ovarian ascorbic acid technique (16).

Conclusions. Androgen sterilization results in the elimination of the multiphasic dose-response curve obtained with the OCD bioassay for LH. The dose-response curve in the androgenized animals is highly reproducible, but the sensitivity of the assay is considerably reduced. The abolition of the multiphasic curve by androgen treatment suggests that endogenous LH release is responsible for the multiphasic type of response in the non-androgenized rat. A linear log dose-response relationship for LH is obtained between 0.0625 μg and 0.25 μg using a 5-hour assay time interval, and between 0.25 μg and 1.0 μg using a 7-hour assay time interval. This response is reproducible and is specific for LH.

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Absorption of Lipids from Mixed Micellar Bile Salt Solutions* (32759)

ABIGAIL G. THORNTON, GEORGE V. VAHOUNY, AND C. R. TREADWELL
Department of Biochemistry, School of Medicine, The George Washington University, Washington, D. C. 20005

It is well established that cholesterol, fatty acids, and monoglycerides can be solubilized in bile salt and lecithin micelles (1–3), and that these lipids are normally present in micellar form in the intestinal lumen (4–6). It has been suggested that bile salts act as intraluminar transport vehicles for lipids, and that the absorption of specific lipids is dependent on their solubility in micellar media (5–7). Simmonds *et al.* (8) recently reported marked differences in the rate of absorption of the individual components of mixed micelles from the intestinal lumen in humans, and suggested that micellar lipids are not absorbed as intact aggregates. However, Johnston and Borgstrom (9, 10) reported that intestinal slices incubated in bile salt micelles containing oleic acid-^{14}C and monopalmitin-^{3}H removed the two labeled components in the same ratio as their concentrations in the incubation media.

The present report provides further evidence that mixed micelles are not absorbed as intact aggregates but rather that the individual components of micelles are absorbed independently by the intestinal mucosa. The basic approach employed was to incubate everted rat intestinal sacs in micellar solutions of bile salt and monoolein, plus cholesterol, oleic acid, and lecithin in various combinations. Each compound, except lecithin, was labeled with either ^{14}C (sodium tauro. etc, monoolein and oleic acid) or ^{3}H (choles-

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TABLE I. Composition of Initial Micellar Media.[a] (Concentrations are in μmoles/4.0 ml)

Medium no.	NaTCDC	MO	C	L	O
I	20.0	1.0			
II	20.0	1.0	1.0		
III	20.0	1.0	1.0	2.5	
IV	20.0	1.0			2.0
V	20.0	1.0	1.0		2.0
VI	20.0	1.0	1.0	2.5	2.0

[a] Abbr.: NaTCD = sodium taurochenodeoxycholate-26-^{14}C; MO = α-monoolein-carboxyl-^{14}C; C = cholesterol-7-^{3}H; L = lecithin; and O = oleic acid-1-^{14}C.

terol). Isotope ratios were determined between compounds in intestinal extracts after incubation and compared to the corresponding ratios in the original incubation medium. If all ratios remained unchanged in the sac from those in the original medium, it would be suggestive that the micelles had penetrated the mucosal wall intact; however, alteration of the isotope ratios in the sac from those of the initial medium would suggest that the micelle was not absorbed as a single aggregate unit.

Materials and Methods. Oleic acid-1-^{14}C, cholic acid-26-^{14}C and cholesterol-7-^{3}H were obtained from Nuclear Chicago Corp. Labeled taurochenodeoxycholic acid was prepared from cholic acid by modification of the method of Norman (11). Monoolein-carboxyl-^{14}C was a generous gift of Dr. Leon Swell.

