Second Session

Chairman : PROFESSOR E. W. H. CRUICKSHANK, *Department of Physiology, University of Aberdeen*

Some aspects of cholesterol metabolism

By G. S. BOYD, *Department of Biochemistry, University of Edinburgh* and
M. F. OLIVER, *Department of Cardiology, Royal Infirmary, Edinburgh*

It has been known for nearly 200 years that cholesterol occurs in mammalian tissues, and this sterol has been shown to be specific to the animal kingdom whereas closely related sterols exist in plants.

Dietary cholesterol

Nutritional studies showed that cholesterol was not essential for rats as these animals grew normally on a sterol-free diet. Carcass analyses of animals existing on such diet revealed a net increase in body cholesterol, and hence endogenous cholesterol biosynthesis from non-sterol precursors must occur in mammalian tissues (Channon, 1925). Furthermore, when cholesterol is included in the diet of mammals it is found that absorption occurs to a variable degree dependent on the species, the percentage of cholesterol in the diet, and other dietary constituents (Cook & Thomson, 1951).

Marked species differences exist in responses to exogenous cholesterol; for example, variations in the dietary cholesterol of rabbits result in parallel changes in the plasma-cholesterol levels, whereas in rats a relationship exists between the cholesterol intake and the concentration of cholesterol in the liver, but the plasma cholesterol is not materially altered. Balance experiments on rabbits (Page & Menschick, 1932) on cats (Menschick & Page, 1933) and on mice (Schoenheimer & Breusch, 1933) have demonstrated that cholesterol 'destruction' occurs within mammals. However, as various micro-organisms occurring in the intestinal flora can destroy cholesterol in vitro (Wainfan, Henkin, Rittenberg & Marx, 1954) it is difficult to separate quantitatively in in vivo experiments katabolism by mammalian tissues from that by micro-organisms.

Two important pathways for the elimination of cholesterol are secretion into the bile and excretion by the small intestine. Thus intestinal cholesterol consists of a mixture of unabsorbed and excreted sterol, and much of this material is excreted in the faeces either unchanged or as the reduction product coprosterol.

Endogenous cholesterol

By classical techniques it was established that all mammalian cells contain cholesterol and that the organism could derive this sterol from the diet, but it was not

https://doi.org/10.1079/PNS19560011 Published online by Cambridge University Press

known whether endogenous synthesis occurred in all or only in certain tissues, nor was the sequence of reactions involved in the biosynthesis understood. The greatest advance in our knowledge of the intermediary metabolism of cholesterol followed the introduction of isotope techniques. Rittenberg & Schoenheimer (1937) introduced into the body water of animals deuterium oxide and found on killing the animals extensive incorporation of deuterium into cholesterol. These authors concluded that cholesterologenesis must proceed by way of multiple condensations of small molecules. In a key experiment Bloch & Rittenberg (1942) showed by administering deuterium-labelled acetic acid to rats and mice that the animal body could utilize acetate in sterol biosynthesis. Furthermore, when the labelled sterol was split into the cyclopentenophenanthrene portion (nucleus) and *iso*-octane moiety (side-chain) by the procedure of Mauthner & Suida (1896), both fragments contained deuterium, and hence acetate must be involved in the total synthesis of the molecule.

Subsequent studies on cholesterol biosynthesis with doubly labelled acetate $^{13}CH_3{}^{14}COOH$ (Little & Bloch, 1950) and experiments with 1-^{14}C acetate and 2-^{14}C acetate followed by elegant stepwise degradations of the sterol molecule have produced almost complete confirmation that all twenty-seven carbon atoms in the molecule are derived from acetate (Wüersch, Huang & Bloch, 1952; Cornforth, Hunter & Popják, 1953*a,b*). These studies have shown that fifteen of the carbon atoms of the sterol molecule originate in the methyl radical of acetate whereas twelve are derived from the carboxyl group of acetate.

