bility, pending more elaborate tests of the gastrointestinal contents to show whether or not the disappearance of the starch grains is attributable to the action of amylolytic enzymes of the fetus itself.

In conclusion, the data show that in the fetal rat with the placenta still functioning, the stomach empties more slowly than might have been anticipated, and they suggest that the fetus has the ability to digest in part such introduced foods as milk and starch. It would seem that the ability to digest meat is less well developed or absent. This exploratory study demonstrates the feasibility of obtaining additional direct experimental data on the digestive abilities of the fetus.

16475

Carcinogenic Effect of Sulfonamides.*

ALF T. HAEREM. (Introduced by G. F. Dick.)
From the Department of Medicine, University of Chicago.

Early workers in the field appreciated the possibility of sulfonamide carcinogenesis because of the similarity in structural formulae between the sulfonamides and known carcinogenic agents of the aniline dye group. Marshall suggested investigation of the possible carcinogenic effects of sulfonamides and Lewis[1] subsequently reported that the injection of sulfanilamide in olive oil was not followed by the appearance of tumors in the strain of mice used. Similar results were later reported when other workers repeated these experiments.[2] Using a slightly different strain of mice and material it was later reported[3] that spindle cell sarcomata developed at the site of injection of 15 mg of sulfanilamide in lard in a small percentage of Swiss albino mice so injected. Since that report the entire experiment has been repeated. Using 20 mice of the same strain, under the same conditions, the same amount of sulfanilamide was injected. Eight (40%) of the group developed a spindle cell sarcoma at the injection site. The mice developing tumors survived from 54 to 269 days (average was 145 days after injection). A similar number of mice injected with lard alone developed no tumors.

In an attempt to further evaluate the sulfonamides in regard to carcinogenic properties, these studies were repeated on albino rats. One group was injected with sulfanilamide in lard and this was compared with a group injected with a known carcinogenic agent (1:2:5:6 dibenzanthracene) in lard. Controls consisted of one group in which lard alone was injected and one group in which no injections were made. Similar observations were made on rats injected with sulfapyridine.

Group I. Sulfanilamide injections (test animals). Two injections of 0.015 g sulfanilamide were given to each of 10 rats. The first injection was made at the age of 14 weeks into the left groin and the second at 23 weeks into the right groin. Nine months after the first injection one of the rats had developed a huge mass in the left groin. Biopsy revealed the tumor to be a spindle cell sarcoma. The tumor was readily transplanted to another rat. Autopsy one month later on the original rat revealed a frankly invasive tumor which had metastasized to both lungs. The cell structure of the original tumor and metastases was identical. Both were composed almost entirely of spindle cells. Mitotic figures were

* Aided by a grant from the Fenger Fund.

1 Lewis, M. R., *Am. J. Cancer,* 1938, **34**, 431.

2 Zamecnik, P. C., and Koletsky, S., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 391.

3 Haerem, A. T., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 536.

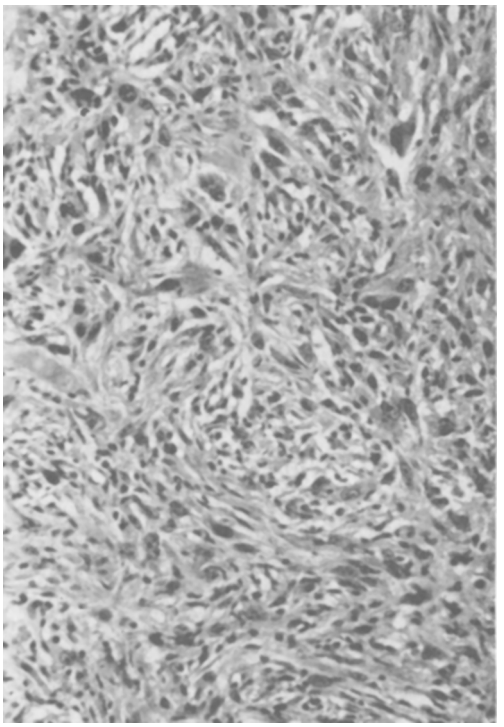

FIG. 1.
Photomicrograph of tumor occurring at injection site of sulfanilamide in lard in an albino rat.

numerous, there were occasional tumor giant cells and direct extension into adjacent tissue was obvious. Fig. 1 shows the microscopic appearance of the tumor. This tumor was transplanted to 14 rat littermates. Seven were fed a stock diet and 7 fed a diet containing 5% sulfanilamide. The controls grew faster than the test group and the tumors were smaller in the sulfanilamide group. However, 6 weeks after the transplant all animals in both groups were grossly deformed by tumors which were identical in structure and resembled closely the original tumor. There was no apparent difference in the 2 groups.

Thirteen months after the first injection a second rat in the group had developed a huge mass in the right groin. This was also a spindle cell sarcoma which was anaplastic, invasive and transplantable. No metastases occurred in this instance however.

