HEPARIN, THE CLEARING FACTOR LIPASE, AND FAT TRANSPORT

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The discovery of the heparin clearing reaction has suggested that heparin, in addition to its well known function as an anticoagulant, may be implicated in the transport of fat. When heparin is given intravenously to an animal which is absorbing fat and which has a visible lipaemia, plasma samples withdrawn a few minutes after the injection are clear (80, 180). Heparin added to lipaemic plasma *in vitro* does not cause clearing of the lipaemia, but, when plasma from an animal previously injected with heparin is mixed with lipaemic plasma clearing does occur *in vitro* (4). Thus after the administration of heparin to an animal the blood contains a so-called clearing factor.

The clearing reaction has now been investigated extensively and comprehensive reviews of the subject are available (117, 151). A complete survey will not be attempted here but particular attention will be given to the following aspects: the measurement of clearing activity, the release and mechanism of action of the clearing factor and the possible physiological significance of the reaction in fat transport.

THE NATURE OF THE CLEARING REACTION

The turbidity of the plasma in alimentary lipaemia is due to the presence of large numbers of visible lipid particles or chylomicra.[2] It is now generally accepted that plasma obtained after heparin injection (post-heparin plasma) clears this turbidity *in vitro* because it contains an enzyme which hydrolyses the triglycerides of chylomicra. The unesterified fatty acids (U.F.A.) liberated combine with plasma albumin and, since the complex formed is soluble and the

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[2] The term 'chylomicron' was introduced by Gage and Fish (65) in 1924 to describe the lipid particles which were responsible for the turbidity of lipaemic plasma. The term has been used here in a wider sense to include lipid particles in both lipaemic plasma and chyle. The source, either plasma or chyle, has been noted wherever this is relevant, since there is evidence that the lipid particles from plasma and chyle are not identical.

chylomicra disappear, the plasma becomes clear. These changes occur *in vitro* without any alteration in total fatty acid content (*cf.* 151).

The clearing factor is therefore a lipase. There is no evidence that it acts directly *in vitro* on any lipid in chylomicra other than triglyceride (171), although it is possible that cholesterol present may be redistributed in association with the U.F.A. released during lipolysis (119, 154).

The changes in blood lipids which follow the injection of heparin in the living animal appear to be explained in terms of the lipase demonstrated *in vitro*, but in addition the conversion of chylomicron triglyceride to U.F.A. increases the rate at which fatty acids leave the blood. The clearing observed *in vivo* is the result of both these effects.

That intravascular lipolysis of triglyceride occurs *in vivo* after heparin is injected is suggested by the changes in the distribution of the low density lipoproteins.[3] There is a fall in the concentration of lipoprotein components of lowest density and highest triglyceride content and a concomitant rise in the denser components, containing less triglyceride (35, 76, 119, 120). The U.F.A. content of the plasma also increases (74, 77, 78, 120, 175).

The increase in the electrophoretic mobility of the lipoproteins found after heparin injection during alimentary lipaemia (*cf.* 117, 151) is probably caused by an association of U.F.A. released on lipolysis with the plasma lipoproteins as well as the plasma albumin (72, 111, 130).

Heparin injection causes a fall in the *total* fatty acid content of plasma during alimentary lipaemia which suggests that fat leaves the circulation at an increased rate (24, 78, 120). This is confirmed by experiments in which the rate of removal from the circulation of injected triglyceride is followed. Heparin causes an increase in the rate of removal of injected chylomicra obtained from plasma (21), of artificial triglyceride emulsions (58) and of homologous fatty chyle (79). This increased rate of removal is probably the result of intravascular lipolysis since U.F.A. when introduced into the circulation are removed very rapidly (11, 53, 55, 57, 113), presumably by dissociation of a fatty acid–albumin complex at the capillary membrane. The mechanism of this dissociation is not understood (*cf.* 61).

The action of a lipase in the blood after the injection of heparin appears, therefore, to be an adequate explanation of the different aspects of the clearing reaction as observed both *in vitro* and *in vivo*.

An enzyme which appears to be identical with the lipase present in the blood after heparin injection has been demonstrated in extracts of heart and adipose tissue (106, 107, 108a, 109). The relationship of the enzyme in the tissues to that in the blood will be considered below.

[3] The lipoproteins can be separated on the basis of their behaviour in the ultracentrifuge into high and low density groups (*cf.* 120). These correspond approximately to the classification of α- and β-lipoproteins as based on chemical fractionation and electrophoresis. Both groups are complex and can be subdivided further. In particular, the low density lipoproteins form a large class of molecules the density of which decreases as their triglyceride content increases.

The measurement of clearing factor lipase activity

A variety of methods has been used to measure the activity of clearing factor lipase in post-heparin plasma and tissue extracts, and a number of general principles have become established which should be taken into account in future work. In the first place it is important wherever possible to make direct measurements of the rate of lipolysis rather than of turbidity change. The turbidity of a lipid emulsion depends not only on its lipid content but also upon the nature of the emulsifying agents present, with the result that lipid preparations with the same triglyceride content can have different turbidities. For example, under certain conditions, non-turbid low density lipoproteins containing triglyceride may be present in a chylomicron preparation and may act as substrates for the clearing factor lipase, yet their breakdown will not result in any turbidity change (25, 107, 120). The clearing of a lipid emulsion may occur also by changes in the physical structure of the emulsion as well as by chemical changes in the lipid components which are being studied. Moreover, a change in turbidity from the chemical reaction alone may be obscured if the turbidity of the suspending medium changes as a result of protein or fatty acid precipitation. For these reasons the rate of turbidity change in a clearing system may not correspond to the rate of lipolysis.

Before the lipolytic nature of the clearing reaction was recognized the rate of change in turbidity was the only measurement made. Whenever subsequent study has shown that the rate of clearing could be correlated with the rate of lipolysis, the main conclusions drawn from the earlier work have been confirmed. However, where this correlation does not exist, the earlier findings may have been misleading. For example, the lipaemia which is produced in the rabbit by excessive bleeding was at first thought to be associated with an inability to produce clearing factor, since it was not abolished by heparin injection (176). However, subsequent study has shown that lipolysis does occur *in vitro* in post-heparin plasma from these animals (152). This suggests that a further investigation should be undertaken of other situations where a lack of response to heparin has been assumed on the basis of clearing studies alone (30, 40, 41, 63).

During clearing factor lipase action *in vitro* lipolysis proceeds rapidly to the monoglycerides and these are then broken down more slowly (16). Further, the plasma albumin becomes saturated as an acceptor of released U.F.A. at a U.F.A./albumin ratio of approximately 6/1 (150). If in any test system the albumin concentration is low initially or if some of its binding sites are already occupied by U.F.A. or other substances, the rate and extent of lipolysis of an added lipid substrate may be reduced. For these reasons it is important when measuring clearing factor lipase activity to add albumin and substrate in adequate amounts and to record the initial rates of lipolysis.

When comparing the response of different individuals to heparin it is essential to work within the linear dose-response range and to measure plasma lipolytic activity at varying intervals after injection. The response of an individual to

heparin increases with the amount of heparin injected up to a maximum, and if this maximum is exceeded the response is no longer quantitative (62, 128). In some individuals the activity in the blood rises and falls rapidly; in others it follows a more prolonged time-response curve (37). Lipolytic activity measured at a single time interval after injection, therefore, can give only incomplete information.

