The Hepatocarcinogenicity of Tannic Acid

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In the group of chemical hepatocarcinogenic agents, the number of which has steadily increased in recent years (37), tannic acid deserves attention. Owing to its astringent effect, this compound was formerly used widely in therapy. The local treatment of thermal burns with tannic acid was suggested by Davidson (1925), and his procedure was generally employed in Hungary during World War II. It was in the course of this war that the hepatotoxic action of tannic acid was recognized (1, 2, 4, 7, 36). Since the results of Anglo-Saxon scientific investigations were, on account of the war, unknown in Central Europe, nobody in Hungary was informed about the hepatotoxic effect of tannic acid before 1946.

Liver damage and cirrhosis.—On the basis of comparative histologic examinations of the liver of individuals who had died of burns which were treated with tannic acid and/or untreated, we suspected the hepatotoxic effect of this substance as early as 1943-44. This suspicion was fully confirmed by animal experiments, but our results could not, because of the war, be presented before 1946, and the experiments had to be repeated, because our files had been lost for the same reason. It was shown in those experiments that tannic acid is a selective poison of liver parenchyma, the effect of which manifests itself, depending on the dose and the route of administration, in acinocentral necrosis of varying extent. The disappearance of glycogen from the cytoplasm of liver cells is an early phenomenon, but it is not followed by fatty degeneration (12).

Next, we demonstrated the cirrhogenous effect of tannic acid. White rats were given repeatedly, at intervals of several days, subcutaneous injections of tannic acid as a 1 per cent watery solution for the first ten doses, and thereafter as a 2 per cent solution, in graded doses of 10–70 mg/rat. This treatment resulted in a gradual transformation of liver architecture (Fig. 1). In the early stages (up to the 40th day) breakdown and subsequent regeneration of the liver cells were the most striking features, with a frequent acinocentral collapse of the argentophilic fibrous meshwork. Another early phenomenon was the swelling and increase of the cells constituting the reticulo-histiocyte system. In the 3d month of treatment, the diffuse proliferation of connective tissue was rather conspicuous. Pseudolobuli appeared, and the proliferation of small bile ducts could frequently be observed. The liver of the rats that survived beyond the 100th day of treatment was shrunken, and its surface was coarsely granular. Thus, the liver changes induced by tannic acid treatment were very similar to the so-called postnecrotic or posthepatitic cirrhosis (16).

Stressor effect of tannic acid.—The parenteral administration of tannic acid also resulted in an intensive systemic stress (25, 27). In the course of protracted administration of tannic acid, the cytologic evidence of increased neurosecretory activity was found in the anterior hypothalamic nuclei (26). Further, male rats were found to be more susceptible than females to the lethal doses of tannic acid. In acute experiments twice as many males as females died before the 60th hour, whereas with chronic treatment 61 per cent of females, but only 22 per cent of the males, were alive on the 300th day. A high casein (30 per cent) diet gave some protection against the lethal and hepatotoxic action of tannic acid: of the rats kept on a low casein (1 per cent) diet, exactly twice as many died, before the 102nd hour, as of those kept on a high casein diet (18). It should be mentioned that acute poisoning with tannic acid may often be attended by gastric erosion (17); in the course of protracted treatment, ulcers are found in nearly 70 per cent of the rats at the pyloric region (33).

Absorption of tannic acid from the gastrointestinal tract.—The question whether tannic acid administered orally is absorbed from the alimentary tract was also studied. Handler and Baker (10) claimed that the gastrointestinal mucosa was, in their experiments on rats, impermeable to tannic acid. We administered to rabbits and dogs aqueous solutions of tannic acid in several concentrations (2.5, 5, and 10 per cent), tea, and claret by stomach tube. We then examined blood samples, taken at various times, by a photometric micro-
method based on the reduction of arsenotungstic acid. The ingestion of the compounds was followed by a rapid increase in the level of tannic acid in the blood. Peak effects were observed at 3 hours (55-110 μg/cu cm plasma); the concentration then gradually diminished, and by the end of 24 hours the blood contained no tannic acid (14). Histological examination revealed that the hepatotoxic effect after oral administration was similar to that observed after parenteral treatment (15). Long-lasting treatment (over 180 days) of rats per os also resulted in cirrhosis, if the dose given per os exceeded by several times the usual parenteral dose (a total amount of 10-15 gm. tannic acid given in 90-120 doses was effective) (11).

Induction of liver tumors.—One drawback of subcutaneous administration is that the toxic effect of tannic acid is great and that the skin undergoes necrosis and subsequent ulceration at the place of injections. For this reason the majority of the animals died early in the experiment. (The lethal effect is influenced by dose, method of administration, age, sex, and diet.) Nevertheless, when 150-200 mg/kg body weight was injected subcutaneously every 5th day in a 1.5 or 2 per cent aqueous solution, 30-50 per cent of the animals survived for 200, and 5-15 per cent for 300, days. Of the animals that survived the 100th day of treatment, hepatomas and/or cholangiomas developed in about 56 per cent. Liver tumors induced by this approach were always multiple and mostly benign, although invasion of the liver veins and an atypical pattern seen in some cases suggest that the possibility of low-grade malignancy should be considered (13, 19, 20).

