Zeitschrift: Bulletin der Schweizerischen Akademie der Medizinischen Wissenschaften = Bulletin de l'Académie suisse des sciences médicales = Bollettino dell' Accademia svizzera delle scienze mediche

Herausgeber: Schweizerische Akademie der Medizinischen Wissenschaften

Band: 13 (1957)

Heft: 1-4: Symposium über Arteriosklerose = Symposium sur l'artériosclérose = Symposium on arteriosclerosis

Artikel: The effect of thyroxine analogues on lipid an lipoprotein metabolism

Autor: Boyd, G.S. / Oliver, M.F.

DOI: https://doi.org/10.5169/seals-307333

D.C. 616.13-002.2:612.123:615.361.44

Department of Biochemistry, University of Edinburgh
and Department of Cardiology, Royal Infirmary, Edinburgh

The Effect of Thyroxine Analogues on Lipid and Lipoprotein Metabolism

By **G. S. Boyd** and **M. F. Oliver**

It is well known that thyroid activity influences the circulating cholesterol and phospholipids, and that in hypothyroid and euthyroid subjects these values can be lowered by the administration of thyroid extract (*Gildea*, *Man* and *Peters* [1939]; *Turner* and *Steiner* [1939]). Associated with this effect there is concomitant elevation of the basal metabolic rate. Similarly, thyroxine reduces the β-lipoproteins (*Malmros* and *Swahn* [1953]) and lowers the concentration of low density lipoproteins, S_f 0–20 (*Strisower*, *Gofman*, *Galioni*, *Almada* and *Simon* [1954]). However, the ratio of cholesterol bound to the α- and β-lipoproteins may not be altered (*Barr* [1955]). The principal compounds secreted by the thyroid are thyroxine and triiodothyronine, which is derived from thyroxine by de-iodination and has similar physiological actions. The mode of action of these thyroid hormones has not yet been elucidated, but triiodothyronine can undergo oxidative deamination to triiodothyropyruvic acid with subsequent oxidative decarboxylation of the latter compound to triiodothyroacetic acid (triac) (Fig. 1). This acetic acid

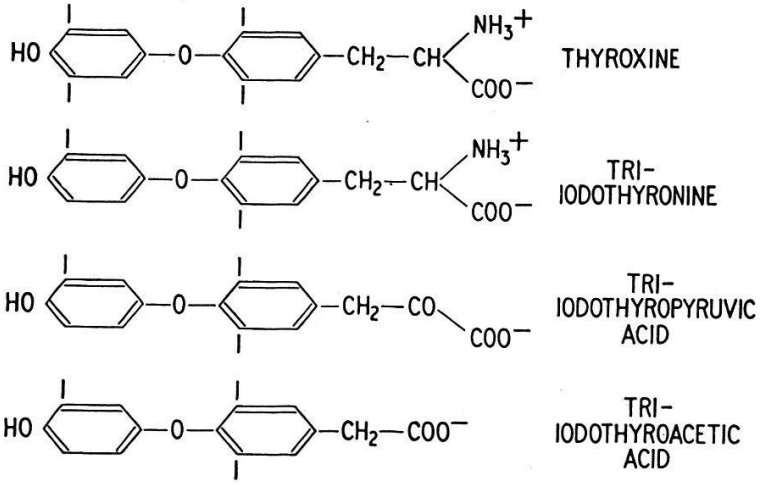

Fig. 1. The formulae of thyroxine and thyroxine analogues.

analogue of triiodothyronine was first synthesised by *Pitt-Rivers* (1953). It has been shown that triac at certain doses depresses the circulating cholesterol in both hypothyroid and euthyroid subjects but there is an apparent dissociation of this action from any increase in basal metabolic rate (*Lerman* and *Pitt-Rivers* [1955]; *Trotter* [1956]). Investigation of the action of these thyroxine analogues on lipid metabolism and oxygen consumption is important and has obvious therapeutic possibilities concerning the correction of the abnormal circulating lipids and lipoproteins found in association with clinical coronary disease.

Clinical Investigations

Methods

The plasma cholesterol was estimated by the Sperry and Webb modification of the Schoenheimer-Sperry procedure (*Sperry* and *Webb* [1950]); the plasma phospholipids were determined by the method of *Allen* (1940); and the concentration of cholesterol attached to the α- and β-lipoproteins was estimated by zone electrophoresis with subsequent elution of cholesterol from the filter paper (*Boyd* [1954]). In each subject the basal metabolic rates were measured on a standard Benedict-Roth apparatus using Aub and Du Bois' linear formula (*Aub* and *Du Bois* [1917]) twice before and twice at the end of the administration of a thyroid derivative.