If the lipolytic activity measured in plasma *in vitro* after heparin injection is reduced it is possible that the amount of lipase induced may be normal but that inhibitors of the enzyme are present in the blood. Such inhibitory substances can be detected by measuring clearing activity in a control sample of post-heparin plasma to which a pre-heparin sample of the plasma under investigation has been added. The presence of an inhibitor of clearing in a protein fraction obtained from normal plasma has been reported (6, 95, 96). Further investigations should be made to determine whether this fraction also reduces the rate of lipolysis. Certain components of the blood, including platelets, white cells and extracts of platelets and white cells, are also reported to inhibit the clearing reaction (50, 129). Although the degree of inhibition may be small under normal conditions it is preferable to remove these components before estimating lipolytic activity. Substances such as bile salts, which inhibit the clearing factor lipase *in vitro*, may be present in the plasma in certain pathological conditions and may cause an apparent reduction in response to heparin. Animals which have been injected with thorium dioxide fail to respond to heparin (116) but this may be due to the presence in the blood of inhibitors of the clearing factor lipase after thorium dioxide injection rather than to a failure to release the enzyme (149).

There are several reports in the literature of deficiencies in the release of clearing factor in response to heparin with increasing age and in certain pathological conditions, notably in atherosclerosis. Most of these studies are difficult to interpret in view of the above considerations. With the possible exception of some (84), though not all (32), cases of essential hyperlipaemia no deficiency in the response to heparin has yet been conclusively demonstrated.

The mechanism of action of the clearing factor lipase

The substrate. A number of different lipid preparations will act as substrates for the clearing factor lipase. They include lipaemic plasma obtained during the absorption of a fatty meal, chylomicra recovered by centrifugation from lipaemic plasma, chylomicra from fatty chyle, and various artificial triglyceride emulsions. The chylomicra obtained from plasma by centrifugation and the chylomicra in chyle are heterogenous and contain turbid lipid particles of varying size and composition. In addition there are in lipaemic plasma and in chyle non-turbid low density lipoproteins which contain triglyceride (39, 119, 139). The relative proportion of such lipoproteins present in any chylomicron sample will increase with the speed of centrifugation used in its preparation.

These lipid preparations will now be considered in relation to the substrate

specificity of the enzyme. Artificial triglyceride emulsions prepared with synthetic emulsifying agents are, in general, adequate substrates for the clearing factor lipase in post-heparin plasma although the rates of lipolysis observed may vary with the agent used and certain of them may be inhibitory. Artificial emulsions do not seem suitable for the study of the clearing factor lipase present in extracts of heart and adipose tissue. Korn (107) and Korn and Quigley (109) showed that an emulsion of coconut oil was attacked at only a slow rate by these extracts unless small quantities of either serum or a high density lipoprotein fraction from plasma were present.

Chylomicra from chyle are suitable substrates for the lipase in post-heparin plasma and in extracts of adipose tissue (62, 149). Such chylomicra contain over 90% of their fatty acids as triglycerides together with small quantities as phosphatides and cholesterol esters (22, 88, 110, 147). The phosphatide component is essential for their stability (147). They also contain a small amount of protein, reported by different investigators to vary from a trace to 2% of the dry weight. The percentage found may depend on the conditions used in separating the chylomicra from subnatant lymph protein (19). If the chylomicra are prepared by flotation at a relatively low centrifugal speed, the percentage protein in the final preparation may be as low as 0.2%; when flotation is carried out at a higher speed, the final protein content is higher. This may be due either to differences in the protein content of different density fractions of chylomicra or to contamination with non-turbid lipoproteins.

There seems little doubt that a small quantity of protein is present in the chylomicron though the amount is not sufficient to form a complete layer over the chylomicron surface (19). This protein is complex and has been separated into three components by end group analysis and other techniques (159). One of these components is indistinguishable from the protein moiety of a high density lipoprotein fraction of plasma. Another is similar to one of the protein components of the low density lipoproteins. The third has not been characterized. While it is unlikely that the high density lipoprotein could be present as a contaminant of the chylomicron fraction, the low density lipoprotein may be. Further study is needed before this component can be accepted as a true constituent of the chylomicron.

Chylomicra which have been prepared from lipaemic plasma or serum, or chylomicra recovered by centrifugation from chyle and subsequently incubated with plasma, differ from chylomicra obtained directly from chyle in their electrophoretic mobility (52), their higher protein content (147, 166) and their immediate hydrolysis by pancreatic lipase preparations (147). They are suitable substrates for both the clearing factor lipase in post-heparin plasma and in extracts of heart and adipose tissue and they contain protein which closely resembles that in a high density lipoprotein fraction from plasma (166).

The non-turbid low density lipoproteins from human plasma serve as substrates for the clearing factor lipase in post-heparin plasma and in extracts of rat heart (107, 120). However, studies have not been carried out on specific

low density lipoprotein fractions of differing triglyceride content; hence, the minimal triglyceride concentration required for lipolytic breakdown of such substrates is unknown.

Since the clearing factor lipase in tissue extracts attacks only coconut oil emulsions which have been activated by a high density lipoprotein from plasma, and since chylomicra from chyle and plasma contain a similar lipoprotein, it has been suggested that the lipoprotein plays an important part in the action of the clearing factor lipase (109). These considerations have led Korn (106, 107) to propose the term 'lipoprotein lipase' for the enzyme. This term has not been adopted here for the following reasons. The requirement for high density lipoprotein is not absolute. Coconut oil emulsions are attacked in the absence of lipoprotein though at a reduced rate (109), and olive oil emulsions, which have been prepared with a mixture of serum phosphatides as emulsifiers, are hydrolysed at a rate up to 50% of the rate of breakdown of chylomicron triglyceride (149). Further, the action of the lipase is not on the lipoprotein but upon the triglyceride of the lipid preparation and, even if the intact lipoprotein molecule is a specific activator, a classification of lipases based on activators alone does not seem justified. The term 'clearing factor' was introduced by Anfinsen, Boyle and Brown (5) before the lipolytic nature of the clearing reaction was appreciated and has been generally accepted in the literature. The term 'clearing factor lipase' seems adequate, therefore, to designate the enzyme.

The function of heparin. The effect of exogenous heparin in inducing clearing activity in the blood was established in the early experiments. Heparin is not unique in this respect and many sulphated polysaccharides act in the same way. Dextran sulphates, sulphated alginic acid, sulphated cellulose, sulphated amylopectin, sulphated hyaluronic acid, Paritol (a polyanhydromannuronic acid polysulphuric acid ester), and Treburon (a sulphated sodium salt of polygalacturonic acid methyl ester methyl glycoside) have all been shown to induce clearing activity on injection (38, 67, 70, 118). The ability of dextran sulphates, of the same molecular weight but different sulphur content, to induce activity increases with an increase in their sulphur content (156). However, the presence of sulphate groups is not essential, since phosphomolybdate, phosphotungstate, silicotungstate, and polymetaphosphate are all active (21, 86). These substances, like heparin, have strongly negatively charged polar groups and it seems that this property may be necessary for the induction of the clearing factor lipase. Exogenous heparin can increase the activity of the enzyme in extracts of heart and adipose tissue (99, 108a). The effect of other substances with negatively charged polar groups has not been investigated.

Heparin may form an integral part of the lipase. Korn (108) has shown that the enzyme in extracts of adipose tissue is progressively inactivated by a bacterial heparinase preparation. It is also inhibited by pyrophosphate and other phosphate derivatives, and this inhibition can be reversed by heparin (106). It has been suggested that such inhibitors competitively replace the heparin which is normally bound to the enzyme. No comparable studies have been made on the enzyme in post-heparin plasma. The inhibition of post-heparin plasma activity by

protamine sulphate, a known heparin antagonist, is inconclusive evidence since protamine sulphate also has a direct effect on the chylomicroe substrate (24a).