Bloch, Borek & Rittenberg (1946) using labelled acetate and surviving rat-liver slices, demonstrated that hepatic cholesterologenesis can occur in vitro, and this result opened up a new approach to the intermediary metabolism of cholesterol. Since all animal cells contain cholesterol it was possible to apply the tissue-slice technique to examination of organs for their ability to synthesize this sterol. It was found that in addition to the liver, cholesterol biosynthesis from acetate occurred in adrenal cortical tissue (Srere, Chaikoff & Dauben, 1948), brain of young rats (Waelsch, Sperry & Stoyanoff, 1940; Srere, Chaikoff, Treitman & Burstein, 1950) kidney, testes, intestine and skin (Srere *et al.* 1950), rat diaphragm (Gould, cited by Gould, 1951) and aorta (Siperstein, Chaikoff & Chernick, 1951). Adult brain, although rich in this sterol, was inactive in cholesterol biosynthesis (Waelsch *et al.* 1940; Srere *et al.* 1950).

Experiments suggest that on a sterol-free diet the plasma cholesterol is derived almost exclusively from hepatic synthesis (Gould, Campbell, Taylor, Kelly, Warner & Davis, 1951) and there is evidence that in some species the testes, spleen, kidney, lung and adrenals, though possessing the mechanism for cholesterol biosynthesis, in fact under physiological conditions seem to withdraw and utilize cholesterol from plasma (Landon & Greenberg, 1954).

Thus the plasma cholesterol appears to exchange with the intestinal cholesterol (some of which is of dietary origin) and with the hepatic cholesterol, whereas the transference of cholesterol from plasma to certain extrahepatic tissues seems to be irreversible. These interrelationships are shown diagramatically in Fig. 1.

https://doi.org/10.1079/PNS19560011 Published online by Cambridge University Press

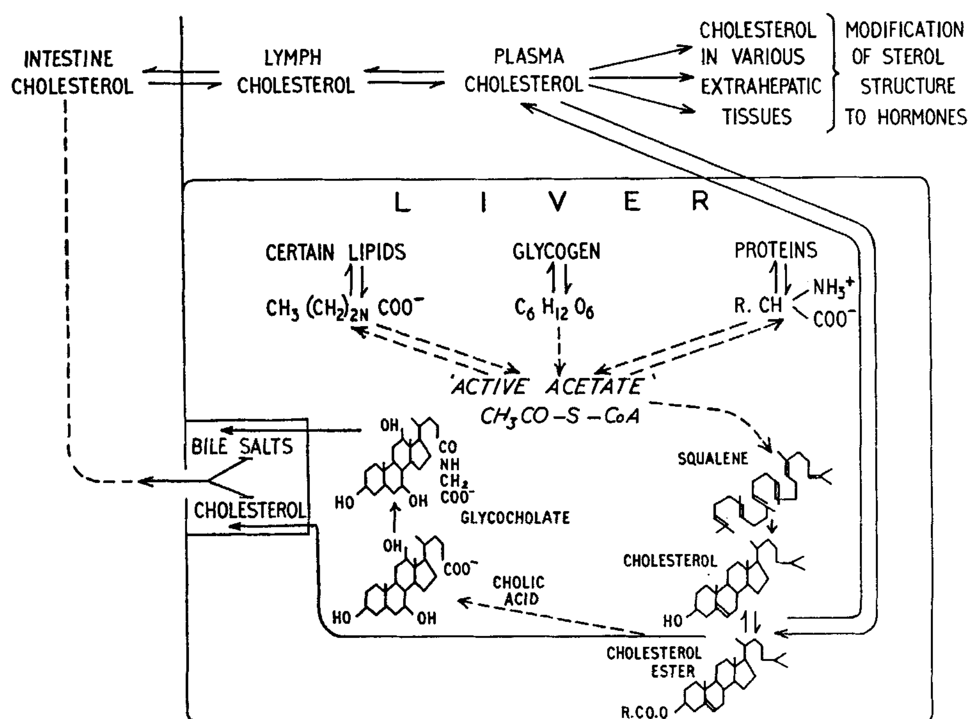

Fig. 1. Some of the interrelationships between exogenous and endogenous cholesterol.

Acetyl coenzyme A (active acetate) has been shown to be a common key intermediate in metabolic pathways of protein, fat, and carbohydrate, and there is evidence that this unit is involved in sterol biosynthesis (Klein & Lipmann, 1953; Boyd, 1953). Considerable effort has been expended in attempts to identify possible intermediates between the C_2 and the C_{27} unit in this complex biosynthesis.