The critical micellar concentrations (CMC), defined as the concentration of a substance at which spontaneous aggregation occurs, was determined in preliminary experiments as follows: Excess concentrations of labeled lipids (monoolein-1-C^{14}, oleic acid-1-^{14}C and/or cholesterol-7-^{3}H) and unlabeled lecithin were mixed with increasing concentrations of taurochenodeoxycholate-26-^{14}C in 0.9% saline. Solvents were evaporated and the mixtures were homogenized and centrifuged at 105,000*g* for 1 hour. The clear infranatant layer, representing micellar bile salts and lipids, was analyzed by liquid scintillation spectrometry following separation of the individual micellar components by thin-layer silicic acid chromatography [solvent system = hexane (83):acetone (16):acetic acid (2)]. Silicic acid areas corresponding to authentic standards were scraped directly into vials for counting, or were exhaustively eluted with methanol:acetone (1:1, v/v) for lecithin phosphorus determinations by the method of Horecker *et al.* (12).

After determination of the critical micellar concentration of bile salt, monoolein, lecithin and oleic acid in various combinations, concentrations were chosen which were appropriate for preparation of 2–5 component micelles, shown in the Tables I–III, and which also were within physiological ranges. Subsequently, all micellar media were prepared by mixing the lipids, in hexane, with the bile salt in physiological saline. After evaporation of solvent, complete solution was achieved by agitation on a test tube spinner for 30–60 sec.

Male Wistar rats (200–300 gm) were fasted 24 hours and sacrificed by decapitation. The proximal half of the jejunum was removed and washed with physiological saline. The intestine was everted and sacs were prepared by the procedure of Wilson and Wiseman (13). These were kept in physiological saline, pH 6.8, until use and subsequently incubated with 4 ml of micellar medium for 10 min under air atmosphere at 37°C. After incubation, each sac was rinsed with 5 ml of distilled water, the washings were added to the remaining incubation medium, and the sac was placed in 10 ml of acetone:alcohol (1:1, v/v) to stop all reactions. The sac was then homogenized, the homogenate was extracted by heating to a boil and, after cooling, was made to 25 ml. After filtration, aliquots were assayed directly by radioactive counting or were subjected to thin-layer chromatography for separation of micellar components. Completeness of extraction and recovery of isotopes were ascertained by counting aliquots of the residue following extraction. The initial and final medium (consisting of the remaining incubation medium and washings) was similarly assayed except that thin-layer chromatography was performed directly on the aqueous sample without prior extraction.

Results and Discussion. The concentrations of lipids in the initial media are shown in Table I and the results of several incubation experiments are shown in Table II. In all cases, the isotope ratios of each

TABLE II. Isotope Ratios in Everted Intestinal Sacs after Incubation with Bile Salt Micelles. (Isotope ratio of the initial media = 1.0)

Medium no.[a]	Total ^{14}C/ Total ^{3}H	NaTCDC/MO	NaTCDC/C	MO/C	MO/O	NaTCDC/O	L/C
I		0.71					
II	0.42[b]	0.48	0.33	0.66			
III	1.72	0.58	1.44	2.48			14.58
IV		0.47			0.98	0.59	
V	0.69	0.40	0.40	1.03	0.99	0.50	
VI	0.95	0.40	0.63	1.54	1.50	0.68	10.16

[a] See text and Table I for details and conditions of experiments. Media designations are the same as in Table I.

[b] Each value is the average of 2 duplicate sacs for Media I, II, and III. For Media IV, V, and VI, each value is the average of 4 duplicate sacs, two of which were incubated in media containing labeled monoolein-^{14}C and unlabeled oleic acid, and two of which were incubated in media containing unlabeled monoolein and labeled oleic acid-1-^{14}C.

component to every other component in the micellar medium were arbitrarily set at 1.0. In experiments with micelles containing both monoolein and oleic acid, four intestinal sacs were incubated in each type of micellar medium (Table I, media IV, V, and VI). Two of the sacs were added to media containing labeled monoolein and unlabeled oleic acid, and two were incubated in micelles containing unlabeled monoolein and labeled oleic acid. The ratios were then calculated for each sac and pooled to give the results shown in Table II.