Korn and Quigley (109) have suggested that heparin provides the link between the enzyme and the lipoprotein moiety of its substrate. It may be relevant that heparin will combine with lipoproteins and has been used as a complexing agent in the purification of the low density fraction (26, 27, 34, 137).

The combination of an enzyme with its substrate is a generally recognized concept in enzymology, and in the case of water-soluble lipases must be achieved either by their association at an oil-water interface or by their penetration into the oil phase. In either case a combination of the enzyme with its substrate should be demonstrable. This has been achieved for the clearing factor lipase as follows. If post-heparin plasma is stored with a chylomicron preparation at 0°C and the mixture then centrifuged at high speed the lipid layer which separates possesses all the enzyme activity originally present in the post-heparin plasma (158). Similarly, if an extract of adipose tissue is incubated with an activated coconut oil emulsion and the mixture then centrifuged at high speed the enzyme activity originally present in the extract can be recovered in the lipid layer (109).

Heparin may have a further function in stabilizing the clearing factor lipase in plasma. After the passage of post-heparin plasma at 0°C down an anion-exchange column which removes at least part of the heparin from the plasma, the clearing activity of the effluent from the column, although initially unchanged, falls rapidly on incubation at 37°C and is completely abolished in 10 minutes (148). The activity of the effluent is maintained at 37°C if heparin is added before incubation but, once the activity has been destroyed, heparin will not re-activate the system.

The inhibitory action on clearing factor lipase of protamine sulphate and other similar substances (*cf.* 117, 151) may be due to their ability to combine with heparin and so reduce the stability of the enzyme. In the presence of protamine sulphate *in vitro* the lipolytic activity of a sample of post-heparin plasma is high initially but is lost on incubation at 37°C for 10 minutes (153). When protamine sulphate is injected in an animal which has been injected previously with heparin, the clearing activity of the plasma is not destroyed immediately, as might be expected if the inhibition were directly on the enzyme, but can be detected in samples withdrawn some three to four minutes after the protamine (174, 175). The activity of samples of plasma withdrawn subsequently is not restored by adding heparin *in vitro*.

In conclusion, it should be emphasized that the functions of heparin have been discussed almost entirely in terms of exogenous heparin. With the exception of Korn's experiments there is no clear evidence that endogenous heparin is a specific component of clearing factor lipase as this normally occurs.

Comparison of the clearing factor lipase with pancreatic lipase and with esterases. The clearing factor lipase is distinct from the esterases normally present in plasma. It is possible, however, that it may hydrolyse such substances as tributyrin, ethylbutyrate and β-naphthyl laurate (*cf.* 117). In this sense it possesses

esterase, in addition to lipase, activity but this can be clearly differentiated from the normal plasma esterase by the use of inhibitors.

Clearing factor lipase resembles pancreatic lipase in its action on triglycerides containing long chain fatty acids. With both enzymes lipolysis proceeds rapidly to the monoglycerides, and then the hydrolysis of these occurs much more slowly (15, 16). The two enzymes can be distinguished by their susceptibility to inhibitors and activators (94, 117, 151). Pancreatic lipase also differs from the clearing factor lipase in requiring no prior activation of its triglyceride substrate by lipoprotein. It acts less rapidly on an emulsion of coconut oil after this has been activated by lipoprotein (107), and acts on chylomicra from chyle only after a distinct lag period, which can be abolished by prior incubation of the chylomicra with a *Cl. welchii* lecithinase preparation (147).

In conclusion, it appears that more detailed information on the mechanism of action of the clearing factor lipase will require study of the purified enzyme in a clearly defined substrate system. Although some success in purifying the enzyme from post-heparin plasma has already been achieved (93, 135), most studies are carried out at present on the crude enzyme in plasma or in tissue extracts. When the pure enzyme is available it should be possible to determine the exact role of heparin in the enzyme system, for example, and to obtain more precise information on the substrate specificity.

The release of clearing factor lipase into the circulation

Although clearing factor lipase is present in various tissue extracts, it is obtained in highest concentration by release into the circulation after heparin injection. The amount of enzyme released rises with the amount of heparin injected up to a maximum, but very small quantities of heparin will produce detectable activity in the blood: less than 1 μg of heparin per kg of body weight is sufficient in the rat (158).

The enzyme is apparently widely distributed in the body. Perfusion of heparin through the circulation of the hind limb, the lung, abdominal viscera or skin causes the liberation of clearing factor lipase into the perfusate (101a, 153, 177, 180). However, activity is not released after the perfusion of heparin through the circulation of the brain or of the isolated liver (132, 177).

After the injection of heparin, lipolytic activity appears in the blood extremely rapidly. In the rabbit it can be detected in blood samples taken from the femoral vein within twenty seconds of injecting heparin into the femoral artery. The time taken for activity to appear is no greater than the time taken for the injected heparin to traverse the capillary bed of the hind limb (153). These observations suggest, either that the enzyme is already in the circulating blood and is activated by heparin, or that the enzyme is released from some binding site immediately accessible to heparin in the circulating blood.

The failure to induce the clearing factor lipase by the addition of heparin to blood, serum or plasma *in vitro* indicates that the former alternative is incorrect. This is supported by the observation that the perfusion of heparin in albumin solution through an isolated hind limb, from which the blood has been washed

by perfusion of albumin alone, induces lipolytic activity in the perfusate (153). The amount of plasma protein, other than albumin, in this system is extremely small; hence, if a plasma factor is necessary, in addition to the albumin required as an acceptor of U.F.A., it need only be present in trace amounts (*cf.* 5).

As further evidence that clearing factor lipase may be normally liberated from some site immediately accessible to substances in the circulating blood, it has been shown that the enzyme appears rapidly after the injection of dextran sulphates of high molecular weight which are thought not to leave the circulation (156).

Extracts of heart, adipose and other tissues contain clearing factor lipase (5, 99, 106, 109, 184) and it has been suggested that, in extracting the enzyme from heart, heparin may release the lipase from its binding site in the tissue (99). The amount of enzyme in heart extracts is considerably greater, per gram of tissue, than that in other tissue extracts so far studied. Although exact quantitative data are not available, it does not seem, however, that the amount of enzyme detectable in extracts of any single tissue could account for the high level of activity in the blood after heparin injection. Moreover, the presence of the enzyme in various tissue extracts provides no information as to the precise site of the enzyme in the tissues concerned. A possibility that could account for the presence of high levels of activity in the blood immediately following heparin injection and the presence of the lipase in extracts of vascular tissues is that the enzyme is normally localized in the blood vessel walls (151). If a histochemical technique, of the type used to demonstrate the cellular localization of other lipases, could be suitably adapted, then it might be possible to determine the site of the clearing factor lipase specifically.

The mechanism whereby heparin releases the enzyme from its binding site is unknown though it appears to depend on the negative charge associated with the heparin molecule. Two possibilities require investigation. Either exogenous heparin may bring the enzyme into solution by combining with it, or heparin may displace the enzyme and itself become bound at the sites which the enzyme previously occupied. There is some histochemical evidence that heparin and related substances have an affinity for a constituent of the blood vessel wall (51, 165).

THE ROLE OF THE CLEARING FACTOR LIPASE IN FAT TRANSPORT

The clearing reaction, as it occurs in the blood after heparin injection, is an artificial phenomenon which does not necessarily have a direct relationship to the physiological role of the clearing factor lipase. This section will be concerned, therefore, with the possible location and functions of the enzyme in the absence of exogenous heparin.