L-thyroxine sodium

a) Hypothyroid subjects: The administration for 8 weeks of 300 μg daily of *l*-thyroxine sodium to 6 subjects with classical myxoedema resulted in a mean decrease in plasma cholesterol of 40% s.c. the plasma cholesterol: phospholipid ratio (the C/P ratio) fell by 6%, the cholesterol in mg% bound to the β-lipoprotein fraction decreased by 29% and the cholesterol in mg% bound to the α-lipoprotein fraction increased by 42%. The mean weight loss was 7 lbs. and the basal metabolic rate increased by 17%.

b) Euthyroid subjects: The administration for 2 weeks of 600 μg daily of *l*-thyroxine sodium to 6 euthyroid hypercholesterolaemic men, who had electrocardiographic evidence of previous myocardial infarction, resulted in a mean decrease in plasma cholesterol of 27% (*Oliver* and *Boyd* [1955]). The C/P ratio fell by 17%, the cholesterol in mg% bound to the β-lipoprotein fell by 29% and there was an associated fall in the cholesterol bound in mg% to the α-lipoprotein of 20%. The mean weight loss after 3 weeks was 4 lbs. The basal metabolic rate increased

by 13% and 3 of these 6 men experienced appreciable reduction of their exercise tolerance from the development of effort angina, which regressed completely a few weeks after thyroxine had been discontinued.

L-Triiodothyronine

a) Large dose: The administration for 2 weeks of 40 μg daily and then for 4 weeks of 100 μg daily of *l*-triiodothyronine to 3 euthyroid hypercholesterolaemic men, who had electrocardiographic evidence of previous myocardial infarction, resulted in a mean decrease in plasma cholesterol of 34% (Fig. 2). The C/P ratio fell by 17%, the cholesterol in mg%

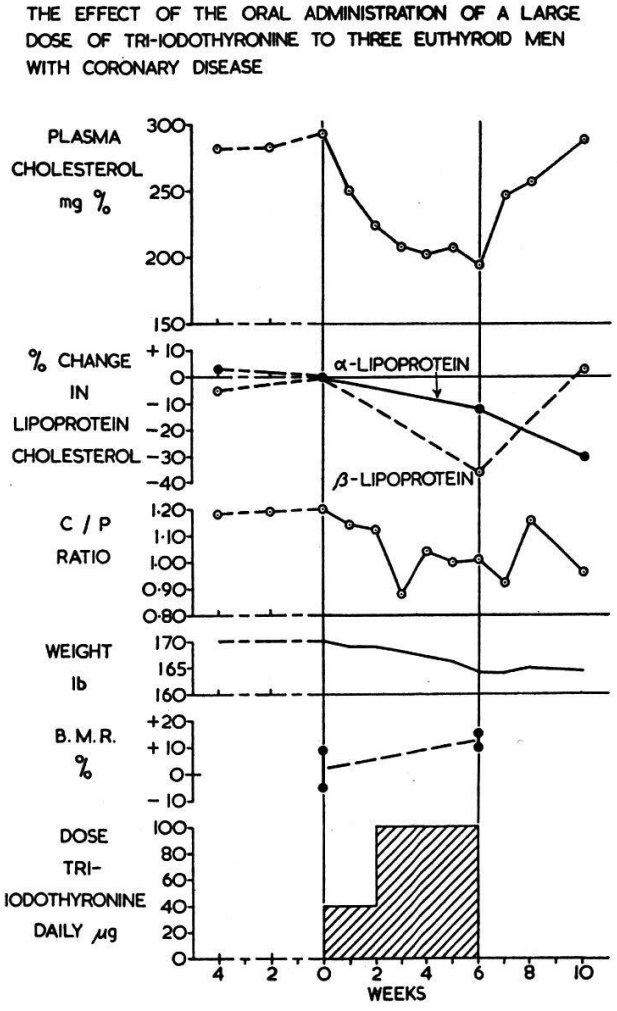

Fig. 2. The effect of the oral administration of a large dose of triiodothyronine to 3 euthyroid men with coronary disease.

bound to the β-lipoprotein fraction fell by 36% and there was an associated fall in the cholesterol in mg% bound to the α-lipoprotein of 12%. The mean weight loss was 6 lbs. The mean basal metabolic rate increased by 11% and 2 subjects experienced significant increase in frequency of anginal pain during administration of triiodothyronine.

