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THE CHOLESTEROL CONTENT OF BLOOD PLASMA AND ERYTHROCYTES AS RELATED TO EXPERIMENTAL VARIATIONS IN THYROID ACTIVITY

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Introduction

The relation of the level of blood cholesterol to thyroid activity has been the subject of numerous clinical and experimental studies, the results of which have been widely divergent.

Clinical studies disagree as to the effects of hyperthyroidism on blood cholesterol. Epstein and Lande (24), Bing and Heckscher (6), Nicholls and Perlzweig (52), Gextman (29), Laroche (42), Mason, Hunt and Hurxthal (47), Hurxthal (37 and 38) and Boyd and Connell (12) found the blood cholesterol decreased in hyperthyroidism; whereas, Denis (22), Luden (45), Castex and Schteingart (17), Gardner and Gainsborough(26), Hinton (34), Bonilla and Moya (9) and Bronstein (16) found the blood cholesterol remained within normal limits. Rothschild and Jacobsohn (57) and Wade (72) concluded that the blood cholesterol was increased in hyperthyroidism.

There are only a few experimental studies, but those that exist show a similar lack of agreement on the relation of hyperthyroidism to the level of blood cholesterol. Leupold and Seisser (44) found that serum cholesterol of the rabbit was decreased following the administration of thyroid substance, whereas Westra and Kunde (74) found that similar treatment sometimes increased and sometimes decreased blood cholesterol. Parhon and Derevici (54), Blinoff (7) and Inglessi (39) found that experimental hyperthyroidism in dogs produced a hypocholesterolemia, but in similar experments Simonds and Helpler (65) found no noteworthy change in the blood cholesterol.

Both clinical and experimental studies are in better agreement as to the effect of hypothyroidism on blood cholesterol. It is well known that myxoedema, the naturally occurring hypothyroidism, is associated with a hypercholesterolemia. This was observed by Denis (22) in 1917 and has been confirmed by Epstein and Lande (24), Nicholls and Perlzweig (52), Gardner and Ganisborough (26), Bronstein (16), Hurxthal (37 and 38) and Boyd (13). Recently, Blumgart and Davis (8) have shown that blood cholesterol is also increased in the myxoedema resulting from total ablation of the thyroid. Similarly, preliminary experiments on dogs (54) and rabbits (74) have shown that hypothyroidism resulting from thyroidectomy is associated with an elevated blood cholesterol.

The discrepancies in the above observations suggested a need for a more complete study of the relations of blood cholesterol to thyroid activity. Such a study in the dog was started 3 years ago in our laboratory and yielded the following result (60): (1) administration of thyroxine, in amounts known to increase basal metabolism 40 to 60 per cent, was without effect on whole blood and plasma cholesterol of normal dogs; (2) thyroidectomy was followed by an increase in whole blood and plasma cholesterol; this increase seemed to be limited to the plasma and was brought about without disturbing the free to total cholesterol ratio normally existing in plasma; (3) administration of thyroxine in amounts which were without effect on the blood cholesterol of normal dogs, abolished the hypercholesterolemia resulting from thyroidectomy.

The present study is a continuation of this earlier work and has considered the following questions: (1) Is the hypercholesterolemia of experimental hypothyroidism limited to the plasma? (2) Does a

hypocholesterolemia follow the administration of massive quantities of thyroxine to either normal or thyroidectomized dogs? (3) How can one explain the changes in blood cholesterol occurring during experimental variations in thyroid activity?

I

Effect of Experimental Variations in Thyroid Activity on the Cholesterol of Plasma and Erythrocytes.

EXPERIMENTAL

Methods

The experiments were organized so as to find out: (1) the effects of massive doses of thyroid substance on the blood cholesterol of the normal dog; and (2) the effects of both small and massive doses of thyroid substance on the blood cholesterol of the thyroidectomized dog. For these purposes 16 animals were used; 6 of these were normal controls; 5 were thyroidectomized and treated subsequently with thyroid substance both before and after thyroidectomy.

Both thyroxine (Hoffman la Roche, synthetic) and desiccated thyroid (Armour) were administered by mouth. In some experiments crystalline thyroxine (Hoffman la Roche, synthetic) was injected subcutaneously.

Total thyroidectomies were performed under aseptic conditions. The completeness of the operation was verified at autopsy, both by gross and microscopic examination of areas adjacent to the site of operation. The parathyroids were also removed at the time of the thyroidectomy. Tetany occurred within 1 to 6 days of the time of operation. This was relieved by the administration of 1 to 3 cc. of parathyroid extract (Lilly) and 30 cc. of a 10 per cent solution of calcium gluconate, both administered intravenously. Recurrence of tetany was prevented by the daily administration of parathyroid extract in such amounts as to keep the animals free from tetany. The amounts required for this

purpose decreased progressively and in most cases it was possible to discontinue therapy within 8 weeks of operation.

All the dogs used were mongrels and were kept in the laboratory for at least 6 weeks prior to starting the experiment. During this time and throughout the experimental period, there were maintained on a constant diet consisting of 300 gm. of salmon bread mixture*, ½ pint of fresh vegetable soup, and ½ pint of milk per day.

Blood samples were obtained by heart puncture following an 18 hour fast and were heparinized. A small portion of the blood was used for determining the cell-plasma ratio according to the technique of Rosahn (55). The remainder was centrifuged at 2,000 R.P.M. for at least 1 hour in order to effect separation of cells and plasma. The white corpusclar layer above the erythrocytes was removed by suction.

Extraction of plasma and erythrocytes--Alcohol-ether extracts of plasma and erythrocytes were prepared according to a slight modification of Boyd's methods (14a, 14b). Eighty cc of 3 to 1 alcohol-ether mixture were quickly added to 3 cc. of plasma and allowed to stand over night at room temperature. The protein precipitate was filtered off, washed throughly with the alcohol-either mixture, and the filtrate and washings diluted to 100 cc. volume. Three cc. of erythrocytes were laked by shaking with an equal volume of distilled water and then extracted by the same technique as for plasma. Aliquot portions of the extracts were analyzed for free and total cholesterol.

* This bread was prepared according to the following formula of Whipple and Robscheit-Robbins (75): 12,000 gm. wheat flour, 6,000 gm. potato starch, 2,000 gm. bran, 3,000 gm. sugar, 1,000 gm. cod liver oil, 2,000 gm. canned tomatoes, 455 gm. compressed yeast, 150 gm. salt mixture, 7,500 gm. water. To each 4,800 gm. of bread were added 1 kg. canned salmon.

Determination of cholesterol—Free cholesterol was determined by the oxidative method of Yasuda (77). The priniciples of this method are as follows: (1) cholesterol is separated from other lipids by precipitation as the digitonide; (2) the cholesterol digitonide is oxidized by an excess of potassium dichromate in the presence of sulphuric acid and a silver dichromate catalyst, the excess dichromate being determined iodimetrically. Our experiments showed the accuracy of the method to be ± 1 per cent.

Total cholesterol was determined colorimetrically by a modification of Bloor's method (10). This method, based on the Liebermann-Burchard reaction, has been criticized because it yields higher results than the classic macro-gravimetric procedure of Windaus (76), and because of the difficulty in obtaining reproducible results (23, 49, 51). This method has been selected, however, because of its simplicity and because experiments by Schmidt and Hughes (61) have shown that reproducible results can be obtained if one excercises rigid control over the temperature of the reaction, the exposure to light, and the time of color development. They have also found that the method gives results comparable to those obtained by the Windaus procedure, if the cholesterol content of the solution used for comparison is standardized on the basis of its content of digitonin precipitable sterol.

The procedure adopted was as follows. Ten cc. of the alcohol-ether extract were evaporated to dryness slowly. Three cc. of anhydrous chloroform were added to the residue, heated to boiling and the resulting solution decanted into a 10 cc. glass-stoppered

volumetric flask. This extraction was repeated twice. The combined extrace was cooled to room temperature, then 2 cc. of acetic anhydride (Merck Blue Label) were added, and the volume of solution made up to 10 cc. with chloroform. A standard solution was prepared as follows: 0.5 mg. of digitonin precipitable sterol in 5 cc. of chloroform were placed in a 10 cc. volumetric flask; 2 cc. of acetic anhydride were added and the volume of solution made up to 10 cc. with chloroform. Two tenths cc. of concentrated sulphuric acid were added to both the standard and the unknown. The flasks were removed immediately to a dark room, shaken well to obtain thorough mixing, and placed in a water bath maintained at 23 C. At the end of 13 minutes the flasks were removed from the bath, and the unknown compared with the standard in a colorimeter, fitted with a Corning red glass filter transmitting light from 6300 to 6100 A°. All colorimetric readings were made in the dark room between 13 and 17 minutes after addition of sulphuric acid. The error of the method was $\pm$ 2 per cent.

Results

The cholesterol content of plasma and erythrocytes of normal dogs.

Plasma. The total cholesterol content of the plasma varied within relatively narrow limits in 5 of the 6 normal dogs. In Dog 0 (Table I) the variations were between 112 and 125 mg. per cent (mean 118, standard deviation ± 5). In Dog 5 (Table II) the limits of variation were 116 and 176 mg. per cent (mean 135, standard deviation ± 15). In Dog 7 (Table III) the limits were 112 and 149 mg. per cent (mean 131, standard deviation ± 10). In Dog 17 (Table V) the limits were 92 to 141 mg. per cent (mean 119, standard deviation ± 14). In Dog 404 (Table VI) variations were between 129 and 159 mg. per cent (mean 141, standard deviation ± 10). These variations are comparable to those found by Bloor (11) in normal dogs studied over a 2 year period.