It would seem probable that clearing factor lipase, with its ability to hydrolyse the triglycerides of chylomicra and low density lipoproteins, has an important function in fat transport. Recent investigations have shown that fat transport is extremely complex and involves the continual intermixing and recycling of fatty acids in the body (17, 43, 54, 114, 115). Since many of the details of these

processes are still unknown, the attempt here to assign a particular role to the clearing factor lipase must be tentative. The subject will be discussed mainly in relation to the role of the lipase in the distribution of chylomicron fat to the tissues and in the mobilization of depot fat during starvation. This division, though helpful in discussing the available data, may not be entirely justified in view of the complex nature of the problem, and may need reinterpretation in the future.

The removal of chylomicron fat from the blood

The chylomicra which carry triglycerides into the bloodstream from the intestinal lymphatics are relatively large particles, 0.1 to 1.5 μ in diameter. Although there is a moderate rise in the total blood fatty acids during fat absorption (123) chylomicron fat is removed very rapidly from the circulation. In the rat, the entry of fat into the blood at a rate of about 1 mg per minute causes a sustained rise of only 20% in the total triglyceride content, indicating that the rate of removal of fat balances its rate of entry (9). Isotopically labelled chylomicron fat has a circulating half-life of only a few minutes when injected intravenously (57, 58, 88). A significant proportion of the fat removed from the blood is taken up by the liver from which it may be redistributed (17, 20, 59): after the injection of labelled chylomicra in the rat, Borgström and Jordan (17) found that the maximum rate of uptake of fatty acids in the liver was 0.4 mg per minute—that is, approximately 40% of the rate of entry of triglyceride into the blood during fat absorption. Approximately 20 to 25% of the injected fat disappearing from the blood can be recovered from the liver, while the remainder either appears as carbon dioxide or is taken up directly by other tissues including the fat depots and skeletal and cardiac muscle (20, 59). The pattern of distribution varies considerably with the nutritional state. In the carbohydrate fed animal for instance, 25 to 30% of the fat can be recovered from the fat depots (20).

The mechanism of this rapid removal of chylomicron fat from the blood is incompletely understood. The triglyceride which reaches the liver cells can do so without prior lipolysis (17, 132), though when lipolysis does occur, the liver takes up U.F.A. readily (91, 132). Whether chylomicron fat is taken up by other tissues as triglyceride or as U.F.A. is at present uncertain. Experiments on adipose tissue incubated *in vitro* have shown an uptake by the tissue of both U.F.A. and triglycerides though the latter only at a slow rate (168, 169). Studies by Borgström and Jordan (17), in which chylomicron triglycerides labelled in both glycerol and fatty acid moieties were injected intravenously, indicate that hydrolysis of the triglyceride precedes uptake by adipose tissue. On the other hand, there is conflicting evidence on the ability of adipose tissue to take up U.F.A. introduced directly into the bloodstream (20, 114). This is clearly an aspect of the problem which demands further investigation.

Removal as triglyceride. To explain the uptake of chylomicron fat as triglyceride by the liver, and possibly by other tissues, it has been suggested that a proportion of the chylomicra reaches the tissue fluids and thence the tissue cells as intact

particles from the bloodstream (*cf.* 61). Lymph collected from various sites in the body contains chylomicra during alimentary lipaemia and these are most abundant in the lymph from the liver (39, 131).

The problem of how chylomicra are transported across the capillary membrane into the tissue spaces in largely an anatomical one. It may perhaps occur most readily in the hepatic circulation where the endothelial lining of the blood sinusoids has a fenestrated structure (49, 81, 162) but this is not a general feature of blood capillaries (8) and there is at present no evidence that the capillaries of adipose tissue, for example, have a specialized structure of this sort (*cf.* 134). The possibility that chylomicra might be fragmented in the blood into smaller lipid complexes, which would penetrate capillary endothelium more readily, has not been investigated.

There is evidence that the reticulo-endothelial system has a role in the removal of fat particles from the blood. When artificial triglyceride emulsions are introduced into the bloodstream they are taken up by Kupffer cells and other cells of this system (66, 100). Dietary cholesterol, which enters the blood with chylomicra, also appears in the Kupffer cells (28) and blockade of the reticulo-endothelial system with colloidal substances causes hypercholesterolaemia (64). On the basis of this type of experiment it has been suggested that Kupffer cells have a function in transferring fat from the blood to the hepatic cells. On the other hand, following the injection of chylomicra or after their perfusion through the isolated liver, fat appears in the parenchymal cells of the liver rather than the Kupffer cells (131, 132, 133). Chylomicra are also taken up much less readily than artificial emulsions by reticulo-endothelial cells at other sites (60). However, the removal of labelled chylomicra from the blood occurs at an exponential rate which is inversely proportional to the initial concentration. These kinetics are similar to those observed with particles known to be removed by the reticulo-endothelial system (58), so that there is the possibility that chylomicra are taken up initially by phagocytosis, though not necessarily by cells forming part of the reticulo-endothelial system (*cf.* 81).

It was thought that direct information on the uptake of chylomicra as particles would be obtained if their phosphatide or protein moiety as well as the triglyceride fatty acid were labelled. If the chylomicra were removed intact, then the phosphatide and protein labels should disappear from the blood at the same rate as the triglyceride label. In such experiments, the rate of removal of the phosphatide and protein was found to be much slower than that of the triglyceride fatty acid (87, 126, 138, 160). However, it was discovered that the phosphatide and protein in chylomicra exchanged with the same components of the plasma lipoproteins so that they could not be considered as fixed components of the chylomicron. For this reason the experiments have proved inconclusive so far. Recently, Hillyard, Cornelius and Chaikoff (91) have found that when chylomicra containing labelled triglyceride and cholesterol are perfused through the isolated rat liver, the cholesterol is removed much more slowly than the triglyceride component. Their findings cannot apparently be accounted for by the rate of exchange of cholesterol between chylomicra and lipoproteins.

If their interpretation is correct then, even in the liver, some disruption of the chylomicron particles may occur before the fat is taken up by the parenchymal cells.

Removal as unesterified fatty acid. The evidence reviewed above for the direct uptake of chylomicron triglyceride by the liver and the possibility that this may occur in other tissues does not exclude alternative mechanisms of removal from the blood. The possibility, which is more relevant to the subject of this review, is that the removal of triglyceride as such is supplemented by a mechanism which involves hydrolysis of triglyceride, either in the blood or at the capillary site of transfer to the tissues. It has been established that the heparin-induced clearing reaction facilitates the removal of chylomicron fat from the bloodstream as a consequence of lipase action. The evidence that a similar process may occur during fat absorption in the absence of exogenous heparin will now be considered.

The orderly sequence of lipoprotein molecules of decreasing triglyceride content found in the blood during alimentary lipaemia and following the injection of low density plasma lipoprotein fractions could represent the products of the partial breakdown of chylomicron triglyceride by lipolysis (69, 103, 119, 141).

A small increase in the U.F.A. content of the plasma occurs during fat absorption (77, 78, 155, 175) and, when chylomicra in which the triglyceride is labelled are injected intravenously, the plasma U.F.A. becomes labelled and its specific activity rises. The extent of this rise in specific activity will be influenced by the concentration and rate of turnover of unlabelled endogenous U.F.A. in the blood at the time. The specific activities observed have in fact varied from 10 to 50% of the specific activity of the injected triglyceride (56, 57, 88).

Mead and Fillerup (127) have shown that the specific activity of the mono- and diglyceride fractions in plasma increases during the ingestion of labelled triglyceride indicating that some lipolysis is occurring. Borgström and Jordan (17), in their experiments with doubly labelled chylomicron fat already referred to, also obtained evidence for partial hydrolysis of chylomicron triglyceride during its removal from the blood.