Channon (1926) observed an increase in liver cholesterol after feeding the hydrocarbon squalene to rats and suggested that the latter substance might be a precursor of cholesterol; in theory the structure of this triterpene lends itself to superimposition on the sterol structure. Since squalene can be considered to be derived from isoprene units and the side-chain of cholesterol appears isoprenoid, the role of squalene in cholesterol biosynthesis was investigated. Langdon & Bloch (1953) showed that ^{14}C labelled acetate was incorporated into the liver squalene of rats previously fed non-isotopic squalene and this evidence suggested that squalene might be an intermediate in cholesterol biosynthesis. This biosynthesized ^{14}C labelled squalene was fed to mice and yielded ^{14}C labelled cholesterol, the finding again supporting the hypothesis that the triterpene may be a precursor of cholesterol. Presumably squalene is produced by the multiple condensations of isoprenoid units but the mechanism involved in the generation of these entities is still obscure.

A major obstacle in this connexion was that though surviving tissue slices could synthesize cholesterol from acetate very efficiently, destruction of the cellular

https://doi.org/10.1079/PNS19560011 Published online by Cambridge University Press

architecture and organization in homogenates as usually prepared resulted in negligible biosynthesis of cholesterol from this substrate. Thus observations had to be made with tissue slices and this procedure severely restricted any serious comparison of, say, a postulated intermediate with acetate. Fortunately cholesterol biosynthesis was ultimately achieved in broken-cell preparations—homogenates (Bucher, 1953)—and also in particle-free aqueous extracts of liver (Rabinowitz & Gurin, 1953). So with isotopes and soluble enzymes in our armamentary we can look forward to the elucidation in the near future of the steps involved in cholesterol biosynthesis.

Nevertheless, it is possible that if an isoprenoid synthesis is involved in cholesterologenesis this synthesis may proceed by reactions analogous to well-known pathways in carbohydrate and fat metabolism. At the moment there is evidence that β-hydroxy-β-methylglutaric acid (Rabinowitz & Gurin, 1954) and possibly dimethylacrylic acid (Bloch, Clark & Harary, 1954) may be on the metabolic pathway, and hence the close similarity to the Krebs tricarboxylic-acid cycle prompted the tentative and highly speculative scheme shown in Fig. 2, to be proposed by the present authors.

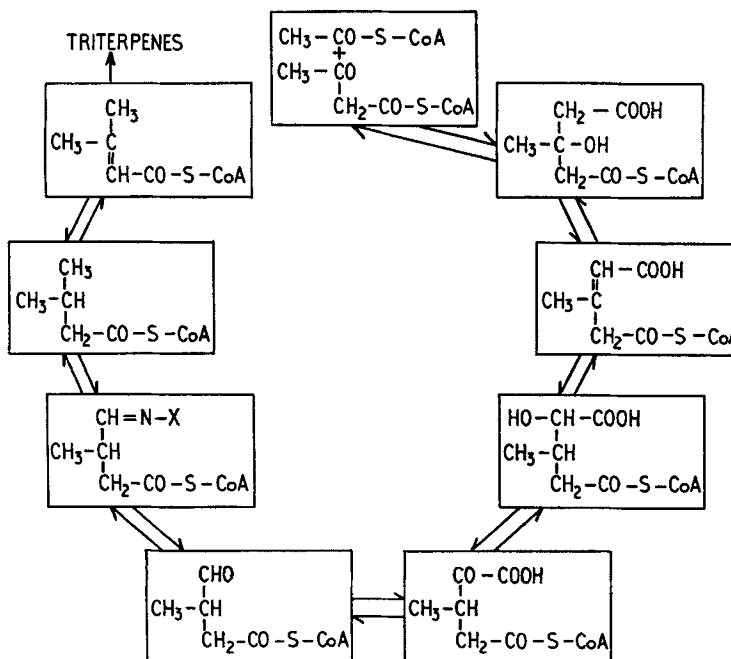

Fig. 2. Speculative scheme (based on the tricarboxylic- and fatty-acid cycles) of possible intermediates in the biosynthesis of cholesterol.

If adequate foundation for this sequence can be demonstrated (even in part) then perhaps certain enzymes involved in the tricarboxylic-acid cycle, or in the fatty-acid cycle (Lynen, 1953) may also be involved in cholesterologenesis. Information on

https://doi.org/10.1079/PNS19560011 Published online by Cambridge University Press

these points would assist in attempts to explain the complex integration which exists between oxidative metabolism and sterol biosynthesis.

