tic esophagitis. They found that these conditions were amenable to treatment with this agent and that satisfactory relief was obtained in a large percentage of trials where standard therapy failed.

While the use of topical anesthetics is not new to gastroenterologists none has previously proven satisfactory in the gastric environment because of either toxicity, or inadequate potency and duration of action. The reason for the failure of anesthetic salts such as procaine, lidocaine or dibucaine can be attributed to the fact that these are ionized (dissociated) at gastric pH and that oxethazaine, a weak base, is relatviely non-ionized. Accordingly, the weaker the base the more free base is released and the greater the anesthetic potency(14). Further, the solubility of oxethazaine salts in organic solvents ($CHCl_3$, CCl_4) at gastric pH indicates that it easily penetrates the lipids of nerve sheaths to effect adequate anesthesia of sufficiently long duration.

Summary. 1. Oxethazaine and several N-substituted bis-acetamide congeners constitute a new series of powerful local anesthetics, exceeding by far the potency of either cocaine, procaine, lidocaine or dibucaine. 2. All are effective by topical application to the cornea, cutaneous infiltration or intraspinally. 3. Since oxethazaine is highly effective topically and has a large margin of safety when administered intragastrically, it has proven to be useful clinically for control of pain due to a variety of gastric disorders. 4. Its effectiveness at the acidity of the gastric environment is due to the fact that oxethazaine, a weak base, is relatively non-ionized at pH 1.

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1. Glassman, J. M., Hudyma, G. M., Seifter, J., *J. Pharmacol. Exp. Therap.*, 1957, v119, 150.

2. Freed, M. E., Bruce, W. F., Hanslick, R. S., Mascitti, A., *J. Org. Chem.*, 1961, v26, 2378.

3. Sollmann, T., *J. Pharmacol. Exp. Therap.*, 1918, v11, 17.

4. Tatum, A. L., *ibid.*, 1931, v42, 276 (Proc.).

5. Bieter, R. N., Cunningham, R. W., Lenz, O., McNearney, J. J., *ibid.*, 1936, v57, 221.

6. Bliss, C. I., *Quart. J. Pharm. and Pharmacol.*, 1938, v11, 192.

7. Glassman, J. M., Seifter, J., *J. Pharmacol. Exp. Therap.*, 1954, v112, 364.

8. Rose, C. L., *J. Lab. Clin. Med.*, 1929, v15, 128.

9. Schaffer, N. K., Loomis, T. A., *Yale J. Biol. Med.*, 1946, v18, 157.

10. Baeder, D. H., Glassman, J. M., Hudyma, G. M., Seifter, J., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 645.

11. Deutsch, E., Christian, H. J., *J. Am. Med. Assn.*, 1959, v169, 2012.

12. Jankelson, I. R., Jankelson, O. M., *Am. J. Gastroenterol.*, 1959, v32, 636.

13. Hollander, E., *ibid.*, 1960, v34, 613.

14. Hirschfelder, A. D., Bieter, R. N., *Physiol. Rev.*, 1932, v12, 190.

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Influence of Propylthiouracil and D- and L-Triiodothyronine on Excretion Of Bile Acids in Bile Fistula Rats. Bile Acids and Steroids 118.* (27301)

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It is well known that the thyroid hormones have hypocholesterolemic effects. In the search for agents with hypocholesterolemic properties similar to those of the thyroid hormones but without their undesirable calorigenic effects, different structural analogues have been tried, *e.g.*, D-thyroxine and D-triiodothyronine. These compounds have been

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shown to possess marked hypocholesterolemic activity but only slight calorigenic potency. Thus D-triiodothyronine has been reported to have a calorigenic activity about one-tenth that of the L-isomer but equivalent hypocholesterolemic activity (for review see ref. 1).

Bile acids are the main metabolic end-products of cholesterol. Factors known to influence cholesterol metabolism, *e.g.*, hormones and diet, have therefore been investigated to some extent with respect to effect on bile acid formation (for review see ref. 2). Eriksson(3), Thompson and Vars(4) and Van Zyl(5) have shown that hyper- as well as hypothyroidism are accompanied by changes in the excretory pattern of bile acids in bile fistula rats.

In view of the intimate relationship between cholesterol and bile acid metabolism and the pronounced influence of thyroid activity on these processes, it was of interest to investigate bile acid formation in rats treated with a thyroid hormone derivative with selective hypocholesterolemic properties.

In the present study male and female rats given DT_3† in doses low enough to bring about minimal calorigenic effects were compared with respect to bile acid formation with eu-, hyper- and hypothyroid rats.

