Lipid metabolism in the fowl under normal and abnormal circumstances

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This paper summarizes current knowledge of certain aspects of the normal metabolism of fatty acids and glycerides, the hormonal control of these processes, the disturbances produced by stress, infection and microbial toxins and the abnormalities present in fatty liver diseases. Differences in the lipid metabolism of avian and mammalian species are noted.

Normal metabolism

Like the mammal, the fowl utilizes fat as its main energy store, taking advantage of the high energy value of fat compared with that of carbohydrate or protein, and its low water content. The lipids present in these species are similar in nature and composition and there appear to be no gross differences in the pathways by which they are metabolized or in the biochemical mechanisms involved (Annison, 1971; Pearce, 1974). The differences which do exist are quantitative rather than qualitative and are expressed physiologically.

Absorption and transport. When a commercial diet is given, the fatty acids and glycerides present in the fowl are derived largely from dietary carbohydrates. However, this type of diet is not obligatory for the chick, and fat can be used to supply over 90% of the 'carbohydrate' energy without affecting growth (Renner, 1964). In the laying hen gluconeogenesis does not appear to be adequate under these circumstances, and egg production and hatchability are reduced (Roland & Edwards, 1972).

Dietary lipids are absorbed mainly from the small intestine, after undergoing partial hydrolysis in the lumen and resynthesis in the mucosa. The mechanism differs from that in mammals in that most of the lipids enter the portal system directly as very-low-density lipoproteins (Noyan, Lossow, Brot & Chaikoff, 1964; Bensadoun & Rothfeld, 1972) whereas in mammals they first pass into the lymph as chylomicrons and then enter the systemic circulation via the thoracic duct. Chylomicrons occur in relatively low concentrations in fowl plasma and are accompanied by the various classes of lipoproteins normally responsible for transporting lipids in mammals (Butler, 1971). The concentrations of the major lipid classes present are similar to those in man.

Lipogenesis. In birds the liver is mainly responsible for lipid synthesis (Goodridge, 1968a; O'Hea & Leveille, 1969), with the adipose tissue and skin making very small contributions (Yeh & Leveille, 1973), whereas in mammals the adipose tissue plays

the major role. The metabolic activity of the liver is therefore extremely high in the fowl, especially during egg production, when the amount of lipid synthesized per year almost equals the body-weight.

The biochemical mechanisms involved appear to be very similar if not identical in these animals. Saturated fatty acids are synthesized from acetyl-CoA, which is produced initially in the mitochondria largely from glycolysis and fatty acid catabolism. At least three systems are involved in liver. In the cytoplasm acetyl-CoA carboxylase (*EC* 6.4.1.2) produces malonyl-CoA for conversion into fatty acids (mostly palmitate) by fatty acid synthetase, which is a multi-enzyme complex requiring $NADPH_2$ as a hydrogen donor (Yun & Hsu, 1972). Chain elongation systems which also require this coenzyme are present in the microsomes and mitochondria, the former requiring malonyl-CoA as a substrate and the latter acetyl-CoA (Donaldson, Mueller & Mason, 1971). In the fowl most of the $NADPH_2$ required for these processes is provided by the malic enzyme system (Goodridge, 1968*b*, *c*) whereas in the mammal this function is performed by the pentose phosphate pathway. The 'non-essential' unsaturated fatty acids such as oleic and palmitoleic acids are formed from the corresponding saturated fatty acids by the action of desaturation systems (Johnson, Fogerty, Pearson, Shenstone & Bersten, 1969). Glycerides, phospholipids and cholesterol esters are produced from the CoA derivatives of fatty acids.

Acetyl-CoA carboxylase is believed to be the most important rate-limiting enzyme in cytoplasmic fatty acid synthesis, with the synthetase enzyme complex probably playing a minor role. The former contains biotin as a prosthetic group (Lane, Edwards, Stoll & Moss, 1970) and its activity is therefore dependent upon the availability of the vitamin (Mason & Donaldson, 1972). It is also regulated by tricarboxylic acids and long-chain fatty-acyl-CoA derivatives, which have stimulatory and inhibitory effects respectively (Lane *et al.* 1970; Goodridge, 1972, 1973), thereby providing fine control of fatty acid synthesis by carbohydrate and lipid intake through feed-forward and feed-back mechanisms.

The synthesis is coarsely controlled in the long term by dietary factors and hormones which influence the rate of enzyme production, but these can have a remarkable effect. For instance, the secretion of ovarian hormones in the pullet at the onset of sexual maturity causes a tremendous increase in lipogenesis in preparation for egg production (Husbands & Brown, 1965) and the transport of large amounts of lipids from the liver to the ovaries produces a marked hyperlipidaemia (McIndoe, 1959; Heald & Badman, 1963). These changes result from an increased synthesis of lipogenic enzymes (Pearce, 1971) and can be simulated by the administration of oestrogens to immature fowls of either sex (Lorenz, 1954) thereby creating a valuable model for experimental work.

