the obese mice. The former findings may be attributed simply to the fact that both groups of obese mothers showed in spite of their greater body weight a tendency toward a decreased food intake in the later period of pregnancy as compared with the non-obese mothers.

It is presumed that teratogenicity of thio-TEPA(10,11) may be attributed to cytotoxic effects inducing an inhibition of nucleic acid metabolism, especially DNA synthesis in embryonic tissues. The reduction of teratogenicity of thio-TEPA in the obese mice is probably caused by interaction of those effects of the compound with certain altered metabolism in the obese mothers and the embryos. It is generally agreed that GTG obese animals are characterized by an increased lipid deposition associated with lesions in the hypothalamus as well as increase in non-lipid constituents of many organs (12). Also, it was reported that those animals show several physiological changes such as increased oxygen consumption, minor elevation of blood glucose, increased inactivation of insulin by the liver, altered function of the kidney and affected pituitary-ovarian axis (12,13). However, the complicated biochemical mechanisms operating in the obese pregnant animals consisting of two different biological systems, mothers and embryos, have not been elucidated.

Summary. Teratogenicity of 3 mg/kg of triethylene thiophosphoramide (thio-TEPA), given intraperitoneally on day 10 of gestation, was examined in obese and non-obese mice. Obesity was induced with goldthioglucose (GTP). No difference was observed in fetal mortality, but frequency of fetuses with malformations caused by thio-TEPA was reduced in the obese mothers. Live fetuses in the obese group showed slight growth retardation.

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Bile Acid Synthesis in Normal and Hypophysectomized Rats: A Rate Study Using Cholestyramine.* (31962)

WILLIAM T. BEHER, BHARATHI RAO, MARGARET E. BEHER, AND JADVYGA BERTASIUS
Edsel B. Ford Insitute for Medical Research, Henry Ford Hospital, Detroit, Mich.

Bile acid and sterol metabolism in hypophysectomized rats differs from that in normals both qualitatively and quantitatively (1,2). Specifically, sterols are metabolized to bile acids in the liver at about one-third the rate found in normal rats; the bile acid spectrum differs; bile acids are eliminated *via* feces more slowly; and sterol synthesis and excretion are retarded. When hypophysectomized rats are fed commercial rat diets which contain little cholesterol, tissue cholesterol concentrations are maintained at about

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normal levels. It is likely that the retarded rates of sterol synthesis and elimination balance out, thus maintaining normal concentrations. However, if hypophysectomized rats are fed diets containing added cholesterol, they accumulate large concentrations in serum and liver(3). The animals, in this case, apparently readily absorb the sterol, but are unable to eliminate it rapidly. Under identical conditions, normal rats accumulate much smaller amounts of cholesterol.

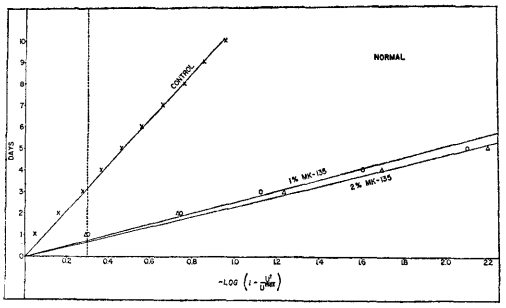
One of the major pathways of tissue sterol elimination is *via* fecal bile acid excretion. This pathway may be divided into two processes: (a) The reactions involved in the conversion of sterols to bile acids in the liver; and (b) The excretion of bile acids from their pool. Since the rate of either of these processes could limit the rate of the whole sequence, we have designed experiments to determine which of these is rate-limiting in normal and hypophysectomized rats. In order to do this, cholestyramine (MK-135), a non-absorbable, bile acid-binding, anionic exchange resin, was used to speed up the turnover of the bile acid pool(4,5).

Methods. Twelve normal and 16 hypophysectomized, 6-month-old, Sprague-Dawley, female albino rats were used. These animals were maintained on ground Rockland rat rations for 3 weeks, during which their general health and weight gains or plateaus were observed. The 12 normal rats were divided into 3 equal groups. One group, the controls, was fed ground Rockland rat rations, while the other 2 groups received the same ground diet supplemented with 1% and 2% MK-135. The 16 hypophysectomized rats were divided into 4 equal groups. Three of these groups received diets corresponding to those fed the normal rat groups. A fourth group received Rockland rat rations supplemented with 0.5% MK-135.

