TABLE I. Total Water Content of Stomach and Intestine in Control; 3 mg/kg Levorphanol; 5 mg/kg Levorphanol and 5 mg/kg Levorphanol, 10 mg/kg Levallorphan Groups.

	Water content: mean ± S.E. (g)			
	Control	Levor 3	Levor 5	Levor 5 Leval 10
Stomach	4.5± .6	5.9±1.1	6.5*± .4	5.1± .1
Intestine	10.2±1.1	10.8±1.2	8.8 ±1.0	10.0±1.0

* Indicates $p < .05$ by "t" test. Each value is based on 5 rats.

1 hour point as described in Methods. The only significant difference in water content was found for the stomach of the 5 mg/kg levorphanol group compared to the stomach of the control. This levorphanol treated group retained more water in the stomach than controls, possibly indicating that less water had been absorbed. Levallorphan apparently antagonized the effect of levorphanol. These conclusions must be made with reservation. Had the stomach and intestine been taken together, it appears that no such difference would have been found.

Summary. Levorphanol altered the control serum osmolality response to a 5% oral water load in rats. The change at a small dose of levorphanol was consistent with retention of water systemically owing to the antidiuretic effect of levorphanol. At a high dose, another component appeared in the osmolality curve suggesting that inhibition of water absorption from the gastrointestinal tract was occurring. All effects of levorphanol were antagonized by levallorphan.

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Effects of Hypophysectomy on Steroid-C^{14} Metabolism in the Rat.* (28623)

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The hypophysectomized rat, in contrast to the normal, easily accumulates large concentrations of serum and liver cholesterol when given a dietary supplement of this sterol(1, 2,3). Either a faster rate of absorption or a slower rate of elimination could account for this difference.

The present study investigates the relative turnover of steroid-C^{14} in normal and hypophysectomized rats. Mevalonic acid-2-C^{14} was used as the sterol precursor because this makes it possible to obtain information on steroid-C^{14} synthesis, as well as on rates of tissue β-sterol-C^{14} elimination and fecal steroid-C^{14} excretion. Use of this precursor also circumvents the problem of choosing a suitable cholesterol-4-C^{14} emulsion.

Methods. Female, albino, Sprague-Dawley rats, normal or hypophysectomized, were fed Rockland rat diet *ad lib.* throughout all experiments. Rats were hypophysectomized† at 3 months (215-225 g), after which their weights and CO_2 production were checked periodically for a 4-month period. Eight hypophysectomized rats, with weights plateaued at 185-197 g, were then selected for the experiment. The 8 intact rats used as controls were 7 months old and weighed 250-290 g.

Four of the hypophysectomized and 4 of the normal rats received intraperitoneal injections of 10 μc dl-mevalonic acid-2-C^{14} (1.2×10^6 counts/min./μc as determined by our instruments). One hour later the animals were sacrificed by decapitation, and samples of blood, liver, small intestine, lung, kidney and muscle removed. These tissues and the remaining carcasses were quick frozen, and subsequently analyzed for cholesterol and cholesterol-X-C^{14} concentrations.

The other 4 hypophysectomized and 4 nor-

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† The Endocrine Laboratories of Madison, Inc., Madison, Wisc.

mal rats were similarly injected and placed in individual metabolism cages. Feces were collected for a 2-week period, before the animals were sacrificed and their tissues collected and analyzed as described above.

Feces were dried over P_2O_5 and extracted with ethyl alcohol for 48 hours in Bailey-Walker apparatus. Aliquots of the alcohol extract were hydrolyzed for 3 hours at 15 lb pressure with 7 N NaOH. Neutral sterols were then extracted for 2 hours with petroleum ether in continuous liquid-liquid extractors. The aqueous fraction was acidified and extracted with petroleum ether to remove fatty acids. Subsequently it was continuously extracted for 6 hours with ethyl ether to remove the acidic fraction containing bile acids. After washing, aliquots of the petroleum ether fraction containing neutral sterols, and of the ethyl ether fraction containing bile acids, were plated in cup planchets and counted. This procedure quantitatively extracts and separates added sterol-C^{14} and bile acid-C^{14}.

Serum cholesterol was determined by the method of Sperry and Webb(4); tissue cholesterol was extracted and determined as previously described(5). Tissue β-sterol-C^{14} was precipitated as the digitonide, plated by the filtration tower technique, and counted. Carcasses were homogenized and aliquots hydrolyzed with alcoholic KOH(6). Aliquots of the hydrolysates were extracted with petroleum ether and suitable portions of the extracts used for cholesterol and β-sterol-C^{14} determinations. All C^{14} activities were determined with a windowless gas flow counter.