Although adipose tissue has little capacity for lipogenesis in the fowl it does form the major storage depot. Lipids are released from circulating lipoproteins and hydrolysed by a lipase present in the capillary walls of the tissue ('clearing-factor lipase') and are resynthesized in the adipocytes. In mammals the lipase is inhibited by hormones which stimulate the production of cyclic AMP.

Lipolysis. Although free fatty acids comprise only about 5% of the lipids circulating in the plasma, they are of great metabolic importance since their turnover rate is high. They are released rapidly from triglycerides stored in adipose tissue in response to hormonal stimulation or a reduction in nutritional status and are transported by plasma albumin to the various tissues where they undergo β-oxidation in the mitochondria (Pugh & Sidbury, 1971).

Their release from adipocytes is brought about by a lipase whose activation by hormones is mediated by cyclic AMP and probably involves phosphorylation by a kinase enzyme. Hormones stimulate lipolysis by accelerating the formation of cyclic AMP from ATP, and in mammals the catecholamines are mainly responsible for this function. In birds, however, glucagon appears to be the dominant lipolytic hormone, as judged by the responses obtained in both in vivo and in vitro experiments (Langslow & Hales, 1971; Freeman & Manning, 1974).

A further indication of the importance of glucagon to the fowl is provided by its high concentration in the pancreas, about ten times greater than that in mammals (Hazelwood, 1973). On the other hand, the concentration of insulin is comparatively low and reflects its relatively minor role in the control of avian metabolism (Hazelwood, 1973). No significant anti-lipolytic effects have been demonstrated in experiments with this hormone, whereas in the mammal it inhibits lipolysis by reducing the intracellular concentration of cyclic AMP.

Effects of stress

As with mammals, exposure to cold or heat stimulates lipolysis in the fowl, as indicated by an increase in the plasma free fatty acid level (Freeman, 1967; Braganza, Peterson & Candella, 1973). Since the injection of adrenocorticotrophic hormone (ACTH) also produces this response (Jaussi, Newcomer & Thayer, 1962), other stressors might be expected to have a similar effect.

The hyperlipidaemic response to ACTH does, however, depend upon the age of the fowl and is not observed in the newly hatched chick. At this age it produces heat in response to cold stress largely by non-shivering thermogenesis and, as in the mammal, the oxidation of free fatty acids makes an important contribution to heat production. However, the site of heat production and the hormone used to stimulate lipolysis differ. In the rabbit and guinea-pig, for example, the heat is produced in the brown adipose tissue in response to the release of noradrenaline from the nerve endings. The young chick, on the other hand, has no specialized or specific tissue for this purpose and shows a reduction rather than an increase in body temperature when injected with this hormone (Freeman, 1970). It may utilize glucagon instead of noradrenaline to provide the primary stimulus and thyroid hormones to augment the response (Freeman, 1971; Palokangas, Vihko & Nuuja, 1973).

Effects of infection and bacterial toxins

Information on the effect of pathogenic agents on the lipid metabolism of the fowl is scanty. Lipid absorption is depressed in coccidial infections, particularly when

the duodenum is affected, as with *Eimeria acervulina* infection (Turk & Stephens, 1967*a*, *b*). Hyperlipidaemia caused by increases in the triglyceride and phospholipid levels has been observed in embryos infected with Japanese encephalitis virus (Grossberg & O'Leary, 1965). The cholesterol level was reduced in these embryos but was increased in chicks infected with Newcastle disease virus (Squibb, Beisel & Bostian, 1971). Reductions in plasma lipid levels have been found in chicks infected experimentally with *Escherichia coli* or injected with its endotoxin (Panigraphy, Waibel & Pomeroy, 1963; M. J. Curtis, B. W. Scott & E. G. Harry, unpublished results) whereas in mammals Gram-negative organisms and their endotoxins produce hyperlipidaemia and hepatic steatosis (Beisel & Fiser, 1970). The reason for this difference in response is not known. More information is obviously needed on the mechanism of this interaction between infection and metabolism.

Fatty liver diseases

The lipids present in the liver are derived from three main sources, dietary fats, adipose tissue depots and hepatic synthesis from carbohydrate. Consequently steatosis can be produced by a variety of physiological and metabolic disturbances, the most important being: (1) decreased lipid oxidation, as in biotin deficiency; (2) enhanced hepatic lipogenesis, produced for example by high carbohydrate intake or essential fatty acid deficiency; (3) decreased formation or release of plasma lipoproteins as in choline or protein deficiency or aflatoxicosis; (4) reduced activity of lipoprotein lipase, due to inhibition by cyclic AMP, for example; and (5) increased lipolysis in adipose tissue, stimulated for example by starvation or stress.

