egg-white proteins had a similar effect on the life span of sperm. Conalbumin and a derived protein, hydroxylamidoovomucoid, were more effective than other proteins from egg white. Purification of the saline diluent alone served to prolong the life span of spermatozoa and correspondingly reduce the effects of glycine and proteins.

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Production of Cirrhosis and Liver Tumors in Male Rats Using High Dosages of 2-Acetylaminofluorene.*† (18974)

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Since Wilson, De Eds, and Cox, Jr.(1) introduced 2-acetylaminofluorene (2-AAF) as a new experimental carcinogen, the compound has been widely used, due to its powerful carcinogenic action and the unusual variety of benign and malignant tumors produced in different organs. 2-AAF appears to be of particular value in studies of the mode of development of cirrhosis and liver tumors. The possibility of correlation of liver changes with lesions produced in other locations seems to confer on 2-AAF an advantage over compounds with strictly hepatotoxic action.

Methods. One hundred and forty-eight rats of hooded strain were used, comprising 73 males and 75 females. The rats were derived from a local strain, inbred since 1933 by continuous brother and sister mating. Two animals of the same sex were kept in each cage. The diet was fully adequate, consisting of a standard Purina meal preparation and water *ad libitum*. The 2-AAF was added to the diet (by mixing) for 35 consecutive weeks. The age of the animals at the start of the experiment was 4 weeks in 128 rats (53 males and 75 females) (Group I), and 16 weeks in 20 males (Group II). The absolute dose of the 2-AAF was 4 mg per day in Group I and 7.5-9 mg per day in Group II for the first 7 weeks; afterwards it was lowered to 3 mg and 6 mg per day respectively for the remaining period of time.

It soon became apparent that male rats receiving higher absolute daily dosages of 2-AAF (Group II) developed extensive liver changes in a short period of time. Twelve of them died within 39 days of the start of administration of the compound. Partial hepatectomies were performed at intervals of 4 to 5 weeks for 7 months on the remaining rats of this group, starting from the third month. Under light ether anesthesia and using sterile technic, 20-25% of the liver volume was removed each time through a mid-line abdominal incision. Although the total amount of liver tissue resected by repeated hepatectomies exceeded the original liver volume, the majority of the animals survived all operations. Control hepatectomies were done on 6 animals kept on the lower absolute daily dosage of 2-AAF (Group I). Hematoxylin-and-eosin and connective-tissue-stain sections were prepared from the resected portions of the liver.

Results. Pathological findings observed by repeated hepatectomies. The earliest gross liver changes in the older group of male rats were observed during the second month by examining the 11 rats which died in this period. The liver was decreased in size with multiple white nodules protruding on the surface. Microscopically, the nodules consisted of circumscribed areas of hyperplasia without specific relation to lobular pattern (Fig. 1).

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† This work was presented at Clinical Congress of American College of Surgeons, Boston, Mass., Oct. 1950. Abstract in *Trans. Surg. Forum*, 1950, v1, 205.

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1. Wilson, R. H., De Eds, F., and Cox, A. J., Jr., *Cancer Res.*, 1941, v1, 595.