After one week each rat received an intraperitoneal injection containing 5 μc (56 mc/mM) of cholic acid-24-C^{14} dissolved in 0.9% sodium bicarbonate. The rats were placed in individual metabolism cages and feces collected for 9 days. They were then killed; and caecums, large and small intestines with their contents, and livers removed. Feces and tissue samples were frozen at collection and dried by lyophylization. Total lipids were separated from each of the ground tissue samples and from feces by continuous extraction for 48 hours with boiling ethanol. The bile acid-24-C^{14} activity in each extract was determined by scintillation counting of aliquots added to 15 ml of phosphor solution (5 g, 2,5-diphenyloxazole and 3 g, 1,4-bis [2-(4-methyl-5-phenyloxazolyl)]-benzene, dissolved in one liter of toluene). To determine bile acid pool sizes, the "acidic fractions" containing the bile acids were isolated from the lipid extracts of the small intestines plus contents(5). Cholic and chenodeoxycholic acids were determined in the "acidic fractions" by densitometry of developed thin-layer chromatograms, sprayed with phosphomolybdic acid in ethanol, and heated(1,6). The total cholic plus chenodeoxycholic acid pool (small intestine + caecum + liver) was calculated according to Strand(7).

Results and discussion. The curves in Fig. 1 and 2 are plots of $-\text{Log}\ (1 - u^t/u^{max})$ against time. The data were obtained subsequent to a single intraperitoneal injection of cholic acid-24-C^{14}. u^t is the cumulative fecal C^{14} activity (counts/min.) excreted up to a given time; u^{max} is the total amount of C^{14} activity recovered in feces, small intestine, large intestine, caecum, and liver(8). Since the curves for the normal, hypophysectomized, and MK-135 treated rats are straight lines, it follows that the elimination of bile acids from their pools follows first-order kinetics in all cases. The dotted vertical lines cut the curves at the points where $u^t/u^{max} = 0.5$; *i.e.*, at the half-life of the bile acids in the pool. It can be seen that feeding MK-135 decreased the half-life; this was true in both normal and hypophysectomized rats. These results were not unexpected, since in previous studies(4,5) MK-135 had been shown to increase bile acid excretion.

To answer the question as to whether it is the conversion of liver sterols to bile acids, or the bile acid pool turnover, that is the rate-limiting step in the conversion of liver sterols to bile acids in normal and hypophysectomized rats, it was necessary to determine the size of the bile acid pools in the various groups.



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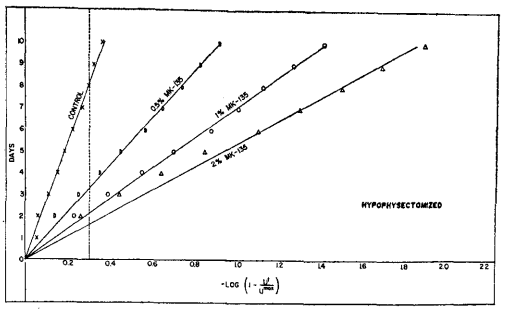

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FIG. 1. Fecal bile acid-24-C^{14} excretion in normal and MK-135 treated normal rats following intraperitoneal cholic acid-24-C^{14} injection. Dotted vertical line intersects the curves at the bile acid pool half-life.

FIG. 2. Fecal bile acid-24-C^{14} excretion in hypophysectomized and MK-135-treated, hypophysectomized rats, following intraperitoneal cholic acid-24-C^{14} injection. Dotted vertical line intersects the curves at the bile acid pool half-life.

This was done by densitometry of developed thin-layer chromatograms. Fig. 3 is a photograph of such a plate. It is evident that the bile acid spectrum of the hypophysectomized rat pool differs from that of the normal rat in only one way: the chenodeoxycholic acid concentration is much lower. MK-135, at the concentrations used in these experiments, had little effect on the bile acid pool spectrum in either normal or hypophysectomized rats. The quantitative data obtained by densitometry of thin-layer chromatograms are presented in Table I. The total determined bile acid pool (cholic plus chenodeoxycholic acids) of hypophysectomized rats is slightly lower than that of normals. This is mainly due to the large decreases in chenodeoxycholic acid in the pools of these rats.