b) Small dose: The administration for 5 weeks of 40 μg daily and then

for 2 weeks of 60 μg daily of *l*-triiodothyronine to 3 euthyroid men, who had electrocardiographic evidence of previous myocardial infarction, resulted in a mean decrease in plasma cholesterol of 16% (Fig. 3). The

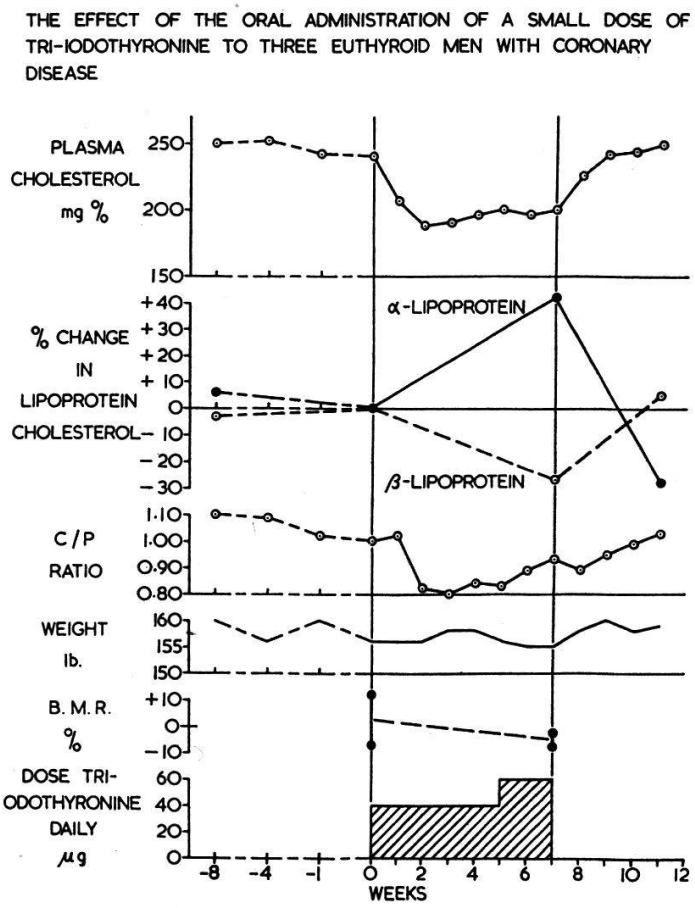

Fig. 3. The effect of the oral administration of a small dose of triiodothyronine to 3 euthyroid men with coronary disease.

C/P ratio fell by 14%, the cholesterol in mg% bound to the β-lipoprotein fraction fell by 27% and the cholesterol in mg% bound to the α-lipoprotein fraction increased by 42%. There was no change in weight and basal metabolic rate but one man who had previously had an excellent exercise tolerance developed effort angina.

Triiodothyroacetic acid

a) Hypothyroid subjects – large dose: The administration for 6 days of 4 mg of triac daily to 2 subjects with classical myxoedema resulted in significant depression of the plasma cholesterol and the cholesterol in mg% bound to the β-lipoprotein fraction and elevation of the cholesterol in mg% bound to the α-lipoprotein fraction (Fig. 4). Within 24 hours there was a clinical response, weight loss and an increase in metabolic rate.