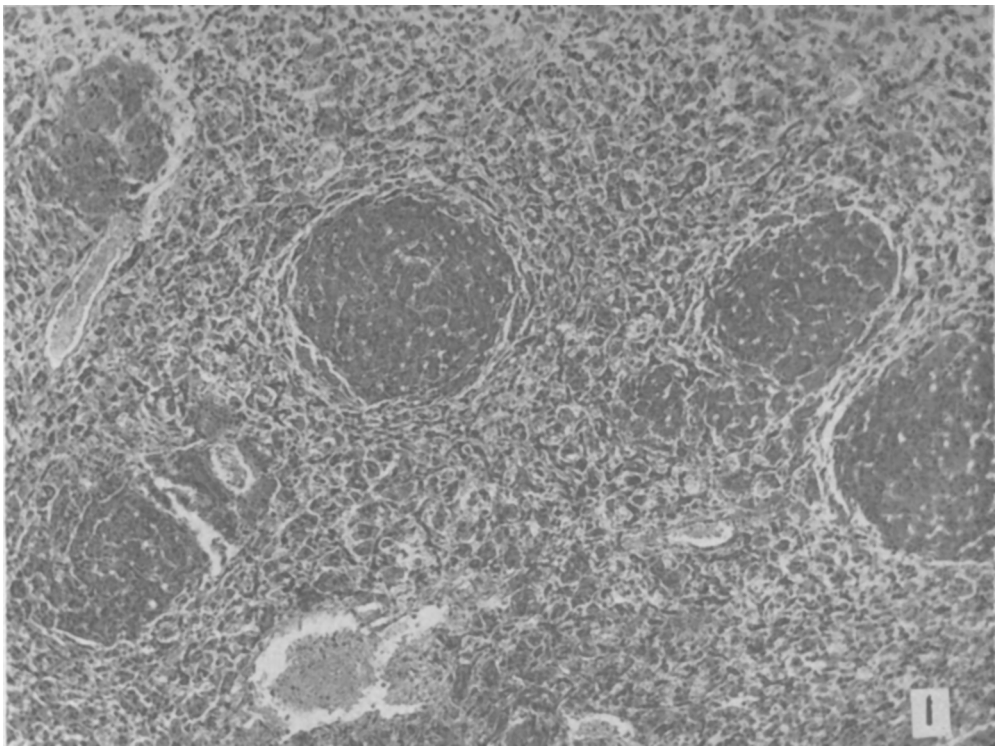

FIG. 1. Irregularly disposed nodules of hyperplastic liver cells, displacing necrotic parenchyma by expansion. H & E. × 105.

The remaining liver tissue showed subacute necrosis, the cells showing disintegrated cytoplasm and degenerative nuclear changes. The general appearance suggested that the degenerated liver tissue was being progressively displaced by multiple nodules of regenerating hyperplastic cells, distorting the normal lobular architecture. In the third month, tissue removed from 8 rats by partial hepatectomies showed complete destruction of the normal lobular pattern (Fig. 2). The first impression, from routine hematoxylin-and-eosin sections, was that of an early cirrhosis, with nodules of regenerating liver cells separated by areas of degenerated tissue. However, connective-tissue stains showed only accentuation of the reticular framework and no definite increase in connective tissue. In the fourth month, active fibroblastic proliferation dominated the histological picture. This, as shown best by Masson and Van Gieson stains, was restricted to spaces between hyperplastic nodules. The cells in the areas of hyperplastic regeneration showed different grades of fatty and glycogenic infiltration. Included in the fibrotic granulation tissue were numerous bile ducts, some of them showing cystic dilatation with flattening of the duct epithelium (Fig. 3). In the fifth month, further increase in the size of the liver was noted in spite of previous resections. Microscopically, this was seen to be due to further increase in size of the hyperplastic nodules. The amount of collagen was denser, and numerous cystically dilated bile ducts were present. In the sixth month, further increase in the collagen was prominent, and associated decrease in cellularity of the fibrotic tissue gave the cirrhosis a more quiescent appearance (Fig. 4). Dilatation of bile ducts had progressed in many cases to cyst formation. In the seventh month, marked increase in hyperplasia was observed. The presence of intermingled fields of normal and hyperplastic cells produced complex "geographic" patterns. In the eighth month, only slight fibrosis was still

