33. CHEMICAL CHANGES IN THE SKIN IN EXPERIMENTAL HYPERTHYROIDISM

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THE thyroid hormone affects a great number of organs and metabolic processes. The actions of the thyroid on glycogen and fat metabolism, on vitamin utilization etc. are familiar and a relationship between the thyroid hormone and the skin has long been recognized. A soft, warm, slightly sweating skin is among the characteristic symptoms of hyperthyroidism, whilst changes of the skin observed in myxoedema were of historic importance for the development of knowledge of thyroid function. Not much is known, however, concerning the chemical changes in the skin produced by lack or excess of thyroid hormone. The following experiments were carried out as a contribution to knowledge of skin metabolism in experimental hyperthyroidism.

EXPERIMENTAL

White rats of 180–250 g., both female and male, were used for the experiments. They were fed on a diet of milk, bread and raw vegetables. To produce hyperthyroidism both thyroid gland and thyroxine were used. After varying times of treatment with thyroid hormone the animals were killed; as much of the skin as possible was removed, freed from fat, connective tissue and hairs and finely minced. After drying for 24 hr. at 20–24° it was analysed.

Determination of total lipin and total cholesterol

1 g. of dried skin was extracted several times with warm alcohol and then with warm ether until the extracts on evaporation left no residue. The extracts were mixed and evaporated and the residue treated several times with chloroform; the chloroform extracts were made up to 25 ml. and aliquot portions of this solution were used for the determination of total lipin and cholesterol. Several checks showed that extraction of fat and cholesterol by this method was complete. Cholesterol and cholesteryl esters were fractionated by means of digitonin; on the average 42.55% of the total cholesterol was found to be esterified. Knudson *et al.* [1938] found on the average 47% cholesterol ester in the skin.

For *determination of water content* the skin was dried at 105° to constant weight.

For *determination of chloride* a carefully weighed sample of skin was ashed with nitric acid after addition of a known quantity of silver nitrate. The excess silver was back-titrated with ammonium thiocyanate.

The experiments on *water imbibition* were carried out on weighed samples of fresh skin freed from hairs. These were immersed 24–48 hr. in buffer solutions of various pH, then dried several times with filter paper and weighed.

In some experiments the cholesterol contents of the lungs and the blood were also determined.

All estimations were carried out in duplicate.

The average normal values for total cholesterol, total lipin, water and dry weight of the skin were at first determined on 13 animals of both sexes. The skin contained on the average 261 mg./100 g. of total cholesterol. Knudson *et al.* [1938] found in the skin of the rat an average of 218 mg./100 ml. total cholesterol. The total fat showed considerable variation with an average of 25.82 %. This figure compares favourably with the data of Matthews *et al.* [1935] who found in rat skin an average of 25.72 % total lipin. The ratio total cholesterol : total lipin varies within a relatively small range and averages 0.008.

The water content of normal skin is 55.72 %.

Oral treatment for 5 days with 0.1 g. thyroid daily brings about marked changes in these figures. The total cholesterol content rises to 471 mg./100 g. on the average, i.e. by about +81 %. A similar rise occurs in the total cholesterol : total lipin ratio, which now averages 0.014 as compared with 0.008 in the normal skin. The average water content of the skin rises by 3 %.

Prolongation of the administration of thyroid to 7 days does not alter these figures considerably, but after 15 days the changes become more pronounced. The average cholesterol content of the skin is then 700.29 mg./100 g., whilst the cholesterol : fat ratio rises to 0.023, i.e. almost 3 times the value in normal animals.

Repeated subcutaneous injections of thyroxine have a similar profound effect on the cholesterol content of the skin; 6 rats were injected subcutaneously with 0.75 mg. thyroxine daily for 24 days. The average cholesterol content of the skin was increased to 649.45 mg./100 g., and the cholesterol : fat ratio rose to 8 times the normal value.

Table 1. *Analysis of the skin of normal rats and of rats after administration of thyroid gland or thyroxine (in brackets number of observations).*

