГЕМОБЛАСТОЗА, ВЫЗВАННОГО У КРЫС ДУБИЛЬНОЙ КИСЛОТОЙ

Б. Корпаши, А. Станоевич и М. Кольтаи

Резюме

Авторы сообщают результаты двух опытов: в первом опыте они давали 20-и белым крысам 4 раза в неделю, а в другим 40-а белым крысам раз в неделю свежий водяной раствор дубильной кислоты через желудочную зонду (аптечный препарат). Опыты продолжались длительное время. В начале крысы получили 1%-ный раствор дубильной кислоты, затем авторы повышали концентрацию раствора через каждые два месяца на 1%. В конце концов концентрация раствора была доведена до 5-и %-ов. Во втором опыте авторы давали 80-и белым крысам длительное время неочищенный заводский препарат дубильной кислоты (»Валекс«) в водяном растворе подкожно. В начале опыта они давали 50 мг/кг, а затем они постепенно повысили дозу до 150 мг/кг.

Среди крыс, получивших средство через рот, 29 крыс переживали 180-ий день опыта. Среди этих животных авторы у трех нашли изменения, характерные, для миэлоидного лейкоза в печени, селезенке, костном мозгу в легких и в головном мозгу. Большинство клеток обнаруженных в костном мозгу и в селезенке принадлежат к самым незрелым клеткам кроветворной ткани, и больше всего подходят клеткам Ферата, т. е. гемогистиобластам. В сосудах всех внутренних органов авторы нашли огромное количество незрелых элементов гранулоцитарьного ряда.

В селезенке и в лимфатических железах крыс, получивших заводскую дубильную кислоту подкожно, авторы нашли гиперплазию разной степени ретикуло-эндотелиальных элементов (особенно плазматических клеток), не раз проявляющуюся в опухолоподобных картинах. Выраженные изменения наблюдались со стороны эндокринных желез, а в ядрах передней подбугровой области авторы нашли кроме увеличения нервных клеток и дегенеративные явления. Эти изменения указывают на то, что при хроническом применении дубильной кислоты изменяется нейро-эндокринная регуляция.

Гиперплазию кроветворной и ретикулярной систем, вызванную длительным применением дубильной кислоты через рот и подкожно, авторы объясняют стимуляцией активной мезенхимы.