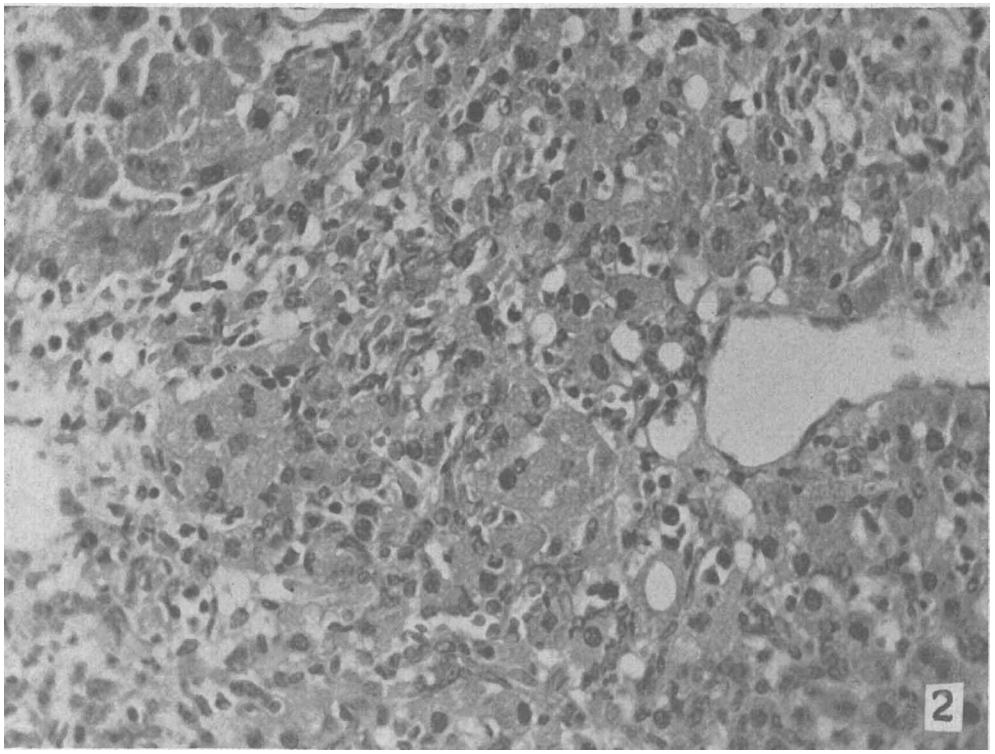

FIG. 2. Beginning fibroblastic proliferation in the necrotic liver parenchyma. Regeneration from intact remaining parenchymal cell groups is evident. H & E. × 415.

present, with narrow, irregular bands of collagen dividing large areas of regenerating parenchyma. In several rats, large areas of proliferated and dilated bile ducts were observed, classified as benign bile-duct adenomas. Liver-cell tumors were present in the majority (Fig. 5).

In the control group of rats, which were maintained on a lower absolute daily dosage of 2-AAF (Group I), the degenerative liver changes were much less extensive. In male rats, the nodules of hyperplastic liver tissue of similar appearance as those described previously, were not as diffuse; the amount of fibroblastic proliferation, as well as parenchymal hyperplasia, was less pronounced. In females of this group, mild degenerative changes appeared considerably later (fifth to seventh months).

Tumors produced by 2-acetylaminofluorene. The average time of occurrence of malignant tumors produced by 2-AAF was between the ninth and twelfth months after the beginning of the experiment. Some of the animals still alive at the end of the twelfth month showed visible or palpable tumors, not included in the results reported here. Ninety rats died after the sixth month, and 35, including 6 males and 29 females, were living at the end of the twelfth month. At autopsies, tumors were found in 70 out of 90 rats; in 43 animals the tumors were classified as malignant on the basis of invasive growth and/or distant metastases. In many cases, multiple primary tumors were present. The most frequent sites of origin were: external auditory canal region (about equal sex incidence), liver (more frequent in males), and breast (only in females). Almost without exception the tumors were of epithelial origin. Out of 31 liver tumors observed, 27 occurred in males and 4 in females. The most frequently appearing tumors were of the liver-cell type with solid, trabecular, or glandular arrangement, often multicentric in origin. Malignant tumors were observed during and after the ninth month, although foci of cells, which might be interpreted as showing incipient malignancy, were