In the sixth animal, Dog 14 (Table IV) the total cholesterol values of the first 2 periods were much higher than those of subsequent periods. These samples of blood were taken 2 and 4 weeks after parturition and contained 193 and 207 mg. per cent cholesterol respectively. It seems probable that these relatively high values were due to parturition and lactation since a post partum rise in plasma cholesterol has been observed in cattle by Shope and Gowen (64), Maynard, Harrison and McKay (48) and in rabbits by Coppola (19). Rejecting the first two values, the remainder varied between 116 and 165 mg. per cent (mean 140, standard deviation ± 16).

The free cholesterol content of the plasma varied to about the same degree as the total cholesterol, but the per cent of cholesterol in the free form was relatively constant. In Dog 0 (Table I) the per cent of free cholesterol varied between 20.2 and 26.5 (average 23.1, standard deviation ± 1.6). In Dog 5 (Table II) the variations were between 23.3 and 28.9 per cent (average 25.6, standard deviation ± 1.6). In Dog 7 (Table III) the variations were from 21.9 to 31.1 per cent (average 24.5, standard deviation ± 2.3). In Dog 14 (Table IV) the per cent of free cholesterol varied between 19.4 and 27.3 (average 23.3, standard deviation ± 2.3). In Dog 17 (Table V) the limits were 21.3 and 27.0 per cent (average 24.3, standard deviation ± 1.5). In Dog 404 (Table VI) the limits were 21.1 and 26.6 per cent (average 24.3, standard deviation ± 1.7).

Erythrocytes. The cholesterol content of the erythrocytes was much more constant than that of the plasma. The amount of cholesterol in the erythrocytes of Dog 0 (Table I) varied between 122 and 150 mg. per cent (average 141, standard deviation ± 7). The erythrocyte cholesterol of Dog 5 (Table II) varied between 129 and 146 mg. per cent (average 138, standard deviation ± 5). In Dog 7 (Table III) the limits were 126 to 149 mg. per cent (average 136, standard deviation ± 7). In Dog 14 (Table IV) the variations were from 131 to 142 mg. per cent (average 137, standard deviation ± 3). In Dog 17 (Table V) the variations were from 125 to 143 mg. per cent (average 134, standard deviation ± 5). In Dog 404 (Table VI) the limits were 126 to 144 mg. per cent (average 138, standard deviation ± 5).

The amount of free cholesterol in the corpuscles closely approximated the total cholesterol content (see Tables I - VI). On the

average, free cholesterol accounted for 95 to 98 per cent of the total. It is probable that the 3 to 5 per cent of esterified cholesterol was due to plasma which had not been completely separated from the corpuscles. We have found that identical free and total cholesterol values are obtained when the corpuscles of the dog are repeatedly washed with physilogical saline so as to free them from the last traces of plasma. This observation agrees with those of Brun (5) and Wacker and Hueck (77) who found that all the cholesterol in human erythrocytes was in the free form.

The effect of thyroid substance on cholesterol content of plasma and erythrocytes of normal dogs.--Administration of large amounts of thyroxine or desiccated thyroid gland caused a slight but definite reduction in plasma cholesterol in Dogs 8, 9, 10 and 13 (Tables VII, VIII, IX and XI) and a very marked reduction in Dog 11 (Table X). The data show that the greatest decreased occurred in those animals having the highest levels of plasma cholesterol prior to treatment. In Dog 11, the plasma cholesterol was 208 mg. per cent prior to treatment; the lowest level reached during treatment with both thyroxine and desiccated thyroid was 109 mg. per cent. Dog 8 had a plasma cholesterol level of 148 mg. per cent prior to treatment; the lowest level found during treatment was 100 mg. per cent. Plasma cholesterol values in Dogs 9, 10 and 13 were 95, 119 and 87 mg. per cent respectively prior to treatment; the lowest levels reached during treatment were 71, 109 and 65 mg. per cent respectively.

In 4 of 5 Dogs (Tables VII-XI) the daily administration of 4 mg. of

thyroxine for 2 weeks reduced the plasma cholesterol to as great an extent as 8 mg. It seemed possible that this lack of effectiveness of increased dosage might be due to incomplete absorption. Clinical study (71) has shown that desiccated thyroid gland has a greater effect on basal metabolism than its thyroxine equivalent when administered orally. The data in Tables VII - XI show that daily administration of 6.7 gm. of desiccated thyroid gland (the equivalent of 8 mg. of thyroxine) did not cause a further decrease in the plasma cholesterol of the dogs previously treated with thyroxine. In 3 of the dogs the desiccated thyroid therapy caused a slight increase in plasma cholesterol.

The date in Tables VII - XI show that administrations of thyroid substance caused a decrease in free cholesterol content of plasma. This decrease was proportional to the change in total cholesterol with the result that per cent free cholesterol remained unchanged. Tables VII - XI also show that the amounts of free and total cholesterol in the erythrocytes were not altered as a result of treatment.

The cholesterol content of plasma erythrocytes following thyroidectomy.--Thyroidectomy was followed uniformily by an increase in plasma cholesterol (Tables IX - XVI). The smallest increase was 45 mg. per cent above the normal level and was found in Dog 12 (Table XIV). The largest increase, 211 mg. per cent, was found in Dog 11 (Table X). It was apparent that the magnitude of hypercholesterolemia was not related to the preoperative level of plasma cholesterol, for in Dogs 11 and 12 (Tables X and XIV) these levels were 109 and 103 mg. per cent respectively. Following thyroidectomy the plasma cholesterol of Dog 11 increased to 320 mg. per cent while that of Dog 12 rose but slightly to 148 mg.

per cent*.

Varying periods of time were required for plasma cholesterol concentrations to reach a maximum. In Dogs 2 and 12 (Tables XIII and XIV) maximum concentrations were reached within 2 weeks following thyroidectomy. In Dogs 1, 11 and 39 (Tables XII, X and XVI) peak values were attained in the 4th post operative week, but 6 weeks were required for Dog 18 (Table XV) and 8 weeks for Dog 13 (Table XI).

The free cholesterol content of the plasma increased in direct proportion to the increase in total cholesterol so that the percentage free cholesterol remained unchanged.

The data show that the amounts of free and total cholesterol in the erythrocytes were not altered significantly by thyroidectomy. The small increase which did occur could be explained by the occlusion of a small amount of plasma. For example in Dog 18 (Table XV) erythrocyte cholesterol rose from an average normal of 137 mg. per cent to an average of 147 mg. per cent following thyroidectomy. Two tenths cc. of plasma, having a cholesterol concentration of 273 mg. per cent (the average cholesterol concentration during the post thyroidectomy period), would account for the 10 mg. per cent rise in erythrocyte cholesterol.

The effect of thyroid substance on the cholesterol content of plasma and erythrocytes of thyroidectomized dogs.--The administration of 1 mg. of thyroxine orally daily for a 2 week period reduced the plasma cholesterol of the thyroidectomized dog to normal or subnormal levels (Tables XII to XVI). Previous study (60) has

* Autopsy showed that this difference was not due to the remnant of a thyroid gland in Dog 12.

shown that twice this amount of thyroxine is without effect on the plasma cholesterol of normal dogs. The 1 mg. daily dose of synthetic thyroxine appeared as effective in reducing plasma cholesterol of the thyroidectomized dog as the 4 mg. daily dose of crystalline thyroxine administered subcutaneously (Tables X and XI). Increasing the oral dosage to 4 mg. and the subcutaneous dosage to 8 mg. produced further slight reductions in plasma cholesterol levels (Tables XII to XVI and X and XI respectively). Increasing the dosage of crystalline thyroxine to 16 mg. daily not only failed to produce a further decrease in plasma cholesterol, but actually produced a significant increase (Tables X and XI).

The daily administration of 3.4 gm. of desiccated thyroid gland* did not lower the plasma cholesterol below the level produced by administration of an equivalent amount of thyroxine (Tables XII - XVI). Moreover, the daily administration of 6.7 gm. (8 mg. thyroxine equivalent) of desiccated thyroid for 4 weeks failed to produce a further decrease in plasma cholesterol content. It was of interest that daily administration of 13.4 gm. of desiccated thyroid for 2 weeks produced a significant increase in plasma cholesterol just as did 16 mg. of thyroxine--the equivalent of 13.4 gm. of desiccated thyroid.

The changes in total cholesterol of plasma just described were accompanied by parallel changes in free cholesterol--the per cent of free cholesterol remaining constant.

* According to Harrington, 3.4 gm. of desiccated thyroid is equivalent to 4 mg. of thyroxine.

The cholesterol content of the erythyrocytes was not altered by administration of thyroid substance (Tables XII - XVI).

Effect of experimental hyperthyroidism and hypothroidism on body weight.--The weights of the control dogs increased slightly during the experiment, probably as a result of normal growth and favorable environment (Tables I - VI). Treatment with thyroxine prior to thyroidectomy caused a definite loss in weight of all animals, the loss amounting to from 6 to 17 per cent of the original body weight (Tables VII - XVI). Thyroidectomy was followed by a weight gain of from 10 to 26 per cent above the preoperative levels (Tables IX - XVI). Subsequent administration of thyroid substance abolished the weight gained during the post-thyroidectomy period (Tables X - XVI).

It is interesting to note that the decrease in weight following the administration of thyroxine did not parallel the reduction in plasma cholesterol. The loss in body weight was progressively greater with continued administration of thyroxine, whereas the maximum reduction inplasma cholesterol occurred during the first period of treatment with thyroid substance. It is also noted that the increase in plasma cholesterol following thyroidectomy did not parrallel the weight gain.

Effects of experimental hyperthyroidism and hypothyroidism on cell-plasma ratio.--The cell-plasma ratios of the control dogs were remarkably constant throughout the experimental period (Tables I - VI). Administration of thyroid substance to normal dogs was without effect on cell volumes (Tables VII - XI). Thyroidectomy was follow-

ed by a decrease in the proportion of cells in all animals except Dog 12 (Tables X - XVI). Administration of thyroid substance subsequent to thyroidectomy restored the cell volume to normal in all except Dog 12 (Tables X to XVI).

