age of 74, the systolic pressure declines slowly in women, but remains essentially constant in men. 4. The mean *diastolic pressure* shows little variation from ages 65 to 80, and tends to decline thereafter. 5. *Frequency distribution curves* of systolic and diastolic pressures, at all ages, have the basic pattern of a bell-shaped curve. In both sexes, the curves have a positive skewness larger and more consistent in the systolic than in the diastolic pressures. 6. A single set of blood pressure standards has been computed for each sex, applicable to the entire apparently healthy population from age 65 to over 100. These computations place the middle 80% range ($\pm$ 1.282 sigma) in males at $\frac{115\text{-}175}{70\text{-}95}$, and females $\frac{120\text{-}192}{65\text{-}102}$; and the middle 95% range ($\pm$ 2 sigma) in males is $\frac{100\text{-}190}{62\text{-}102}$, in females $\frac{100\text{-}212}{55\text{-}112}$.

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Turnover and Nature of Fecal Bile Acids in Germfree and Infected Rats Fed Cholic Acid-24-^{14}C. Bile Acids and Steroids 41.*† (22981)

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The turnover of bile acids has been studied in normal rats(2) and in rats treated with chemotherapeutics(3). It was found that the rate of excretion was much slower in rats treated with chemotherapeutics than in normal animals. The purpose of the present investigation was to study excretion of cholic acid in rats reared in germ-free conditions from birth and in rats with a known intestinal flora. Furthermore, the nature of the fecal acids excreted after feeding 24-^{14}C-cholic acid was to be studied as a complement to earlier experiments carried out on normal rats(4), on rats treated with chemotherapeutics(5) and *in vitro* with microorganism isolated from rat feces(6).

Methods. The germfree rats used had been delivered into the germfree unit through cesarian section and hand-fed for the first 20 days according to the technic of Gustafsson (7) with slight modifications. Litter mates were reared outside the units on the same sterilized diet. The animals used were 3-6 months old. 3 germfree and 3 control animals were studied. The diet contained casein 22%, wheat starch 63%, arachis oil 10%, salt mixture 4% and sufficient amounts of vitamins. The diet was mixed with 50% water and autoclaved at 121°C for 20 minutes. 1-2 mg of sodium salt of cholic acid-24-^{14}C(8) (3.9 μc/mg) was autoclaved in water solution, transferred to the apparatus and given by mouth to the animals. The rats were kept in metabolic cages and their feces collected every 24 hours. The

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feces were crushed and boiled twice in 80% ethanol for 3 hours. The isotope content was determined on a small aliquot of the combined extracts as described earlier(3). Prior to the chromatographic separations the ethanol extracts were evaporated *in vacuo* and extracted with butanol from an acidified water solution. The butanol extracts were subjected to reversed phase partition chromatography on hydrophobic Supercel(9,10). Phase system C: Stationary phase: isooctanol/chloroform 1:1; Moving phase: methanol/water 1:1. 4 ml of the stationary phase was used per 4.5 g of supporting medium. Fecal excretion of cholic acid in germfree rats during a 10 day period was determined. The combined ethanol extracts were saponified and cholic acid isolated with column chromatography and then quantitatively determined by the method of Sjövall(11).

Results. 1. *Nature of fecal bile acids after feeding 24-^{14}C-cholic acid.* When labelled cholic acid was fed the acids appearing in the feces of rats in normal environments were found to consist of a variety of compounds (4), while in rats treated with chemotherapeutics(5) only taurocholic acid was found, *i.e.* the same compound that was found in the bile when labelled cholic acid had been injected(12). The changes brought about by the intestinal microorganisms were a splitting of the peptide bond of taurocholic acid and alterations on the steroid nucleus. Some strains of microorganisms capable of splitting

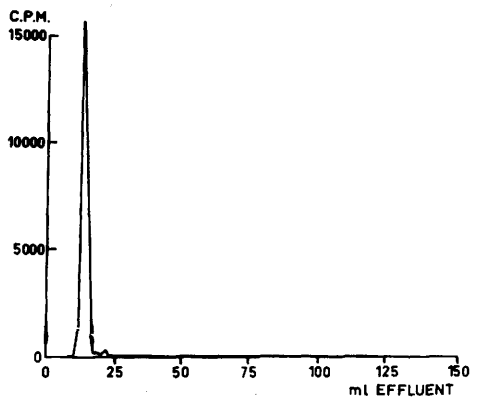

FIG. 1. Chromatogram of labelled compounds in feces of rat 82 × 1 under germfree conditions. Phase system C.