Experimental. White male and female rats of the Sprague-Dawley strain, weighing 180-250 g, were fed a commercial rat diet (Anticimex, Stockholm, Sweden). The following 5 groups of each sex were included, each group consisting of 6 animals.

	♂	♀	
Group	I	VI	Control
	II	VII	Given 40 μg LT_3/kg body wt and day
	III	VIII	Given 40 μg DT_3/kg body wt and day
	IV	IX	Given 400 μg DT_3/kg body wt and day
	V	X	Given 0.1% propylthiouracil‡ in drinking water.

‡ Pharmacia, Sweden.

The hormones were solubilized in slightly alkaline 0.9% sodium chloride solution and daily subcutaneous injections were given for 14 days. Rats were made hypothyroid by giving drinking water containing 0.1% propylthiouracil for 30 days. Control and hypothyroid rats were given daily injections of alkaline 0.9% sodium chloride solution of the same volume and alkalinity as that administered to the groups treated with LT_3 or DT_3.

Body weight change and food intake were followed until time of operation. Bile fistulas were made in the usual manner. After operation the animals were given 0.9% sodium chloride solution to drink to which in the case of the hypothyroid rats 0.1% propylthiouracil was added. All rats received daily hormone injections also after bile duct cannulation. Bile was collected in 3 fractions during the first day and thereafter in 24-hour portions for 7-8 days. The samples were weighed, diluted with distilled water and stored at −24°C until analyzed. Prior to quantitative estimation of the bile acids in the bile samples, which was performed according to the paper chromatographic method of Sjövall(6), a scanning chromatogram was run to exclude the existence of appreciable quantities of bile acids other than TC and TCD. That cholic acid and chenodeoxycholic acid constituted the main part of the bile acids was also confirmed by gas liquid chromatography after hydrolysis of the bile acid conjugates.

Results. In the rats given LT_3 in doses of 40 μg/kg/day and DT_3 in doses of 400 μg/kg/day (Groups II, IV and VII, IX respectively) the body weight gain during 2 weeks after start of hormone administration was somewhat lower than for control rats and rats given DT_3 in doses of 40 μg/kg/day (Groups III and VIII). Food intake in the former groups was, however, from 4th day after start of administration 40-50% higher than for the euthyroid control rats and the rats given DT_3 in doses of 40 μg/kg/day. These latter groups had about the same food consumption. The hypothyroid rats (Groups V and X)

† Abbreviations used are 3,5,3′-triiodo-L-thyronine, LT_3, 3,5,3′-triiodo-D-thyronine, DT_3, taurocholic acid, TC, taurochenodeoxycholic acid, TCD.

LT_3 (Cytomel Sodium) and DT_3 (S.K.F. No. D-2623) were generous gifts from Smith Kline & French Labs., Philadelphia.

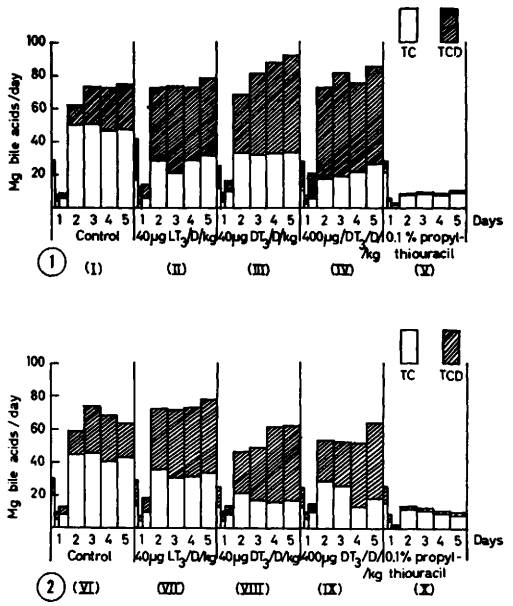

FIG. 1. Male bile fistula rats. Excretion of taurocholic (TC) and taurochenodeoxycholic acid (TCD) in 5 experimental groups. Values are expressed as mg/6 hr during first day and as mg/24 hr during following days. Each column in diagram represents mean value of 3 animals.

FIG. 2. Female bile fistula rats. For explanation of diagram see legend to Fig. 1.

showed a slight body weight reduction during the first week after initiation of propylthiouracil treatment, and thereafter the body weight stayed at a roughly steady level during the following 3 weeks. In these latter groups food intake was just about half that of control rats.