Plasma cholesterol

Much of the cholesterol synthesized in the liver is discharged into the plasma, and within recent years the importance of the plasma lipid and lipoprotein levels in the aetiology of atherosclerosis has been generally recognized. A positive correlation seems to exist between the incidence of the clinical manifestations of atherosclerosis and a tendency towards hypercholesterolaemia (Steiner, Kendall & Mathers, 1952; Oliver & Boyd, 1953, 1955), and furthermore the lipids of the atherosclerotic plaques are derived at least in part from the circulating cholesterol (Biggs, Kritchevsky, Colman, Gofman, Jones, Lindgren, Hyde & Lyon, 1952).

Evidence that the circulating lipids do not exist free in plasma but are attached to protein has been summarized by Russ, Eder & Barr (1951) and it has been shown that the plasma cholesterol is largely associated with two globulin fractions, yielding the so called α- and β-lipoproteins. It is possible to separate these lipoproteins by zone electrophoresis as shown in Fig. 3 and assess the distribution of cholesterol

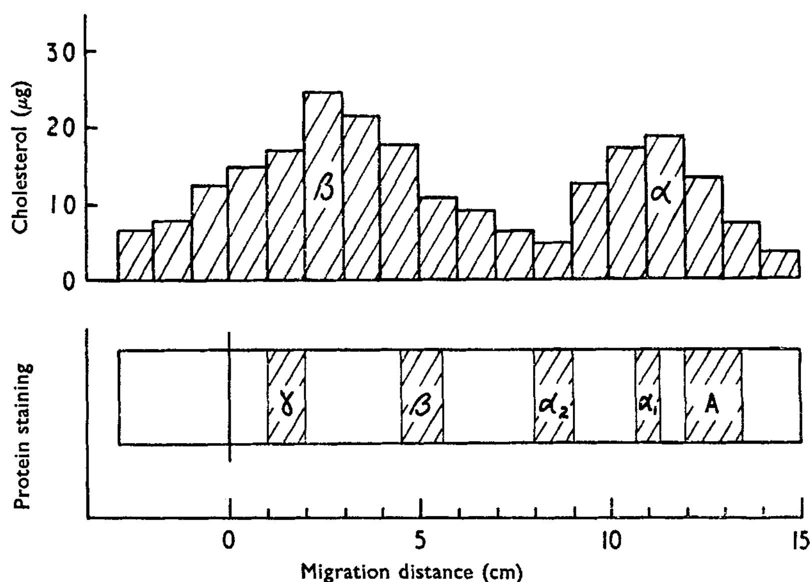

Fig. 3. Separation and estimation of the α- and β-lipoproteins by zone electrophoresis.

between the fractions (Boyd, 1954). With this method it can be shown that very marked species differences exist in the distribution of cholesterol between the plasma lipoproteins. In the rat it is difficult to raise the plasma cholesterol by dietetic measures. This species seems to be immune from atheroma, and the distribution of cholesterol between the α- and β-lipoproteins is approximately 70 : 30. By contrast the corresponding ratio in normal rabbit plasma is 50 : 50, which upon moderate

https://doi.org/10.1079/PNS19560011 Published online by Cambridge University Press

cholesterol feeding (producing mild hypercholesterolaemia with the sequelae of atherosclerosis) may alter to about 2 : 98. In normal young male human subjects this plasma ratio is about 28 : 72, whereas in young men of comparable age who have evidence of atherosclerosis this plasma ratio is altered to about 9 : 91, the difference being highly significant (Oliver & Boyd, 1955).

Thus, without implying a causal relationship, there appears to be a correlation between the elevated concentration of cholesterol in the β-lipoprotein fraction and atheroma formation. The circulating lipoproteins, which appear to be produced in the liver, have different chemical constitutions, iso-electric points, molecular weights and possibly turnover rates. The hepatic rates of synthesis, the hepatic or extrahepatic rates of degradation and the permeability of the capillary endothelium to these molecular species are only a few of the many factors governing the survival of lipoproteins in plasma. These parameters must be evaluated before a rational approach to the role of lipoproteins in atherogenesis can be expected.