There appears, therefore, to be good evidence that partial hydrolysis of chylomicron fat occurs during its removal from the circulation. What is the evidence that this is brought about by the clearing factor lipase? In the first place, substances such as protamine sulphate or Triton WR 1339 (an aryl alkyl polyether of phenol, equivalent to Triton A20), which inhibit the lipase *in vitro*, cause a prolongation of alimentary lipaemia and delay the rate of removal of injected chylomicra from the blood (21, 24, 58, 105, 173). These results are consistent with the proposed role of clearing factor lipase, but there is some difficulty in interpreting them since it is not known that the substances act solely as specific inhibitors of the enzyme *in vivo*. They may affect the behaviour of chylomicra in other ways. It has been found that Triton WR 1339 alters the distribution of injected chylomicron fat in the intact animal and increases the uptake of chylomicra by reticulo-endothelial cells, presumably by altering the

surface properties of the particles (59, 60); protamine sulphate will combine with and cause the flocculation of chylomicra *in vitro* (24a).

More direct evidence for the activity of clearing factor lipase in the blood during alimentary lipaemia has been obtained in the rat. Plasma samples obtained after a fatty meal clear added chylomicra spontaneously by a reaction which appears to be identical with the clearing induced by heparin (101, 157, 158). Under optimal conditions it has been estimated that up to 30% of the total chylomicron triglyceride entering the blood could be hydrolysed by this spontaneous clearing reaction. The same phenomenon, but at a lower level of activity, has been demonstrated in several other animal species (102). In man, while some lipolysis does occur in blood withdrawn after a fatty meal, the level of activity is much smaller than in the rat and accounts for the hydrolysis of only a small percentage of the dietary fat entering the blood (46, 47, 48, 155).

The absence during alimentary lipaemia of high levels of clearing factor lipase in the blood of certain species, including man, has given rise to the concept that, although the enzyme may be concerned in the removal of chylomicron fat from the blood, its main action is not in the circulating blood but at the binding site of the enzyme (151). The possibility that this site may be in the blood vessels has already been mentioned; if this is so, then the main action of the enzyme may occur during the passage of chylomicron fat through the vessel walls. The appearance of some enzyme activity in the blood could be explained by the binding of the enzyme at the surface of chylomicra which continue to circulate (see above). The various lipolytic products of chylomicron fat which have been identified in the blood during alimentary lipaemia may then represent the products of the action of this circulating enzyme. On the other hand, they may be formed at the binding site where, in addition to the products which pass directly to the tissues, some may leak back into the bloodstream.

There is considerable uncertainty about the quantitative role of the mechanisms which have been proposed for the disposal of chylomicron fat and about their relative importance at different sites in the body. It appears unwise at present to interpret changes in the rate of removal and pattern of distribution of dietary fat in terms of these mechanisms. However, many interesting observations will need to be fitted into any scheme which may be proposed. For example, the degree of alimentary lipaemia is greater and more prolonged in males than in females (68), and is increased in certain pathological states such as atherosclerosis, nephrosis and diabetes (13, 31, 90, 92, 98, 125, 132a, 143, 182). There may also be effects of carbohydrate metabolism and hormonal stimuli on the removal and pattern of distribution of dietary fat (1, 2, 3, 20).

The mobilization of depot fat

The fat depots form 10 to 20% of the body weight in a normal adult animal and are composed largely of triglycerides. They are not metabolically inert. Most of the biochemical reactions occurring in other tissues, and particularly those concerned with lipid metabolism, occur in the depots (170, 181) and there appears to be a constant movement of fatty acids, as well as of other metabolites,

to and from adipose tissue (82, 168, 167). However, under conditions where carbohydrate utilization by the tissues is minimal, as for instance in starvation and in diabetes, there is a net movement of fatty acids away from the depots which seems to be under hormonal and nervous control (54, 168). The possible importance of the clearing factor lipase in this mobilization of depot fat will now be considered.

It is generally assumed that all mobilized fat is transported directly from the depots to the blood, and the subject will be discussed from this viewpoint. The alternative possibility, that mobilized fat enters the blood indirectly via the lymph, was suggested by Rony, Mortimer and Ivy (161) but at present has little experimental support. Analyses of lymph from the thoracic duct of the rat during starvation show no elevation of the total fatty acid content (149). However, since it is known that fat particles introduced into tissue spaces are taken up in the lymph (183) this aspect of the subject merits further investigation.

Mobilized fatty acid is transported in the blood either as triglyceride or as U.F.A. The view that phosphatides and cholesterol esters carry a large proportion of the mobilized fatty acids is no longer tenable, though these lipids may have important subsidiary functions in fatty acid transport (82, 122, 142).

The importance of the role of the U.F.A. fraction is based on several types of observations. Firstly, under conditions of fat mobilization, for example, in starvation, in diabetes and after the injection of adrenaline and noradrenaline, there is a rise in the concentration of the plasma U.F.A. (42, 74, 112, 175). This rise is promptly reversed by glucose administration in starvation or by insulin treatment in diabetes. The reversal appears to be caused by a diminution in the output of mobilized fatty acids by the fat depots rather than by an enhanced utilization by the peripheral tissues (11). Preparations of growth hormone administered to hypophysectomized rats also raise the level of the plasma U.F.A. and will increase the release of U.F.A. from adipose tissue *in vitro* (45a, 105a, 144a). On the other hand, substances such as glucagon and tolbutamide (1-butyl-3-*p*-tolyl-sulphonylurea) cause a fall in the level of the plasma U.F.A. (9a, 10).

Secondly, it has been shown in man and in the dog that the uptake of U.F.A. by the heart is sufficient to provide for the caloric needs of the cardiac muscle in starvation (73, 136). This uptake is accompanied by a rise in the concentration of U.F.A. in blood draining areas rich in adipose tissue. The uptake by the myocardium and the output by the areas rich in adipose tissue is abolished by carbohydrate ingestion.

Turnover of the plasma U.F.A. is rapid in the fed animal and increases further during starvation (11, 53, 55, 57, 88, 113, 114). The turnover of U.F.A. in the blood in man has been calculated to be sufficient during starvation for the transport of up to 5000 calories as fatty acid per day (53, 113). Although the U.F.A. transported is not all utilized immediately, data on the production of carbon dioxide during starvation in man suggests, nevertheless, that approximately 50% of the caloric needs are satisfied by the oxidation of the U.F.A fraction (55).

Fatty acids are stored in the depots as triglycerides and it is evident that a lipase must be involved at some stage in their mobilization as U.F.A. It has been recognized for many years that lipases are present in the fat depots (144, 145) though the nature of these lipases and their exact site of action in the tissue are unknown. More recently the clearing factor lipase has been identified in extracts of adipose tissue (109).

Intact adipose tissue from starved rats releases U.F.A. when incubated *in vitro* and the rate of release is decreased by glucose and insulin and increased by adrenaline and by growth hormone (75, 105a, 146). Wadström (179) found an increase in the proportion of mono- and diglycerides in adipose tissue after the injection of adrenaline.

These observations are consistent with lipase action in the cells of adipose tissue. However, the rate of release of U.F.A. *in vitro* by intact adipose tissue from starved rats is far too low to account for the known rate of transport of U.F.A. during mobilization (75). Moreover it is not influenced by the presence of such inhibitors of the clearing factor lipase as sodium pyrophosphate, sodium chloride in high concentrations and protamine sulphate (149). Nor does intact adipose tissue from starved rats hydrolyse added chylomicron triglyceride from chyle *in vitro*. There is the further possibility that the U.F.A. released on the incubation of adipose tissue from starved rats *in vitro* is derived in part from a pool of U.F.A. in the tissue (114, 115, 146).

