Liver Tumors Following Cirrhosis Caused by Selenium in Rats

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It is generally believed that carcinoma of the human liver is frequently preceded by cirrhosis (4). In the case of experimental tumors of the liver, such as those produced by feeding *p*-dimethylaminoazobenzene [1] (1) to rats or feeding buckwheat to mice (5) or by the oral administration of a dose of carbon tetrachloride 2 or 3 times weekly (2) to mice, the livers often develop a cirrhotic appearance more or less concurrently with the early stages of tumor formation, which often takes place from 2 to 6 months after feeding of the agent has been begun. To our knowledge no one has reported the development of tumor in an experimentally cirrhotic liver in which the cirrhosis has been present without tumor for a long time; Moon's exhaustive review (6) of experimental cirrhosis does not mention this.

In this paper we are reporting the development of hepatic cell adenoma and low grade carcinoma in rat livers, beginning 18 months after the rats were placed on a seleniferous diet; this diet, after a 3 month period of a subacute type of liver damage, produced a chronic nodular cirrhosis without necrosis.

MATERIAL

Seven groups of 18 rats each of our inbred Osborne-Mendel strain were fed selenium in organic combination in corn and wheat and in a mixed inorganic selenide [2] beginning at 3 weeks of age at levels of 5, 7, and 10 parts per million of diet. The primary purpose of the experiment was to determine the lower level of selenium necessary to produce chronic toxicity. Among the 53 rats fed the seleniferous diet for periods of at least 18 months, up to the end of the 24 month experimental period tumors of the liver, some as large as 3 cm. in diameter, diagnosed as hepatic cell adenoma and low grade hepatic cell carcinoma developed in 11, while 4 others had pronounced adenomatoid hepatic cell hyperplasia that could be interpreted as a transition to tumor. In the 73 rats that died or were sacrificed before 18 months there were no tumors and no advanced adenomatoid hyperplasia, although cirrhosis was fairly frequent (after 3 months).

The diet for the 126 experimental and 18 control rats contained 12 per cent protein and consisted of 49 per cent corn, 44 per cent wheat, 3 per cent yeast, and 1 per cent each of cod liver oil, calcium carbonate, sodium chloride, and dried whole liver; seleniferous and nonseleniferous grains were combined in the proper proportions to give the desired level of selenium, while for the group on the inorganic selenium the material was simply added to the diet composed of nonseleniferous materials. The rats were all females, which are slightly more susceptible to selenium toxicity than males (9, 11). Seventy-one of the 126 experimental rats and 14 of the 18 control rats were examined microscopically.

Survival Periods by Groups

Table I gives the number of rats in each group that died, or that were sacrificed because of poor condition, during various intervals of time.

On a dosage level of 10 parts per million of selenium obtained from corn and wheat, 25 of 36 rats died during the first 3 months and only 4 lived the 2 year experimental period, while on a level of 5 parts per million only 2 of the 36 died during the first 3 months and 19 lived the full 2 years, nearly as many as in the selenium control group and our various other control groups, in which about two-thirds survived this period. Survival on 7 parts per million from corn and wheat and 10 parts per million from the selenide was intermediate between the groups previously mentioned.

Description of Liver Damage by Selenium

Only a brief summary will be given here. The liver damage was of two types, depending on whether death occurred before or after 3 months on the seleniferous diet; in the period from 2 to 4 months features of both types were seen. Rats that died during the first 3 months, generally between the 3rd and 6th

[1] Although this dye is commonly called butter yellow it should be emphasized that it is not used as a food color.

[2] A solution of ammonium potassium sulfide and ammonium potassium selenide, containing 48 gm. of Se per liter of solution.

weeks, usually had bloody or occasionally clear yellow fluid in the abdomen, and dark red granular livers with a hemorrhagic appearance. Microscopically there was a subacute type of damage with varying degrees of hepatic cell necrosis, atrophy, hyperplasia, cystic dilatation of sinusoids, and focal myelosis. Fibrosis, pigmentation, and bile duct proliferation were not striking. The gross abdominal hemorrhage appeared to originate by rupture of distended subcapsular sinusoids; often there was an organizing, pericapsular hemorrhagic exudate. Periportal or other intrahepatic hemorrhage was negligible.