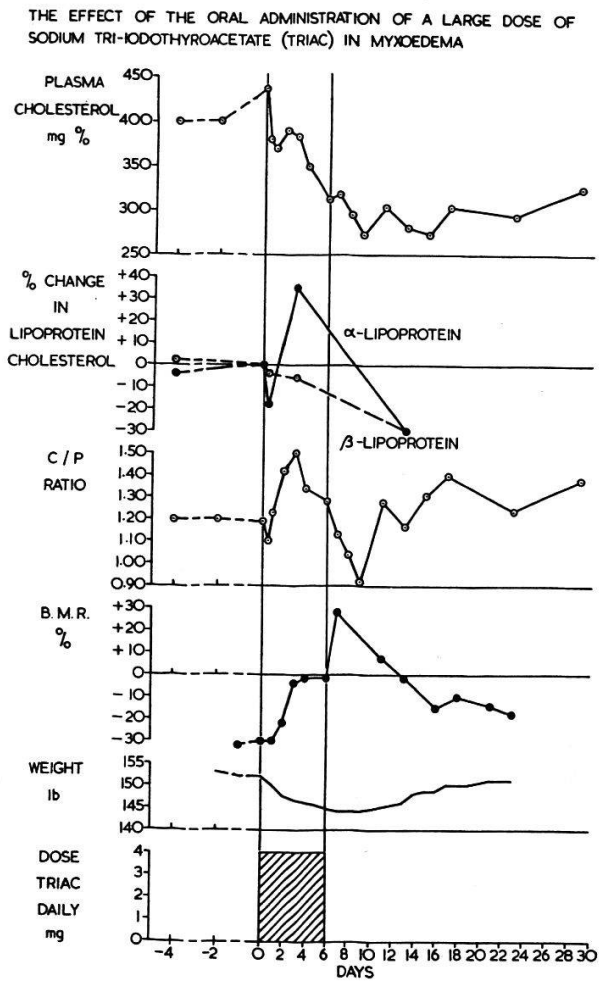

Fig. 4

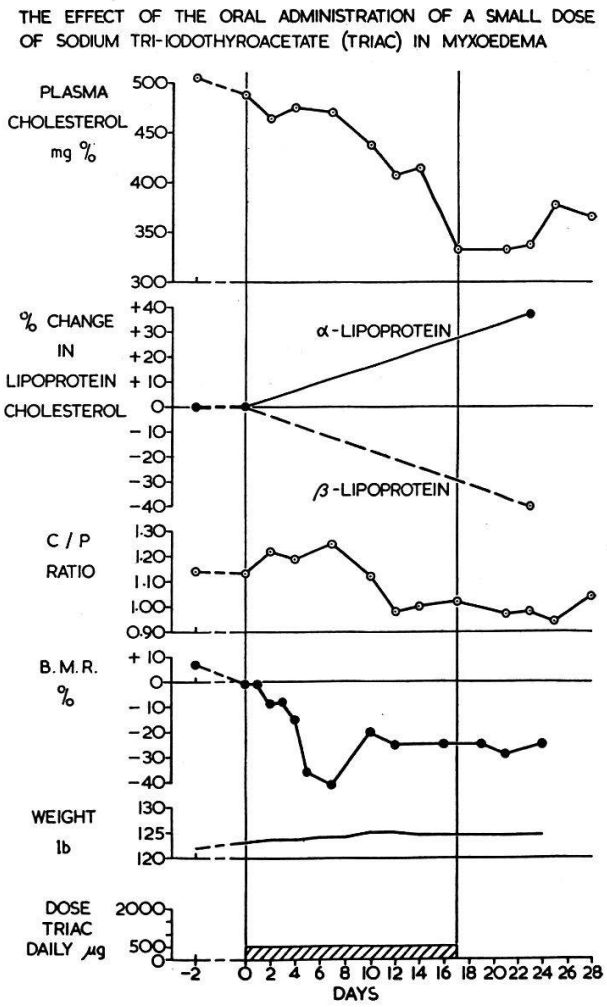

Fig. 5

Fig. 4. The effect of the oral administration of a large dose of triiodothyroacetic acid in myxoedema.

Fig. 5. The effect of the oral administration of a small dose of triiodothyroacetic acid in myxoedema.

b) Hypothyroid subject – small dose: The administration of 0.5 mg of triac daily to a 69 year old woman with classical myxoedema resulted in significant depression of the plasma cholesterol and the cholesterol in mg% bound to the β-lipoprotein fraction (Fig. 5). Over 17 days there was no weight loss and no increase in the basal metabolic rate.

c) Euthyroid subjects: Triac was administered over a period of 95 days in divided and increasing doses from 0.5 mg daily to 4 mg daily to 6 euthyroid hypercholesterolaemic men who had electrocardiographic evidence of previous myocardial infarction (Fig. 6). There was no significant alteration in the levels of the circulating lipids and lipoproteins until 3 mg of triac was administered daily. After 4 mg daily for 10 days there was a mean decrease of plasma cholesterol of 21% but the C/P ratio was not significantly altered. The cholesterol in mg%