Relationship between dietary, endogenous and plasma cholesterol

Experimental results suggest that in adult animals of any one particular species under physiological conditions, the levels of cholesterol in plasma, liver and carcass are maintained within fairly narrow limits despite the rapid turnover of this compound in most tissues of the body with the exception of the central nervous system. This homeostatic control of cholesterol metabolism involves equation of the variable cholesterol intake and hepatic biosynthesis with the changing cholesterol requirements of the organism and excretion of sterol.

As cholesterol is not essential in the diet of mammals the intake may vary from almost nothing to over 0·1% and hence, to maintain *status quo*, cholesterol biosynthesis must be under some fine control. As the plasma cholesterol originates in the liver whereas the absorbed cholesterol enters the plasma, it is tempting to implicate the plasma cholesterol as a controlling factor, but as the circulating cholesterol fluctuates little in healthy mammals it seems unlikely that this entity can serve as the controlling 'feed back' mechanism in hepatic biosynthesis.

In support of this thesis Gould & Taylor (1950) using dogs, and Tomkins, Sheppard and Chaikoff (1953) using rats, have shown in feeding experiments with cholesterol that hepatic cholesterol biosynthesis is drastically reduced, and it has also been shown that extrahepatic biosynthesis is unaffected by this constraint (Gould, Taylor, Hagerman, Warner & Campbell, 1953). Furthermore, effective inhibition can be achieved by the use of a sterol-containing diet for a very brief period before the experiment is performed, and this inhibition can be accomplished without a detectable rise in the concentrations of cholesterol in plasma or liver. It seems therefore that the dietary cholesterol can influence hepatic (and hence plasma) cholesterol biosynthesis in certain species in some obscure manner. Some possible mechanisms through which this homeostatic control may be exerted are as follows.

An indirect control might be postulated through exogenous cholesterol at the intestinal phase, or after absorption into some other tissue resulting in the release

https://doi.org/10.1079/PNS19560011 Published online by Cambridge University Press

of a 'hormone like' substance which might inhibit cholesterol biosynthesis selectively in the liver, but at the present time there is no direct evidence to support this hypothesis.

On the other hand, a direct effect of exogenous cholesterol *per se* on hepatic cholesterol biosynthesis would appear to be a more simple explanation. The physical state in plasma of exogenously derived cholesterol is probably different from that of cholesterol associated with the major plasma lipoproteins, and as such this plasma-cholesterol increment (which might be quite minute at any given time) is taken up in part by the liver. It is known that in some species such as the rat, excess cholesterol in the diet results in deposition of esterified cholesterol in the liver, the amount of unesterified sterol in this organ remaining fairly constant.

In our laboratory, male rats were maintained on diets containing from none to 0·25% cholesterol for several weeks. They were then killed, and the percentage incorporation of 1-^{14}C acetate into cholesterol was studied by a liver-slice technique. Simultaneous determinations were made of hepatic esterified and unesterified cholesterol, and as the latter fraction remained fairly constant, the measure of hepatic cholesterologenesis was plotted against the other variable, the liver esterified cholesterol, as shown in Fig. 4.

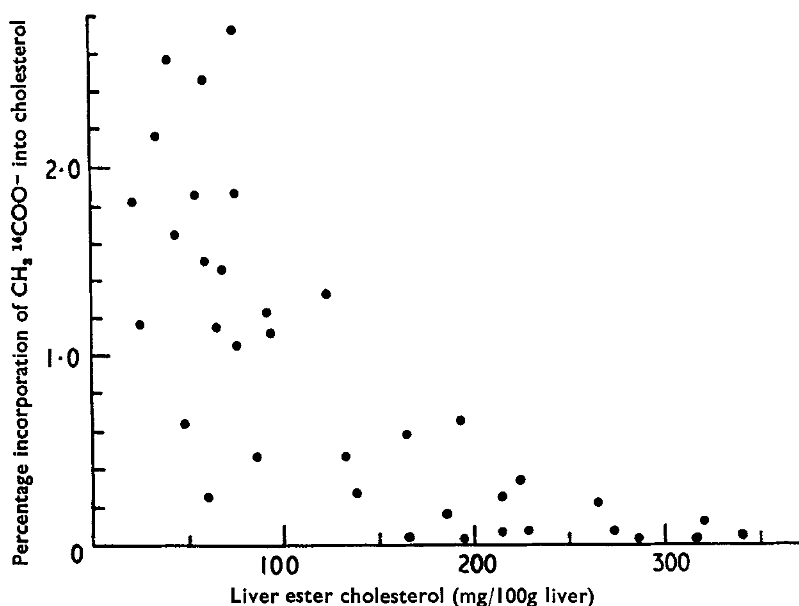

Fig. 4. Percentage incorporation of $CH_3{}^{14}COO^-$ into cholesterol by liver slices, plotted against the ester-cholesterol content of the livers.