Most of the rats living through, and presumably not as much affected during, this first phase of selenium toxicity developed a chronic type of liver damage without intrahepatic hemorrhage or necrosis, with less of cystic sinusoids and myeloid foci, and with increasing portal fibrosis, distortion of architecture, focal capsular retraction, and focal hepatic cell hyperplasia. These changes expressed themselves as varying degrees of a nodular cirrhosis (Table II), which in its advanced stages is a portal or Laënnec's cirrhosis according to the criteria of Moon (6). Nodularity was sometimes so advanced that the smaller lobes resembled miniature bunches of grapes. Atrophy was relatively uniform among the different lobes, while regenerative hyperplasia and tumors tended to be more noteworthy in the left lateral and caudate lobes.

Hemosiderin pigmentation was usually slight. Ascites was occasionally, and some degree of hemoperitoneum rarely (3 times), present; the source of the hemoperitoneum was not determined.

Lesions caused by selenium in viscera other than the liver were not extensive and none was very characteristic. With the control group as a base line (and this showed few spontaneous lesions) there were slight hyperplasia and hemosiderosis of the splenic pulp, slight hyperplasia of the bone marrow, slight focal myocardial fibrosis, and minor renal changes. There were no gastrointestinal, pancreatic, pulmonary, adrenal, or gross cutaneous alterations, and osseous lesions were occasional and slight. In the younger rats there was slight Kupffer cell and splenic reticulum cell hyperplasia, and some testicular atrophy.

TABLE I: SURVIVAL PERIODS BY GROUPS

Level of selenium in diet	Period of survival in months					Number in group
	3 or less	3½–11½	12–17½	18–23½	24	
5 p.p.m. (corn)	2	1	5	1	9	18
5 p.p.m. (wheat)	0	1	3	4	10	18
7 p.p.m. (corn)	7	0	1	3	7	18
7 p.p.m. (wheat)	9	2	3	1	3	18
10 p.p.m. (corn)	13	0	0	2	3	18
10 p.p.m. (wheat)	12	1	2	2	1	18
10 p.p.m. (selenide)	2	6	3	1	6	18
Total experimental	45	11	17	14	39	126
Control	0	2	2	2	12	18