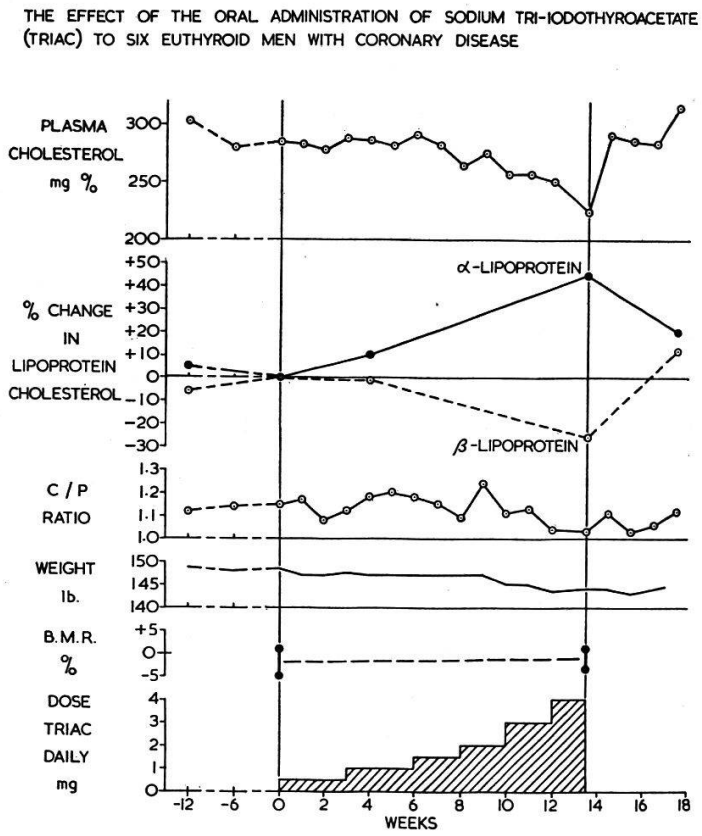

Fig. 6. The effect of the oral administration of triiodothyroacetic acid to 6 euthyroid men with coronary disease.

bound to the β-lipoprotein fraction decreased by 26% and the cholesterol in mg% bound to the α-lipoprotein fraction increased by 45 per cent. There was no weight loss and the mean basal metabolic rate was not significantly altered. There was no electrocardiographic change during the administration of triac. In 2 men, in whom the lipid and lipoprotein changes were less than the mean, there was elevation of the basal metabolic rate by 20% and by 13% respectively. The latter subject complained of a marked reduction of his exercise tolerance due to effort angina. Another man whose basal metabolic rate actually decreased during triac administration also developed effort angina. When triac was withdrawn both these men experienced rapid regression of their symptoms and have now returned to their normal exercise tolerance.

Animal Investigations

Parallel with and complementary to the clinical investigations, experiments have been performed on rats to study the effect of triac on lipid metabolism and basal metabolic requirements.

Methods

The subjects were male inbred albino Wistar strain rats maintained on rat cake, diet 41 (*Bruce* [1950]). In all cases triac was administered

by subcutaneous injection in saline solution containing 0.005 N-NaOH. The basal metabolic rates were determined by a close circuit respirometer in which the oxygen consumption was recorded on a kymograph drum. Tissue slice respiration was studied in the conventional Warburg apparatus employing 100% oxygen as the gas phase at 110 oscillations per min. at 37.5° C. *In vitro* cholesterol biosynthesis by surviving tissue slices was studied by incubation of the slices in Krebs-phosphate-Ringer (pH 7.4) in the presence of 3 μM acetate-1-^{14}C for 3 hours under 100% oxygen at 37° C. After alkaline hydrolysis of the tissue, petrol-ether extraction and cholesterol precipitation, the cholesterol digitonide was combusted to CO_2 and the percentage incorporation of ^{14}C into cholesterol was determined.

Serum cholesterol and basal metabolic rate

Triac was administered at six dose levels from 50 to 2000 μg/kg to different groups of 6 intact male euthyroid rats for periods up to 17 days. The serum cholesterol and the basal metabolic rate were determined on 4 occasions during this time: both the serum cholesterol and the basal metabolic rate were elevated when large doses were administered (Fig. 7).

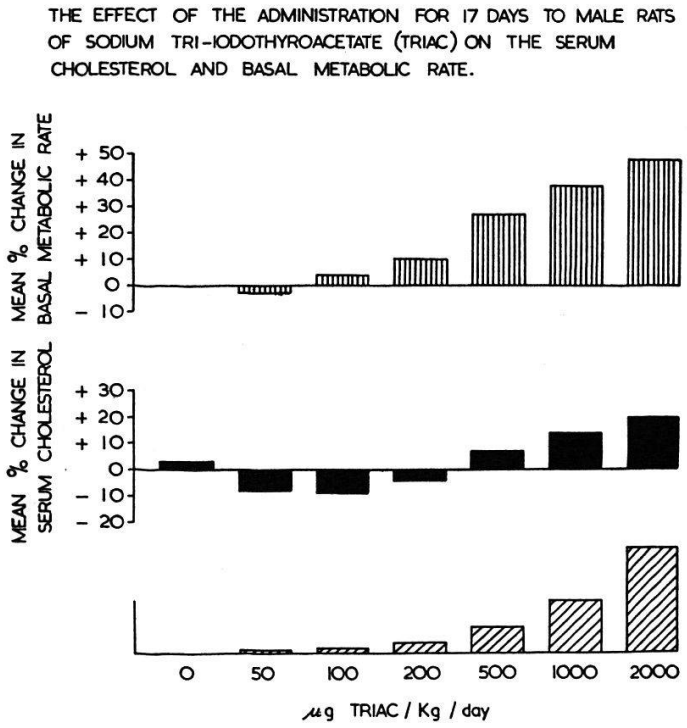

Fig. 7. The effect of the subcutaneous administration of triiodothyroacetic acid to male euthyroid rats.

