tiary, the tests were performed in a similar manner, except that the gastric content was collected by continuous Wangensteen suction for a period of 60 minutes after the subcutaneous injection of the standard dose of histamine. The postoperative tests were performed from 3 to 6 weeks following the gastric vagotomy when the patients had entirely recovered from the operation. The effect of gastric vagotomy on the secretory response to histamine in the 15 ulcer patients studied at the University of Chicago Clinics is illustrated in Table III. In this series of patients, the vagotomy produced a decrease of 41% in the volume of gastric juice secreted in response to the histamine injection and a decrease of 62% in the total amount of hydrochloric acid produced. The free acid concentration for the 15 tests decreased from an average of 93 clinical units to 67 following the vagus section. Table IV illustrates the results secured in the 18 patients with peptic ulcer studied at the Illinois State Penitentiary. In these patients, the average volume of gastric juice secreted in response to a standard dose of histamine decreased 56%, and the total acid output 74% after the vagotomy. The average free acid concentration of the histamine stimulated gastric juice averaged 110 clinical units before vagotomy and 48 clinical units after the operation.

Conclusions. 1. The gastric secretory response to a standard dose of histamine in dogs with a totally isolated stomach pouch is markedly reduced by bilateral vagotomy.

2. Atropine similarly reduces the gastric secretory response to histamine in these animals.

3. Gastric vagotomy in patients with peptic ulcer also markedly reduces the gastric secretory response to a standard dose of histamine.

16298 P

Effect of Bile Preparations on Fat Absorption in Bile Fistula Dogs.*

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That bile is concerned in the absorption of dietary fat has been qualitatively established by the observation of steatorrhea in animals[1] and in man[2] when bile fails to reach the intestine. The effectiveness of human whole bile in correcting this absorptive defect has been shown in only 2 patients,[3] and bile acid preparations have not been tested directly in man or experimental animals. Inconclusive and contradictory results from studies of blood fat after a fatty meal,[4] absorption from isolated intestinal loops,[5,6] and *in vitro* diffusion[7] or solubilization[8] constitute the basis for the belief that bile acids are essential for fat absorption, and that

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TABLE I.
Fecal Fat Excretion in Bile Fistula Dogs.

Regime	No. dogs compared	Means of changes from fecal fat output of 27.4 g/day on no-bile regime, g fat/day	Each regime compared with no-bile, t-ratio
3 g dehydrocholic acid	8	+ 1.61	0.71
3 " iron ppt'd ox bile*	7	— 3.20	1.61
3 " desoxycholic acid	5	+ 0.14	0.06
3 " desiccated ox bile†	7	— 1.47	0.72
6 " " " "	6	— 7.50	3.58‡
90 cc " " "	7	—14.90	7.60§

* Contains 47% cholic and 48% desoxycholic acids.
† Contains about 50% cholic acid and undetermined desoxycholic acid.
‡ Significant at 5% level.
§ Significant at 1% level.

desoxycholic acid is particularly active. In view of the inadequacy of the indirect, and the lack of direct evidence, studies were undertaken to evaluate the relative effectiveness of various bile acids in restoring normal fat absorption in bile fistula dogs.

Methods. Fecal fat excretion was determined in 9 dogs before and after cholecystonephrostomy, in the latter with and without administration of various bile preparations. The daily diet consisted of 335 g "Pard" with 25 g added lard (36 g total fat). Food was given once daily, and was completely eaten. Each regime consisted of a 7-day period; during the last 5 days feces were pooled and analyzed for total fat.[9] When given, bile preparations were mixed with the meal (pills, capsules, or fresh bile). Following operation the feces were free of urobilinogen. Neither jaundice nor diarrhea developed, although stools were bulky.

The plan to subject each dog to each regime was not completed because 3 dogs developed duodenal ulcers and 2 dogs did not tolerate 3 g doses of desoxycholic acid. Statistical analyses were made by comparing 2 regimes in the same animal.