Summary of preceding observations.—These experiments have shown: (1) that thyroidectomy in the dog was followed by an increase in plasma cholesterol; (2) that administration of 4 to 8 mg. doses of thyroxine to normal dogs lowered the plasma cholesterol—the extent of the reduction depending upon the level of cholesterol prior to treatment; (3) that administration of a 1 mg. dose of thyroxine, which was without effect in the normal dog reduced the plasma cholesterol of the thyroidectomized dog to the normal level; (4) that prolonged administration of 4 to 8 mg. of thyroxine to the thyroidectomized dog reduced the plasma cholesterol little more that did the administration of the 1 mg. dose; (5) that prolonged administration of the 16 mg. dose of thyroxine produced a significant increase in the plasma cholesterol of those dogs which had been treated with 1 to 8 mg. doses of thyroxine subsequent to thyroidectomy; (6) that the administration of desiccated thyroid gland had an effect on the plasma cholesterol similar to that of equivalent amounts of thyroxine; (7) that the free cholesterol content of the plasma increased following thyroidectomy and decreased following treatment with thyroid substance in direct proportion to changes in total cholesterol so that the per cent of free cholesterol remained unchanged; (8) that the total and free cholesterol contents of the erythrocytes were unaffected by thyroidectomy or by treatment with thyroid substance.

Table I

VARIATIONS IN BLOOD CHOLESTEROL OF NORMAL DOG O

Date	Weight	Plasma cholesterol			R.B.C. hemat-ocrit	R.B.C. cholesterol		
		T[1]	F[2]	PF[3]		T[1]	F[2]	PF[3]
	kg.				per cent			
12--4-37	26.0	125	29.5	23.6	52.0	122	122	100
12-18-37		123	27.8	22.6	52.5	142	136	96
12-31-37		120	31.8	26.5	53.5	137	140	102
1-15-38		120	24.3	20.2	53.0	137	137	100
1-29-38		116	27.2	23.5	52.0	146	138	95
2-10-38		119	27.2	22.9	52.0	141	138	98
2-24-38		125	25.9	21.7	52.0	139	137	99
3-10-38		115	25.2	21.9	52.0	145	133	92
3-24-38		112	24.3	21.7	50.0	149	145	98
4--7-38		113	25.4	22.5	51.0	150	136	97
4-21-38	27.0	113	26.9	23.8	51.0	140	136	97
Average		118	25.9	23.1		141	136	97

T[1]--mg. per cent cholesterol

F[2]--mg. per cent free cholesterol

PF[3]--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscle

Table II

VARIATIONS IN BLOOD CHOLESTEROL OF NORMAL DOG 5

Date	Weight	Plasma cholesterol			R.B.C. hemat-ocrit	R.B.C. cholesterol		
		T^1	F^2	PF^3		T^1	F^2	PF^3
	kg.				per cent			
12--4-37	14.8	116	28.0	24.1	51.0	129	125	97
12-18-37	15.2	128	33.6	26.4	52.0	140	130	93
12-31-37	15.6	176	41.4	23.5	50.5	136	131	96
1-15-38	16.0	133	35.8	26.9	46.5	130	124	95
1-29-38	16.3	132	36.1	27.3	47.5	139	135	97
2-10-38	16.1	125	36.2	28.9	47.0	137	135	99
2-24-38	16.5	129	32.2	25.0	48.0	137	138	101
3-10-38	16.9	140	34.7	24.8	46.0	146	136	93
3-24-38	16.8	133	35.0	26.3	48.0	144	133	93
4--7-38	16.9	147	37.4	25.4	45.0	141	131	93
4-21-38	17.3	127	29.6	23.3	45.0	137	125	91
Average		135	34.5	25.6		138	131	95

T^1--mg. per cent cholesterol

F^2--mg. per cent free cholesterol

PF^3--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscle

Table III

VARIATIONS IN BLOOD CHOLESTEROL OF NORMAL DOG 7

Date	Weight	Plasma cholesterol			R.B.C. hemat-ocrit	R.B.C. cholesterol		
		T[1]	F[2]	PF[3]		T[1]	F[2]	PF[3]
	kg.				per cent			
12--4-37	11.5	112	27.1	24.2	35.0	126	119	94
12-18-37	12.0	128	39.9	31.1	42.0	149	139	93
12-31-37	12.6	134	29.8	22.2	47.5	133	137	103
1-15-38	13.0	126	30.8	24.5	50.7	134	134	100
1-29-38	13.7	133	31.1	23.4	53.0	140	143	102
2-10-38	14.2	127	32.6	25.7	54.0	124	120	97
2-24-38	13.6	130	32.0	24.6	53.0	135	137	101
3-10-38	15.0	142	33.4	23.5	53.0	137	132	97
3-24-38	16.2	149	32.6	21.9	53.0	143	145	101
4-7-38	16.1	121	29.5	24.4	53.0	136	125	92
4-21-38	15.9	139	34.0	24.4	55.0	143	137	96
Average		131	32.1	24.5		136	135	98

T[1]--mg. per cent cholesterol

F[2]--mg. per cent free cholesterol

PF[3]--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscle

Table IV

VARIATIONS IN BLOOD CHOLESTEROL OF NORMAL DOG 14

Date	Weight	Plasma cholesterol			R.B.C. hematocrit	R.B.C. cholesterol		
		T[1]	F[2]	PF[3]		T[1]	F[2]	PF[3]
	kg.				per cent			
12--4-37	11.2	195	52.8	27.3	47.5	139	130	94
12-18-37	11.3	207	49.6	23.9	52.0	142	129	99
12-31-37	11.3	165	39.0	23.7	53.0	133	133	100
1-15-38	11.2	133	35.3	26.5	51.5	131	129	99
1-29-38	11.3	143	27.7	19.4	53.5	141	133	94
2-10-38	11.3	135	27.8	21.6	50.0	135	133	99
2-24-38	11.6				50.0			
3-10-38	11.9	161	33.4	20.7	53.0	137	132	96
3-24-38	12.0	116	26.6	22.9	54.0	138	132	96
4--7-38	12.2	125	30.0	24.0	53.0	135	135	100
4-21-38	12.5	140	32.3	23.1	52.0	138		
Average		140	35.3	23.3		137	132	97

T[1]--mg. per cent cholesterol

F[2]--mg. per cent free cholesterol

PF[3]--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscle

Table V

VARIATIONS IN BLOOD CHOLESTEROL OF NORMAL DOG 17

Date	Weight	Plasma cholesterol			R.B.C. hematocrit	R.B.C. cholesterol		
		T[1]	F[2]	PF[3]		T[1]	F[2]	PF[3]
	kg.				per cent			
12--4-37	15.9	135	32.1	23.8	53.0	128	130	102
12-18-37	16.1	129	32.1	24.9	48.0	135	129	96
12-31-37	16.5	123	27.6	22.4	46.5	138	136	99
1-15-38	16.7	102	27.6	27.0	44.5	127	124	98
1-29-38	16.6	92	23.0	25.3	48.0	136	136	100
2-10-38	16.8	107	25.7	24.0	47.0	125	129	103
2-24-38	16.6	112	26.3	23.5	47.0	133	131	99
3-10-38	16.9	124	27.8	24.4	48.0	135	128	95
3-24-38	17.5	112	29.8	26.6	47.0	143	134	94
4--7-38	17.7	130	31.6	24.3	51.0	136	126	93
4-21-38	17.6	141	30.0	21.3	52.0	135	131	97
Average		119	28.4	24.3		134	130	98

T[1]--mg. per cent cholesterol

F[2]--mg. per cent free cholesterol

PF[3]--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscle

Table VI

VARIATIONS IN BLOOD CHOLESTEROL OF NORMAL DOG 404

Date	Weight	Plasma cholesterol			R.B.C. hematocrit	R.B.C. cholesterol		
		T^1	F^2	PF^3		T^1	F^2	PF^3
	kg.				per cent			
12--4-37	11.6	152	32.1	21.1	51.5	126	119	95
12-18-37	11.7	159	40.3	25.3	49.0	140	138	99
12-31-37	11.8	155	37.8	24.4	48.5	138	142	103
1-15-38	11.9	129	30.4	23.6	46.5	132	128	97
1-29-38	12.1	134	35.2	26.2	47.0	144	136	95
2-10-38	12.0	136	31.5	23.2	47.0	137	135	99
2-24-38	12.2	135	31.8	23.5	50.0	144	141	98
3-10-38	12.4	140	37.3	26.6	50.0	136	128	94
3-24-38	12.8	130	28.3	21.8	52.0	139	134	96
4--7-38	13.0	143	37.5	26.2	52.0	141	138	98
4-21-38	13.0	139	34.0	25.1	53.0	140	137	98
Average		141	34.2	24.5		138	134	97

T^1--mg. per cent cholesterol

F^2--mg. per cent free cholesterol

PF^3--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscle

Table VII

VARIATIONS IN BLOOD CHOLESTEROL OF DOG 8

Date	Treatment	Weight	Plasma cholesterol			R.B.C. hematocrit	R.B.C. cholesterol		
			T^1	F^2	PF^3		T^1	F^2	PF^3
		kg.				per cent			
12--4-37	No treatment	12.6	149	37.8	25.4	51.5	146	139	95
12-18-37	No treatment	13.5	148	39.1	26.4	51.5	159	148	96
12-31-37	4 mg. thyroxine orally daily from 12-18 to 12-31	13.2	115	31.6	27.4	53.0	136	143	105
1-15-38	8 mg. thyroxine orally daily from 12-31 to 1-15	13.1	100	23.7	23.7	52.0	137	130	95
1-29-38	6.7 gm. desiccated thyroid orally from 1-15 to 1-29	12.6	103	26.8	26.8	51.0	139	142	102
2-10-38	6.7 gm. desiccated thyroid orally from 1-29 to 2-10	12.7	117	22.1	22.1	53.0	131	132	100
		Average			25.3		141	139	99

