The fact that methylcholanthrene (MC) was first synthesized from cholic acid (5, 28) and the structural resemblance of the hydrocarbon to estrogens and to steroids of the adrenal cortex have prompted several investigations into the possible role of MC as an intrinsic carcinogenic substance. It was found that MC may induce the development of mammary tumors in certain mouse strains in which such tumors do not occur spontaneously (11), and it accelerates the development of certain spontaneously occurring malignancies such as mammary carcinoma (5, 8, 14, 22), lung tumors (1), and leukemias (10, 15). The administration of MC also enhances the occurrence of virus papillomas (16). Mammary tumors can be produced in rats by the gastric instillation of MC (17). In comparison with other carcinogenic hydrocarbons like benzpyrene or dibenzanthracene, the fate of MC in the body is relatively little known. By use of the fluorescence method it was found to be excreted in the bile (4). The stomach of the rat is able to absorb and retain MC for a considerable period. It is absorbed also in the intestine and can be detected in the blood and in the liver (13). Using radioactively labeled MC, Dauben and Mabee (6) found no accumulation of MC in the liver of mice. Radioactive substances were eliminated mainly in the feces, and to a lesser degree in the urine. No attempt was made to determine whether the urinary and fecal excretion represented unchanged MC. In analogy to other carcinogenic hydrocarbons, it might be assumed that MC is being detoxicated in the liver. The influence of dietary liver damage on the detoxication of the estrogens is well established (2, 18). It seemed likely that the detoxication and activity of MC might be affected in a similar manner. The experiments presented were undertaken to study the effect of a prolonged oral administration of small doses of MC to rats in which the liver was damaged by a diet low in protein.

METHODS
Sixty male and 40 female Wistar rats weighing about 100 gm. at the beginning of the experiment were kept on the diet described in Table 1. To the diet of 40 male and 20 female rats MC was added in the amount of 10 mg/100 gm food. After being fed MC for 22 weeks, ten male and ten female rats were transferred to the basic diet without MC. From about the 50th week onward rats were killed at intervals. All rats were autopsied; the organs were weighed and fixed in formalin or alcohol for histological investigation. Rats which were found dead and in a state that, due to cannibalism, made investigation impossible, are not accounted for.

RESULTS
The growth of all rats was reduced to 1.0 gm/week on the average, but individual rats weighed more than 350 gm at the end of the experiment. The mortality among the rats receiving MC was very high during the first 4 months when twelve males and seven females died in this group, whereas only one female and two males died in the control group. After this time the mortality was low in both groups. The effective number of rats, therefore, was taken to be the number alive after 16 weeks; it was 50 both in the control group and in the groups receiving MC. Since no appreciable differences were found in the lesions of the rats fed MC for only 5 months and in those of rats receiving MC during the whole experimental period, the findings for these two groups are treated together. In the group treated with MC the post mortem findings for rats dying during the first 12 weeks did not reveal any lesion which could be attributed to the dietary regime; five males autopsied between the 12th and 15th weeks showed incipient cirrhosis. Papillomatosis and ulcers were present in the stomach of three rats. In one control male, which died during this period, cirrhosis of
the liver and an ulcer in the stomach were found.

Table 2 summarizes the results with regard to survival and the pathological changes in the liver and stomach of all rats. Fatty changes and cirrhosis with the deposition of ceroid and cholangiofibrosis were found in the livers of rats fed the basic diet with and without MC. Although these changes were manifest in all rats, their severity was enhanced by the feeding of MC. While among the rats killed after the 15th week moderate cirrhosis was present in thirteen on the basic control diet and in nine receiving MC, very severe cirrhosis was found in only one on the basic diet and in six receiving MC. Cystic cholangiomas were present in the liver of one rat on the basic diet, and cystic and solid cholangiomas in the livers of five rats receiving MC. None of these tumors metastasized or showed definite malignant characteristics.

From about the 18th week, the squamous epithelium of the forestomach of the rats underwent hyperplasia, ulceration, and marked papillomata at the junction of glandular and squamous epithelium. Metaplasia and dyskeratosis occurred in several instances. The reaction of the stomach contents to litmus was acid in all rats. In one rat fed MC and killed 73 weeks after the beginning of the experiment the stomach wall was markedly thickened. Histologically, the lesion consisted of atypical metaplasia of the epithelium with a marked inflammatory reaction in the connective tissue and gave the impression of a premalignant change. A malignant tumor was found in the stomach of another rat of the same group killed after 88 weeks. This was localized at the squamoglandular junction. The cellular characteristics

TABLE 1

<table>
<thead>
<tr>
<th>EXPERIMENTAL PERIOD (WEEKS)</th>
<th>GROUP</th>
<th>NO. RATS</th>
<th>NPC</th>
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<th>Cirrhosis</th>
<th>Tumor</th>
<th>NPC</th>
<th>Ulcer</th>
<th>Papillomas</th>
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<td>1</td>
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<td>23</td>
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</table>

* MC = Rats fed methylcholanthrene with the basic diet.
E = Controls, rats fed the basic diet only.
NPC = No pathological changes.
The number of + signs designate the severity of the lesion.
† One lesion apparently is precancerous.
of the tumor suggested sarcoma, possibly leiomyosarcoma. The tumor cells stained yellow with the Van Giesen stain, and the Sudan IV stain showed that they contained fine fat droplets. Some areas suggested glandular spaces. No mucus-containing secretion could be demonstrated by the periodic acid Schiff stain. A diagnosis of mixed carcinoma or a tumor arising from the vessel wall must also be considered (Fig. 1).