At present, therefore, although lipases occur in adipose tissue, the evidence for their action, and specifically for the action of the clearing factor lipase, in the fat cells during mobilization is inconclusive. The possibility remains that triglycerides are mobilized as such and that the hydrolysis which accounts for their appearance in the blood occurs at some later stage in their transport.

Changes in the level of the plasma triglycerides do occur during fat mobilization. In starvation the concentration of triglycerides, and of low density lipoproteins rich in triglyceride, rises in the plasma; carbohydrate administration causes the levels to return to normal (33, 85, 163). In diabetes similar rises occur with a return to normal levels after insulin treatment (12, 29, 71, 83, 124, 140, 178). Pancreatectomy in the rat causes a rise in the level of plasma triglycerides unless the fat depots have been depleted by starvation, and the rise is reversed by insulin injection (36). The elevations of plasma triglycerides which follow adrenaline injection (44, 45, 104), repeated haemorrhage (14, 97), irradiation (89), the injection of certain non-ionic surface active agents of the Triton (polyether alcohol) and Tween (complex esters such as Tween 80, polyoxyethylene sorbitan monooleate) (105) types, and which occur during pregnancy (18), may be due in part to the mobilization of lipid from the depots as triglyceride. The injection of protamine sulphate in starved animals also produces a marked and rapid rise in the concentration of triglycerides in the plasma (21), and the lipid mobilizing substances of both posterior and anterior pituitary origin, which have been reported recently, increase the concentration of the plasma triglycerides (164, 172); their effect on the plasma U.F.A. level has not been studied.

The rate of turnover of the triglyceride fatty acids in plasma is high. As deduced from studies on the incorporation of labelled acetate into the triglyceride fatty acid molecule, it is much more rapid than that of the cholesterol ester and phosphatide fatty acids (82, 121, 122, 142). Bates (7) has reported a turnover of injected plasma triglyceride fatty acids in the dog of approximately 1.5 g per hour; but in her experiments the turnover curves were complex and the plasma triglyceride and U.F.A. fractions were not distinguished. Laurell (115) has measured the turnover of plasma triglyceride in starving rats and found it to be approximately 20% of the turnover of the plasma U.F.A.

These findings suggest an important function for the plasma triglycerides in fat mobilization. However, they are difficult to interpret since the rises in concentration which occur may represent the recycling as triglyceride of fatty acids originally mobilized as U.F.A. (119). There is little evidence for the direct release of triglyceride from isolated adipose tissue *in vitro* (146) and if it does occur *in vivo* the mechanism is unknown. There appear to be three possible forms of fat mobilization which would be consistent with the observed elevations in plasma triglyceride and U.F.A.

1. Triglyceride is hydrolysed within the adipose tissue cells and passes through the capillary membrane into the blood as U.F.A. Recycling of mobilized U.F.A. may occur as triglyceride.

2. Triglyceride leaves the adipose tissue cells as such but is hydrolysed in the course of its transit across the capillary membrane and enters the blood as U.F.A. Some triglyceride may pass into the blood unchanged or mobilized fatty acids may be recycled as triglyceride.

3. Mobilized fatty acid enters the blood as triglyceride and is distributed to the tissues by mechanisms analogous to those already discussed in relation to the disposal of dietary fat.

It is impossible at present to decide between these possibilities and hence to know at what point in mobilization a lipase is involved. The clearing factor lipase does not appear to be restricted to adipose tissue and this makes it unlikely that the main function of the enzyme is to hydrolyse triglycerides within adipose tissue cells. Such hydrolysis if it occurs during mobilization, as seems probable, may depend on the action of other lipases. If, on the other hand, hydrolysis occurs during the passage of triglyceride fatty acids across the capillary membrane, then this may be a function of clearing factor lipase in fat mobilization analogous to that which has aready been proposed in discussing the disposal of dietary fat.

The redistribution of triglyceride

A further possible function of the clearing factor lipase must be considered briefly. It is that the enzyme is active in the distribution of triglyceride fatty acids which enter the blood from organs other than the fat depots. This may occur when triglycerides are synthesized in particular organs or when triglycerides taken up by the liver, for example, during fat absorption or mobilization, are recycled in the blood. Some recent interesting studies by Laurell

(115) have suggested that in the glucose-fed animal, when presumably the synthesis of fatty acids in the tissues is taking place, a considerable proportion of the plasma triglyceride, present largely in low density lipoprotein complexes, is derived from the liver. Further, the plasma triglyceride is the source of most of the plasma U.F.A. under these conditions. It is evident that, since the clearing factor lipase is active on triglycerides present in low density lipoprotein complexes, it could be responsible for this production of U.F.A. so facilitating the transfer of fatty acids to the peripheral tissues. Further investigation of this aspect of fat transport is needed.

CONCLUSION

Since Hahn's first observation on the clearing reaction a considerable amount of work on the clearing factor lipase has been carried out. Although many of the properties of the enzyme are now known, the precise role of heparin in its function and its substrate specificity still need to be defined further.

The main problems in determining the physiological role of the enzyme in fat transport are that neither the exact site of action of the enzyme nor the significance of lipolysis in the various stages of fat transport has been fully established.

The weight of evidence indicates that clearing factor lipase is not normally active in the blood and that its appearance there in high concentration is an artificial consequence of heparin injection. However, the rapidity of this appearance in the blood suggests that the normal site is readily accessible to the circulation and on these grounds a location in the vessel wall has been proposed.

Lipase activity must be involved at some stage in the utilization of dietary and depot triglycerides, since before the triglyceride fatty acids can be oxidized in the tissues they must be released as U.F.A. The observation that clearing factor lipase acts readily on chylomicra and low density lipoproteins containing triglycerides suggests that these transport forms of triglyceride are the natural substrate for the enzyme. Further, since there is considerable difficulty in understanding how these particles and macromolecules can cross the capillary membrane readily without prior hydrolysis in organs other than the liver, the probable role of the enzyme at this stage in fat transport has been emphasized. There is no definite evidence that clearing factor lipase is specifically implicated at other stages of fat transport, namely in the hydrolysis of triglyceride in the fat cells during mobilization or in the tissue cells where the utilization of triglyceride fatty acids occurs. It may well function, however, in the redistribution of triglyceride fatty acids to the peripheral tissues.

The transport of fatty acids to and from the tissues *via* the blood is a complex process involving the continual intermixing of dietary, synthesized and stored fatty acids and the recycling of triglyceride fatty acid as U.F.A. and *vice versa*. Until these various processes are more clearly understood it must remain uncertain whether the proposed role of clearing factor lipase in facilitating the passage of triglyceride fatty acids across the capillary membrane will provide a complete assessment of the functions of this enzyme.