The hepatocarcinogenic effect was inhibited by a high casein-low fat diet (25 per cent casein, 5 per cent oil) and promoted by a diet containing little casein and much fat (8 per cent casein, 20 per cent sunflower oil). On the other hand, the cirrhogenous effect of tannic acid could not be influenced by the casein and fat content of the food. Although the survival of the rats maintained on a high casein-low fat diet was somewhat longer than that of the animals fed a low casein-high fat diet, liver tumors occurred in the latter group twice as frequently (67.5 per cent) as in the former (29.7 per cent). The diet containing little casein and much fat exerted, by itself, no carcinogenic or cirrhogenous effect (21, 22). Vitamin B12 (Berubigen, Upjohn), 1 μg/100 gm twice weekly given intramuscularly, exerted no influence on the hepatocarcinogenic effect of tannic acid.1

Although subdermal application of tannic acid gave rise to ulceration of the skin, these ulcers rapidly and completely healed if the treatment was stopped temporarily or if the injections were given at another site. In no case did a tumor arise from the margin of an ulcer or from the healed scars. Local carcinogenic effects were not observed even when the skin ulcers of rats produced by thermocauterization were painted with tannic acid for 1 year (19). Local carcinogenic effects were not observed with white mice either, when their skin was treated with a tannic acid solution for a longer time. These mice showed no hepatic tumors.2

The simultaneous administration of tannic acid and 2-acetylaminofluorene (tannic acid parenterally, AAF in the food) showed that tannic acid greatly enhanced the hepatocarcinogenic action of 2-acetylaminofluorene. On the 180th day of the experiment, 92 per cent of the rats treated with both substances, but only 28 per cent of the rats treated only with AAF, developed liver tumors, aside from the fact that in the first group metastases were also present. The incidence in rats treated with tannic acid alone was about 50 per cent. Among the animals treated with both compounds, advanced liver cirrhosis frequently occurred, in contrast to the mild hepatic changes found in the animals treated only with AAF. These experiments suggest that liver cirrhosis is an important promoting factor in liver carcinogenesis (29).

In the liver of rats treated with tannic acid per os or parenterally for a long time, the appearance of small foci of myeloid metaplasia was a common phenomenon. At the same time, hypercellular bone marrow was found. In a few animals treated for a long time, leukemic myelosis developed, as seen from the tremendous enlargement of the spleen and the disappearance of its normal pattern, as well as the myeloid infiltration in various organs. The high grade of anaplasia and the mass of mitotic nuclei indicated stem-cell leukemia.

Massive deposition of a yellowish-brown, iron-containing pigment, especially in the spleen, may be considered a sign of intravascular hemolysis. Thus, the occurrence of extramedullary hematopoietic foci may be regarded as a compensatory process. It is supposed that the leukemoid reaction elicited by long-lasting tannic acid treatment may, in exceptional cases, result in a true leukemia (24). It should be noted that in the strain of rat which we have used for 10 years spontaneous tumors rarely occurred; those observed were of

1 Korpássy and Mosonyi, unpublished experiments.

2 Korpássy and Bartók, unpublished experiments.
mesenchymal origin, but no spontaneous leukemia was observed in untreated animals.

Histogenesis.—It seems that tumor formation induced by tannic acid treatment in the liver is related to the regenerative liver cell and bile duct hyperplasia occurring as a sequel of repeated breakdown of the parenchyma. The cholangio-fibrosis observed, in the presence of the reparative epithelial proliferation after the 100th day of the experiment, is, in our view, a preneoplastic change. The intense reticuloendothelial hyperplasia of the early phase later became less conspicuous. In our opinion cirrhosis is an important promoting factor in hepatocarcinogenesis; tumor formation, however, is not a necessary sequel to this process.

When the action of tannic acid on liver is compared with that of substances of quite different chemical composition (dimethylaminoazo-benzene, 2-acetylaminofluorene, ethionine, carbon tetrachloride, alkaloids of Senecio jacobaea, etc.), one may be greatly impressed by the resemblance in the sequence of pathologic changes. From this point of view, the statement of Farber (5) deserves to be quoted: "It therefore appears that many different chemical compounds, capable of producing liver tumors in rats and mice, induce a similar sequence of histological changes in which oval cell hyperplasia, presumably of bile duct epithelial origin, is prominent." We arrived, earlier, at the same conclusion, with the difference that we believed the "oval cells" to be of reticuloendothelial origin (16, 19). It is noteworthy that myeloid metaplasia was also found in the liver in the course of carcinogenesis by p-dimethylaminoazo-benzene (31). This phenomenon is, in the opinion of W. Fischer (6), rather characteristic.

Mechanism of action.—The metabolic changes associated with tannic acid carcinogenesis are not known. Thunberg (34) showed in 1936 that tannic acid inhibited the hydrogenase activities in certain crude seed and animal tissue extracts. In an examination of endocrinologic relationships tannic acid exhibited a strong stimulatory effect, manifesting itself in the activation of the adrenocortical function of the pituitary and in the hypertrophy of the zona fasciculata in the adrenal cortex. Other authors (9, 32) believe that a pituitary-adrenal interrelationship may be a prerequisite for liver cancer formation.