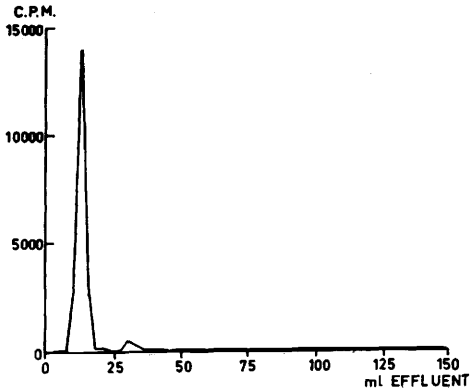

FIG. 2. Chromatogram of labelled compounds in feces of rat 82 × 1 infected with *Aspergillus niger*. Phase system C.

the peptide bond *in vitro* have been isolated (6), while the nature of the microorganisms effecting the attack on the steroid molecule is still largely unknown.

In Fig. 1 a chromatogram with phase system C of the fecal acids from a germfree rat (82 x 1) fed labelled cholic acid is shown. The activity is confined within one single peak appearing at the place of taurocholic acid. The material constituting this peak has been collected, hydrolyzed and re-run with carrier cholic acid in phase system C, where all of the activity appeared at the place of cholic acid. The results are identical with those earlier obtained in rats treated with chemotherapeutics(5), thus definitely showing that intestinal enzymes do not hydrolyze the conjugated bile acids. Identical results were obtained with the 2 other germfree animals studied.

One of the germfree rats (82 x 1) was accidentally infected with *Aspergillus niger*. 15 days later a dose of labelled cholic acid was given. Fig. 2 shows a chromatogram of the fecal acids of this rat. It is identical with that obtained from the same rat during germfree conditions, demonstrating the inability of the mold to attack either the peptide bond or the steroid part of the molecule. To study the effect of a strain of bacteria known to produce enzymes capable of hydrolyzing the peptide bond *in vitro*(6) we infected the same rat with *Clostridium perfringens type E.* After infection the animal had a slight diar-

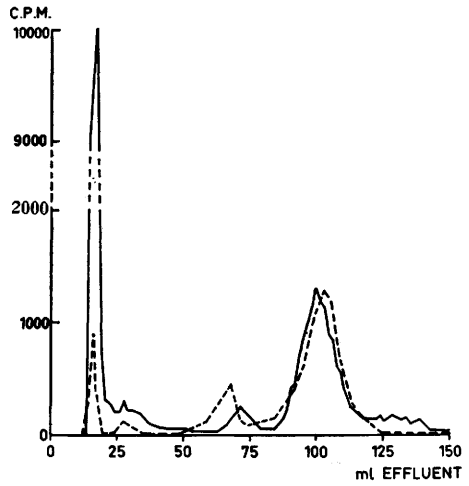

FIG. 3. Chromatograms of labelled compounds in feces of rat 82 × 1 infected with *Aspergillus niger* + *Clostridium perfringens* type E. Dotted line: Result of *in vitro* experiment with the same microorganism (redrawn from Norman and Grubb (5)). Phase system C.

rhea for a few days. The feces contained a large number of living microorganisms of the two strains in question.. Fifteen days after the infection with *Clostridium perfringens* labelled cholic acid was administered as earlier. The chromatogram of the fecal acids is shown in Fig. 3 together with the result of an *in vitro* experiment with the same strain of bacteria. The peak that is seen at 80-110 ml effluent appears at the place of free cholic acid, while a small peak just before (50-75 ml) is caused by an unknown metabolite.

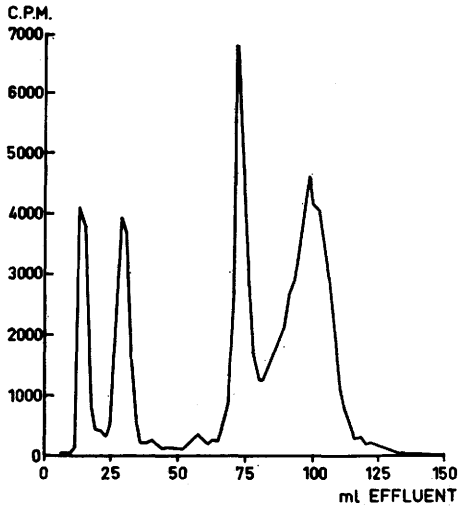

FIG. 4. Chromatograms of labelled compounds in feces of rat 82 × 1 in normal environments. Phase system C.