Considerable variation has been experienced in the results obtained in cholesterol-biosynthesis experiments by the liver-slice technique. Thus the spread of results obtained in any one series from an apparently homogeneous group of animals is

https://doi.org/10.1079/PNS19560011 Published online by Cambridge University Press

very considerable and serves to accentuate the existence of other (uncontrolled) factors in this complex biosynthesis. Nevertheless, a relationship between the esterified cholesterol content of the organ and cholesterologenesis appears to exist, a finding in general agreement with that of Frantz, Schneider & Hinkelman (1954), who related the total cholesterol content to the rate of synthesis.

This problem may be considerably clarified when studies on the distribution of cholesterol in liver-cell fractions such as those of Rice, Schotz, Alfin-Slater & Deuel (1953) are extended and coupled to biosynthesis experiments performed by sub-cellular techniques similar to those of Bucher (1953) and Frantz & Bucher (1954). By this approach it should be possible to test whether the apparent inverse relationship between esterified cholesterol content and cholesterol biosynthesis persists at the subcellular level.

Apart from the influence of a specific dietary constituent such as cholesterol on hepatic sterol biosynthesis, the act of withholding food from some species for a brief period has a dramatic effect on synthesis of cholesterol in the liver. Tomkins & Chaikoff (1952) fasted rats for periods varying from 24 to 72 h, and found by in vitro studies that hepatic cholesterol synthesis was very low in all their fasted animals when contrasted with the control animals.

This biosynthesis only proceeds under aerobic conditions and is an endergonic process requiring energy from high-energy phosphate or acylmercaptan bonds. If carbohydrate, as one of the principal available donors of metabolic energy, is depleted, as it would be in the livers of fasted rodents, then cholesterol biosynthesis might be expected to suffer this partial inhibition.

Reasoning along these lines prompted us to re-examine data for control rats from other experiments in which we had available results both for liver glycogen

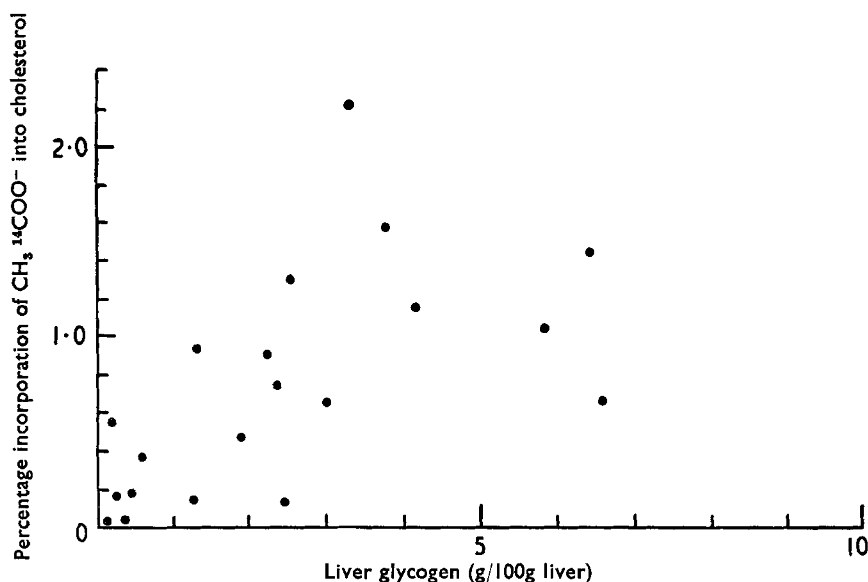

Fig. 5. Percentage incorporation of $CH_3{}^{14}COO^-$ into cholesterol by liver slices, plotted against the glycogen content of the livers.

https://doi.org/10.1079/PNS19560011 Published online by Cambridge University Press

content, and for in vitro hepatic cholesterol biosynthesis. This information, together with additional data on animals fasted for periods up to 24 h (Boyd & McGuire, unpublished) is shown in Fig. 5. Although the biosynthesis data are again very variable, there is a certain degree of correlation between the available tissue carbohydrate and the rate of cholesterol biosynthesis in liver slices.