At present, the most common diseases of the fowl that are characterized by hepatic steatosis are known as fatty liver and kidney syndrome, which affects young chicks, and fatty liver–haemorrhagic syndrome, which occurs in laying hens. Their aetiologies involve dietary, environmental and genetic factors, but appear to be unrelated.

Fatty liver and kidney syndrome. This disease, which is sometimes known as 'fat nephrosis', occurs in several countries, particularly in broiler breeds (Laursen-Jones, 1971). The affected chicks are usually aged between 10 and 30 d but are sometimes younger. They become lethargic and appear to be paralysed, and usually die within a few hours, the mortality reaching 20% in some instances. Their growth rate is not appreciably depressed. Outbreaks appear to be precipitated by stress such as may be caused by low or high temperatures, a lighting failure or vaccination.

Excess fat is deposited in the liver, the kidney tubules and in other tissues, and the myocardial adipose tissue is often pink due to the congestion of the small blood vessels. Lesions occur constantly in the epithelium of the proximal convoluted tubules of the kidney and frequently there are small haemorrhages on the periphery of the liver.

The disease is frequently associated with wheat-based diets containing low levels of fat and protein (Husbands & Laursen-Jones, 1969; Blair, Whitehead, Bannister & Evans, 1973; Whitehead & Blair, 1974*a*) and can be prevented by supplementing

the diet with molasses or the residue from their fermentation (Hemsley, 1973a, b), or with biotin (Whitehead & Blair, 1974b). Disturbances occur in both carbohydrate and lipid metabolism (Evans & Bannister, 1974). The affected chicks are hypoglycaemic, plasma pyruvate levels are elevated and hepatic gluconeogenesis and glycogenesis are reduced. Hepatic lipogenesis is also impaired and the presence of excess lipid in the plasma and organs such as the liver and kidney appears to be due largely to a reduction in lipoprotein lipase activity in adipose tissue. The role of biotin in the aetiology of this disease is not yet clear.

Fatty liver–haemorrhagic syndrome. Outbreaks of this condition occur sporadically in Europe and the USA, particularly in caged flocks of the heavier breeds during hot weather (Couch, 1956; Ringer & Sheppard, 1963; Greuel & Hartfiel, 1968; Nesheim, Ivy & Norvell, 1969; Wolford, Ringer, Sheppard, Barton & Flegal, 1971; Wolford & Polin, 1972). Steatosis is accompanied by haemorrhage from the liver, causing death in extreme cases, and by the presence of excessive amounts of abdominal fat. Egg production is sometimes depressed but often there are no clinical signs of disease until mortality occurs.

Large amounts of lipids ($>$100 mg/g fresh weight) accumulate in the liver cells, disorganizing their internal structure and imparting a putty colour to the liver. The whole organ is very friable, probably owing to the degeneration of reticulin (Hall, 1974). The fat present has abnormally high contents of neutral lipids, mainly triglycerides, and oleic and palmitic acids, and reduced contents of linoleic and arachidonic acids (Greuel & Hartfiel, 1968; Ivy & Nesheim, 1973; A. W. Pearson, unpublished results). Since there is no evidence that the syndrome is associated with a dietary deficiency of a lipotropic factor, such as choline, this pattern, together with the hyperlipidaemia which is present, indicates that the excess fat in the liver arises mainly from increased lipogenesis rather than from dietary lipids and that there is probably no major defect in the transport mechanism.

The increased lipogenesis is probably due partly to an excessive intake of carbohydrate brought about by lack of exercise and high environmental temperatures. Similar changes in lipid metabolism can be produced by force-feeding (Ivy & Nesheim, 1973). The cereal used as the carbohydrate source may also have an effect (A. W. Pearson & A. P. Laursen-Jones, unpublished results), and there is now evidence that the F-2 toxin of *Fusarium graminearum*, which frequently infects cereals, behaves as an oestrogen in stimulating lipogenesis (A. W. Pearson, W. R. Rosser, A. Hacking & J. Lacey, unpublished results). Force-feeding also increases the incidence of liver haemorrhage (Wolford & Polin, 1974) and since a similar syndrome can be reproduced in immature fowls by giving repeated injections of oestradiol (Wolford & Polin, 1973; A. W. Pearson, unpublished results) there is considerable support for the view that the haemorrhage can result from the steatosis.

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