T^1--mg. per cent cholesterol

F^2--mg. per cent free cholesterol

PF^3--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscle

Table VIII

VARIATIONS IN BLOOD CHOLESTEROL OF DOG 9

Date	Treatment	Weight	Plasma cholesterol			R.B.C. hemat-ocrit	R.B.C. cholesterol		
			T^1	F^2	PF^3		T^1	F^2	PF^3
		kg.				per cent			
12--4-37	No treatment	13.5	95	22.7	33.9	42.0	128	117	92
12-18-37	No treatment	13.9	97	29.4	30.3	42.0	146	132	90
12-31-37	4 mg. thyroxine orally daily from 12-18 to 12-31	13.9	86	16.5	19.2	44.5	131	127	97
1-15-38	8 mg. thyroxine orally daily from 12-31 to 1-15	13.9	77	16.8	21.8	39.0	127	122	96
1-29-38	6.7 gm. desiccated thyroid orally daily from 1-15 to 1-29	13.4	71	14.8	20.9	39.0	139	122	88
2-10-38	6.7 gm. desiccated thyroid orally daily from 1-29 to 2-10	12.9	87	17.0	19.5	41.0	134	123	92
		Average			25.8		138	129	93

T^1--mg. per cent cholesterol

F^2--mg. per cent free cholesterol

PF^3--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscle

Table IX

VARIATIONS IN BLOOD CHOLESTEROL OF DOG 10

Date	Treatment	Weight	Plasma cholesterol			R.B.C. hematocrit	R.B.C. cholesterol		
			T^1	F^2	PF^3		T^1	F^2	PF^3
		kg.				per cent			
12--4-37	No treatment	15.8	131	33.7	25.7	44.0	147	133	91
12-18-37	No treatment	15.8	119	31.8	26.7	43.5	139	140	100
12-31-37	4 mg. thyroxine orally daily from 12-18 to 12-31	14.8	109	28.9	26.5	48.0	132	134	101
1-15-38	8 mg. thyroxine orally daily from 12-31 to 1-15	14.9	115	33.0	28.7	48.5	131	130	98
1-29-38	6.7 gm. desiccated thyroid orally daily from 1-15 to 1-29	13.4	120	31.1	25.9	49.0	146	136	93
2-10-38	6.7 mg. desiccated thyroid orally daily from 1-29 to 2-10	13.0	124	28.3	20.2	44.0	135	131	97
2-24-38	Thyroidectomized 2-15-38	14.3	161	47.6	29.6	36.0	158	149	95
3-10-38	No treatment	15.7	187	47.5	25.4	33.0	155	154	99
		Average			26.1		143	138	97

T^1--mg. per cent cholesterol

F^2--mg. per cent free cholesterol

PF^3--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscle

Table X - VARIATIONS IN BLOOD CHOLESTEROL OF DOG 11

Date	Treatment	Weight	Plasma cholesterol			R.B.C. hematocrit	R.B.C. cholesterol		
			T^1	F^2	PF^3		T^1	F^2	PF^3
		kg.				per cent			
12--4-37	No treatment	18.2	226	55.4	24.5	45.0	118	112	95
12-18-37	No treatment	18.7	208	51.4	24.6	46.0	128	119	93
12-31-37	4 mg. thyroxine orally daily from 12-18 to 12-31	18.4	135	34.3	25.4	48.5	132	125	95
1-15-38	8 mg. thyroxine orally daily from 12-31 to 1-15	17.7	127	31.3	24.6	47.0	122	117	96
1-29-38	6.7 gm. desiccated thyroid daily from 1-15 to 1-29	17.3	119	28.1	23.6	42.0	130	121	93
2-10-38	6.7 gm. desiccated thyroid daily from 1-29 to 2-10	17.0	109	26.0	23.8	44.0	119	112	94
2-24-38	Thyroidectomized 2-15-38	18.8	236	64.0	27.1	45.0	143	138	97
3-10-38	No treatment	21.5	320	72.7	22.7	40.0	139	126	91
3-24-38	No treatment	22.6	255	62.1	24.3	39.0	141	133	94
4--7-38	No treatment	22.0	265	66.8	25.2	38.0	135	126	93
4-21-38	4 mg. thyroxine subcutaneously daily from 4--7 to 4-21	18.5	107	28.5	26.6	37.0	117	119	101
5--5-38	8 mg. thyroxine subcutaneously daily from 4-21 to 5--5	17.5	93	23.4	25.1	39.0	129	126	98
5-19-38	16 mg. thyroxine subcutaneously daily from 5--5 to 5-19	16.5	134	31.5	23.5	46.0	134	125	94
		Average			24.7		130	123	95

T^1--mg. per cent cholesterol

F^2--mg. per cent free cholesterol

PF^3--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscles

Table XI - VARIATIONS IN BLOOD CHOLESTEROL OF DOG 13

Date	Treatment	Weight	Plasma cholesterol			R.B.C. hematocrit	R.B.C. cholesterol		
			T[1]	F[2]	PF[3]		T[1]	F[2]	PF[3]
		kg.				per cent			
12--4-37	No treatment	13.5	87	26.4	30.3	49.0	127	122	96
12-18-37	No treatment	14.4	104	29.2	28.1	45.5	135	136	100
12-31-37	4 mg. thyroxine orally daily from 12-18 to 12-31	14.3	84	20.7	24.6	48.5	134	129	96
1-15-38	8 mg. thyroxine orally daily from 12-31 to 1-15	14.1	65	19.4	29.9	43.0	124	127	102
1-29-38	6.7 gm. desiccated thyroid orally daily from 1-15 to 1-29	13.0	77	17.3	22.5	45.0	136	130	96
2-10-38	6.7 gm. desiccated thyroid orally daily from 1-29 to 2-10	13.1	74	15.2	20.6	45.0	129	131	102
2-24-38	Thyroidectomized 2-16-38	13.3	155	47.2	30.4	42.0	140	146	104
3-10-38	No treatment	14.0	163	43.2	26.4	39.0	146	150	101
3-24-38	No treatment	15.1	205	52.7	25.7	35.0	145	139	96
4--7-38	No treatment	15.4	249	68.2	27.4	41.0	153	139	91
4-21-38	4 mg. thyroxine subcutaneously daily from 4--7 to 4-21	13.9	71	17.3	24.4	38.0	130	128	98
5--5-38	8 mg. thyroxine subcutaneously daily from 4-21 to 5--5	12.9	58	14.9	25.7	42.0	129	127	99
5-19-38	16 mg. thyroxine subcutaneously daily from 5--5 to 5-19	12.0	96	22.1	23.0	50.0	128	130	101
		Average			26.0		135	133	99

T[1]--mg. per cent cholesterol

F[2]--mg. per cent free cholesterol

PF[3]--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscles

Table XII - VARIATIONS IN BLOOD CHOLESTEROL OF DOG 1

Date	Treatment	Weight	Plasma cholesterol			R.B.C. hematocrit	R.B.C. cholesterol		
			T[1]	F[2]	PF[3]		T[1]	F[2]	PF[3]
		kg.				per cent			
12--4-37	No treatment	14.5	107	27.3	25.5	44.0	136	124	91
12-18-37	No treatment	15.1	136	34.5	25.4	51.5	146	133	91
12-31-37	Thyroidectomized 12-30-37								
1-15-38	No treatment	16.0	249	65.2	26.2	42.0	140	133	95
1-29-38	No treatment	16.6	196	52.6	26.8	42.0	142	135	95
2-10-38	1 mg. thyroxine orally daily from 1-29 to 2-10	16.2	101	24.3	24.1	40.0	132	128	97
2-24-38	4 mg. thyroxine orally daily from 2-10 to 2-24	15.8	84	24.0	28.5	40.0	132	125	95
3-10-38	3.4 gm. desiccated thyroid orally daily from 2-24 to 3-10	15.2	93	21.3	22.9		134	124	93
3-24-38	6.7 gm. desiccated thyroid orally daily from 3-10 to 3-24	14.7	96	22.1	23.0	42.0	132	125	95
4--7-38	6.7 gm. desiccated thyroid orally daily from 3-24 to 4--7	14.6	97	22.3	23.0	43.0	128	124	97
4-21-38	13.4 mg. desiccated thyroid orally daily from 4--7 to 4-21	15.1	135	32.5	24.1	43.0	139	135	97
		Average			24.9		136	129	95

T[1]--mg. per cent cholesterol

F[2]--mg. per cent free cholesterol

PF[3]--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscle

Table XIII - VARIATIONS IN BLOOD CHOLESTEROL OF DOG 2

Date	Treatment	Weight	Plasma cholesterol			R.B.C. hematocrit	R.B.C. cholesterol		
			T^1	F^2	PF^3		T^1	F^2	PF^3
		kg.				per cent			
12--4-37	No treatment	14.3	59	16.6	28.4	43.0	123	116	94
12-18-37	No treatment	14.5	72	22.5	31.4	44.0	132	126	96
12-31-37	Thyroidectomized 12-21-37	13.9	185	55.3	30.0	39.0	147	136	93
1-15-38	No treatment	15.0	137	36.9	26.9	34.5	138	140	101
1-29-38	No treatment	16.0	170	44.5	26.2	32.5	140	144	102
2-10-38	1 mg. thyroxine orally daily from 1-29 to 2-10	15.7	74	14.7	19.8	31.0	143	141	99
2-24-38	4 mg. thyroxine orally daily from 2-10 to 2-24	14.9	64	14.1	22.5	32.0	125	120	96
3-10-38	3.4 gm. desiccated thyroid orally daily from 2-24 to 3-10	14.6	74	16.1	21.8	44.0	133	120	90
3-24-38	6.7 gm. desiccated thyroid orally daily from 3-10 to 3-24	14.1	74	14.6	20.4	43.0	127	121	95
4--7-38	6.7 gm. desiccated thyroid orally daily from 3-24 to 4--7	12.9	91	21.6	23.8	42.1	133	122	92
4-21-38	13.4 mg. desiccated thyroid orally daily from 4--7 to 4-21	13.0	138	31.0	22.5	41.0	132	123	93
		Average			25.8		134	128	96