Oxygen uptake of tissue slices

At intervals throughout the above experiment certain animals from each dosage category were sacrificed and the oxygen uptake of liver

and heart tissue slices was determined. At the higher doses of triac (1000–2000 μg/kg) the oxygen uptake of liver slices was slightly increased (10%), while the oxygen uptake of heart muscle was considerably increased (21%).

Hepatic cholesterol biosynthesis

In view of the hepatic origin of plasma cholesterol and the reported *in vitro* stimulation of the respiration of tissue slices by triac (*Thibault* and *Pitt-Rivers* [1955]), the influence of triac on hepatic cholesterol biosynthesis was examined as follows. Liver slices from euthyroid and thyroidectomised rats were incubated in the presence of acetate-1-^{14}C in Ringer containing triac at concentrations of 0.01, 0.1, 1.0 or 10 μg/ml. The percentage incorporation of ^{14}C into cholesterol was determined. Triac did not produce any significant change in the rate of hepatic cholesterol biosynthesis (Fig. 8). Concurrently with these experiments, the respirations of tissue slices from the same livers were studied and again there was no significant change after triac (Fig. 8).

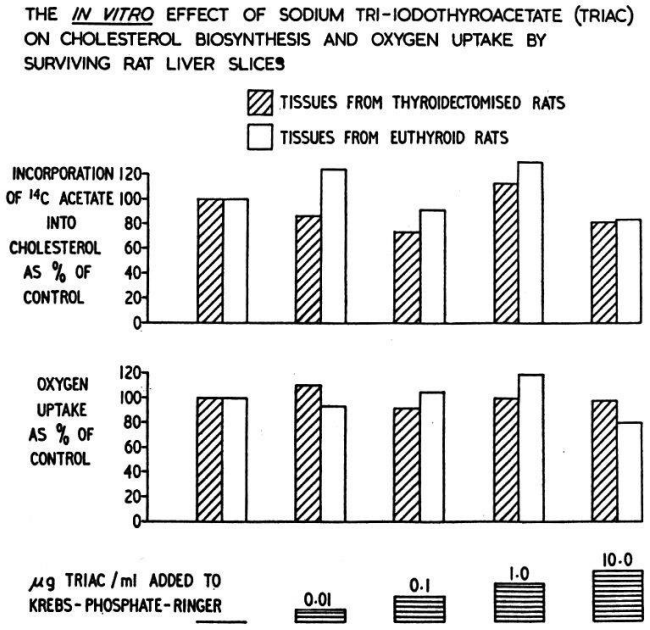

Fig. 8. The *in vitro* effect of triiodothyroacetic acid on cholesterol biosynthesis and oxygen uptake by surviving rat liver slices.

Discussion

In hypothyroid and euthyroid subjects small doses of triiodothyroacetic acid decrease the serum cholesterol and the cholesterol in mg% bound to the β-lipoproteins, without elevating the basal metabolic rate. Triiodo-

thyronine has a similar effect in euthyroid subjects. There is an individual variation in the minimal dose which will produce this dissociated effect and it is easy to overstep the optimum and produce elevation of the basal metabolic rate. Depression of circulating cholesterol in dissociation from any basal metabolic rate response can be achieved in hypothyroid subjects at a dose which is much less than that required to produce a similar action in euthyroid subjects. Since triac has this dissociation of effect in humans, the mechanism of this action on lipogenesis obviously required further investigation and this was carried out in animals. In euthyroid rats small doses of triac produced little effect on the serum cholesterol and basal metabolic rate but larger doses elevated both values. Moreover, in liver slices from hypothyroid and euthyroid rats the rate of cholesterol biosynthesis was not influenced by the *in vitro* addition of triac, and the oxygen uptake of slices from the same livers was also not significantly affected. This result seemed surprising in view of the observation of *Thibault* and *Pitt-Rivers* (1955) that triac raised the oxygen consumption of rat-kidney slices *in vitro* without any latent period of action. However, triac does not stimulate the oxygen uptake of the liver of our particular strain of rats, and it is the liver which is probably of greater interest in so far as it is the chief source of plasma cholesterol.