	Total cholesterol of dry skin mg./100 g.	Total lipin of dry skin %	Fat content of dry skin %	Chole- sterol : fat ratio	Water content of fresh skin %	Chloride content of dry skin mg./100 g.
Normal						
Highest	363.64	47.33	46.96	0.013	64.21	917.97
Lowest	181.80	13.57	13.39	0.06	50.37	690.58
Mean	261.00 (13)	25.82 (10)	25.56 (11)	0.008 (9)	55.72 (9)	808.91 (4)
0.1 g. thyroid for 5 days						
Highest	520.50	45.05	44.63	0.017	60.91	992.10
Lowest	423.28	28.26	27.78	0.010	56.33	653.51
Mean	471.51 (4)	34.60 (4)	34.11 (4)	0.014 (4)	58.76 (4)	788.69 (4)
0.1 g. thyroid for 7 days						
Highest	641.80	43.75	43.32	0.016	62.82	1061.10
Lowest	294.10	19.04	18.74	0.008	46.60	596.26
Mean	437.61 (13)	32.23 (13)	31.80 (13)	0.014 (13)	59.74 (10)	778.83 (5)
0.1 g. thyroid for 10 days						
Highest	498.67	22.35	21.82	0.025	—	—
Lowest	336.06	20.15	19.67	0.022	—	—
Mean	447.76 (3)	20.89 (3)	20.43 (3)	0.021 (3)	—	—
0.1 g. thyroid for 15 days						
Highest	752.70	43.25	42.61	0.184	68.41	1185.80
Lowest	629.26	3.77	3.08	0.015	57.24	877.53
Mean	700.29 (11)	20.17 (11)	19.47 (11)	0.023 (11)	62.94 (11)	1057.16 (6)
0.75 mg. thyroxine daily subcutaneously for 24 days						
Highest	788.17 (1)	35.49 (1)	35.17 (1)	0.123	66.51	1169.00
Lowest	316.60	6.06	5.32	0.009	59.35	875.86
Mean	649.45 (6)	15.65 (6)	15.00 (6)	0.064 (6)	63.78 (6)	961.19 (6)

The chloride and water contents of the skin are closely correlated physiologically. Both values increase in pronounced hyperthyroidism. After 15 days' administration of 0.1 g. thyroid daily the average chloride content of the skin rises from 808.91 mg./100 g. to 1057.16 mg./100 g. The average water content of the skin rises simultaneously by 7%. The skin of the animals treated for 24 days with thyroxine contains 961.19 mg./100 g. chloride and 63.78% water on the average, which means an average increase of 152.28 mg./100 g. in the chloride and of 8.06% in the water content; 5 days' administration of thyroid has little or no effect on the chloride and water contents of the skin.

The results are summarized in Table 1, and those of the cholesterol determinations are represented graphically in Fig. 1.

Fig. 1. Average cholesterol content of the skin (mg./100 g.) of normal rats and of rats after administration of thyroid gland or thyroxine.

The rise of the chloride and water content of the skin during hyperthyroidism suggests an increased water-binding power of the constituents of the skin.

To examine this point, swelling experiments were carried out. Carefully weighed samples of fresh skin were immersed in buffer solutions of pH 2.92, 3.67, 4.41, 6.10, 8.92, 11.06 and 12.97. Swelling is most pronounced at pH 3.67 and 12.97. On the acid side, at pH 2.92, normal skin still swells measurably. At this pH the swelling of the skin of the hyperthyroidic animal, however, is so marked that complete gelation occurs and reliable determinations become impossible. Each swelling experiment lasted 24-48 hr. The figures below illustrate the results obtained.

*Average increase of weight (%) of skin samples during 24-48 hr.
swelling in buffer solutions*

	pH 3.67	pH 12.97
Animal No. 11 (normal)	+ 29.82	+ 55.65
Animal No. 12 (normal)	46.68	53.26
Animal No. 13 (normal)	48.00	80.71
Animal No. 35, after 14 days' administration of thyroid	+ 157.5	+ 96
Animal No. 36, after 14 days' administration of thyroid	140.0	192.5
Animal No. 37, after 14 days' administration of thyroid	112.5	101.0
		15-2

Analyses were also made of the cholesterol of the lungs and blood of normal and hyperthyroidic rats. The lungs of groups of 6 animals gave the following results in mg./100 g.: (a) normal animals: 461.90, 465.22, 392.17, 420.70, 486.43, 337.10; average: 427.10; (b) animals after 16 days' administration of 0.1 g. thyroid daily: 469.82, 479.17, 550.06, 457.15, 485.19, 480.76; average: 487; (c) animals after 24 days' administration of 0.75 mg. thyroxine daily: 387.05, 423.80, 419.20, 439.00, 285.30, 359.87; average: 384.10.

The changes are thus slight and irregular, and indeed scarcely outside the range of physiological variation.

The cholesterol of human blood in cases of hypo- or hyper-function of the thyroid has been studied by several workers. The data in the literature are divergent, but the majority of authors agree that in cases of hypo-function the cholesterol content of the blood is increased; in hyperthyroidism, irregular variations have been observed. A decrease of blood cholesterol is certainly not as constant a finding in hyperthyroidism as is an increase in myxoedema [cf. Schmidt & Hughes, 1938]. In the experiments reported here experimental hyperthyroidism did not cause marked changes in the cholesterol content of the blood.