T^1--mg. per cent cholesterol

F^2--mg. per cent free cholesterol

PF^3--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscle

Table XIV - VARIATIONS IN BLOOD CHOLESTEROL OF DOG 12

Date	Treatment	Weight	Plasma cholesterol			R.B.C. hematocrit	R.B.C. cholesterol		
			T^1	F^2	PF^3		T^1	F^2	PF^3
		kg.				per cent			
12--4-37	No treatment	13.5	118	24.7	21.0	41.0	128	120	94
12-18-37	No treatment	14.0	103	21.3	20.7	42.5	135	128	95
12-31-37	Thyroidectomized 12-22-37	14.3	148	36.1	24.4	46.0	137	129	94
1-15-38	No treatment	15.3	131	36.0	27.4	41.5	134	138	103
1-29-38	No treatment	16.0	113	28.6	25.3	47.5	138	126	92
2-10-38	1 mg. thyroxine orally daily from 1-29 to 2-10	16.1	86	17.0	19.8	42.0	130	125	96
2-24-38	4 mg. thyroxine orally daily from 2-10 to 2-24	16.1	75	16.7	22.2	40.0	126	125	99
3-10-38	3.4 gm. desiccated thyroid orally daily from 2-24 to 3-10	15.5	69	13.8	20.0	43.0	116	118	101
3-24-38	6.7 gm. desiccated thyroid orally daily from 3-10 to 3-24	15.5	72	15.5	21.6	37.0	132	124	94
4--7-38	6.7 gm. desiccated thyroid orally daily from 3-24 to 4--7	15.1	74	21.6	29.2	40.0	132	125	95
4-21-38	13.4 mg. desiccated thyroid orally daily from 4--7 to 4-21	14.3	83	22.8	27.5	40.0	131	128	98
		Average			25.6		131	126	96

T^1--mg. per cent cholesterol

F^2--mg. per cent free cholesterol

PF^3--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscle

TABLE XV - VARIATIONS IN BLOOD CHOLESTEROL OF DOG 18

Date	Treatment	Weight	Plasma cholesterol			R.B.C. hemat-ocrit	R.B.C. cholesterol		
			T^1	F^2	PF^3		T^1	F^2	PF^3
		kg.				per cent			
12--4-37	No treatment	8.2	150	32.1	21.4	44.0	132	125	95
12-18-37	No treatment	8.8	173	42.1	24.3	40.5	142	129	91
12-31-37	Thyroidectomized 12-22-37	9.1	241	59.6	24.7	44.5	152	150	99
1-15-38	No treamtnet	9.0	274	77.1	28.1	38.0	145	132	91
1-29-38	No treatment	10.1	304	65.8	21.6	33.5	144	138	96
2-10-38	1 mg. thyroxine orally daily from 1-29 to 2-10	9.5	144	25.8	19.8	34.0	127	127	100
2-24-38	4 mg. thyroxine orally daily from 2-10 to 2-24	9.1	103	21.8	21.2	35.0	130	125	96
3-10-38	3.4 gm. desiccated thyroid orally daily from 2-24 to 3-10	8.7	82	22.3	27.2	38.0	134	121	91
3-24-38	6.7 gm. desiccated thyroid orally daily from 3-10 to 3-24	8.5	81	17.3	21.3	42.0	123	127	103
4--7-38	6.7 gm. desiccated thyroid orally daily from 3-24 to 4--7	8.4	94	28.6	30.4	42.0	132	130	99
4-21-38	13.4 gm. desiccated thyroid orally daily from 4--7 to 4-21	8.4	124	29.4	23.7	43.0	142	139	98
		Average			24.0		137	131	96

T^1--mg. per cent cholesterol

F^2--mg. per cent free cholesterol

PF^3--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscle

Table XVI - VARIATIONS IN BLOOD CHOLESTEROL OF DOG 39

Date	Treatment	Weight	Plasma cholesterol			R.B.C. hematocrit	R.B.C. cholesterol		
			T^1	F^2	PF^3		T^1	F^2	PF^3
		kg.				per cent			
12--4-37	No treatment	17.7	212	52.6	27.1	33.5	123	123	100
12-18-37	No treatment	18.3	240	55.7	23.2	35.0	133	126	95
12-31-37	Thyroidectomized 12-21-37	19.0	278	77.0	27.7	33.5	150	141	94
1-15-38	No treatment	21-	312	87.9	28.2	32.0	138	128	93
1-29-38	No treatment	21.8	268	67.6	25.2	30.0	145	137	94
2-10-38	1 mg. thyroxine orally daily from 1-29 to 2-10	20.9	153	33.1	21.5	31.0	136	129	95
2-24-38	4 mg. thyroxine orally daily from 2-10 to 2-24	20.5	106	23.1	21.8	36.0	131	131	100
3-10-38	3.4 gm. desiccated thyroid orally daily from 2-24 to 3-10	19.5	106	25.2	23.8	37.0	123	127	103
3-24-38	6.7 gm desiccated thyroid orally daily from 3-10 to 3-24	18.4	112	25.0	22.3	40.0	138	132	96
4--7-38	6.7 gm. desiccated thyroid orally daily from 3-24 to 4--7	18.0	112	33.1	29.5	39.0	133	136	102
4-21-38	13.4 gm. desiccated thyroid orally daily from 4--7 to 4-21	17.1	157	35.9	22.9	39.0	143	140	98
		Average			24.8		136	132	97

T^1--mg. per cent cholesterol

F^2--mg. per cent free cholesterol

PF^3--per cent of cholesterol as free cholesterol

R.B.C.--red blood corpuscle

DISCUSSION

Our observations in the thyroidectomized dog justify the conclusion that experimental hyperthyroidism is associated with a plasma hypercholesterolemia. This not only confirms our earlier work (60) but also agrees with the experimental observations of Parhon and Derevici (54) and Westra and Kunde (74) and the clinical findings of Denis (22), Epstein and Lande(24), Nicholls and Perlzweig (52), Gardner and Gainsborough (26), Bronstein (16), Hurxthal (37, 38), Boyd (13) and Blumgart (8).

Our observations on normal dogs and on the effects of administration of thyroid substance on the plasma cholesterol of normal and thyroidectomized dogs offer possible explanations for many of the conflicting results of earlier investigations.

Before comparing our results with those of other investigators, it is important to note the wide variations in the plasma cholesterol levels of supposedly normal individuals. This is true of both dogs and humans. Our plasma cholesterol values for normal dogs varied between 60 and 240 mg. per cent; Glusker (31) found variations ranging from 68 to 188 mg. per cent. (Bloor has suggested that the variations noted by Glusker were probably the result of dietary variations, but such was not the case in our experiments since the same diet was used throughout the study.) Gardner and Gainsborough (27) found that the cholesterol content of normal human plasma varied from 78 to 227 mg. per cent; Page and associates (53a) found variations from 109 to 376 mg.

per cent and Sperry (66) found values ranging from 132 to 392 mg. per cent.

Several investigators of the relationship between thyroid activity and the level of blood cholesterol have not seriously considered this wide variation in the cholesterol content of normal plasma. Thus Parhon and Derevici (54) concluded that administration of thyroxine to dogs produced a hypocholesterolemia, but their experimental data show an average serum cholesterol level of 134 mg. per cent--an essentially normal value according to our own and Glusker's observations. Mason, Hunt and Hurxthal (47) concluded a hypocholesterolemia to be associated with clinical hyperthyroidism although their data on 47 cases of exophthalmic goitre show 22 cases having plasma cholesterol values above 140 mg. per cent--values well within the normal limits defined by Gardner (27), Page (53) and Sperry (66). The same criticism applies to the conclusions of Wade (72).

Futhermore, the exclusive use of whole blood for cholesterol determinations as in the studies of Wade (72), Epstein and Lande (24) and by Bing and Heckscher (6) is open to objection, since according to our observations and those of Gardner and Gainsborough (26) and Boyd (15) the changes occurring in blood cholesterol in thyroid dysfunction are limited to the plasma.

Our experiments show that administration of large amounts of thyroid substance reduced the plasma cholesterol level appreciably when the levels in the untreated dog were either average normal or higher than average. Thyroxine administration did not lead to a plasma hypocholesterolemia in the true sense, i.e., the plasma

cholesterol was not reduced significantly below the levels found in some normal animals. Critical examinstions of the clinical data of Bonilla and Maya (9), Gardner and Gainsborough (26), Mason, Hunt and Hurxthal(47) Parhon and Ornstein (54) and Boyd and Connell (12), indicates this is also true in clinical hyperthyroidism. The average plasma cholesterol level in a group of cases of clinical hyperthyroidism is lower than the average of normal humans, but individual levels of hyperthyroid patients are within the range of normal values.

Epstein and Lande (24) and Hurxthal (37, 38) have concluded that in hyperthyroidism there is an inverse relation between blood cholesterol level and the basal metabolic rates. Thompson and Thompson (71) have shown that the basal metabolism increases in proportion to the amount of thyroxine or thyroid gland being administered. Therefore, it should follow that ingestion of increasing amounts of thyroid substance should produce progressive decreases in the plasma cholesterol level. This was not true in our dogs--the maximum changes in plasma cholesterol were produced by relatively small doses of thyroxine and 8 times that amount produced no further significant change. This observation does not support the generalizations of Epstein (24) and Hurxthal (37, 38), but it does agree with Cutting's (20) observation--that the inverse relation between basal metabolism and serum cholesterol level held only when the metabolic rates fell between 0 and -30 per cent.

Having demonstrated that experimental variations in thyroid activity produce well defined changes in the level of plasma cholesterol it was important to consider the mechanism of these changes.