The basal metabolic rate is derived from the nett oxygen uptake of a subject in a supposedly basal state, and it is well known that this observation has a coefficient of variation of $\pm$ 5%. The total oxygen consumption of a subject represents the arithmetical sum of the oxygen requirements of all the body tissues and hence, after the administration of a substance which elevates the basal metabolic rate, it must not be assumed that all the tissues are influenced uniformly. Conversely, the administration of a substance which produces a biological response, without influencing the basal metabolic rate, may well affect the oxygen requirement of certain specific tissues. Since the body is composed of tissues of different histological structure and function it would be surprising if they did react towards a metabolic stimulant in the same way. This concept receives support from the work of *Gordon* and *Heming* (1944) and *Ullrick* and *Wihtehorn* (1952), who have shown that there is a marked differential response in oxygen uptake between liver, kidney, diaphragm, skeletal muscle and heart tissue following the administration of desiccated thyroid to euthyroid rats. In the hyperthyroid state produced by desiccated thyroid the greatest increase in metabolic rate of excised tissues occurred in cardiac tissue. Furthermore, our observations, which support those of *Barker* and *Lewis* (1956), indicate that in hypo-

thyroid and euthyroid rats triac increases the oxygen uptake of heart tissue to a greater extent than that of liver tissue.

It is widely, although not universally accepted that anginal pain is anoxaemic in nature and results from the discrepancy between oxygen supply and demand. In our investigation of subjects who were known to have clinical coronary disease, those who experienced angina while receiving thyroxine analogues fell into two groups. First, in some subjects the dose was sufficient to elevate the basal metabolic rate and hence increase their myocardial oxygen requirements (*Leight*, *DeFazio*, *Talmers*, *Regan* and *Hellems* [1956]); the development of angina in this group is readily understandable. Second, in three out of nine euthyroid subjects the dose of thyroxine analogues produced no detectable rise in basal metabolic rate, but their exercise tolerance was reduced by effort angina. It is possible, as already suggested, that this small dose may in fact have increased the oxygen demand by the myocardium.

Considerable care must be employed with the administration of triac for reduction of hypercholesterolaemia, particularly as the individual dose response is so variable. Nevertheless, triac can be administered to selected cases of clinical coronary disease as a hypocholesterolaemic agent without side effects and therefore merits fuller careful clinical investigation.

Summary

1. The administration of triiodothyroacetic acid (triac) to hypothyroid and euthyroid subjects resulted in depression of the plasma cholesterol and of the cholesterol bound to the β-lipoproteins without increase in basal metabolic rate. Triiodothyronine produced a similar effect in euthyroid subjects. Larger doses overcame this dissociated action and produced elevation of basal metabolic requirements.

2. The administration of large doses of triac to euthyroid rats caused elevation of serum cholesterol and the basal metabolic rate. Triac added *in vitro* to liver slices from hypothyroid and euthyroid rats failed to influence cholesterol biosynthesis from acetate or oxygen uptake.

3. Following the injection of triac to hypothyroid rats, the percentage increase in oxygen uptake of heart tissue was considerably greater than the percentage increase in oxygen uptake of liver tissue.

4. It is suggested that triac may increase the oxygen consumption of the myocardium in the human without any apparent change in the basal metabolic rate. This possibility is considered in view of the reduction of exercise tolerance by effort angina which developed in some of the subjects during triac administration.

Zusammenfassung

1. Die Verabreichung von Trijodthyroessigsäure (Triac) an Hypothyroide und Euthyroide ergab eine Verminderung des Plasmacholesterins und des an die β-Lipoproteide gebundenen Cholesterins ohne Zunahme des Grundumsatzes. Tri-jodothyronin zeigte bei Euthyroiden eine ähnliche Wirkung. Größere Dosen bringen diese dissoziierte Wirkung zum verschwinden und steigern den Grundumsatz.

2. Die Verabreichung von größeren Dosen von Triac an euthyroide Ratten verursacht eine Hebung des Serumcholesterinspiegels und des Grundumsatzes. Triac, das Leberschnittpräparaten von hypo- und euthyroiden Ratten in vitro zugesetzt wird, beeinflußt weder die Cholesterinbiosynthese aus Azetat noch die Sauerstoffaufnahme des Gewebes.

3. Die prozentuale Zunahme der O_2-Aufnahme nach Triac-Injektion bei hypothyroiden Ratten erwies sich im Herzgewebe als beträchtlich höher als im Lebergewebe.

