

Factors Affecting Steroid Excretion in the Rat

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Previous work reported by us (7) and others (4) has suggested that the presence of a normal complement of intestinal flora exerts a major influence on the sterol metabolism of the rat. Swedish workers have shown that the time required for fecal excretion of 50% of an injected dose of C¹⁴ cholic acid is five times as long in the germfree as in the conventional rat (4). Wostmann, Wiech and Kung (8) reported that the *in vivo* conversion of cholesterol-26-C¹⁴ into bile acid is 50% faster in the conventional as opposed to the germfree rat. The absence of coprostanol, the major fecal neutral sterol of the conventional rat, has also been noted in the germfree rat (1, 2). The above studies indicate that major differences exist in sterol excretion between rats differing only in the presence or absence of intestinal microflora. This paper reports on one of a series of studies initiated to determine the qualitative and quantitative aspects of the microbiological influence on sterol metabolism.

Materials and Methods

Germfree Wistar male rats 90-120 days of age were housed in plastic metabolism cages in germfree isolators of the flexible plastic Trexler type. Diets were of a semi-purified casein-rice starch type (L-474E12) (6). The rats were placed on diet in the metabolism cages for two weeks prior to a five day fecal collection period. The fecal pellets and the diet were assayed for bile acids (3) and neutral sterols (5). After the germfree collection period the rats were monocontaminated with *Clostridium perfringens* type A and after two weeks another collection was taken and assayed as above.

Results and Discussion

Table 1 gives the total bile acid and endogenous neutral sterol excretion of the rats studied. The literature values for conventional rats reported by others (3, 5) using the same techniques is also reported. The germfree bile acid excretion of 1.82 mg/kg body wt/24 hrs is only one-tenth of the bile acid excretion reported by others for the conventional rat. The germfree bile acids have been identified as approximately 90% cholic and β -muricholic acids. Trace amounts of other bile acids have been isolated and are currently under study.

The neutral sterols of the germfree rat contain no coprosterols whereas coprostanone, coprostanol, and the copro analogs of dietary plant sterols are predominant in the conventional rat's excreta. The absence of coprostanol in the germfree rat has been reported previously by others (1, 2). Monocontamination of the germfree rat with *Clostridium perfringens* type A results in a doubling of endogenous neutral

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sterol excretion without the appearance of any sterols not seen in the germfree excreta. This monocontamination appears to have no effect on the bile acid excretion either quantitatively or qualitatively. However, the integrity of conjugate bonds to taurine or glycine has not been investigated.

These data indicate that the intestinal microflora is responsible for a nearly tenfold increase in bile acid elimination and a 50% increase in neutral sterol elimination.

The existence of agents which can cause such a marked change in the metabolism of compounds known to be of pathological significance emphasizes the need for detailed investigations of the factors influencing them. The mechanism for these changes are currently under study.

TABLE 1. Bile acid and neutral sterol excretion of germfree, monocontaminated, and conventional adult male rats.

	mg/kg body wt/day		
	Germfree	Monocontaminated	Conventional
Bile acids	1.82 (3)*	2.1 (3)*	17.3 (10)*(3)+
Endogenous neutral sterols	5.90 (3)*	11.1 (3)*	8 (10)*(5)+

* number of animals

+ reference number

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