a single dose of 50r produced tumors in 70% of the mice. Daily exposure to 0.11r (from radium) with an accumulated total dose of 90r produced ovarian tumors in about 70% of the mice.[4] No hematological changes have been detected with a daily exposure of 0.1r of gamma radiation in animals that subsequently developed ovarian tumors.[10] An increase in the ovarian tumor incidence in mice exposed to single doses of 26n to 90n of fast neutrons is on record.[5] This is probably much above the minimal tumor producing dose. If mutation is the basis of tumor induction by x-rays, with an increase in dosage an increase in the number of tumors should be anticipated. The present experiments do not exclude this possibility in the range of about 25 to 200r while earlier experiments in the dose range above 87r[1] and those of Lorenz and associates[4] failed to show such a trend.

The relation of age to tumor induction was studied by Kaplan[3] who irradiated 2 weeks to 6 months old mice with 600r administered in daily doses of 50r on 12 consecutive days. The greatest ovarian tumor incidence occurred in mice irradiated at 1 month. None was noted in 7 mice irradiated at 2 weeks but intercurrent mortality was high in this group. The present studies show that mice are susceptible even at 1-3 days of age.

A follow-up of these pilot experiments is desirable, even though each requires about 3 years. The relation of the dose of x-rays and age of hosts to the rate of ovarian tumor induction requires a closer check. The hormonal state of mice preceding tumor development and the incidence of pregnancies and abortions should be determined more accurately. The histological changes in ovaries following irradiation ante- and postpartum deserve further consideration, particularly the alleged postnatal ovogenesis and the regenerative phase which leads to neoplasms. Does irradiation induce a chromosomal change and what initiates and sustains the hormonal imbalance are questions to be answered.

Summary. Mice exposed to 50-200r of x-rays can have one or several normal pregnancies and still be liable to the development of ovarian tumors in a stock in which this neoplasm is practically non-existent.

Irradiation at 1-3 days of life with 150r sterilized only 1/3 of the mice while ovarian neoplasms appeared in about 76%. Irradiation at 1-3 days did not hasten the onset of ovarian neoplasms as compared to irradiation at 4-10 weeks. In both groups the tumors developed in middle aged and old mice. The growth rate of the tumors was likewise slow, hardly interfering with the normal life span of these animals.

It is postulated that a specific delayed x-ray effect coupled with a hormonal imbalance provoked by x-rays leads to the development of ovarian tumors.

These experiments were begun at Cornell University Medical College and assisted by Thelma Weaver Mold.

[10] Jacobson, L. O., and Marks, E. K., *Radiology*, 1947, **49**, 286.

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17162. Correction of Steatorrhea in Bile Fistula Dogs by Frequent Return of Bile.*

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It was previously reported that 3 to 6 g daily doses of various bile acid preparations mixed with a fatty meal were at best only partially effective in correcting the steatorrhea of bile fistula dogs.[1] This was attributed to one or more of the following possibilities: *a*,

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[1] Heersma, J. R., and Annegers, J. H., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 339.

a physiologically inactive bile preparation; *b*, an inadequate total dosage; *c*, improper time of administration. In subsequent studies, *a* and *b* were minimized by returning all of animals' own bile to the intestine instead of giving commercial bile preparations. As to *c*, flow of concentrated gallbladder bile into the intestine during gastric evacuation and digestion of a fatty meal was shown to be unnecessary for normal fat absorption, since no steatorrhea developed in cholecystectomized dogs.[2] The present experiments involving the return of bile fistula dogs' bile at different intervals throughout the day were designed to determine whether bile must be present continuously for normal fat absorption.

Methods. Rous-McMaster external bile fistula dogs[3] were prepared with an additional rubber tube for return of bile into the duodenum.[4] Beginning 7 to 10 days after operation, diets containing 335 g of commercial dog food ("Pard") and 25 g of lard (36 g of total fat) were fed once daily. All meals were completely consumed. Total fecal fat excretion was determined[5] on 5-day stool collections when no bile was returned and when the animals' own bile was returned. Cholic acid in the bile returned during 5-day periods was determined[6] on pooled aliquots. The bile collected during 8, 4, or 1-hour intervals was introduced into the duodenum at the rate of 5 cc per minute.

Results. When no bile was returned, fecal fat excretion averaged 25.7 g per day in 6 dogs. This value is in agreement with fecal fat excretion of 27.3 g per day obtained in a previous study in 9 cholecystonephrostomized dogs receiving the same diet.[7]

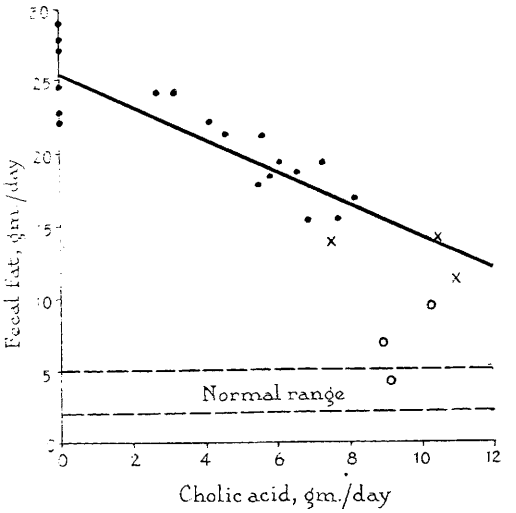

FIG. 1.