Source and chemistry.—A variety of tannic acids is found in nature. These acids are polymers of various hydroxybenzoic acids. The acid commonly referred to as tannic acid is gallotannic acid. It is usually obtained from nutgall, an excrescence on the young twigs of various species of Quercus (Goodman and Gilman, 1955). Even purified pharmaceutical preparations of gallotannic acid contain, however, in addition, pentadigallaglycol glucoside and several other known or partly known organic substances in small quantities, for example, ellagic acid, quercitol, and quercic acid.

Oenotannic acid (contained in red wine) and gallotannic acid (obtained from nutgall) are not identical, the former belonging to the group of condensed tannic acids or pyrocatechins, the latter to the tannic acids having an ester binding. To our knowledge, oenotannin is not available commercially.

The tannic acid used in all our experiments was Acid. tannic. U.S.P. 3

Human-pathologic relationships.—Since tannic acid is no longer applied locally to thermal burns, the substance is now ingested only with food. As is known, certain beverages (tea, coffee, cocoa, claret) contain varying amounts of tannic acid: there are 94–475 mg. in a cup of tea, 215–371 mg. in a cup of cocoa, and 90–187 mg. in a cup of coffee (28). "Light" clarets contain 0.1–1.15 per cent, "heavy" ones 0.2–0.3 per cent, those obtained from Southern Europe 0.5 per cent or more (30). Thus, at least 1–2 gm. tannic acid is ingested with 1 liter claret. It is not known whether the tannic acid of red wine and that obtained from nutgall act on the liver in the same manner.

Among the so-called antioxidants used for the preservation of fruits, vegetables, etc., there are also derivatives of gallic acid. The British Committee for Alimentary Standards suggested as an upper limit 0.01 per cent for pyrogallate and 0.02 per cent for butylhydroxyanisole (35, 38).

In our opinion, the possibility that the excessive consumption of tea, coffee, cocoa, or claret, associated with the absorption of their tannic acid content may, especially if certain favorable dietetic factors be present, result in hepatic damage should become a subject of deliberation.

In considering the carcinogenicity of tannic acid, the following properties are of particular interest: (a) it is water-soluble; (b) it is weakly carcinogenic and acts at a site distant from its application; (c) its action on the liver is influenced by dietetic and endocrinologic factors; and (d) it enters the human organism via the food.

REFERENCES


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**Fig. 1.—**Numerous mitoses in the regenerating liver parenchyma of a 105-gm. rat, treated 4 times with 2.5 per cent aqueous tannic acid solution (5 ml.) at 2-hour intervals by stomach tube. The rat was killed after 50 hours. Hematoxylin & eosin. X400.

**Fig. 2.—**Proliferation of reticulendothelial (oval) cells in the liver of a 120-gm. rat treated with 4 ml./5 per cent tannic acid solution by stomach tube once daily for 3 days. The rat was killed on the 7th day. Hematoxylin and eosin. X400.

**Fig. 3.—**Completely transformed architecture of the liver, with the formation of pseudo-lobules. The rat, weighing 187 gm., was treated with 750 mg. tannic acid administered subcutaneously in 28 doses. The rat was killed on the 141st day. Gomori’s reticulum stain. X100.

**Fig. 4.—**Coarse nodular cirrhosis. The rat was treated with 9,700 mg. tannic acid/kg body weight, administered subcutaneously in 48 injections, and died on the 278th day.
Fig. 5.—Polymorphism of the liver cells with an enormous binucleate cell and proliferated oval cells. The rat was given sixteen subcutaneous injections, or a total of 320 mg. tannic acid; it was killed on the 71st day. Hematoxylin & eosin. X400.

Fig. 6.—Invasion of a large blood vessel in the liver. The rat was treated with 4,250 mg/kg body weight tannic acid, administered in 22 doses, and died on the 192nd day. X400.

Fig. 7.—Atypical pattern in cholangioma simulating low-grade adenocarcinoma. The rat was given a total of 9950 mg. tannic acid/kg body weight in 49 injections, and died on the 294th day. Hematoxylin & eosin. X360.

Fig. 8.—Cholangiocarcinoma invading hepatic tissue of rat (see Fig. 6). Hematoxylin & eosin. X400.
The rat was killed after 50 hours. Hematoxylin and eosin. X400.

FIG. 1.--Numerous mitoses in the regenerating liver parenchyma of a 105-gm. rat, treated 4 times with 2.5 per cent aqueous tannic acid solution (3 ml.) at 2-hour intervals by stomach tube.

FIG. 5.--Numerous mitoses in the regenerating liver parenchyma of a 75-gm. rat, treated with 5,700 mg. tannic acid/kg body weight, administered subcutaneously in 48 injections, and died on the 278th day.

FIG. 6.--Completely transformed architecture of the liver, with the formation of pseudo-lobules. The rat, weighing 187 gm., was treated with 750 mg. tannic acid administered subcutaneously in 28 doses. The rat was killed on the 141st day.

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