The rat (82 x 1) was finally removed from the unit and placed in the laboratory for 10 days to acquire a "normal" intestinal flora. The result (Fig. 4) is similar to that found in normal animals(4) with extensive splitting of the conjugates and further modifications of the free bile acids.

2. *Turnover of bile acids in germfree rats.* Elimination of cholic acid in rats treated with chemotherapeutics is much slower than in control animals(3). Rats treated with chemotherapeutics might not represent a physiologically steady state owing to the fairly short time of treatment and the possible toxic effect of the drugs(3). In the present work, however, no such objection can be raised and rate of elimination should therefore be considered equal to rate of synthesis. Fig. 5 shows a plot of $-\log\left(1-\frac{U_t}{U_{max}}\right)$ *vs* time for germfree and control animals. (U_t = amount of activity excreted at time t. U_{max} = plateau level reached in control animals—85-100% of injected amount—and total amount administered to germfree rats, where no plateau level was reached in the experimental period.) For a discussion of this way of plotting the result see ref. 2. The half-lives that can be read from this figure/normal: 2 days (1.2-2.1); germfree: 11.4 (8-14.5)/ show that the half-lives of germfree rats are of the same

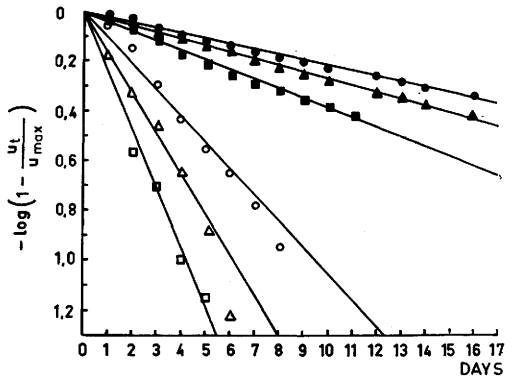

FIG. 5. Semilogarithmic plot of elimination of cholic acid in normal (□ △ ○) and germfree (■ ▲ ●) rats.

order of magnitude as in rats treated with chemotherapeutics.

To obtain the daily synthesis of bile acids one would have to know the size of the bile acid pool of the germfree rats. It has not been possible to determine this by direct methods as done in normal rats(13). However, as taurocholic acid is excreted unchanged in germfree rats it was possible to determine the daily excretion with the quantitative paper chromatographic method of Sjövall(11). This was done for a 10 day period in each rat. The mean daily excretion of cholic was 0.9 mg 100 g body weight corresponding to a pool of 15.4 mg 100 g body weight. While the size of the pool is considerably larger than found in normal animals by Bergström and Eriksson(13) daily excretion of cholic acid is less than in normal animals(2).

Germfree animals have been found to have a greatly distended coecum(7). The possibility that the bile acids are trapped in the coecum was excluded by an experiment where the distribution of activity in different parts of the intestinal tract was determined 24 hours after administration of labelled cholic acid. The result (Table I) shows that more than 80% of the recovered activity is located in the small intestine. This distribution is similar to that found in normal animals.

It was further shown by X-ray studies that a barium meal reached the colon in approximately the same time in a germfree and a control animal. Thus after 4 hours 2 fecal pellets were observed in both the control and the germfree animal.

3. *Influence of different infections on rate of turnover of bile acids.* Fig. 6 shows a semilogarithmic plot of excretion of isotope in rat 82 x 1 during periods with different conditions in the intestine. When the bile acids of this

TABLE I. Distribution of Isotope 24 Hours after Oral Administration of Cholic Acid-24-^{14}C to a Germfree Rat.