4. Es wird die Hypothese aufgestellt, daß Triac den O_2-Verbrauch des Myokardes des Menschen ohne augenscheinliche Veränderung des Grundumsatzes steigern kann; diese Möglichkeit wurde im Zusammenhang mit der Toleranzverminderung gegenüber Anstrengungen bei «Effort-Angina», die sich in einigen Fällen während einer Triac-Medikation entwikkelte, in Erwägung gezogen.

Résumé

1. L'administration d'acide triiodothyracétique à des sujets hypothyroïdiens et euthyroïdiens a provoqué une dépression dans le taux du cholestérol plasmatique et du cholestérol lié au β-lipoprotéines, sans augmentation du métabolisme de base. La Tri-iodothyronine produisit un effet similaire chez des sujets euthyroïdes. De plus fortes doses ont fait disparaître cette action dissociée et ont produit une élévation du métabolisme basal.

2. L'administration d'une forte dose de triac à des rats euthyroïdiens a produit une élévation du taux du cholestérol sérique et du métabolisme basal. Du triac, additionné *in vitro* à des tranches de foie provenant de rats hypothyroïdiens et euthyroïdiens, n'a pas d'influence, ni sur la biosynthèse du cholestérol, après adjonction d'acétate, ni sur l'absorption d'oxygène du tissu.

3. Après l'injection de triac à des rats hypothyroïdiens, l'augmentation de l'utilisation d'oxygène par le tissu cardiaque a été plus grande que l'augmentation de l'utilisation d'oxygène par le tissu hépatique.

4. On émet l'hypothèse que le triac peut augmenter la consommation d'oxygène du myocarde chez l'homme sans modifier le taux du méta-

bolisme basal de façon apparente, car la tolérance à l'effort est diminuée chez quelques sujets, qui, lorsque ils absorbent du triac, souffrent d'angine de poitrine d'effort.

Acknowledgments

We wish to thank Professor *G. F. Marrian*, F.R.S., and Dr. *Rae Gilchrist*, of our respective departments, for their advice and encouragement; Dr. *A. G. Macgregor* of the Department of Therapeutics for access to three cases of myxoedema; Dr. *H. M. Walker* of Glaxo Laboratories, London for supplies of triiodothyroacetic acid and triiodothyronine; and Mr. *Fraser Syme* and Mr. *William Cooper* for skilled technical assistance. We are grateful to the Scottish Hospitals Endowments Research Trust for defraying the expenses incurred through these investigations.

Allen, R. J. L.: Biochem. J. **34,** 858 (1940). – *Aub, J. C.*, and *Du Bois, E. F.:* Arch. intern. Med. **19,** 823 (1917). – *Barker, S. B.*, and *Lewis, W. J.:* Proc. Soc. exp. Biol. (N. Y.) **91,** 650 (1956). – *Barr, D. P.:* Symposium on Arteriosclerosis, University of Minnesota, Minneapolis, p. 58 (1955). – *Boyd, G. S.:* Biochem. J. **58,** 680 (1954). – *Bruce, H. M.:* J. Hyg. (Camb.) **48,** 171 (1950). – *Gildea, E. F., Man, E. B.*, and *Peters, J. P.:* J. clin. Invest. **18,** 739 (1939). – *Gordon, E. S.*, and *Heming, A. E.:* Endocrinology **34,** 353 (1944). – *Leight, L., DeFazio, V., Talmers, F. N., Regan, T. J.*, and *Hellems, H. K.:* Circulation **14,** 90 (1956). – *Lerman, J.*, and *Pitt-Rivers, R.:* J. clin. Endocrin. Metab. **15,** 653 (1955). – *Malmros, H.*, and *Swahn, B.:* Acta med. scand. **145,** 361 (1953). – *Oliver, M. F.*, and *Boyd, G. S.:* Symposium on Arteriosclerosis, University of Minnesota, Minneapolis, p. 64 (1955); Lancet **1,** 124 (1957). – *Pitt-Rivers, R.:* Lancet **2,** 234 (1953). – *Sperry, W. M.*, and *Webb, M.:* J. biol. Chem. **187,** 97 (1950). – *Strisower, B., Gofman, J. W., Galioni, E. F., Almada, A. A.*, and *Simon, A.:* Metabolism **3,** 218 (1954). – *Thibault, O.*, and *Pitt-Rivers, R.:* Lancet **1,** 285 (1955). – *Trotter, W. R.:* Lancet **1,** 885 (1956). – *Turner, K. B.*, and *Steiner, A.:* J. clin. Invest. **18,** 45 (1939). – *Ullrick, W. C.*, and *Whitehorn, W. V.:* Amer. J. Physiol. **171,** 407 (1952).