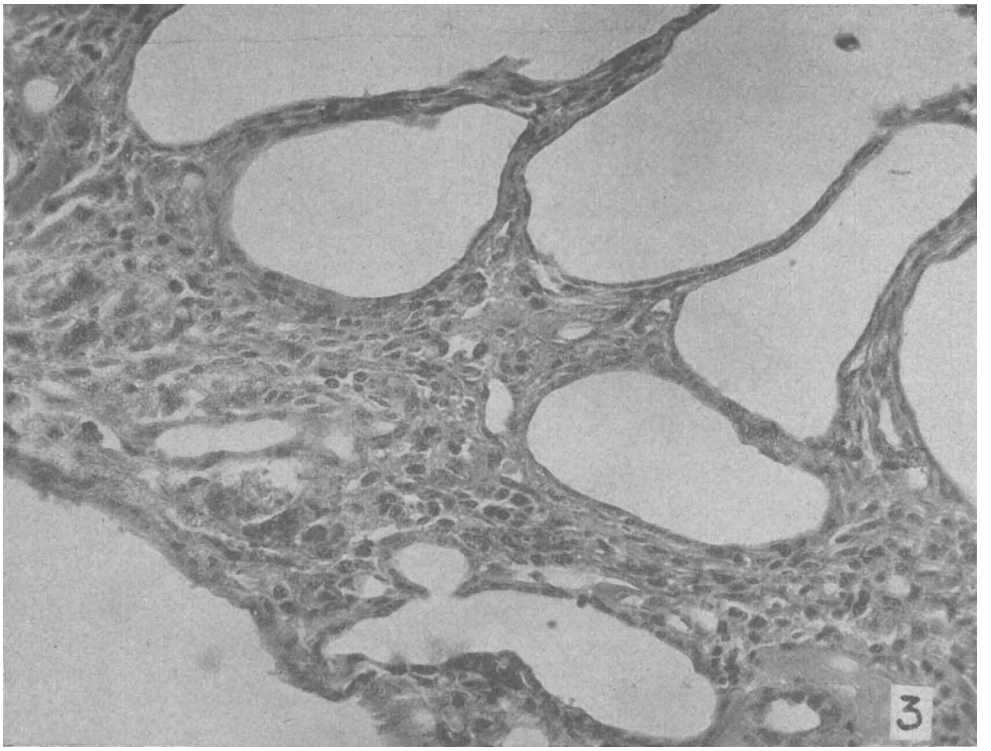

FIG. 3. Cystic dilatation of the bile ducts with fibrosis of the stroma (cholangiofibrosis). H & E. × 440.

observed in the seventh and eighth months. Frequent peritoneal and pulmonary metastases were found. Multiple cysts, sometimes reaching huge sizes, were often present on the tumor and liver surfaces, occasionally giving rise to intra-abdominal hemorrhage. No malignant tumors of bile-duct origin were observed.

Discussion. Studies by repeated partial hepatectomies indicate a sequence of changes in liver structure similar to the "compensatory process following destruction of liver tissue" described by Opie(2) upon administration of azo dyes to rats. The initial lesion observed during the second month of administration of 2-AAF might be summarized as subacute necrosis with nodular hyperplasia. As suggested by the microscopic appearance of the lesion (Fig. 1), the hyperplastic regenerative nodules seem to exert pressure on the surrounding degenerated liver parenchyma by means of expansive growth. This might be considered as the primary cause of accumulation of pre-existing reticular framework fibers in the degenerated liver tissue. These findings, and the fact that nodular hyperplasia precedes fibroblastic proliferation, seem to substantiate the views of Kelty, Baggenstoss, and Butt(3), that the regenerative phase plays a determining role in the production of cirrhosis.

The rapidly progressing hyperplasia of liver cells occurring in later months of administration of 2-AAF caused a relative decrease in the amount of cirrhosis. The newly formed parenchyma seems to develop resistance to the toxic action of the compound when its administration is continued. Thus the type of cirrhosis produced by 2-AAF does not comply with the definition of liver cirrhosis by Kretz(4) as "a chronic degenerative process with inclusion of regenerative periods." Simi-