The fate of the rest of the group is not as significant. Two died of lung abscesses at 8 months and 13 months after the first injection. The remainder were killed at 16 months after the first injection and no abnormalities noted at autopsy at this date.

Group II. Sulfapyridine injections (test animals). Injections exactly similar to Group I were made with 0.015 g of sulfapyridine in 40 rats. Three rats developed spindle cell sarcomata at the injection site. They were sacrificed at 16, 19, and 20 months. A considerable number of rats died of other causes during this period. Fifteen rats were still alive at 21 months. These were sacrificed and no tumors were found at autopsy.

Group III. Benzanthracene injections (positive controls). Ten rats were injected in an exactly identical manner as described in Group I using 0.015 g of 1:2:5:6 dibenzanthracene in 0.5 cc lard. Six of these animals developed sarcomata at the injection site. There were lung metastases in 2 of the animals. The tumors were all anaplastic, invasive and transplantable. The animals were killed when the tumor became obvious. The first animal to develop a tumor in this group was sacrificed at 6 months after the first injection, and the last occurred at 15 months. The remaining animals died of other causes.

Group IV. Lard injection (lard control). Thirty rats were injected with 0.5 cc of lard in the same manner as listed above. No deaths had occurred in this group at 16 months when the animals were sacrificed and autopsied. No abnormalities were found in this group.

Group V. No injections (negative controls). Thirty rats of the same strain were observed for a period of 16 months for the spontaneous development of tumors. All the rats were alive at the end of that period and were sacrificed. No tumors were noted at autopsy.

Discussion. Since the artificial production of tumors in animals is a resultant of numerous factors such as species, susceptibility, potency of the inducing agent and the age of the animal, it has always been difficult to obtain unequivocal results in evaluating any substance for possible carcinogenicity. The species variance to sulfanilamide is probably great and it would be fallacious to apply these meager data to higher species. Are the sul-

TABLE I.
Summary of Injection Experiments

	Material inj.	Amt	Vehicle	No. of rats	Tumors	%
I.	Sulfanilamide	30 mg	Lard	10	2	20
II.	Sulfapyridine	30 "	"	40 (18)*	3	7.5 (16⅔)*
III.	Dibenzanthracene	30 "	"	10	6	60
IV.	Lard	0.5 cc		30	0	0
V.	No injection			30	0	0

* Only 18 rats survived the entire experiment (see text).

fonamides carcinogenic for man? This question which occurred to the early workers remains as yet unanswered. Some analogy may perhaps be drawn from the experience with aniline dyes and carcinoma of the bladder in man. Hueper[4] has summarized the studies and experience in this field and it would seem that aniline dye workers develop bladder tumors in a statistically significant higher incidence than other people their age who are not exposed to these dyes. The average time between exposure and appearance of the tumor was 18 years. So many concomitant factors operate in such a period that conclusive evidence can be obtained only by statistical methods. However, if sulfonamides exert a similar influence and have the same latent period, it may be that an increase in the incidence of bladder tumors may be seen in or about 1955. Although highly speculative, this possibility deserves consideration because of obviously grave implications.

Conclusions. 1. Under the conditions listed it was observed that albino rats injected with sulfanilamide or sulfapyridine in lard developed sarcomata at the injection site. This incidence was less than the number of tumors occurring following the injection of dibenzanthracene under identical conditions. 2. The occurrence of tumors in Swiss albino mice following the injection of sulfanilamide in lard is reported in confirmation of previous work.

[4] Hueper, W. C., *J. Indust. Hyg.*, 1934, **16**, 255.

16476

Lipids of the Fasting Mouse. V. Liver Phospholipid, Lecithin and Cephalin.

H. C. HODGE, P. L. MACLACHLAN, W. R. BLOOR, E. WELCH, S. L. KORNBERG, AND M. FALKENHEIM.

From the Department of Biochemistry and Pharmacology, University of Rochester, School of Medicine and Dentistry, Rochester, N.Y.

The young, adult mouse has been shown to mobilize available carcass fat in the first 2 days of a fasting period.[1] The liver total lipid increases during this period perhaps due to the failure of a phosphorylating mechanism to meet the emergency by converting the neutral fat to phospholipid prior to burning or transporting it. The incorporation of radio phosphorus (P32) into liver phospholipid metabolism is increased during fasting: this increase has been found in a series of experiments in which the total phospholipid, lecithin and cephalin fractions of the livers of mice fasted 5 days were studied.

Experimental. Three months old, male, albino mice were fasted as described previously.[1] Twenty-four hours before sacrifice, a small volume of a solution containing P32 as Na_2HPO_4 was injected intraperitoneally;

[1] Hodge, H. C., MacLachlan, P. L., Bloor, W. R., Stoneburg, C. S., Oleson, M. C., and Whitehead, R., *J. Biol. Chem.*, 1941, **139**, 897.