Results. The mean fecal fat excretion of the 9 dogs was increased from 3.0 g/day before operation to 27.4 g/day after diversion of bile from the intestine. Three-gram daily doses of bile acids were given in an effort to correct this steatorrhea. This dose is the maximum that is tolerated for some of the preparations, and is also the 8-hr output of cholic acid by healthy bile fistula dogs when their bile is returned every 8 hours.[10] The failure of iron-precipitated ox-bile (Bilron, Lilly) and desoxycholic acid (Degalol, Ames) to reduce fat excretion was wholly unexpected. Dehydrocholic acid (Ketochol, Searle) was expected to have less effect than the other products tested (Table I). On the basis that the above bile acids may have been altered in their purification, desiccated ox bile and fresh ox bile (kept frozen until use) were administered. Three-gram doses of desiccated ox bile were ineffective; 6-g doses produced a slight but significant reduction in fecal fat. Ninety-cc doses of fresh ox bile, equivalent to 6 g desiccated material, produced a significantly greater, but still incomplete improvement in fat absorption.

Discussion. These results indicate that 3-g doses of certain bile preparations are completely ineffective in replacing the fat-absorptive function of normal whole bile in the dog. It appears that desiccation reduces the ability of fresh ox-bile to promote fat absorption. These results agree with an earlier report[6] that fresh bile, but not sodium glycocholate or taurocholate, can facilitate the disappearance of sodium oleate from isolated intestinal loops.

No convincing explanation can be offered for these results at present. They are con-

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trary to general beliefs concerning the role of bile acids in fat absorption, but many speculative possibilities await study. Further direct experimentation is required to determine (a) whether the absorptive defect consequent to bile exclusion can be completely corrected, (b) the identity of the active constituent(s) in bile (c) the effect, if any, of the manner of entry of active material into the intestine, (d) the role, if any, of species differences, and (e) the mechanism by which fat absorption is facilitated.

Summary. 1. Three-gram doses of certain bile preparations (including desoxycholic acid) proved completely ineffective in reducing the steatorrhea of bile fistula dogs.

2. Ninety-cc doses of fresh ox bile reduced the steatorrhea by 50%, but equivalent doses (6 g) of desiccated ox bile were only half as effective.

16299

Increased Hemolytic Potency of Mouse Mammary Carcinoma Extracts Following Incubation with Tumor Cells.*

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It was recently observed in this laboratory that filtered or centrifugated extracts prepared from spontaneous mouse mammary carcinomas possess the ability to hemolyze mouse erythrocytes *in vitro*.[1]

In course of subsequent experiments it was found that tumor extracts that had been separated from the tumor cells by centrifugation, and have been left, in absence of cells, in test tubes, at room temperature, lost practically all hemolytic potency after 5 hours; the decrease in the hemolytic potency of the tumor extracts was even more rapid at 37°C.[2] This rather striking lack of resistance of the hemolytic factor contained in the tumor extracts, but separated from the tumor cells, to exposure for a few hours to either room, or incubator temperature, was further investigated. One of the first questions requiring clarification was whether the tumor extracts would also lose their hemolytic potency if incubated, at 37°C, in presence of tumor cells.

Accordingly, a series of experiments was performed in which tumor cell suspensions of 20% concentration were freshly prepared from spontaneous mouse (C3H) mammary carcinomas.[2] Fresh samples of such suspensions were tested for their hemolytic activity.[1] The suspensions were immediately divided into 2 groups of tubes: one was left unchanged as a control, and the other was centrifugated twice at 3,000 t.p.m. for 10 minutes each, to separate the extracts from the tumor cells; following centrifugation, the final supernatant fluid was used. Both, the tumor cell suspensions, and the cell-free extracts, were then placed in an incubator, at 37°C, for 5, and 24 hours. After incubation, samples removed from both groups of tubes were tested for their hemolytic activity by serial titration. Eight different mouse mammary carcinomas were used for the preparation of extracts, and 8 successive series of experiments were performed under apparently identical conditions. The results were as follows:

All 8 fresh tumor extracts were found to possess the usual hemolytic potency[1] when tested before exposure to the incubator temperature. After exposure for 5, or 24 hours, to 37°C, the centrifugated tumor extracts incubated without cells, were found to have lost their hemolytic potency, when tested in

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