Results and discussion. Table I compares tissue cholesterol concentrations in normal and hypophysectomized rats. In all tissues except small intestine and kidneys, there were very small but significant increases in cholesterol concentration in the hypophysectomized rats. Thus, the hypophysectomized rat is able to maintain a nearly normal homeostatic balance between cholesterol synthesis and elimination. However, it does not necessarily follow that the absolute rates of these processes are the same.

TABLE I. Tissue Cholesterol Concentrations in Normal and Hypophysectomized Rats.

	Normal	Hypophysectomized	P
	(mg/100 ml)		
Serum	99.8 ± 8.4	111.0 ± 9.2	.03
	(mg/g)		
Liver	2.69 ± .18	3.07 ± .23	<.01
Small intestine	2.79 ± .21	2.87 ± .07	.34
Lung	5.42 ± .38	6.84 ± .75	<.01
Kidney	4.93 ± .39	4.66 ± .16	.10
Muscle	.81 ± .10	1.08 ± .14	<.01
Carcass*	1.35 ± .10	1.91 ± .11	<.01

* Body minus blood, liver, lung, kidneys, small intestine and intestinal contents.
± standard deviations.

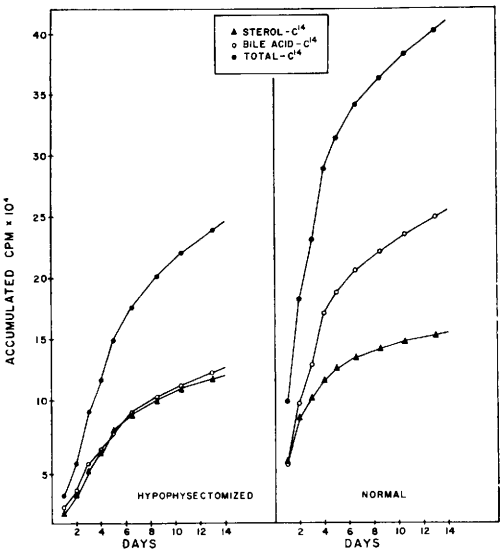

FIG. 1. Time-course of steroid-C^{14} excretion in hypophysectomized and normal rats.

Fig. 1 shows that the excretion of total steroid-C^{14} in the normal rat was approximately double that in the hypophysectomized at all time intervals. Rates of excretion of both bile acid-C^{14} and sterol-C^{14} were decreased by hypophysectomy; however, the larger decrease was in the bile acid-C^{14} fraction. In the normal rat 62% of the excreted steroid-C^{14} was in the bile acid fraction, whereas in the hypophysectomized rat 51% of the activity appeared in this fraction.

Table II contains a balance sheet of the activity excreted in the feces in 14 days or retained in the tissues. The hypophysectomized rat retained a greater amount of β-sterol-C^{14} in all the tissues except the kidneys.

It can be seen that the difference between normal and hypophysectomized rats in total retained tissue β-sterol-C^{14} is approximately equal to the difference in the total excreted

TABLE II. Total Tissue β-Sterol-C^{14} Concentrations in Normal and Hypophysectomized Rats.

	Normal		Hypophysectomized	
	1 hr	14 days	1 hr	14 days
	Total β-Sterol-C^{14}, counts/min./μc injected			
Serum	9,787 ± 668	1,341 ± 107	7,033 ± 1975	3,050 ± 272
Liver	24,887 ± 1742	1,205 ± 145	30,909 ± 7310	3,397 ± 742
Small intestine	1,347 ± 249	459 ± 133	1,352 ± 115	2,175 ± 201
Lung	688 ± 156	442 ± 15	681 ± 228	2,689 ± 490
Kidney	6,893 ± 945	33,194 ± 7620	1,044 ± 131	21,330 ± 987
Carcass*	6,214 ± 1178	10,491 ± 1253	6,333 ± 1806	34,808 ± 2043
Total tissue β-Sterol-C^{14}	49,816	47,132	47,352	67,449
Total excreted fecal Steroid-C^{14}		40,244		23,906
Total Steroid-C^{14} synthesized		87,376		91,355

* Body minus blood, liver, lung, kidney, small intestine and intestinal content.
± Standard deviation.

fecal steroid-C^{14} in the two groups. Consequently the total steroid-C^{14} synthesized during the 14 days by the two groups is about equal. Table II also shows that the incorporation of dl-mevalonic acid-2-C^{14} into various tissues (except kidneys) in one hour was incomplete but the same for the two groups.

It was noted above that cholesterol concentrations were only slightly elevated as a result of hypophysectomy. This occurred in spite of the fact that steroid turnover is much slower, and mevalonate incorporation into tissue sterol proceeds at the same rate as in the intact rat.