Since hepatic glycogen stores may fluctuate considerably throughout the day, it would appear to be advisable to co-ordinate data for liver biosynthesis with carbohydrate estimations when investigating factors involved in hepatic cholesterologenesis.

The liver-glycogen and blood-glucose levels are closely related, and recent evidence points to the arterio-venous blood-glucose difference probably exerting an influence over energy balance and food intake (Mayer & Bates, 1952; Mayer, 1953) through a hypothalamic mechanism. Lesions in the hypothalamus are known to produce obesity due to hyperphagia with a consequent increase in neutral fat in adipose tissue. There is also evidence that the hypothalamus functions in the integration of a multiplicity of metabolic activities ranging from carbohydrate, fat, and electrolyte metabolism to temperature regulation. Since this control may be mediated in part through the anterior pituitary and the secretions of this endocrine gland are known to influence cholesterologenesis (Tomkins, Chaikoff & Bennett, 1952), the nutritional and endocrinological aspects of cholesterol metabolism may be integrated at the hypothalamic level.

REFERENCES

Biggs, M. W., Kritchevsky, D., Colman, D., Gofman, J. W., Jones, H. B., Lindgren, F. T., Hyde, G & Lyon T. P. (1952). *Circulation*, **6**, 359.
Bloch, K., Borek, E. & Rittenberg, D. (1946). *J. biol. Chem.* **162**, 441.
Bloch, K., Clark, L. C. & Harary, I. (1954). *J. biol. Chem.* **211**, 687.
Bloch, K. & Rittenberg, D. (1942). *J. biol. Chem.* **145**, 625.
Boyd, G. S. (1953). *Biochem. J.* **55**, 892.
Boyd, G. S. (1954). *Biochem. J.* **58**, 680.
Bucher, N. L. R. (1953). *J. Amer. chem. Soc.* **75**, 498.
Channon, H. J. (1925). *Biochem. J.* **19**, 424.
Channon, H. J. (1926). *Biochem. J.* **20**, 400.
Cook, R. P. & Thomson, R. O. (1951). *Quart. J. exp. Physiol.* **36**, 61.
Cornforth, J. W., Hunter, G. D. & Popják, G. (1953*a*). *Biochem. J.* **54**, 590.
Cornforth, J. W., Hunter, G. D. & Popják, G. (1953*b*). *Biochem. J.* **54**, 597.
Frantz, I. D. & Bucher, N. L. R. (1954). *J. biol. Chem.* **206**, 471.
Frantz, I. D., Schneider, H. S. & Hinkelman, B. T. (1954). *J. biol. Chem.* **206**, 465.
Gould, R. G. (1951). *Amer. J. Med.* **11**, 209.
Gould, R. G., Campbell, D. J., Taylor, C. B., Kelly, F. B., Warner, I. & Davis, C. B. (1951). *Fea Proc.* **10**, 191.
Gould, R. G. & Taylor, C. B. (1950). *Fed. Proc.* **9**, 179.
Gould, R. G., Taylor, C. B., Hagerman, J. S., Warner, I. & Campbell, D. J. (1953). *J. biol. Chem.* **201** 519.
Klein, H. P. & Lipmann, F. (1953). *J. biol. Chem.* **203**, 95.
Langdon, R. G. & Bloch, K. (1953). *J. biol. Chem.* **202**, 77.
Landon, E. J. & Greenberg, D. M. (1954). *J. biol. Chem.* **209**, 493.
Little, H. N. & Bloch, K. (1950). *J. biol. Chem.* **183**, 33.
Lynen, F. (1953). *Fed. Proc.* **12**, 683.
Mauthner, J. & Suida, W. (1896). *Mh. Chem.* **17**, 41.
Mayer, J. (1953). *New Engl. J. Med.* **249**, 13.
Mayer, J. & Bates, M. W. (1952). *Amer. J. Physiol.* **168**, 812.
Menschick, W. & Page, I. H. (1933). *Hoppe-Seyl. Z.* **218**, 95.
Oliver, M. F. & Boyd, G. S. (1953). *Brit. Heart J.* **15**, 387.