REFERENCES

1. ALBRINK, M. J., FITZGERALD, J. R. AND MAN, E. B.: Effect of glucagon on alimentary lipemia. Proc. Soc. exp. Biol., N. Y. **95:** 778–780, 1957.
2. ALBRINK, M. J., FITZGERALD, J. R. AND MAN, E. B.: Reduction of alimentary lipemia by glucose. Metabolism **7:** 162–171, 1958.
3. ALBRINK, M. J. AND MAN, E. B.: Effect of carbohydrate ingestion on postprandial lipemia. Amer. J. dig. Dis. **2:** 649–653, 1957.
4. ANDERSON, N. G. AND FAWCETT, B.: An antichylomicronemic substance produced by heparin injection. Proc. Soc. exp. Biol., N. Y. **74:** 768–771, 1950.
5. ANFINSEN, C. B., BOYLE, E. AND BROWN, R. K.: The role of heparin in lipoprotein metabolism. Science **115:** 583–586, 1952.
6. ANGERVALL, G. AND HOOD, B.: Studies on heparin and lipaemia clearing factor. Inhibition towards clearing in normal and atherosclerotic individuals. Acta med. scand. **157:** 407–415, 1957.
7. BATES, M. W.: Turnover rates of fatty acids of plasma triglyceride, cholesterol ester and phospholipid in the post-absorptive dog. Amer. J. Physiol. **194:** 446–452, 1958.
8. BENNETT, H. S., LUFT, J. H. AND HAMPTON, J. C.: Morphological classifications of vertebrate blood capillaries. Amer. J. Physiol. **196:** 381–390, 1959.
9. BERGSTRÖM, S., BORGSTRÖM, B. AND ROTTENBERG, M.: Intestinal absorption and distribution of fatty acids and glycerides in the rat. Acta physiol. scand. **25:** 120–139, 1952.

9a. BIERMAN, E. L., DOLE, V. P. AND ROBERTS, T. N.: An abnormality of non-esterified fatty acid metabolism in diabetes mellitus. Diabetes **6:** 475–479, 1957.

10. BIERMAN, E. L., ROBERTS, T. N. AND DOLE, V. P.: Effect of tolbutamide (Orinase) on plasma non-esterified fatty acids. Proc. Soc. exp. Biol., N. Y. **95:** 437–439, 1957.
11. BIERMAN, E. L., SCHWARTZ, I. L. AND DOLE, V. P.: Action of insulin on release of fatty acids from tissue stores. Amer. J. Physiol. **191:** 359–362, 1957.
12. BLIX, G.: Studies on diabetic lipemia. Acta med. scand. **64:** 234–259, 1926.
13. BLOOR, W. R., GILLETTE, E. M. AND JAMES, M. S.: Fat metabolism in diabetes. J. biol. Chem. **75:** 61–83, 1927.
14. BOGGS, T. R. AND MORRIS, R. S.: Experimental lipemia in rabbits. J. exp. Med. **11:** 553–560, 1909.
15. BORGSTRÖM, B.: On the mechanism of pancreatic lipolysis of glycerides. Biochim. biophys. Acta **13:** 491–504, 1954.
16. BORGSTRÖM, B. AND CARLSON, L. A.: On the mechanism of the lipolytic action of the lipaemia-clearing factor. Biochim. biophys. Acta **24:** 638–639, 1957.
17. BORGSTRÖM, B. AND JORDAN, P.: Metabolism of chylomicron glyceride as studied by C^{14}-glycerol-C^{14}-palmitic acid labeled chylomicrons. Acta Soc. Med. Upsaliensis **64:** 185–193, 1959.
18. BOYD, E. M.: The lipemia of pregnancy. J. clin. Invest. **13:** 347–363, 1934.
19. BRAGDON, J. H.: On the composition of chyle chylomicrons. J. Lab. clin. Med. **52:** 564–570, 1958.
20. BRAGDON, J. H. AND GORDON, R. S.: Tissue distribution of C^{14} after the intravenous injection of labelled chylomicrons and unesterified fatty acids in the rat. J. clin. Invest. **37:** 574–578, 1958.
21. BRAGDON, J. H. AND HAVEL, R. J.: *In vivo* effect of anti-heparin agents on serum lipids and lipoproteins. Amer. J. Physiol. **177:** 128–133, 1954.
22. BRAGDON, J. H., HAVEL, R. J. AND BOYLE, E.: Human serum lipoproteins. J. Lab. clin. Med. **48:** 36–42, 1956.
23. BRAGDON, J. H., HAVEL, R. J. AND GORDON, R. S.: Effects of carbohydrate feeding on serum lipids and lipoproteins in the rat. Amer. J. Physiol. **189:** 63–67, 1957.
24. BROWN, W. D.: Reversible effects of anticoagulants and protamine on alimentary lipaemia. Quart. J. exp. Physiol. **37:** 75–84, 1952.

24a. BROWN, W. D.: Protamine, chylomicrons and clot formation. Science **118:** 46–47, 1953.

25. BROWN, R. K., BOYLE, E. AND ANFINSEN, C. B.: The enzymatic transformation of lipoproteins. J. biol. Chem. **204:** 423–434, 1953.
26. BURSTEIN, M.: Précipitation des β-lipoprotéines sériques par l'héparine et les héparinoïdes de synthèse à pH 5. C. R. Acad. Sci., Paris **245:** 586–588, 1957.
27. BURSTEIN, M. AND PRAVERMAN, A.: Sur la précipitation des β-lipoprotéines du sérum par l'héparine en présence de certains cations bivalents. C. R. Acad. Sci., Paris **245:** 2558–2560, 1957.
28. BYERS, S. O., MIST-ST. GEORGE, S. AND FRIEDMAN, M.: Hepatic reticulo-endothelial cells as participants in the normal disposition of exogenous cholesterol in the rat. In: Physiopathology of the Reticulo-Endothelial System, ed. by B. Benacerraf and J. F. Delafresnaye, pp. 128–146. Blackwell Scientific Publ., Oxford, 1957.
29. CAGAN, R. N., SOBEL, A. E., NICHOLS, R. A. AND LOEWE, L.: Serum lipids in normal and alloxan diabetic rats. Metabolism **3:** 168–172, 1954.
30. CAIRNS, A. AND CONSTANTINIDES, P.: Endocrine effects on the heparin-induced lipemia-clearing activity (LCA) of rat plasma. Canad. J. Biochem. Physiol. **33:** 530–538, 1955.
31. CAMELIN, A., RAMEL, P., SCLEAR, F. AND MINDUS, A.: Hyperlipidémie provoquée et athérosclérose. Pr. méd. **62:** 1124–1125, 1954.
32. CARLSON, L. A. AND OLHAGEN, B.: Studies on a case of essential hyperlipemia. Blood lipids, with special reference to the composition and metabolism of the serum glycerides before, during and after the course of a viral hepatitis. J. clin. Invest. **38:** 854–862, 1959.
33. CARLSON, L. A. AND WADSTRÖM, L. B.: On the occurrence of tri-, di-, and monoglycerides in human serum. Third Internat. Conf. on Biochemical Problems of Lipids, pp. 123–135. Brussels, 1956.
34. CHARGAFF, E.: Lipoproteins. Advanc. Protein Chem. **1:** 1–24, 1944.