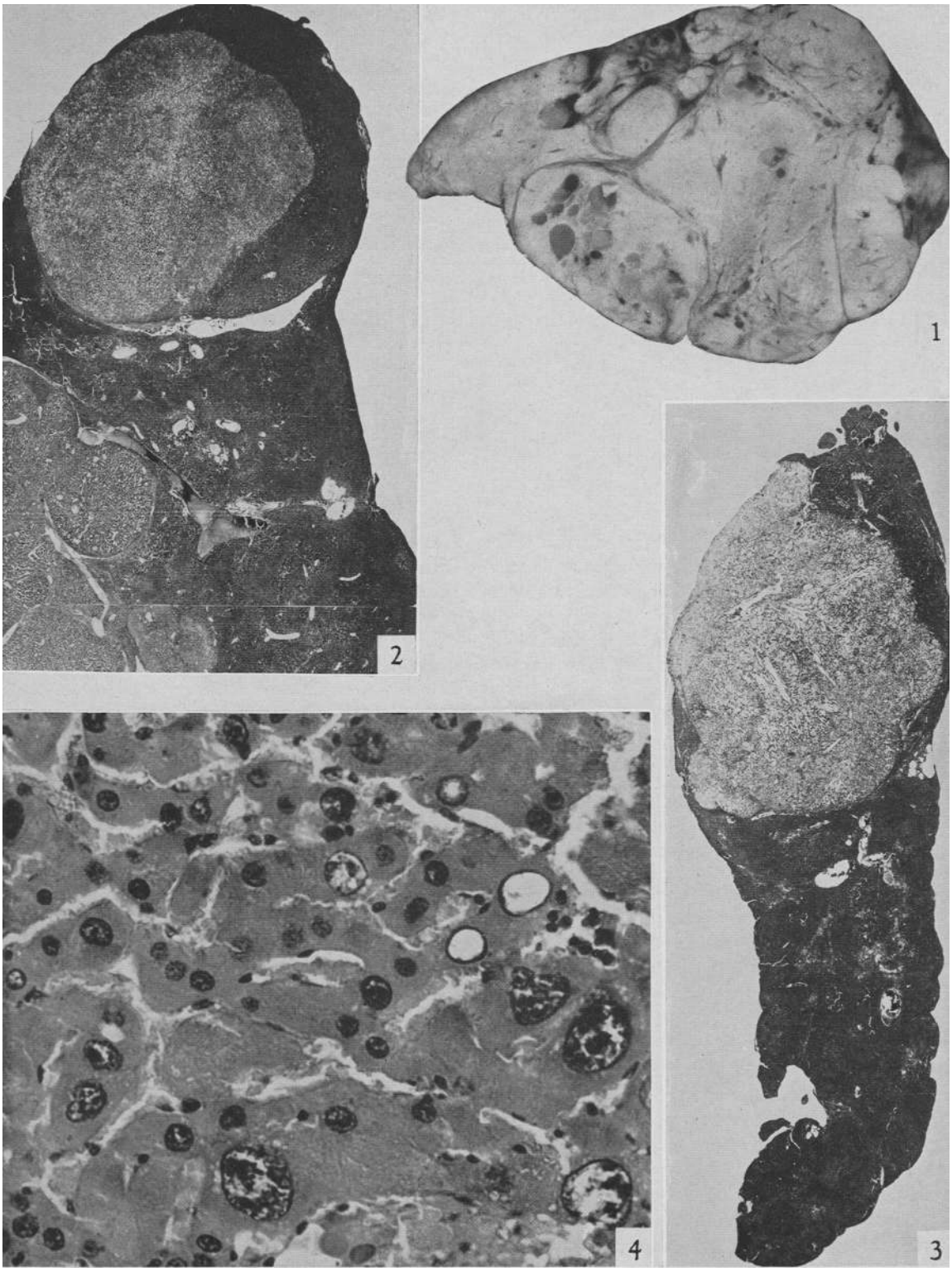
TABLE II: CIRRHOSIS IN RATS SURVIVING MORE THAN 3 MONTHS

Degree of cirrhosis	Months on experiment				Total
	3½–11½	12–17½	18–23½	24	
None	4	6	3	7	20
Slight	2	2	5	11	20
Moderate	3	2	2	9	16
Advanced	1	6	3	9	19
Extreme	0	0	1	3	4
Undetermined	1	1	0	0	2
Total	11	17	14	39	81

DESCRIPTION OF ADENOMA AND CARCINOMA IN CIRRHOTIC LIVERS

After ingesting the seleniferous diets for 18 months, 11 of the 53 surviving rats, 43 of which had cirrhosis, developed definite liver tumors up to 3 cm. in diameter, and 4 others advanced adenomatoid hyperplasia (Table III).

The table shows that the tumors were somewhat more frequent in the left lateral than in the other lobes of the liver, and that both single and multiple tumors occurred, the latter more frequently. There



FIGS. 1–4

was no parallelism between the degree of cirrhosis and the presence of tumor, except that there were no tumors in the 10 rats of this age group (18 to 24 months) without cirrhosis. The tumors were usually well circumscribed (Figs. 1, 2, 3) and paler and more granular than the surrounding liver tissues (after formalin fixation). Some of the larger ones had a moderately lobulated appearance (Fig. 1). No encapsulation was noted upon gross examination and no metastases were seen.

Upon microscopic examination, portions of the peripheries of the tumors were found separated from the rest of the liver by collagenous fibers, but there was no complete encapsulation. The growths were composed of fairly regular to irregular cords of large hepatic cells (Figs. 4 to 7), usually more oxyphilic than the surrounding liver in the adenomas and less oxyphilic in the carcinomas. Some tumors showed no mitoses after several minutes' search and in others a few or even a moderate number were seen in a shorter time. Bile duct proliferation was slight except in one instance (Path. 1550), where the peripheral 1 to 2 mm. of a 3 cm. tumor was composed of small bile ducts. Hemosiderin pigmentation and fibrosis within the tumors were not striking; focal necrosis was seen rarely, and hemorrhage not at all. Fatty degeneration was slight and was the same as, or a little less than, in the surrounding liver; the livers of the selenium control rats also showed a slight fatty degeneration, which was not found in our other control groups on more adequate diets. A few of the tumors enclosed small foci of myeloid cells, and in a few there were