2. Opie, E. L., *J. Exp. Med.*, 1944, v80, 231.

3. Kelty, R. H., Baggenstoss, A. H., and Butt, H. R., *Proc. Mayo Clin.*, 1950, v25, 18.

4. Kretz, R., *Wien. klin. Woch.*, 1900, v12, 272.

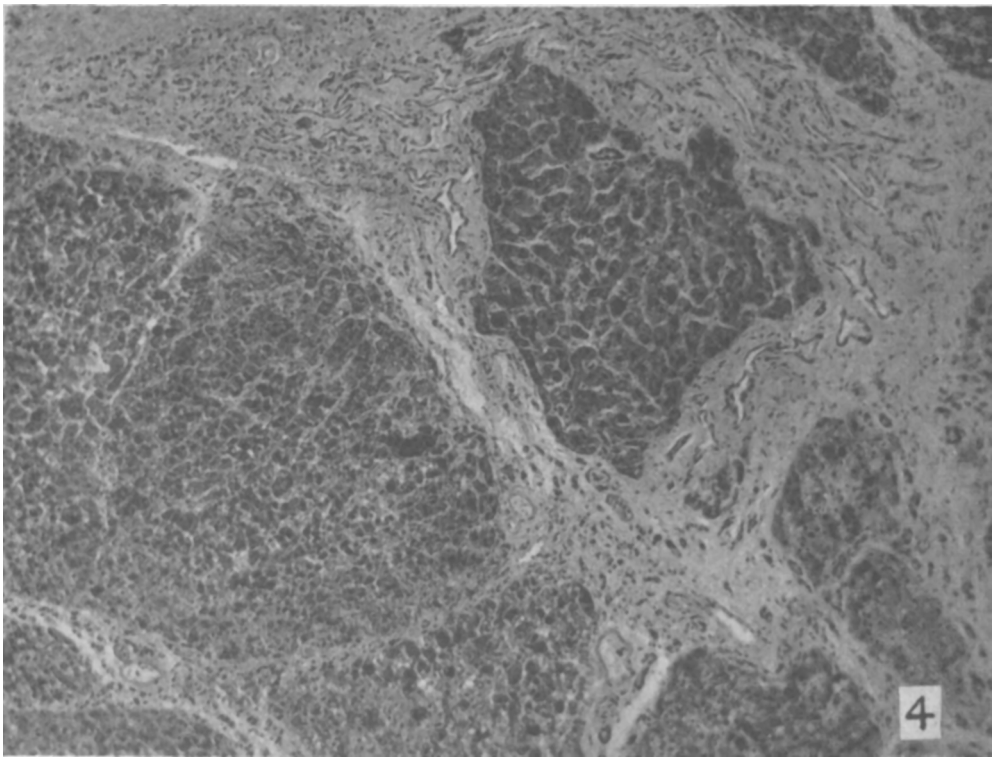

FIG. 4. Advanced cirrhosis with coarse bands of dense collagen containing numerous bile ducts, many of which show ectasia. Masson trichrome. × 110.

larly, Orr(5) expressed doubt that irreversible cirrhosis could be produced by the administration of p-dimethylaminoazobenzene.

Orr(5) drew attention to the fact that cirrhosis produced by certain agents, such as carbon tetrachloride, reaches a much greater density than that produced by p-dimethylaminoazobenzene, although the latter has more potential carcinogenic effect. The same can be stated with regard to the action of 2-AAF. Thus the frequency of liver tumor occurrence cannot be regarded as paralleling the extent of cirrhotic liver changes. Frequent association of cirrhosis with tumors can be explained by the fact that cirrhosis indicates previous liver damage and irregular regeneration. It is then necessary (1) to regard cirrhosis as a secondary process replacing the degenerated liver parenchyma; (2) to presume that a focal liver damage pre-existed in cases of tumors arising in non-cirrhotic livers. From the results of this experiment it is apparent that the initial parenchymal necrotic changes in the liver produced by the hepatotoxic action of 2-AAF invariably occurred prior to tumor formation. The percentage of liver tumors was considerably higher in males, which also were more susceptible than female rats to the hepatotoxic action of the compound. The incidence of liver tumors in male rats was higher when a larger dose was administered, although the total number of tumors in all organs was not appreciably changed. Therefore, it appears probable that the initial hepatotoxic action of higher dosages of 2-AAF has a localizing effect on the carcinogenic action of the compound.