https://doi.org/10.1079/PNS19560011 Published online by Cambridge University Press

Oliver, M. F. & Boyd, G. S. (1955). *Brit. Heart J.* **17**, 299.
Page, I. H. & Menschick, W. (1932). *J. biol. Chem.* **97**, 359.
Rabinowitz, J. L. & Gurin, S. (1953). *Biochim. biophys. Acta*, **10**, 345.
Rabinowitz, J. L. & Gurin, S. (1954). *J. biol. Chem.* **208**, 307.
Rice, L. I., Schotz, M. C., Alfin-Slater, R. B. & Deuel, H. J. Jr. (1953). *J. biol. Chem.* **201**, 867.
Rittenberg, D. & Schoenheimer, R. (1937). *J. biol. Chem.* **121**, 235.
Russ, E. M., Eder, H. A. & Barr, D. P. (1951). *Amer. J. Med.* **11**, 468.
Schoenheimer, R. & Breusch, F. (1933). *J. biol. Chem.* **103**, 439.
Siperstein, M. D., Chaikoff, I. L. & Chernick, S. S. (1951). *Science*, **113**, 747.
Srere, P. A., Chaikoff, I. L. & Dauben, W. G. (1948). *J. biol. Chem.* **176**, 829.
Srere, P. A., Chaikoff, I. L., Treitman, S. S. & Burstein, L. S. (1950). *J. biol. Chem.* **182**, 629.
Steiner, A., Kendall, F. E. & Mathers, J. A. L. (1952). *Circulation*, **5**, 605.
Tomkins, G. M. & Chaikoff, I. L. (1952). *J. biol. Chem.* **196**, 569.
Tomkins, G. M., Chaikoff, I. L. & Bennett, L. L. (1952). *J. biol. Chem.* **199**, 543.
Tomkins, G. M., Sheppard, H. & Chaikoff, I. L. (1953). *J. biol. Chem.* **201**, 137.
Waelsch, H., Sperry, W. M. & Stoyanoff, V. A. (1940). *J. biol. Chem.* **135**, 297.
Wainfan, E., Henkin, G., Rittenberg, S. C. & Marx, W. (1954). *J. biol. Chem.* **207**, 843.
Wüersch, J., Huang, R. L. & Bloch, K. (1952). *J. biol. Chem.* **195**, 439.

Experimental cholesterol atherosclerosis

By I. Rannie, *Department of Pathology, King's College, University of Durham and Royal Victoria Infirmary, Newcastle upon Tyne*

Since Anitschkow (1913) first produced cholesterol atherosclerosis in rabbits his experiments have been repeated by many investigators who, though confirming his original findings, have failed to agree in the interpretation of the condition. Briefly stated, the result of prolonged cholesterol feeding in rabbits, as far as the blood vessels are concerned, is the production of changes in their walls commencing after a latent period of from 3 to 6 weeks. The changes are essentially focal accumulations of lipid-bearing phagocytes in the intima which becomes progressively thicker and eventually shows evidence of fibrosis with degeneration and sometimes calcification in the deeper layers. The lesions thus closely resemble human atheroma.

Interpretation

Anitschkow, up to 1933 (Anitschkow, 1933), held that the lesions were produced by the infiltration of cholesterol from the plasma through the endothelium and that its focal distribution in the vessel wall was due to local differences in the permeability of the endothelium. Duff (1936), on the other hand, said that the initial lesions were in the media due to some unrecognized metabolic product, and that the site of the intimal lesion was determined by the medial damage.

The origin of the foam cells or lipophages which appear in the intima has been a matter of controversy. The majority of investigators have regarded them as local endothelial or connective-tissue cells which have picked up cholesterol and other lipids from the imbibed plasma; but Leary (1941, 1949) looked on them as cells of the reticulo-endothelial system, most probably Küpfer cells, which have detached themselves from the sinusoidal walls of the liver and entered the circulation. He

https://doi.org/10.1079/PNS19560011 Published online by Cambridge University Press