TABLE III: DATA ON LIVER TUMORS AND ADENOMATOID HYPERPLASIA IN RATS FED SELENIUM

Pathology No.	Months on experiment	Dosage, Se, p.p.m.	Cirrhosis, degree	Size and location of tumor	Microscopic diagnosis
1450	23	7 (wheat)	Slight	5 mm.: left lateral lobe	Adenoma
1498	24	10 (corn)	Advanced	8, 7, and 3 mm.; caudate lobe	Adenoma and low grade carcinoma
1520	24	10 (corn)	Advanced	Numerous scattered; to 7 mm.	Low grade carcinoma and adenomatoid hyperplasia
1524	24	5 (wheat)	Moderate	$3\frac{1}{2} \times 3\frac{1}{2} \times 3$ cm. in left lateral lobe; few elsewhere to 9 mm.	Adenoma
1549	24	7 (corn)	Advanced	$1.6 \times 1.6 \times 1.0$ cm.; left lateral lobe	Adenoma
1550	24	5 (corn)	Slight	$3.2 \times 2.7 \times 2.0$ cm., left lateral; few elsewhere to 4 mm.	Adenoma
1559	24	5 (wheat)	Slight	1.3 cm.; median lobe	Adenoma
1561	24	5 (corn)	Slight	Several up to 1 cm.; chiefly left lateral	Adenoma
1564	24	10 (selenide)	Advanced	About 10 up to $1\frac{1}{2}$ cm.; chiefly median and left lateral	Carcinoma, low grade
1565	24	10 (selenide)	Moderate	One dozen up to $1\frac{1}{2}$ cm.; right and left lateral	Carcinoma, low grade
1596	24	7 (corn)	Advanced	Multiple in left lateral lobe $3\frac{1}{2} \times 3 \times 2\frac{1}{2}$ cm.	Carcinoma, low grade
1006	18	10 (selenide)	Extreme		Adenomatoid hyperplasia
1201	$20\frac{1}{2}$	10 (corn)	Advanced		Adenomatoid hyperplasia
1543	24	7 (wheat)	Advanced		Adenomatoid hyperplasia
1560	24	7 (wheat)	Slight		Adenomatoid hyperplasia

DESCRIPTION OF FIGURES 1 TO 4

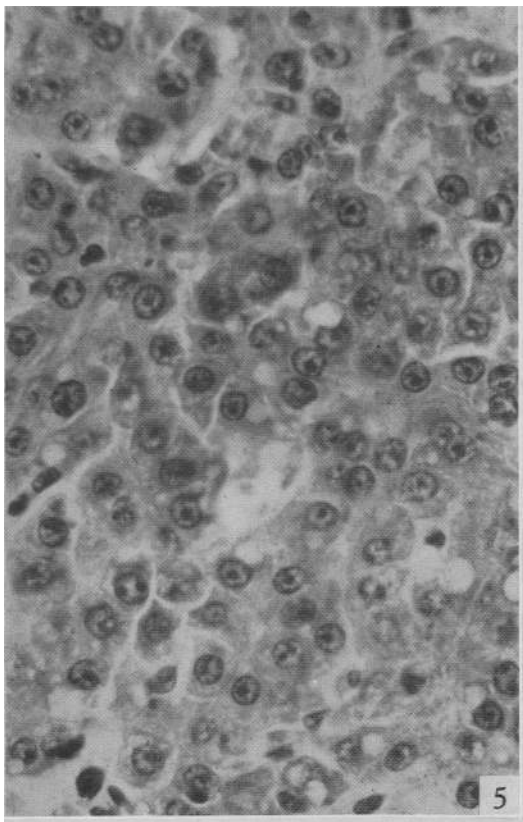
The photographs were made by Mr. M. L. Foubert of the U. S. Department of Agriculture and Mr. F. H. Meiller of the Food and Drug Administration.

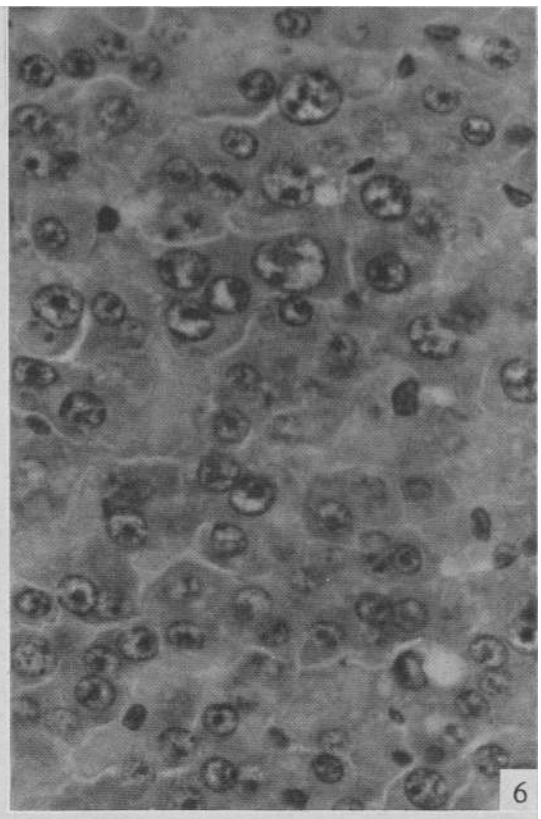
FIG. 1.—One of the larger tumors, in the left lateral lobe of rat 1524, showing moderate lobulation, small cystic areas in one lobule, and a portion of nonneoplastic liver to one side of the tumor. Mag. × 2.