Summary and conclusions. (1) Histological changes in the liver during the administration period of 2-acetylaminofluorene were studied by means of repeated partial hepatectomies. (2) It was observed that malignant liver tumors produced by 2-AAF are preceded by a certain pattern of changes in liver structure, beginning with subacute necrosis, nodular

5. Orr, H. W., *J. Path. and Bact.*, 1940, v50, 393.

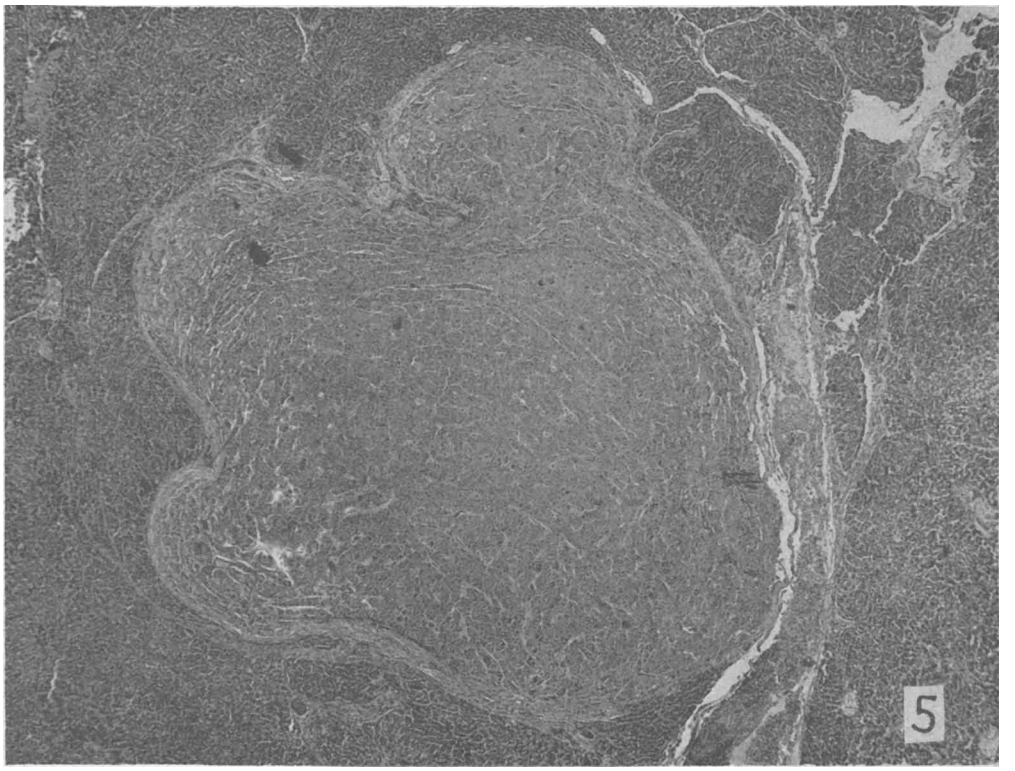

FIG. 5. Benign trabecular hepatoma. H & E. × 31.

hyperplasia, and cirrhosis. (3) Although these findings confirm the frequency of hyperplasia preceding malignant tumor formation, the actual conversion to malignancy does not seem to follow necessarily the condition of hyperplasia and/or benign neoplasia. (4) Repeated partial hepatectomies did not increase the incidence or accelerate the occurrence of hepatic tumors. (5) The percentage of liver tumors was considerably higher in males, which were found to be more susceptible than female rats to the hepatotoxic action of 2-AAF. (6) The incidence of liver tumors apparently depends on the production of hepatic damage, which has a localizing effect on the carcinogenic action of 2-AAF.

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Difference between Autoclaved and Sterile Filtered Medium in Culturing Fragments of a Marine Hydroid.* (18975)

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Invertebrates, especially those from the lower phyla which possess a high regenerative capacity, present interesting material for ex-

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