As shown in Table II, the ratios in the sac after incubation were markedly altered from the arbitrary ratio of 1.0 in the initial media. The alterations in ratios of bile salt and lipids are evidence for the disruption of micelles prior to entrance into or at the mucosal surface. Johnston and Borgstrom (9, 10) have reported that oleic acid and monoolein, dissolved in sodium taurodeoxycholate micelles, entered intestinal slices in the same proportions as they existed in the original incubation medium. Similar results were obtained in the present study using sodium taurochenodeoxycholate–monoolein–oleic acid micelles, with and without cholesterol; however, when lecithin was also included in the micelle, the ration of monoolein/oleic acid (MO/O) in the sac was 1.50 as compared to the ratio of 1.0 in the initial medium. Moreover, when ratios of other components in the micelles, such as bile salt to either fatty acid, monoolein, or cholesterol, or fatty acid to cholesterol, are examined, the ratios are not 1.0. Thus, when each component in the micelle is considered, it seems improbable that intact micelles penetrated the mucosa. Further evidence for this can be seen in Table III, which presents data on the differential uptake by everted intestinal sacs of the lipid components of the same micellar solutions shown in Table I. Similar results have been found with a different bile salt, sodium taurocholate, in two-, three-, four-, and five-component complex micelles.

The validity of this method of the comparison of ratios in initial media and intestinal sacs for determination of micelle absorption is further supported by data from experiments

TABLE III. Intestinal Uptake of Lipid Components from Simple and Mixed Micelles.

Medium no.[a]	Sodium taurochenodeoxycholate (%)	Monoolein (%)	Cholesterol (%)	Lecithin (%)
I	8.66	12.19		
II	6.97	14.39	22.70	
III	4.10	7.10	2.84	39.84

[a] Media designations are the same as in Table I.

designed to study the reverse process, or efflux from the intestine. When sacs were incubated for 10 min in micellar media containing labeled bile salt and cholesterol, and were subsequently transferred to unlabeled micellar media of the same composition, little exchange of isotope occurred; these data indicate that efflux or exchange of isotopes were not responsible for the observed ratios in the intestinal sac.

Summary. Data are presented on the absorption of cholesterol and other lipids by everted rat intestinal sacs from micellar solutions of sodium taurochenodeoxycholate. It appears that neither simple, two-component nor complex, multiple-component micelles penetrate the intestinal mucosal wall intact, but rather that the lipids and the bile salts are absorbed individually by rat intestine.

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Toxohormone (32760)

RALPH F. KAMPSCHMIDT AND HERBERT UPCHURCH
Biomedical Division, The Samuel Roberts Noble Foundation, Inc., Ardmore, Oklahoma 73401

Toxohormone was the name given by Nakahara and Fukuoka (1) to a toxic material extracted from tumor tissue. This toxic extract from tumor tissue would, upon injection into normal animals, lower liver catalase activity, decrease plasma iron concentration, and produce several other changes which are characteristic of the tumor-bearing animal (2, 3). Nakahara and Fukuoka were able to prepare this toxic extract from all growing cancer tissues tested but not from normal tissues (1). A number of investigations have indicated that active extracts can be prepared from several different normal tissues (4–7) and that all tumor tissues may not have greater activity than some normal tissues (7, 8). We observed that bacterial products gave activities similar to toxohormone and that extracts prepared from normal or tumor tissues were equally active in the absence of bacteria (7, 9). The toxic activity of bacteria carried through the toxohormone extraction procedures has been confirmed (10); but in several studies, it has been reported that toxohormone activity could be prepared from tumor tissues in the absence of bacteria (8, 10–12). These studies, however, did not include similar preparations made from normal tissues. Olivares *et al.* (13) showed that autolysis of normal tissues will give an extract with "toxohormone-like" activity. The extraction procedures employed for the preparation of toxohormone make it difficult to standardize and avoid the possibility of autolysis. In the experiments reported in this investigation, normal tissue was processed simultaneously with each tumor tissue preparation.

Materials and Methods. The tissues used were: Walker carcinoma 256 carried intramuscularly in Holtzman rats, 20-methylcholanthrene tumors induced intramuscularly in Fisher rats, normal liver, and normal tissue preparations which consisted of the whole rat minus skin, bones, and intestines. A portion of each tissue was inoculated into nutri-