Fig. 1 and 2 show daily excretion of TC and TCD in the bile fistula in the various groups. In the control groups total daily excretion of conjugated bile acids amounted to 60-80 mg for both male and female rats except for the first collection day when approximately 50 mg of bile acids were excreted. During the first day bile was collected in 3 fractions and it was found that more than half the quantity of bile acids or about 30 mg were excreted during the first 6 hours. In both male and female rats approximately 30% of the total bile acids consisted of TCD, the remainder being TC. In the hyperthyroid groups (II, IV and VII, IX, respectively) total daily bile acid excretion was roughly the same as in control groups while the ratio between TCD and TC was substantially changed so that TCD accounted for 60-80% of total daily output of bile acids, in agreement with the findings of Eriksson(3).

In the 2 groups receiving DT_3 in noncalorigenic doses as judged by food intake (III, VIII), the excretion of bile acids showed the same pattern as in hyperthyroid rats with TCD accounting for 60-80% of total bile acid output.

In the 2 female groups receiving DT_3 (VIII and IX) the ratio of TCD/TC in the bile samples collected during the first 2 or 3 days was somewhat lower than in the male rats, TCD in this case representing about 50% of total bile acids. However, in the following days TCD excretion increased to 40-50 mg per day or 60-80% of total daily bile acid synthesis, *i.e.*, the same figures as found for male rats.

In the 2 propylthiouracil treated groups bile acid excretion during the first 6 hours did not differ much from normal rats with respect to total output and TCD/TC ratio. Thereafter, however, the daily excretion of bile acids was only about 10 mg, *i.e.*, 15-20% of that of the control rats. In the hypothyroid rats 10-20% of the total bile acids excreted was accounted for by TCD which is somewhat less than in control rats.

Discussion. It has been repeatedly demonstrated that thyroid activity influences the excretion of bile acids in bile fistula rats (for review see ref. 2). Eriksson(3) has shown that hyper- as well as hypothyroidism are accompanied by a decreased excretion of TC in bile fistula rats as compared to euthyroid animals. In hyperthyroid rats, however, there was a considerable increase of the TCD synthesis so that the total quantity of bile acids formed per day was about equal to that found in euthyroid rats. In hypothyroid rats on the other hand TCD synthesis was diminished as compared to euthyroid rats, leading to a considerably reduced total bile acid output. In hyperthyroidism the TCD/TC ratio was thus increased while in hypothyroidism it was diminished as compared to euthyroid rats.

The results obtained in the present study on male eu-, hyper- and hypothyroid bile fis-

tula rats are on the whole in agreement with those of Eriksson. In our euthyroid rats of both sexes the TCD fraction was somewhat greater than that earlier reported, being 30-35% as compared to 10-20% found by previous workers. This difference might depend on the strain of rats used. Furthermore, our hypothyroid rats excreted about 10 mg of bile acids or only 15-20% of the quantity formed in euthyroid bile fistula rats. This figure is lower than that obtained by Eriksson, who found a daily excretion of 20-30 mg of bile acids in hypothyroid bile fistula rats. The reason for this discrepancy is unknown but it might be due to the length and mode of propylthiouracil-treatment. Thus Eriksson gave propylthiouracil as 0.5% of diet for 3 weeks while the drug in our case was given for one month as 0.1% of drinking water.

The male and female groups receiving DT_3 in doses of 40 μg/kg/day showed minimal signs of hyperthyroidism with respect to weight gain and food intake. Interestingly enough, however, the pattern of excretion of bile acids in these animals is indistinguishable from that found in clearly hyperthyroid rats with 60-80% of the total bile acid output in the form of TCD.

Recently(1) it has been demonstrated that certain structural analogues of thyroxine, especially DT_3 and D-thyroxine, have a marked depressive effect on the serum cholesterol level with only a fraction of the calorigenic effects of the natural thyroid hormones, indicating that this effect of thyroid hormones (and its analogues) is not secondary to general metabolic stimulation but represents a specific action, the mechanism of which, however, is not known.

The results of the present investigation, in conjunction with those of Eriksson, demonstrate that LT_3 and thyroxine as well as DT_3 have a pronounced influence on the degradation of cholesterol to bile acids in the bile fistula rat, causing a reversal of the normal ratio of TCD to TC. Thus, it appears likely that the thyroid hormones exert a specific action on one or more of the enzymic reactions concerned with formation of bile acids. As in this process the transformations of the steroid nucleus precede the degradation(2) of the side-chain, 2 major sites of action appear possible: either the 12α-hydroxylation is inhibited or oxidation of the side-chain is stimulated or both of these reactions are influenced simultaneously. Efforts are now being made to determine more precisely the site(s) of action by studies of bile acid formation *in vitro*.