Several possible explanations suggest themselves; three in particular have been investigated. These were the following: (1) decreases in plasma cholesterol following ingestion of thyroid substances might be the result of starvation; (2) increases and decreases in plasma cholesterol might be the result of changes in circulating blood volume; (3) the changes in plasma cholesterol concentrations might reflect similar variations in the cholesterol content of all tissues. These possibilities have been studied and the results are presented in the following sections.

II

Effects of Starvation on the Cholesterol Content of Plasma and Erythrocytes.--According to the clinical investigations of Man and Gildea (46), Rosenthal and Patrzek (56), and the animal studies of Wendt (73), hypocholesterolemia is frequently associated with malnutrition. Since hyperthyroidism is also associated with inanition, it seemed possible that the fall in blood cholesterol which frequently occurs in hyperthyroidism might be attributed to the general effects of malnutrition and not to any direct action of the thyroid principle on cholesterol metabolism.

EXPERIMENTAL

Methods

The effect of starvation on blood cholesterol were studied in the 6 dogs previously used as normal controls. Two of these animals, Dog 0 and 5, continued to serve as controls and were maintained on the ordinary diet. Throughout the experimental period, the body weights of these dogs remained constant and the free and total cholesterol content of both plasma and erythrocytes fluctuated within the limits established in the preceding 22 week control period.

The food intake of the remaining animals, Dogs 7, 14, 17 and 404 was reduced by $\frac{1}{2}$ for a period of 3 weeks. This reduction produced no significant loss in weight but a slight fall in plasma cholesterol in Dogs 7, 14 and 404 (Tables XIX, XX and XXII). Further reduction of the food intake to $\frac{1}{4}$ of the maintenance diet was followed by a prompt loss in

body weight without a further decrease in plasma cholesterol except in Dog 17. Increasing the food intake to ½ the maintenance diet checked the weight loss but did not lead to an increase in plasma cholesterol.

Following these periods of starvation the dogs were returned to the standard diet. After a recovery period of 1 week, thyroxine was administered orally in 8 mg. daily doses for a 2 week period. This treatment produced a slight reduction in body weight and a much more marked reduction in plasma cholesterol level than did the restricted food intake (Tables XIX to XXII).

Increasing the food intake to the maximum amount the animals would ingest (approximately one and one half times the normal consumption) and continuing the 8 mg. daily dose of thyroxine led to an increase in body weight in Dogs 7, 17 and 404 and to a slight increase in the plasma cholesterol of these animals. Neither the weight nor the plasma cholesterol of Dog 14 was changed by this treatment. In none of the animals was the plasma cholesterol increased sufficiently to reach the lowest levels found during the starvation periods.

During starvation and during intensive thyroxine treatment, the free cholesterol content of the plasma decreased in proportion to the decrease in total cholesterol so that the free cholesterol percentage remained constant. No significant deviation from the normal was noted in the free or in the total cholesterol content of the erythrocytes.

These observations indicate that starvation is not per se the sole cause for the decrease in plasma cholesterol resulting from administration of thyroxine although it may be one of the contributing factors.

Table XVII

VARIATIONS IN BLOOD CHOLESTEROL OF NORMAL DOG O

Date	Weight	Plasma cholesterol			R.B.C. hemat-ocrit	R.B.C. cholesterol		
		T^1	F^2	PF^3		T^1	F^2	PF^3
	kg.				per cent			
12--4-37 to 4-21-38 /4	27.0	118	25.9	23.1	51	141	136	97
5--5-38		108	25.2	23.3	51	140	140	100
5-19-38		110	25.0	22.7	52	146	138	95
6--2-38		110	24.6	22.4	50	136	133	98
6-16-38	27.3	118	27.6	23.4	50	146	142	97
6-29-38		121	29.6	24.4	50	141	141	99
Average		114	26.3	25.2		142	138	98

T^1--mg. per cent cholesterol

F^2--mg. per cent free cholesterol

PF^3--per cent of cholesterol as free cholesterol

4--average values of 11 determinations

R.B.C.--red blood courpuscles

Table XVIII

VARIATIONS IN BLOOD CHOLESTEROL OF NORMAL DOG 5

Date	Weight	Plasma cholesterol			R.B.C. hemat-ocrit	R.B.C. cholesterol		
		T^1	F^2	PF^3		T^1	F^2	PF^3
	kg.				per cent			
12--4-37 to 4-21-38/4	17.5	135	34.5	25.6	45	138	131	95
5--5-38	17.5	153	38.3	25.0	45	134	128	96
5-19-38	17.6	146	36.3	24.8	43	135	129	96
6--2-38	17.7	145	39.4	26.2	44	134	135	101
6-16-38	17.5	136	33.1	24.3	48	143	135	95
6-29-38	17.0	122	30.7	25.1	47	133	128	96
Average		140	35.4	25.2		136	131	97

T^1--mg. per cent cholesterol

F^2--mg. per cent free cholesterol

PF^3--per cent of cholesterol as free cholesterol

4--average values of 11 determinations

R.B.C.--red blood corpuscles

Table XIX - VARIATIONS IN BLOOD CHOLESTEROL OF DOG 7

Date	Treatment	Weight	Plasma cholesterol			R.B.C. hematocrit	R.B.C. cholesterol		
			T[1]	F[2]	PF[3]		T[1]	F[2]	PF[3]
		kg.				per cent			
12—4-37 to 4-21-38/4	No treatment	15.9	131	32.1	24.5	55	136	135	98
5—5-38	200 gm. food daily from 4-15 to 5—5	15.6	115	29.5	25.6	52	134	127	95
5-19-38	100 gm. food daily from 5—5 to 5-19	13.8	101	23.4	23.2	52	136	127	94
6—2-38	200 gm. food daily from 5-19 to 5-27	13.4	96	25.3	26.6	47	130	132	101
6-16-38	400 gm. food daily from 5-27 to 6-16 8 mg. thyroxine orally daily from 6—2 to 6-19	12.6	82	20.9	25.5	51	135	133	99
6-29-38	600 gm. food daily from 6-19 to 6-29 8 mg. thyroxine orally daily from 6-19 to 6-29	13.2	94	22.0	23.4	50	131	128	98
		Average			24.8		134	130	98

T[1]—mg. per cent cholesterol

F[2]—mg. per cent free cholesterol

PF[3]—per cent of cholesterol as free cholesterol

4—average values of 11 determinations

R.B.C.—red blood corpuscles

Table XX - VARIATIONS IN BLOOD CHOLESTEROL OF DOG 14

Date	Treatment	Weight	Plasma cholesterol			R.B.C. hematocrit	R.B.C. cholesterol		
			T^1	F^2	PF^3		T^1	F^2	PF^3
		kg.				per cent			
12--4-37 to 4-21-38/4	No treatment	12.5	142	35.3	23.3	52	137	132	97
5--5-38	200 gm. food daily from 4-15 to 5--5	12.0	129	32.9	25.5	54	134	132	99
5-19-38	100 gm. food daily from 5--5 to 5-19	11.0				51			
6--2-38	200 gm. food daily from 5-19 to 5-27	10.9	115	25.8	22.4	47	132	126	96
6-16-38	400 gm. food daily from 5-27 to 6-16 8 mg. thyroxine orally daily from 6--2 to 6-19	10.5	83	19.4	23.4	50	133	128	96
6-29-38	600 gm. food daily from 6-19 to 6-29 8 mg. thyroxine orally daily from 6-19 to 6-29	10.5	86	18.8	22.0	51	135	128	95
		Average			23.3		134	129	96

T^1--mg. per cent cholesterol

F^2--mg. per cent free cholesterol

PF^3--per cent of cholesterol as free cholesterol

4--average values of 11 determinations

R.B.C.--red blood corpuscles

Table XXI - VARIATIONS IN BLOOD CHOLESTEROL OF DOG 17

Date	Treatment	Weight	Plasma cholesterol			R.B.C. hemat-ocrit	R.B.C. cholesterol		
			T^1	F^2	PF^3		T^1	F^2	PF^5
		kg.				per cent			
12--4-37 to 4-21-38/4	No treatment	17.6	119	28.4	24.3	52	134	130	98
5--5-38	200 gm. food daily from 4-15 to 5--5	17.2	114	29.6	25.9	54	134	135	101
5-19-38	100 gm. food daily from 5-15 to 5-19	15.9	115	26.1	22.7	50	130	129	99
6--2-38	200 gm. food daily from 5-19 to 5-27	15.3	117	31.7	27.1	53	130	127	98
6-16-38	400 gm. food daily from 5-27 to 6-16 8 mg. thyroxine orally daily from 6--2 to 6-19	14.7	71	18.2	25.6	50	133	128	96
6-29-38	600 gm. food daily from 6-19 to 6-29 8 mg. thyroxine orally daily from 6-19 to 6-29	16.3	89	22.0	24.6	42	136	133	98
		Average			25.5		133	130	98

T^1--mg. per cent cholesterol
F^2--mg. per cent free cholesterol
PF^3--per cent of cholesterol as free cholesterol
4--average values of 11 determinations
R.B.C.--red blood corpuscles

Table XXII - VARIATIONS IN BLOOD CHOLESTEROL OF DOG 404

Date	Treatment	Weight	Plasma cholesterol			R.B.C. hematocrit	R.B.C. cholesterol		
			T^1	F^2	PF^3		T^1	F^2	PF^3
		kg.				per cent			
12--4-37 to 4-21-38/4	No treatment	13.0	141	34.2	24.3	53	138	134	97
5--5-38	200 gm. food daily from 4-15 to 5--5	12.8	117	32.7	27.9	53	132	127	96
5-19-38	100 gm. food daily from 5-15 to 5-19	11.2	113	27.0	23.9	53	128	128	100
6--2-38	200 gm. food daily from 5-19 to 5-27	10.9	105	22.7	21.6	50	130	129	99
6-16-38	400 gm. food daily from 5-27 to 6-16 8 mg. thyroxine orally daily from 6--2 to 6-19	10.2	73	18.4	25.2	50	138	133	96
6-29-38	600 gm. food daily from 6-19 to 6-29 8 mg. thyroxine orally daily from 6-19 to 6-29	11.1	91	23.3	25.6	47	133	133	100
		Average			24.7		133	131	98

T^1--mg. per cent cholesterol

F^2--mg. per cent free cholesterol

PF^3--per cent of cholesterol as free cholesterol

4--average values of 11 determinations

R.B.C.--red blood corpuscles

III

Are the Changes in Plasma Cholesterol in Experimental Hyperthyroidism and Hypothyroidism Due to Changes in Blood Volume?--Certain clinical and experimental observations have indicated that the increase in plasma cholesterol following thyroidectomy and the decrease following the administration of thyroid substance might be the result of plasma concentration or dilution. The clinical studies of Chang (18), Goldbloom and Libin (32), and Gibson and Harris (30) show that there is an abnormally high circulating blood volume in hyperthyroidism. Thompson (70), Blumgart (8), Gargill and Gilligan (28), Holbell (35) and Gibson and Harris (30) show that there is an abnormally low blood volume in myxoedema. Frielander, Laskey and Silbert (25) found that thyroidectomy in the male cat led to a decrease in blood volume, but in the female produced no significant change.