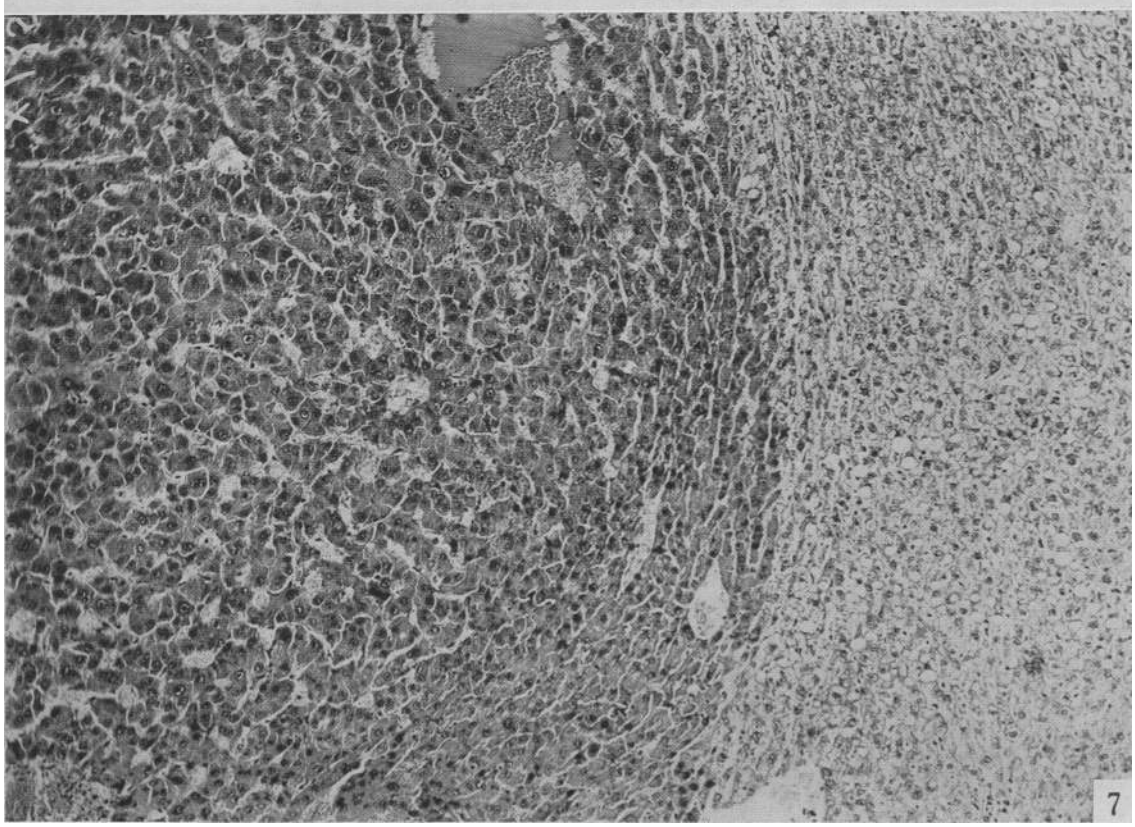
FIG. 2.—Two of the tumors in the left lateral lobe of the liver of rat 1596. Mag. × 8.

FIG. 3.—One of the tumors in the caudate lobe of the liver of rat 1498. Mag. × 8.

FIG. 4.—Area of adenoma in the median lobe of the liver of rat 1559, showing the short irregular cords of hepatic cells, with more variation in nuclear size than is usually seen, and a few vacuolated nuclei. Mag. × 125.







FIGS. 5-7

small cystic areas. Since sections were not made of every tumor, some livers listed as showing adenoma may have contained carcinoma also, and *vice versa*.