Part of intestinal tract	% of isotope recovered
Duodenum	3.5
Small intestine	83
Coecum	11.2
Colon	2.3

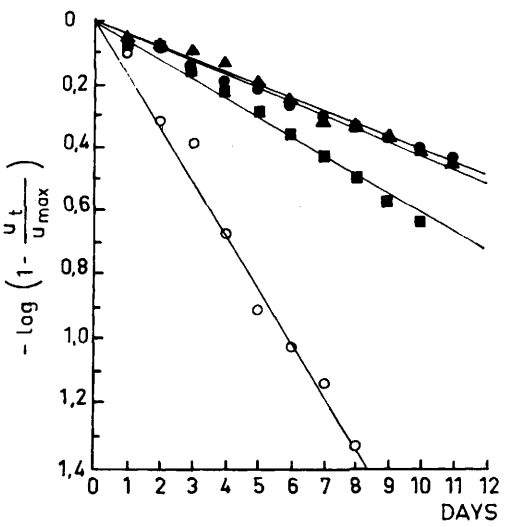

FIG. 6. Semilogarithmic plot of elimination of cholic acid in rat 82 × 1 during different conditions (●—● germfree, ■—■ infected with *Aspergillus niger*, ▲—▲ infected with *Aspergillus niger* + *Clostridium perfringens*, ○—○ totally infected).

rat in germfree surroundings had a half-life of 7 days monoinfection with *Aspergillus niger* did not produce any certain change in the half-life (5.2 days) or in fecal bile acid composition, nor did a superimposed infection of the same individual rat with *Clostridium* change the half-life of the cholic acid (8 days) in spite of a considerable splitting of the conjugates. When the infected rat finally was brought outside the apparatus and had acquired a "normal" intestinal flora the half-life of the cholic acid was reduced to that of a normal rat, *i.e.* 1.8 days.

Summary. 1. Taurocholic acid-24-^{14}C was the only metabolite found in feces of germfree rats fed cholic acid-24-^{14}C. 2. The half-life of cholic acid in germfree rats is 11.4 days as compared with 2 days in control animals. Daily excretion of cholic acid in germfree rats is 0.9 mg/100 g body weight. 3. Monoinfection of a germfree rat with *Aspergillus niger* did not change the half-life nor the composition of the bile salts in feces. Infection of the same rat with *Clostridium perfringens* resulted in free cholic acid in feces but no change in turnover time. When the rat was taken out of the germfree rearing ap-

paratus turnover time and bile acid picture changed to that of "normal" rats.

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Antral Inhibition of Gastric Secretion.* (22982)

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Edkins(1) proposed that presence of secretagogues in the stomach promoted gastric secretion by release of a hormone, "gastrin," from the antral mucosa. Although his experiments were inadequate to prove this hypothesis, subsequent studies have demonstrated that the gastric phase of gastric secretion is mediated by a hormonal mechanism which is initiated by chemical(2) and mechanical(3) stimulation of the antral mucosa. The presence of acid of sufficiently strong concentration within the antral portion of stomach will diminish the rate of gastric secretion which results when antral gastrin mechanism is stimulated by secretagogues within the antrum(4). Whether the acid environment is unsuitable for release of gastrin or whether it results in production of a substance which actively inhibits production of HCl is not clearly established. Woodward (4), interested in the possible existence of a gastric secretory inhibitor factor from the antrum, was unable to inhibit the secretory effect of histamine by perfusion of the isolated, innervated antrum with acid and concluded that such a factor did not exist. Recently Harrison *et al.*(5) utilizing the technic of a divided antrum have presented evidence for existence of a gastric secretory inhibitor mechanism within the antrum. We have attempted to prove the existence of this mechanism by using the divided antral pouch technic in such a way that the environment of each antral segment may be altered simultaneously at will.

Method and procedure. Four healthy adult mongrel dogs were prepared as follows: The gastric antrum was removed from the gastrointestinal continuity and divided longitudinally forming 2 small antral pouches which were marsupialized. A Heidenhain pouch was constructed and drained by a

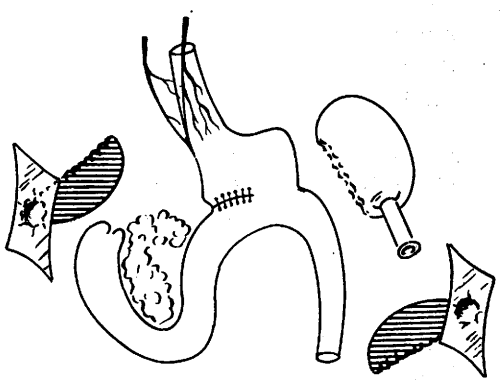

FIG. 1. Experimental preparation of stomach consisted of a Heidenhain pouch, 2 marsupialized antral pouches and a gastrojejunostomy.

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