The possible bearing of the present results on the mechanism of the serum cholesterol lowering effect of thyroid hormones requires further investigation. This should include determination of rate of formation of bile acids in intact LT_3- and DT_3-treated animals as compared to untreated animals. The observed similarities in rate of excretion of bile acids in hyper- and euthyroid bile fistula rats do not exclude the presence of substantial differences in intact animals, as the formation of bile acids in the bile fistula rat is maximally stimulated by the broken enterohepatic circulation and the liver thus operates at maximal capacity(7).

Summary. The effects of L- and D-triiodothyronine (LT_3 and DT_3) administration and of propylthiouracil treatment on excretion of bile acids in male and female bile fistula rats have been studied. It has been shown that DT_3 administered in doses with minimal calorigenic effects gives the same bile acid picture as is obtained in clearly hyperthyroid rats with the normal taurochenodeoxycholic/taurocholic acid ratio (TCD:TC) of about 1:3 shifted to 3:1. In the propylthiouracil-treated rats total daily production of bile acids after the bile acid pool had been excreted was only about one-fifth that found for normal and thyroid hormone-treated rats and the TCD:TC ratio was 1:9. No significant differences between male and female rats regarding bile acid excretion could be seen.

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1. Boyd, G. S., *J. Atheroscler. Res.*, 1961, v1, 26.

2. Bergström, S., Danielsson, H., Samuelsson, B., in *Lipide Metabolism* (ed., K. Bloch), John

Wiley and Sons, Inc., New York, 1960, p320.
3. Eriksson, S., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 582.
4. Thompson, J. C., Vars, H. M., *ibid.*, 1953, v83, 246.
5. Van Zyl, A., *J. Endocrinol.*, 1957, v16, 213.
6. Sjövall, J., *Clin. Chim. Acta*, 1959, v4, 652.
7. Bergström, S., Danielsson, H., *Acta physiol. Scand.*, 1958, v43, 1.

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Prolactin, Immunologic Evidence of Species Specificity.* (27302)

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Previous studies from this laboratory demonstrated the antigenicity of ovine prolactin in rabbits and indicated that the antiserum produced seemed to be relatively specific for prolactin when immunization was done with a purified prolactin preparation(1). The present study was designed to test the antigenicity of a relatively crude preparation of human prolactin and to determine its immunologic cross-reaction with ovine prolactin. Such information would assess the feasibility of an immunoassay for human prolactin.

Materials and methods. Ovine Prolactin: A purified preparation of lactogenic hormone with a stated potency of 15 I.U./mg was used.†

Human Prolactin: A preparation from human anterior pituitary glands with a stated potency of 10 I.U./mg was used.‡

Immunization: Two adult white rabbits were injected subcutaneously with saline solutions of ovine and human prolactin, respectively, suspended in equal volumes of Freund's complete adjuvant. Each dose contained approximately 50 I.U. of prolactin activity. Seven weeks later intravenous booster injections of 0.20 mg of the particular prolactin in saline solution were given.

Sampling: Peripheral blood samples were obtained from the rabbits just prior to immunization and one week after the booster injections.

Hemagglutination titers: The technic employed was that described previously(1), except that each 1.0 ml volume of a 2.5% suspension of washed, tannic acid treated sheep erythrocytes was sensitized with only 0.10 mg of prolactin.

Absorption of antiserum: 1.0 ml of human growth hormone§ was added to 4.0 ml of the antiserum daily. The mixture was refrigerated for 24 hours, centrifuged, and the precipitate removed. This procedure was repeated until no further precipitate was visible.

Results. Both animals lacked demonstrable anti-prolactin antibodies before immunization; both developed such antibodies after immunization. Table I summarizes the results of hemagglutination studies with the antisera. When reacted with the homologous antigen, significant titers occurred; hemagglutination was absent when the sensitized

TABLE I. Prolactin, Anti-prolactin Hemagglutination.

Type of anti-prolactin antiserum	Source of prolactin, erythrocyte sensitization	Hemagglutination titers*
Ovine	Ovine	1/640
"	Human	0
Human	"	>1/10,000†
"	Ovine	0

* The same titers were observed in duplicate experiments done on different days.
† Higher dilutions of antiserum were not tested.

* Supported in part by grant from Nat. Inst. Health, P.H.S.

† NIH prolactin, Lot U 10303, supplied through the kindness of the Endocrine Study Section, Nat. Inst. Health, U.S.P.H.S.

‡ Prepared and bioassayed by Dr. W. H. McShan, Univ. of Wisconsin, from acetone dried human anterior pituitaries.

§ Purified human growth hormone (Lot Homo No. 9), prepared by Dr. M. Raben(2), and diluted to a concentration of 10 μg/ml.