Since it has been shown(7,8) that acetate incorporation into liver sterols is greatly retarded by hypophysectomy, it appears that some step between acetate and mevalonate must be retarded in these animals.

Sterol metabolism in the kidneys of hypophysectomized animals behaved somewhat differently than in other tissues. The incorporation of mevalonic acid-2-C^{14} proceeded more slowly, and at the end of 14 days less β-sterol-C^{14} remained in the kidneys of hypophysectomized rats than in the kidneys of intact rats. The lower retention of activity in the kidneys of hypophysectomized rats could be due to the slower rate of incorporation. Or as Duncan and Best(9) have suggested, the disappearance rate of kidney cholesterol-C^{14} could be somewhat greater in hypothyroid rats than in normals. It would be necessary to run a time-specific activity study to decide this point.

The data suggest that the reason for the sensitivity of hypophysectomized rats to cholesterol accumulation is a slow rate of elimination of absorbed cholesterol from the tissues via the feces.

Summary. Tissue cholesterol concentrations, sterol-C^{14} elimination rates, and fecal steroid-C^{14} time-patterns were determined in intact and hypophysectomized rats. Tissue cholesterol was slightly elevated in the hypophysectomized animals. The rate of tissue sterol-C^{14} elimination was greatly reduced in hypophysectomized rats. Hypophysectomized rats excreted fecal steroid-C^{14} at one-half the rate of normal animals, with the greater reduction in the bile-acid-C^{14} fraction. The incorporation of mevalonic acid-2-C^{14} into tissue cholesterol at the end of one hour was about the same in both groups.

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Effect of Colicine Production on *Escherichia coli* in the Normal Human Intestine. (28624)

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Microorganisms which cause disease in the human intestine and the host-parasite mechanisms involved have long been under investigation, but less attention has been given to the normal intestine and the factors which influence its flora. The influence of one of these factors, the antibiotic "colicine"(1) which is produced by *Escherichia coli,* has been investigated, but results were inconclusive. The present study was undertaken to reevaluate existing information by utilizing more extensive and definitive methods to demonstrate production and activity of colicines of *E. coli* strains recovered from 5 normal individuals for a 6-month period.

Materials and methods. Fecal samples were collected monthly from June through November from 5 persons for a period of 6 months. Each sample was streaked immediately onto eosin methylene blue and blood agar plates, and after incubation for 18 hours at 37°C, a total of 10 typical *E. coli* colonies was picked from the 3 plates when possible. Biochemical characterization of each isolate was based on lactose, sucrose, and mannitol fermentations, indol, methyl red, Voges-Proskauer and citrate reactions. To assure that typical *E. coli* were selected for study, only those isolates which displayed the IMViC formula ++ −− were included. Somatic (O) serotypes were determined according to the methods of Edwards and Ewing(2) using antisera for *E. coli* serotypes 01 through 025 and for enteropathogenic *E. coli* types 026, 055, 086, 0111, 0112ab, 0112ac, 0125ab, 0125ac, 0126, 0127, 0128ab, 0128ac. Isolates not belonging to any of these serotypes were used for production of antiserum prepared according to the technique of Ewing(3) for the purpose of identifying all similar serotypes. Cross absorptions were done when necessary to determine the identity of some isolates.

All 10 *E. coli* isolated from each of the 5 individuals during a one-month period were tested for colicine activity against *E. coli* isolated during the previous month. In parallel all isolates were assayed for colicine activity against the colicine indicator strain of Gratia(4), *E. coli* phi.

Two methods were employed for demonstration of colicine activity. The first is essentially the simultaneous pour plate method of Blackford *et al.*(5). A thin layer of Proteose no. 3 agar (Difco) was poured into a Petri dish, allowed to harden and covered with a second layer of agar which had been seeded with approximately 4×10^7/ml of the substrate organism(6). Twenty-four hour cultures of the isolates to be tested for colicine activity (producer strains) were then spot inoculated on the surface and the plates incubated for 24 hours at 37°C.

The second method is a modification of the chloroform plate diffusion technique of Gratia and Fredericq(4). Twenty-four hour agar slants of *E. coli* isolates to be tested for colicine production were spot inoculated with a sterile cotton swab onto thick Proteose no. 3 agar plates. The plates were then incubated for 48 hours at 37°C, flooded with chloroform and allowed to stand for one hour to assure death of the organisms. The chloroform was decanted and the plates allowed to remain with the lids ajar for one hour for the residual chloroform to evaporate. Growth from 24-hour agar slants of the substrate