These observations suggested the necessity of determinining whether the changes in plasma cholesterol which we have observed in experimental hyperthyroidism and hypothyroidism are the result of plasma dilution and concentration.

EXPERIMENTAL

Methods

Four dogs, litter mates, were used in this study. Two animals, Dogs 20 and 140, served as normal controls. The other two, Dogs 110 and 170, were thyroidectomized and 2 weeks later were fed varying amounts of thyroxine for a 4 week period. Tetany was controlled by administration of parathyroid extract as in previous experiments.

Fasting blood samples were obtained weekly by cardiac puncture and were heparinized. Cell--plasma ratios, and free and total cholesterol content of plasma and erythrocytes were determined by the methods outlined above.

Blood volume determinations were made at weekly intervals according to the method of Keith, Rowntree and Geraghty (40), as modified by Hooper, Smith, Belt and Whipple (36). A known amount of vital red dye was injected in the saphenous vein. Four minutes later a sample of blood was drawn from the corresponding vein of the opposite leg and mixed with a known volume of isotonic sodium oxalate solution. The plasma was separated from the cells by centrifugation and the concentration of vital red therein determined by colorimetric comparison with a standard solution of the dye.

Results

During the 2 months of study, the plasma volume, the plasma cholesterol concentration and the total amount of cholesterol in plasma in the control animals, Dogs 20 and 140, remained remarkably constant. In Dog 20 (Table XXIII), the plasma cholesterol concentration varied between 145 and 167 mg. per cent and the total amount of plasma cholesterol varied between 851 and 940 mg. In Dog 140 (Table XXIV), the plasma cholesterol concentration varied between 184 and 202 mg. per cent, while the total amount of plasma cholesterol varied between 1548 and 1662 mg.

The data in Tables XXV and XXVI show that changes in circulating plasma volume do not entirely account for the changes in plasma cholesterol. In Dog 110, the plasma volume decreased from 609 cc. to 559 cc.

following thyroidectomy. The plasma cholesterol concentration increased to a much greater degree, from 165 to 280 mg. per cent, so that the total amount of cholesterol in circulating plasma increased from 1005 to 1565 mg. After treatment with thyroxine for 3 weeks, plasma volume increased to 699 cc. while the plasma cholesterol concentration fell to 86 mg. per cent so that total amount of cholesterol in plasma was only 600 mg. The variations in Dog 170 were similar to those just described. The post-thyroidectomy increases in concentration and in total amount of plasma cholesterol were not as great as in Dog 110. The effects of thyroxine treatment were more marked.

These data justify the conclusion that changes in plasma volume cannot account entirely for the changes in plasma cholesterol observed during experimental variations in thyroid activity. This conclusion was anticipated in a previous study (60).

Table XXIII

VARIATIONS IN BLOOD VOLUME AND BLOOD CHOLESTEROL OF NORMAL DOG 20

Date	Total plasma volume	R.B.C. hemat-ocrit	Total blood volume	Plasma cholesterol per 100 cc.	Total plasma cholesterol
	cc.	per cent	cc.	mg.	mg.
11-16-38	548	48	1054	167	915
11-30-38	587	50	1174	151	884
12-14-38	584	50	1168	150	876
12-21-38	582	50	1164	157	914
12-28-38	584	50	1168	161	940
1- 4-39	587	50	1174	145	851
1-18-39	572	50	1144	150	858

Table XXIV

VARIATIONS IN BLOOD VOLUME AND BLOOD CHOLESTEROL
OF NORMAL DOG 140

Date	Total plasma volume	R.B.C. hemat-ocrit	Total blood volume	Plasma cholesterol per 100 cc.	Total plasma cholesterol
	cc.	per cent	cc.	mg.	mg.
11-30-38	823	45	1515	202	1662
12-14-38	802	48	1542	199	1596
12-21-38	845	47	1594	184	1555
12-28-38	837	48	1609	185	1548
1- 4-39	802	48	1609	199	1596
1-18-39		50		201	

Table XXV - VARIATIONS IN BLOOD CHOLESTEROL OF DOG 110

Date	Treatment	Total plasma volume	R.B.C. hemat-ocrit	Total blood volume	Plasma cholesterol per 100 cc.	Total plasma cholesterol
		cc.	per cent	cc.	mg.	mg.
11-16-38	No treatment	609	47	1149	165	1005
11-30-38	No treatment		47		151	
12-14-38	Thyroidectomized 2--1-38	559	44	998	280	1565
12-21-38	No treatment	579	42	998	207	1198
12-28-38	2 mg. thyroxine orally daily from 12-21 to 12-28	595	42	1026	179	1065
1--4-39	2 mg. thyroxine orally daily from 12-28 to 1--4	662	42	1141	135	894
1-18-39	8 mg. thyroxine orally daily from 1--4 to 1-18	699	44	1249	86	600
2--1-39	No treatment	602	40	956	166	999
2--8-39	No treatment	614	37	990	158	970
2-15-39	No treatment				250	

Table XXVI - VARIATIONS IN BLOOD CHOLESTEROL OF DOG 170

Date	Treatment	Total plasma volume	R.B.C. hemat-ocrit	Total blood volume	Plasma cholesterol per 100 cc.	Total plasma cholesterol
		cc.	per cent	cc.	mg.	mg.
11-16-38	No treatment	618	42	1066	233	1440
11-30-38	No treatment	643	42	1109	202	1299
12-14-38	Thyroidectomized 2--1-38	570	40	950	287	1636
12-21-38	No treatment	572	38	923	251	1436
12-28-38	2 mg. thyroxine orally daily from 12-21 to 12-28	579	39	950	172	976
1--4-39	4 mg. thyroxine orally daily from 12-28 to 1--4	625	39	1024	115	719
1-18-39	8 mg. thyroxine orally daily from 1--4 to 1-18	596	43	1044	68	405
2--1-39	No treatment	603	43	1060	172	1037
2--8-39	No treatment	594	42	1024	214	1271
2-15-39					287	

IV

Is the Decrease in Plasma Cholesterol Observed in Experimental Hyperthyroidism a Reflection of a General Decrease in Cholesterol Content of the Body?--The possibility suggested itself that the decrease in plasma cholesterol might reflect a general decrease in the cholesterol content of the entire organism. To investigate this possibility, a study was made of the influence of thyroxine on the cholesterol content of the mouse body.

EXPERIMENTAL

Methods

For this study, 64 full grown male, albino mice were used. Thirty served as normal controls. The remaining 34 received varying amounts of crystalline thyroxine administered subcutaneously for varying periods of time. Since sensitivity to thyroxine varied considerably in different animals, it was necessary to divide the mice into groups, according to the amounts of thyroxine required to produce a 20 to 25 per eent loss of weight (Table XXVIII). During the experiment the animals were fed oats in an unlimited quanity, unless othervise stated.

The entire group was sacrificed at the end of the experimental period. Blood was obtained from the heart of each animal, heparinized and pooled with that from the other animals. The cells and plasma were separated by centrifugation, extracted with alcohol-ether mixture and the free and total cholesterol contents determined as dexcribed previously.

The animal body was digested and the cholesterol extracted from the neutralized digest according to the method of Schoenheimer and Breusch

(63). Cholesterol was determined by the oxidative method of Yasuda (78).

Results

Following administration of thyroxine the plasma cholesterol of the mouse decreased to even a greater extent than in the dog (Table XXVII). The plasma cholesterol of groups of normal control mice varied between 127 and 148 mg. per cent, whereas that of the mice treated with thyroxine varied from 44 to 74 mg. per cent. As was the case in dogs, thyroxine had no effect on per cent of free cholesterol in the plasma or the cholesterol content of the erythrocytes.

The cholesterol content of the control mouse body was relatively constant varying from 0.19 to 0.24 per cent (Table XXVIII). The per cent cholesterol in the thyroxine treated mice seemed to be higher than that of the normal controls when calculations were based on the final weights of the mice. When cholesterol percentages were calculated on the basis of initial weights, it became evident that thyroxine treatment produced no change in body cholesterol content and that the apparent increase was only a relative one, due to loss in body weight.

In connection with these studies, the effects of starvation on plasma cholesterol concentrations and on body cholesterol content were also studies. A group of 20 mice was placed on a diet of oats, restricted to an amount that produced a 20 per cent body weight loss in 14 days.

The reduced food intake produced a reduction in plasma cholesterol levels (Group VII, Table XXVIII). However, this reduction was very slight as compared to that produced by thyroxine treatment (Groups III - VI, Table XXVII).

The percentage of cholesterol in the bodies of the starved animals was identical with that of the normal controls, when calculations were based on final weight, but was significantly lower when based on initial weights. This effect is in sharp contrast to the effect observed in thyroxine treated mice.