The microscopic study of the other organs revealed no significant pathologic change in the heart, brain, or adrenals. The lungs of most rats after 15 weeks showed patches of lobular pneumonia which seemingly did not interfere with the general well-being of the rats. In the kidneys of all rats, irrespective of whether or not they received MC, calcium deposition was found in the epithelium of the collecting tubules, and calcium-containing amorphous material was present in some of them. The spleens and thymi of all rats were small; the decrease in the lymphoid elements and the deposition of pigment in the spleen were more marked in rats receiving MC. The testes were markedly atrophied in all groups; however, no significant changes were found in the ovaries.

DISCUSSION

This experiment was undertaken as a part of a series of investigations on dietary influences on hepatic carcinogenesis. From all the experiments connected with the problem, it seems to evolve that certain diets, deficient in proteins or in vitamins, may provoke tumor formation in the liver if administered for a sufficiently long period of time. When a substance is added which, by its metabolic requirements, might put a special strain on the liver, tumor induction is enhanced. In such a manner the effects of feeding MC on the liver of rats might be understood, particularly since the livers of rats were found to be extremely resistant to the direct carcinogenic activity of MC (7). The production of tumors in the liver by feeding 1,2-benzanthracene (24) or chillies (9) to rats kept on a low protein diet may have a similar explanation. In all the dietary experiments the male rats seem to be more prone to dietary liver damage than the females.

The many experiments designed to produce carcinomas of the stomach in animals are reviewed up to 1941 in an excellent paper by Klein and Palmer (12) and recently by Stewart et al. (19). In 1942, Stewart and co-workers (20) succeeded in inducing adenocarcinoma in the stomach of certain pure-strain mice by intramural introduction in the stomach of carcinogenic polycyclic hydrocarbons dissolved in oil or impregnated on a cotton thread. In 1953, Stewart reported (19) that a single intramural injection of MC in the stomachs of five different strains of mice produced malignant tumors with a large percentage of adenocarcinomas or mixed adenocarcinoma-sarcomas. The DBA strain of mice was found refractory to the induction of malignant neoplasm in the stomach. A probably remote induction of gastric carcinomas was observed by Strong and co-workers (21) in mice of the NHO strain injected with MC subcutaneously at an age of 60 days.

From the present experiment it seems that the changes provoked in the stomach of rats by a low protein diet might be augmented by the feeding of MC, so that malignant tumors would eventually arise. Experiments are in progress to confirm these findings.

SUMMARY

Methylcholanthrene at a level of 0.01 per cent was fed to rats in a low protein basic diet over a long period.

In the livers of rats fed the basic diet only, fatty changes and cirrhosis developed; in one rat a cystic cholangioma was found. When rats received MC in their diet, the cirrhosis was more severe, and five rats developed cystic and solid cholangiomas. Male rats were found to be more prone to the development of liver damage than were females.

Ulcers, squamous hyperplasia, and papillomas developed in the stomachs of rats fed the basic diet, as well as in those receiving MC. In the latter group the changes were again more pronounced; one premalignant lesion occurred, and in one case a malignant tumor was present.

REFERENCES

7. DUNNING, W. F.; CURTIS, M. R.; and SEGALOFF, A. Methylcholanthrene Squamous Cell Carcinoma of the Rat Prostate with Skeletal Metastases, and Failure of the


**Fig. 1.**—Transverse section of the stomach of a rat fed MC in the basic diet for 88 weeks, showing the gross appearance of a tumor.

**Fig. 2.**—Section of the tumor showing the replacement of the submucosa. There are small epidermal inclusion cysts in the hyperplastic squamous epithelium. X30.

**Fig. 3.**—The same tumor showing spindle-shaped cells and areas of glandlike spaces. X250.

**Fig. 4.**—Section through the stomach of a rat fed MC in the basic diet for 78 weeks, showing thickening of the submuco- sa. X30.

**Fig. 5.**—The same lesion showing spindle-shaped cells and inflammatory infiltration. X250.
Effect of Prolonged Feeding of Methylcholanthrene to Rats Kept on a Low Protein Diet

Cornelia Hoch-Ligeti

*Cancer Res* 1954;14:749-752.