MK-135 failed to have any significant effect on the size of the bile acid pools in any of the animals. Since MK-135 had no effect on the bile acid pool size, and at the same time greatly increased the half-life of these pools, the conversion of sterols to bile acids must have been much more rapid in MK-135 treated animals. Since in one half-life, half of the bile acids in a given pool are excreted, and we know the half-life and the pool size, the daily rate of bile acid synthesis can be calculated. These data are presented in Table I. It can be seen that the relative rate of increase of bile acid synthesis, as effected by MK-135, is about the same in normal and hypophysectomized rats.

To decide which process—the conversion of sterols to bile acids or the elimination of bile acids from their pool—is rate limiting, we must ask what should be the result of the action of MK-135 under each of these conditions. If the conversion of sterols to bile acids is rate-limiting, the turnover of cholic acid-24-C^{14} would be increased and the size of the bile acid pool would drop precipitously. On the other hand, if the bile acid pool turnover is rate-limiting, the cholic acid-24-C^{14} elimination rate would increase and the pool size would remain at nearly the same level.

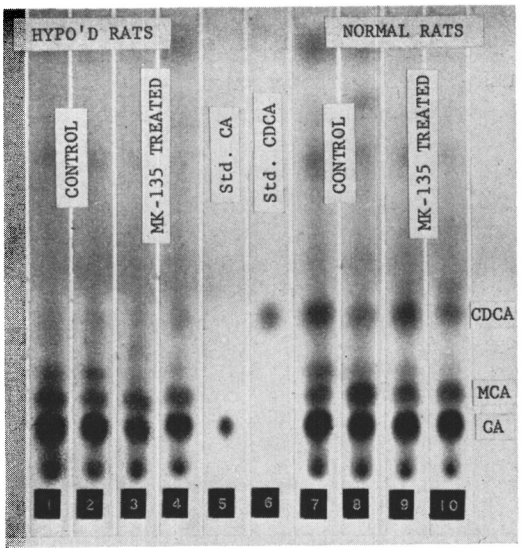

FIG. 3. Separation of bile acids in the "acidic fraction" isolated from small intestine (plus contents) of normal, hypophysectomized, and MK-135 treated rats. CA, cholic acid; CDCA, chenodeoxycholic acid; MCA, muricholic acid. Chromatography plates coated with silica gel G and developed with isooctane: ethyl acetate:acetic acid, 10:10:2 (v/v).

TABLE I. Bile Acid Pool Sizes, Half-Lives, and Synthesis Rates in Normal and Hypophysectomized Rats Treated with Cholestyramine (MK-135).

Group	Cholic acid (mg/100 g rat)	Chenodeoxy-cholic acid (mg/100 g rat)	Total bile acids (mg/100 g rat)	Bile acid half-life (days)	Bile acid synthesis rate (mg/day/100 g rat)
Normal	7.04 ± .94	2.02 ± .61	9.06	3.25	1.39
1% MK-135	5.90 ± .60	1.89 ± .50	7.79	.75	5.20
2% MK-135	7.00 ± .50	3.21 ± .85	10.21	.75	6.81
Hypophysectomized	6.75 ± 1.20	.05 ± .02	6.80	8.00	.43
.5% MK-135	6.47 ± 2.10	.14 ± .03	6.61	3.25	1.02
1.0% MK-135	4.99 ± .58	.15 ± .08	5.14	2.00	1.28
2.0% MK-135	5.82 ± 1.54	.46 ± .10	6.28	1.50	2.09

It is therefore evident that, in both normal and hypophysectomized rats, the rate of elimination of bile acids from their pool is rate-limiting. Thus hormonal changes brought about by hypophysectomy would appear to have little influence on the rates of reaction in the sequence involved in the conversion of liver sterols to bile acids. This would include thyroid, gonadal, adrenal and primary pituitary hormones. It is also clear that one or more of the hormones has great influence on the rate of elimination of bile acids from their pool, and in this way has an important effect on the rate of sterol turnover in this species. The thyroid hormone is capable of speeding up bile acid turnover in hypophysectomized rats(1), but preliminary experiments have shown the effect not to be primary since thyroidectomized rats have nearly normal bile acid turnover rates.