Table XXVII -- VARIATIONS IN BLOOD CHOLESTEROL OF NORMAL, HYPERTHYROID AND STARVED MICE

Group No.	Treatment	No. mice in sub-groups	Plasma cholesterol			R.B.C. cholesterol
			T[1]	F[2]	PF[3]	T[1]
I	Untreated controls - 2 weeks	5	136	33.7	24.8	143
		5	137	32.7	23.9	143
II	Untreated controls - 3 weeks	5	148	31.8	21.5	138
		5	146	33.9	23.2	138
		5	128	32.1	25.1	147
		5	127	32.7	25.8	145
III	Each received 3.7 mg thyroxine subcutaneously in 2 weeks	6	44	9.6	21.8	143
IV	Each received 5.3 mg thyroxine subcutaneously in 2½ weeks	5	64	14.4	22.5	142
		6	60	13.5	22.5	143
V	Each received 3.5 mg thyroxine subcutaneously in 3½ weeks	5	74	19.2	25.9	131

Table XXVIII - VARIATIONS IN BODY CHOLESTEROL OF NORMAL, HYPERTHYROID AND STARVED MICE

Group No.	No. of mice in group	Treatment	Average weight		Per cent cholesterol in mouse body					
			Initial	Final	Calc. on initial weight			Calc. on final weight		
			gm.	gm.	max.	min.	mean	max.	min.	mean
I	10	Untreated Controls 2 weeks	22.3	23.4				0.24	0.21	0.23
II	20	Untreated Controls 3 weeks	21.6	23.0				0.24	0.19	0.22
III	6	Each received 3.7 mg. thyroxine subcutaneously in 2 weeks	22.7	18.4	0.23	0.21	0.22	0.28	0.25	0.27
IV	11	Each received 5.3 mg. thyroxine subcatene-ously in $2\frac{1}{2}$ weeks	22.7	19.5	0.24	0.22	0.23	0.29	0.22	0.26
V	9	Each received 3.5 mg. thyroxine subcutaneously in $3\frac{1}{2}$ weeks	21.6	16.3	0.24	0.21	0.23	0.35	0.29	0.31
VI	8	Each received 5.9 mg. thyroxine subcutaneously in 4 weeks	23.0	19.8	0.23	0.20	0.22	0.25	0.24	0.25
VII	20	Restricted food intake for 2 weeks	21.9	17.8	0.20	0.15	0.18	0.25	0.19	0.22

Discussion

The preceding experiments warrant the conclusion that the decreases in plasma cholesterol observed in experimental hyperthyrodism are not entirely the result of either starvation or increases in circulating plasma volume; nor are they merely a reflection of a decrease in the cholesterol content of the entire organism. One may also conclude that the hypercholesterolemia, developing subsequent to thyroidectomy, is not due to a decrease in plasma volume.

These facts limit the possible explanations for the changes in plasma cholesterol, but the real explanation is not yet apparent. Certain possibile explanations may be suggested. These are: (1) that the changes in plasma cholesterol are the result of altered excretion of cholesterol in either bile or large intestine; (2) that they are due to altered absorption of cholesterol from the small intestine; (3) that they represent the retention of cholesterol by specific organs of the body or mobilization from these organs; (4) that they reflect increases or decreases in the transport of fatty acids, induced by heightened or lowered metabolic activity; (5) that they result from stimulation or inhibition of the activity of some organ regulating the level of plasma cholesterol; (6) that they are the result of increased synthesis or decreased destruction or decreased synthesis and increased destruction.

1. Are the changes in plasma cholesterol due to altered excretion of cholesterol in either the bile or large intestine?-- It is very un-

likely that the increase in plasma cholesterol, following thyroidectomy, results from a retention of cholesterol which would normally be eliminated in the bile. Thyroidectomy in dogs causes a slight increase in the excretion of biliary cholesterol while administration of thyroxine to the normal animal causes a small decrease in biliary cholesterol (4 a and 4 b). Even if these observations could be neglected, the retention of the entire output of biliary cholesterol (33) could not cause as large changes in plasma cholesterol as were observed in the dog following thyroidectomy.

There are no observations on the effects of variations in thyroid activity on the excretion of cholesterol in the large intestine. It has been observed by Sperry (67, 68 and 69) Beumer and Hepner (3) that in the absence of bile from the intestine enormous quantities of sterol are excreted from the large intestine. According to Schoenheimer and Sperry (62) this sterol is not unabsorbed food cholesterol but represents a secretion of the large intestine. Numerous workers have found that administration of thyroxine or clinical hyperthyroidism leads to liver injury and it is not unlikely that this liver injury is associated with a lowered secretion of bile salts in the bile. Were such the case the decrease in plasma cholesterol resulting from administration of thyroxine might be due to an increased excretion of sterol by the large intestine.

2. Are the changes in plasma cholesterol due to altered absorption of cholesterol from the small intestine?--The available evidence does not favor this possibility. In the first place our experiments were conducted on fasting animals which should rule out the absorption

factor. Secondly, studies on alimentary lipemia show that there is greater post absorptive increase in plasma cholesterol in thyroxine treated than in normal dogs. This observation is in accord with that of Althausen and Stockholm (1), who found that the rate of carbohydrate absorption was increased following administration of thyroxine.

3. Are the changes in plasma cholesterol due to retention of cholesterol by specific organs of the body or mobilization from these organs?-- It seems quite possible that there is a redistribution of cholesterol as a result of increased or decreased thyroid activity. Our observations on mice showed that the cholesterol of the entire mouse body was not altered following administration of thyroxine, notwithstanding the 50 per cent decrease in cholesterol of blood plasma. Sasaki (58) found that the cholesterol content of cerebrum, cerebellum and cord was the same in groups of normal and thyroxine treated rabbits. Schmidt (59) examined the livers and skeletal muscles of these same rabbits and found that the cholesterol levels in the livers and muscles of the group receiving thyroxine was 15 to 20 per cent higher than those of the normals. Page and Pasternak (53b) made a similar observation in both rabbits and rats. Thus one might conclude that the reduction in plasma cholesterol in experimental hyperthyroidism was due to a "taking up" of the cholesterol by liver and muscle.

Whereas this explanation might be satisfactory for the observations in experimental hyperthyroidism, it is not applicable to the plasma cholesterol increases in hypothyroidism. For, Artom (2) has found that thyroidectomy leads to a 2 fold increase in the cholesterol content of the liver, an even greater increase than Schmidt and Page found after adminis-

stration of thyroxine.

4. Do the changes in plasma cholesterol reflect the participation of cholesterol in fatty acid transport?--The fact that the changes in blood cholesterol were confined to the plasma argues in favor of a disturbance in the transport of cholesterol. Whether the changes result from a disturbance in fatty acid transport may be questioned seriously. In the first place there is considerable question whether plasma cholesterol participates in fatty acid transport. Secondly, the evidence offered in support of the transport theory is the increase in proportion of esterified cholesterol during fatty acid absorption. No change in proportion of esterified cholesterol was found in our experiments. This would be an argument against the explanation that the changes in plasma cholesterol were the result of a disturbance in fatty acid transport.

5. Are the changes the result of inhibition or stimulation of the activity of some organ regulating the level of plasma cholesterol?--The liver, large intestine, and kidney seem to be the 3 organs most intimately connected with regulation of plasma cholesterol level. The manner in which changes in thyroid activity alter the functioning of these organs is not known. Certain possibilies connected with liver and large intestine have been discussed before.

One observation on the role of the kidney in cholesterol metabolism is of particular interest. Disease of the kidney such as nephrosis or nephritis are invaribly associated with an elevation in plasma cholesterol. Miyazaki (50) found that the kidney normally contained a hormone which would lower the plasma cholesterol of either normal or nephrectomized

animals. If his observations are correct, it may be that increased circulatory rate in hyperthyroidism leads to overproduction of this hormone and a reduction in plasma cholesterol. The reduced circulatory rate in hypothyrodisism would have an opposite effect.

6. Are the changes in plasma cholesterol the result of increased synthesis or destruction?--The experiments of Schoenheimer and Breusch (63) and Dam (21) have shown that cholesterol is both synthesized and destroyed in the animal body. The decrease in plasma cholesterol in hyperthyroidism might well be due to an increased destruction of this substance, or to a decreased synthesis. The reverse condition might be true in hypothyroidism. If such did occur we would have a logical explanation for our changes in plasma cholesterol. At present balance experiments are not available to prove this point, but it is one which must be seriously considered.

Summary

1. Thyroidectomy in the dog was followed by an increase in blood cholesterol content, and this increase was limited to the plasma.

2. The administration of thyroid substance promptly reduced the hypercholesterolemia of experimental hypothyroidism, and small doses were as effective as large doses in producing this reduction.

3. Massive doses of thyroid substance produce a decrease in the plasma cholesterol of the normal dog, while small doses were without effect.

4. The magnitude of reduction in plasma cholesterol of both the normal and the thyroidectomized dog following thyroid administration appeared to be a function of pretreatment plasma cholesterol levels--the higher this level, the greater the percentage reduction.

5. The free cholesterol content of the plasma increased following thyroidectomy and decreased following thyroid treatment in direct proportion to changes in total cholesterol so that the ratio normally existing between free and total cholesterol remained unchanged.

6. The cholesterol content of the erythrocytes was unaffected by thyroidectomy or by thyroid treatment.

7. The hypocholesterolemia of experimental hyperthyroidism cannot be solely ascribed to inanition.

8. The hypocholesterolemia of experimental hyperthyroidism and the hypercholesteremia of experimental hypothyroidism cannot be explained by concomitant changes in the circulating blood volume.

9. The hypocholesterolemia of experimental hyperthyroidism in the mouse is not a reflection of a general decrease in body cholesterol content.

10. Other possible explanations for the changes occurring in plasma cholesterol in thyroid dysfunction are discussed.

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