Relationship between fecal fat excretion and cholic acid of whole dog bile introduced into the intestine.

● Bile returned every 8 hr
x " " " 4 "
o " " " 1 "

The straight line represents the regression equation fitted to the filled circles only.

In 4 dogs bile was returned every 8 hours for 2 to 10 consecutive 5-day periods. The daily quantity of bile acid secreted and returned increased to reach a steady-state level by the third period, in agreement with the results of Berman *et al.*[4] The average daily quantity of cholic acid secreted and returned during the 5-day tests, and the corresponding daily excretion of fecal fat are plotted for each dog in Fig. 1 (filled circles). As the dose of cholic acid increased, the fecal fat output decreased in linear fashion; the regression equation (correlation coefficient was –0.88) indicated that for each gram increase in cholic acid return fecal fat excretion decreased by 1.17 g. However, at the steady-state level of cholic acid secretion and return (6 to 8 g/day), the defect in the fat absorption was only about 50% corrected. Extrapolation of the regression line implied that 20 g of cholic acid per day would be required for normal fat absorption when the bile is returned every 8 hours. This amount greatly exceeds the steady-state output obtained in this and a previous study,[4] and hence indicates that a time factor as well as a dose factor must be concerned.

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[2] Heersma, J. R., and Annegers, J. H., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 140.

[3] Rous, P., and McMaster, P. D., *J. Exp. Med.*, 1923, **37**, 11.

[4] Berman, A. L., Snapp, E., Ivy, A. C., and Atkinson, A. J., *Am. J. Physiol.*, 1941, **131**, 776.

[5] Fowweather, F. S., and Anderson, W. N., *Biochem. J.*, 1946, **40**, 350.

[6] Irvin, J. L., Johnson, C. G., and Kopola, J., *J. Biol. Chem.*, 1944, **153**, 439.

[7] Heersma, J. R., and Annegers, J. H., *Am. J. Physiol.*, 1948, **153**, 143.

Accordingly, bile was returned every hour in 3 dogs for 6 days. Fat absorption during the last 5 days was within normal limits[7] in one dog and considerably improved in the others. Since the plotted points (open circles) are well below the regression line in Fig. 1, the improvement appeared to be due largely to the continuous presence of bile, and not to the increased quantity of cholic acid secreted and returned. To check this point further, bile was returned every 4 hours in the same 3 dogs. The quantity of cholic acid returned did not change, but fecal fat excretion increased so that the plotted values (crosses) fell near the original regression line.

Finally, fecal fat excretion was measured in 3 dogs after cessation of bile return, in order to determine the rapidity with which the fat absorptive defect develops when bile is excluded. From the third through the seventh day after bile return was stopped the fecal fat excretion averaged 17.8 g per day; from the eighth through the thirteenth day, 22.0 g per day. The former value is significantly less, by group comparison analysis,[8] than the mean fecal fat excretion obtained in the present and previous[7] study during 5-day periods begun 10 days or more after the operation. The results indicate that impaired fat absorption develops rapidly when the bile is first excluded, then more slowly to a maximum after about 7 days. The presence of bile acids in the scraped intestinal mucosa of dogs 24 hours, but not 1 month after bile exclusion,[9] suggests a possible reason for the delay in development noted.

Discussion. The present results demonstrate that previous failure to correct steatorrhea in bile fistula dogs by feeding them whole ox-bile containing 6 g of cholic acid with the single daily meal was probably due to the failure of this procedure to provide adequate amounts of the bile in the intestine over the period required to complete fat absorption. Although continuous hourly administration of bile was employed successfully in this study to restore normal fat absorption, this does not imply that hourly return must be continued *throughout a 24-hour day* to complete the absorption of a single daily fat meal. However, the establishment of the fact that bile must be present in the intestine continuously for at least most of the day now makes it possible to investigate further the bile component(s) and dosages necessary for normal fat absorption, and to assay bile replacement measures.

Summary. The steatorrhea of 3 bile fistula dogs given a fatty meal once daily was nearly completely corrected when the animals' own bile was returned to the duodenum every hour. The steatorrhea persisted, however, when bile was returned every 4 or 8 hours. Since the total quantity of cholic acid returned during the 1- and 4-hour regimes was the same, the importance of continuous presence, apart from total daily quantity present, in the intestine is demonstrated.

[8] Snedecor, G. W., *Statistical Methods*, Iowa State College Press, 1946.

[9] Verzar, F., and Von Kuthy, A., *Biochem. Z.*, 1931, **230**, 451.

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17163. Observations on Experimental Dental Caries. XI. Influence of Pregnancy.

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The results of the clinical investigations on the relationship between pregnancy and dental caries have been contradictory. However, most of the controlled studies made in the last years negate the validity of the old popular idea that pregnancy increases the susceptibility to dental caries. Day,[1] for example,

[1] Day, C. D. M., *Indian J. Med. Research*, 1947, **35**, 101.