The mechanisms responsible for the slower bile acid turnover rates in hypophysectomized rats are unknown but several possibilities exist. The bacterial spectrum, bile acid end-product spectrum, intestinal motility, rate of bulk passage, and/or control of enterohepatic recirculation are possibilities which are being investigated. Another important point should be mentioned. The results of many experiments suggest that the rate of conversion of liver sterols to bile acids is regulated by the concentration of recirculating bile acids *via* feedback(9,10,11). The present experiment confirms this concept. There is, however, no general agreement on which bile acid, or acids, is important in the feedback. Our experiments indicate that cholic acid and its conjugates are regulators in the mouse(11). This is reasonable since cholic acid is the only bile acid present in any quantity in this species (5). In the rat, recent experiments suggest that chenodeoxycholic acid (or its conjugates) is the regulatory acid, and that it acts by uncoupling oxidative phosphorylation(12). The results of the present experiment appear to be at variance with this concept, since hypophysectomized rats have very low concentrations of chenodeoxycholic acid in their bile acid pools, coupled with a decreased conversion of liver sterols to bile acids.

Summary. Two sequences are involved in the elimination of tissue sterol *via* the bile acid pathway: the conversion of the sterols to bile acids, and the elimination of these bile acids from their pool. Since either of these could be rate-limiting, we investigated this point in normal and hypophysectomized rats. Cholestyramine (MK-135) was used to increase the turnover rate of the bile acid pool. Since the bile acid pool sizes did not decrease when faster turnover rates were induced, the rate of bile acid elimination and not the rate of conversion of liver sterols to bile acids was shown to be the rate-limiting sequence in both types of rats. It thus appears that thyroid, adrenal, gonadal or pituitary hormones do *not* directly influence the rate of conversion of sterols to bile acids in this species.

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Transformation of Bovine Cells *in vitro* by Polyoma Virus, and the Properties of the Transformed Cells. (31963)

H. DIDERHOLM (Introduced by L. Philipson)
Department of Virology, Institute of Medical Microbiology, University, Uppsala, Sweden

Polyoma virus is capable of inducing tumors in a variety of species: Mouse, rat, hamster, guinea-pig, rabbit, ferret, and *Mastomys* (for references see 1,2). Transformation *in vitro* by polyoma virus has been demonstrated in cell cultures of mouse(3), hamster(3,4), and rat (4,5).

This report describes *in vitro* transformation of bovine embryonic lung cells by polyoma virus. The transformed cells are studied with regard to the presence of infectious polyoma virus, the presence of complement-fixing "tumor" antigen, and the susceptibility to various viruses. The characteristics of the polyoma-transformed cells are compared with those of SV40-transformed bovine cells derived from the same source.

Materials and methods. Cell cultures and media. Bovine cells for the transformation experiments were obtained from the lungs of an embryo. They were trypsinized and grown in monolayers in 4-oz prescription bottles. The medium consisted of Eagle's minimal essential medium (MEM) with 10% lamb serum. The cultures were split 1:1 or 1:2 by trypsinization twice weekly. Mouse cells were prepared from embryos using MEM with 10% calf serum as growth medium. Maintenance medium was MEM with 10% horse serum. BS-C-1 cells(6) were grown in MEM with 10% calf serum. The same medium with 2% calf serum was used for maintenance. Bovine cells to be used for virus titrations were prepared from kidneys of newborn calves using Hanks' salt solution with 0.5% lactalbumin hydrolysate and 10% calf serum as growth medium. The same medium, without serum, was used for maintenance.

Viruses. T polyoma virus (kindly supplied by Prof. Stoker, University of Glasgow) and a strain of SV40 isolated from a kidney culture of rhesus monkey were used.

The viral susceptibility of transformed cells was tested with the following viruses: Foot-and-mouth disease virus (FMDV) type C strain Detmold and its attenuated derivate; bovine enterovirus (BEV) strain V 100/60; infectious bovine rhinotracheitis virus (IBRV); pseudorabies virus (PRV) strain Z; parainfluenza virus type 3 (PIV-3) strain 23B; Newcastle disease virus (NDV) strain 205; and bovine viral diarrhoea virus (VDV) strains Oregon C24V and Ug59.

Virus assay. Titrations of infectivity were carried out in roller tube cultures. Serial 10-fold dilutions were inoculated in 0.1-ml amounts into each of 4 or 5 cultures. The titers were calculated according to Kärber and expressed as $\log_{10}$ units of TCD_{50}/0.1 ml. Polyoma virus was titrated in mouse cells, SV40 in BS-C-1 cells, and the other viruses in calf cells.

In tests for the presence of small amounts of polyoma virus and SV40, cultures were frozen and thawed, and the suspensions of cell debris were inoculated in 0.2-ml amounts into tube cultures. If the cultures did not show any changes characteristic of polyoma