The cholesterol content of the blood of the normal rats used in these experiments averaged 40 mg./100 ml. After 7 days' administration of 0.1 g. dried thyroid daily the following values (mg./100 ml.) for blood cholesterol were found: 53.13, 44.94, 39.47, 35.84, 41.44; average: 42.97. After 2 weeks of the same treatment the values of blood cholesterol content were: 42.12, 47.26, 43.02, 53.19, 44.94, 39.47, 35.84, 41.44; average: 43.40.

DISCUSSION

Among the characteristic symptoms of hyperthyroidism is an increased liberation of energy and correspondingly an increased loss of heat. As is well known this loss occurs mainly by means of radiation, heat conduction and evaporation of water from the skin. These regulating mechanisms are used to a large extent during thyrotoxicosis.

Cardiac output is also increased during hyperthyroidism, the small blood vessels being dilated. By the increased circulation in the skin more heat is transported to the surface of the body, thus facilitating the loss of heat to the surroundings. As shown in the present investigation, administration of thyroid gland also diminishes the fat content of the skin. Fat is a poor heat conductor and, under physiological conditions, protects the organism against undue loss of heat. Its decrease during hyperthyroidism will therefore facilitate the increased loss of heat.

Another process of importance for the increased loss of heat through the skin is the evaporation of water in the form of both visible and invisible perspiration. The increased cholesterol content in the skin during hyperthyroidism may play a part in this connexion, since cholesterol enhances the imbibition of water by the tissues. Apart from the absolute cholesterol content the cholesterol : fat ratio is particularly important; this ratio (called "coefficient lipocylique" by French authors [cf. Mayer *et al.* 1938]) actually rises during hyperthyroidism up to 10 times its normal value. The skin can then take up considerably larger quantities of water, the evaporation of which will contribute to the heat loss. Increases in both water content and swelling power of the skin after prolonged thyroid administration are, as shown above, actually demonstrable.

Water and chloride metabolism usually change in parallel fashion. In the present experiments this rule is followed, the increase of water content of the

skin being accompanied by a corresponding increase of chloride content. These changes, however, occur only during pronounced hyperthyroidism; administration of small quantities of thyroid hormone scarcely affects the normal values, as shown in Table 2.

Table 2

	Chloride content of the skin mg./100 g.	Water content of the skin %
Normal	808.91	55.72
After 5 days' administration of thyroid powder	788.70	56.90
After 7 days' administration of thyroid powder	973.54	58.76
After 15 days' administration of thyroid powder	1057.16	62.94
After 24 days of thyroxine	1005.53	63.78

The source of the great quantities of cholesterol which appear in the skin during experimental hyperthyroidism is unknown. Whether this material is mobilized or newly synthesized can only be decided by further experiments. The cholesterol content of the blood, however, did not change during thyroid administration in the present experiments, which does not favour the assumption of cholesterol mobilization from other organs; moreover, in the lungs no decrease of cholesterol could be demonstrated. It is, however, possible that the cholesterol comes from other organs, perhaps from the liver, and the possibility of new formation of cholesterol in the organism (particularly in the liver) is not excluded.

The skin contributes not only to the excretion of water, but also of iodine. During experimental hyperthyroidism the skin of rats is considerably richer in iodine than is that of normal animals [Abelin & Wehren, 1940], and it gives off more iodine to the surroundings than the normal skin. Whether the changed chemical composition of the skin, particularly the increased cholesterol content, is related to its iodine content is however not yet clear.

SUMMARY

The skin of rats after administration of thyroid gland or thyroxine over a considerable period shows the following changes in its chemical composition: its cholesterol, chloride and water contents rise, while the neutral fat content diminishes. The skin of rats in experimental hyperthyroidism has an increased water-binding power.

By facilitating the loss of heat from the surface of the skin these chemical changes are related to heat regulation.

The low fat content enhances the heat conduction and the high cholesterol content (up to 300 % of the normal values) favours the water imbibition of the skin and the succeeding evaporation of water. Possibly also loss of iodine through the skin is favoured.

No definite changes occur in the cholesterol content of the lungs and the blood during thyroid administration.

REFERENCES

- Abelin & Wehren (1940). *Arch. int. Pharmacodyn.* **64**, 91.
 Knudson, Sturges & Bryan (1938). *J. biol. Chem.* **126**, 547.
 Matthews, Newton & Bloor (1935). *J. biol. Chem.* **108**, 145.
 Mayer, Schaeffer & Terroine. Cf. Cristol (1938). *Précis de chimie biologique médicale*, Paris.
 Schmidt & Hughes (1938). *Endocrinology*, **22**, 474.