The differentiation between adenoma and low grade carcinoma was difficult to make in this series of tumors; the latter showed greater irregularity of liver cell cords, decreased oxyphilia of liver cells, more mitotic figures, and an invasive tendency at their margins. They were very similar to many of the low grade carcinomas of the rat liver seen after the ingestion of *o*-aminoazotoluene, and anyone who has

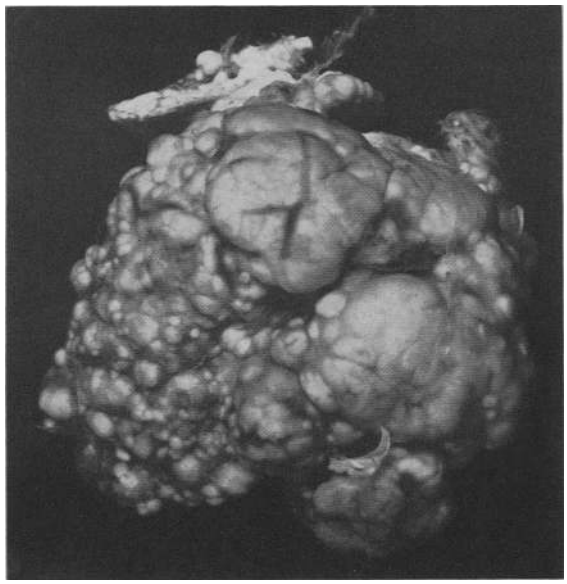


FIG. 8.—Liver of rat 1523, showing a high grade of nodular cirrhosis resulting from the feeding of 10 parts per million of selenium for 24 months. Mag. × 13.

studied a series of the latter tumors will appreciate the difficulty of deciding just when the borderline between nonmalignant and malignant tumor has been passed, and also just when hyperplasia has passed into tumor. The differentiation into adenoma and low grade carcinoma was made chiefly because it was an orthodox procedure; perhaps the tumors could as well have been grouped under the term "hepatoma" or "hepatoma of low malignancy."

INCIDENCE OF SPONTANEOUS TUMORS OF THE LIVER

The incidence of spontaneous adenoma and low grade carcinoma of the liver in our control rats and in rats fed substances other than selenium was low; in 350 rats finishing a 2 year experimental or control period there have been 4 such tumors in 3 rats, in 200 rats from 18 to 24 months there has been one, and in nearly 1,000 younger rats none. These spontaneous growths were from 1 to 2 cm. in diameter and none had metastasized; there was no associated hepatic cirrhosis. The incidence of lymphosarcoma, leukemia, and spontaneous tumors of viscera other than the liver, for which careful records are kept, is not different in the rats reported upon in this paper from that in our entire group.

DISCUSSION

Liver damage in rats fed seleniferous grain was first reported by Franke (3) in 1934, although at that time it was not certain that selenium was the offending agent. For reviews of selenium toxicity in general and of the pathological manifestations of selenium poisoning, the reader is referred to the publications of Moxon (7), who carried on Franke's work after the latter's death, and of Smith (10).

As has been stated in the preceding tables and text, rats coming to autopsy at all ages up to 18 months had no advanced adenomatoid hyperplasia or tumors of the liver. In view of the relatively large percentage showing tumors at 24 months, which is the length of time chosen as the standard for ending our chronic toxicity experiments, there may be indicated the reaching of a "tumor age" or added susceptibility to certain carcinogenic influences at some time between 18 and 24 months for our colony of rats. This is very similar to the age condition necessary for the occurrence of spontaneous pulmonary lymphosarcoma (8) in our rats. Another possibility is that age as such is not a factor and that this length of time is required for selenium to produce its carcinogenic effect.

SUMMARY

Eleven of 53 rats developed adenoma or low grade carcinoma in cirrhotic livers, and 4 others advanced adenomatoid hyperplasia, after having survived for 18 to 24 months on diets containing 5, 7, or 10 parts per million of selenium; no tumors occurred in 73 rats surviving less than 18 months, although after 3 months cirrhosis was frequent. In control rats 18 to 24 months of age the incidence of spontaneous hepatic tumors was less than 1 per cent.

DESCRIPTION OF FIGURES 5-7

FIG. 5.—Low grade carcinoma in the liver of rat 1565. A greater departure from normal hepatic architecture is seen than in the adenoma shown in Fig. 4. Mag. × 512.

FIG. 6.—Low grade carcinoma in the liver of rat 1564. Mag. × 512.

FIG. 7.—Edge of the adenoma (darker portion of print) in the left lateral lobe of the liver of rat 1450. Mag. × 107.

This appears to be the first report of tumors arising in experimentally cirrhotic livers after the cirrhosis had been present without tumor for a relatively long period of time.

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