35. CHANDLER, H. L., LAWRY, Y., POTEE, K. G. AND MANN, G. V.: Spontaneous and induced variations in serum lipoproteins. Circulation **8**: 723–731, 1953.
36. CHERNICK, S. S. AND SCOW, R. O.: Early effects of 'total' pancreatectomy on fat metabolism in the rat. Amer. J. Physiol. **196**: 125–131, 1959.
37. COHEN, H.: Personal communication.
38. CONSTANTINIDES, P., CAIRNS, A. AND WERNER, A.: Anti-lipemic activity of sulfated polysaccharides. Arch. int. Pharmacodyn. **99**: 334–345, 1954.
39 COURTICE, F. C. AND MORRIS, B.: The exchange of lipids between plasma and lymph of animals. Quart. J. exp. Physiol. **40**: 138–148, 1955.
40. DAY, A. J. AND PETERS, J. A.: The effect of uranium acetate on heparin induced lipemia clearing. Aust. J. exp. Biol. med. Sci. **36**: 39–48, 1958.
41. DAY, A. J. AND PETERS, J. A.: Observations on clearing factor inhibitor elaborated by cortisone in rabbits. Aust. J. exp. Biol. med. Sci. **36**: 121–132, 1958.
42. DOLE, V. P.: A relation between non-esterified fatty acids in plasma and the metabolism of glucose. J. clin. Invest. **35**: 150–154, 1956.
43. DOLE, V. P.: Transport of non-esterified fatty acids in plasma. In: Chemistry of Lipids as Related to Atherosclerosis, ed. by I. H. Page, pp. 189–204. Thomas, Springfield, Ill., 1958.
44. DURY, A.: Effects of epinephrine on lipid partition and metabolism in the rabbit. Circulation Res. **5**: 47–53, 1957.
45. DURY, A. AND TREADWELL, C. R.: Effect of epinephrine on plasma lipid components and interrelationships in normal and epileptic humans. J. clin. Endocrin. **15**: 818–825, 1955.
45a. ENGEL, H. R., HALLMAN, L., SIEGEL, S. AND BERGENSTAL, D.: Effect of growth hormone on plasma unesterified fatty acid levels of hypophysectomized rats. Proc. Soc. exp. Biol., N. Y. **98**: 753–755, 1959.
46. ENGELBERG, H.: Human endogenous lipemia clearing factor (active factor). Amer. J. Physiol. **181**: 309–312, 1955.
47. ENGELBERG, H.: Human endogenous lipemia clearing activity. Studies on lipolysis and effects of inhibitors. J. biol. Chem. **222**: 601–610, 1956.
48. ENGELBERG, H.: Human endogenous plasma lipemia clearing and lipolytic activity after tricalcium phosphate adsorption. Proc. Soc. exp. Biol., N. Y. **95**: 394–397, 1957.
49. FAWCETT, D. W.: Observations on the cytology and electron microscopy of hepatic cells. J. nat. Cancer Inst. **15**: 1475–1503, 1955.
50. FEKETE, L. L., LEVER, W. F. AND KLEIN, E.: Inhibition of lipemia clearing activity by human white blood cell and platelet components. J. Lab. clin. Med. **52**: 680–686, 1958.
51. FLOREY, H. W., POOLE, J. C. F. AND MEEK, G. A.: Endothelial cells and "cement" lines. J. Path. Bact. **77**: 625–636, 1959.
52. FRAZER, A. C.: Blood plasma lipo-proteins, with special reference to fat transport and metabolism. Disc. Faraday Soc. **6**: 81–97, 1949.
53. FREDRICKSON, D. S. AND GORDON, R. S.: Metabolism of albumin-bound labelled fatty acids in man. Fourth Internat. Conf. on Biochemical Problems of Lipids, pp. 195–204, Oxford, 1957.
54. FREDRICKSON, D. S. AND GORDON, R. S.: Transport of fatty acids. Physiol. Rev. **38**: 585–630, 1958.
55. FREDRICKSON, D. S., GORDON, R. S., ONO, K. AND CHERKES, A.: The metabolism of albumin-bound C^{14}-labelled unesterified fatty acids in normal human subjects. J. clin. Invest. **37**: 1504–1515, 1958.
56. FREDRICKSON, D. S., MCCOLLESTER, D. L., HAVEL, R. J. AND ONO, K.: The early steps in transport and metabolism of exogenous triglyceride and cholesterol. In: Chemistry of Lipids as Related to Atherosclerosis, ed. by I. H. Page, pp. 205–221. Thomas, Springfield, Ill., 1958.
57. FREDRICKSON, D. S., MCCOLLESTER, D. L. AND ONO, K.: The role of unesterified fatty acid transport in chylomicron metabolism. J. clin. Invest. **37**: 1333–1341, 1958.
58. FRENCH, J. E. AND MORRIS, B.: The removal of ^{14}C-labelled chylomicron fat from the circulation in rats. J. Physiol. **138**: 326–339, 1957.
59. FRENCH, J. E. AND MORRIS, B.: The tissue distribution and oxidation of ^{14}C-labelled chylomicron fat injected intravenously in rats. J. Physiol. **140**: 262–271, 1958.
60. FRENCH, J. E. AND MORRIS, B.: The uptake and storage of lipid particles by reticulo-endothelial cells in lymph nodes in the rat. J. Path. Bact. **79**: 11–19, 1960.
61. FRENCH, J. E., MORRIS, B. AND ROBINSON, D. S.: Removal of lipids from the blood stream. Brit. med. Bull. **14**: 234–238, 1958.
62. FRENCH, J. E., ROBINSON, D. S. AND FLOREY, H. W.: The effect of the intravenous injection of heparin on the interaction of chyle and plasma in the rat. Quart. J. exp. Physiol. **38**: 101–111, 1953.
63. FRENCH, J. E., ROBINSON, D. S. AND HARRIS, P. M.: The role of cholesterol in the interaction of chyle and plasma. Quart. J. exp. Physiol. **40**: 320–330, 1955.
64. FRIEDMAN, M., BYERS, S. O. AND ROSENMAN, R. H.: Observations concerning the production and excretion of cholesterol in mammals. XII. Demonstration of the essential role of the hepatic reticulo-endothelial cell (Kupffer cell) in the normal disposition of exogenously derived cholesterol. Amer. J. Physiol. **177**: 77–83, 1954.
65. GAGE, S. H. AND FISH, P. A.: Fat digestion, adsorption, and assimilation in man and animals as determined by the dark-field microscope, and a fat-soluble dye. Amer. J. Anat. **34**: 1–85, 1924.
66. GILBERT, A. AND JOMIER, J.: La cellule étoilée du foie. Arch. Méd. exp. **20**: 145–158, 1908.
67. GILLMAN, T., HATHORN, M. AND PENN, J.: Lipaemia clearing action of sulphated animal hyaluronate. Nature, Lond. **177**: 894–895, 1956.

68. GILLMAN, T., NAIDOO, S. S. AND HATHORN, M.: Sex differences in plasma fibrin, fibrinolytic capacity and lipids as influenced by ingested fat, gonadectomy and hormone implants. Clin. Sci. 17: 393–408, 1958.
69. GITLIN, D., CORNWELL, D. G., NAKASATO, D., ONCLEY, J. L., HUGHES, W. L. AND JANEWAY, C. A.: Studies on the metabolism of plasma proteins in the nephrotic syndrome. II. The lipoproteins. J. clin. Invest. 37: 172–184, 1958.
70. GLAZKO, A. J., STRONG, J. AND KAZENKO, A.: Some factors influencing the lipemia-clearing activity of rat plasma. Fed. Proc. 13: 55, 1954.
71. GOFMAN, J. W., DELALLA, O., GLAZIER, F., FREEMAN, N. K., LINDGREN, F. T., NICHOLS, A. V., STRISOWER, B. AND TAMPLIN, A. R.: The serum lipoprotein transport system in health, metabolic disorders, atherosclerosis and coronary heart disease. Plasma 2: 413–484, 1954.
72. GORDON, R. S.: Interaction between oleate and the lipoproteins of human serum. J. clin. Invest. 34: 477–484, 1955.
73. GORDON, R. S.: Unesterified fatty acid in human blood plasma. II. The transport function of unesterified fatty acid. J. clin. Invest. 36: 810–815, 1957.
74. GORDON, R. S. AND CHERKES, A.: Unesterified fatty acid in the human blood plasma. J. clin. Invest. 35: 206–212, 1956.
75. GORDON, R. S. AND CHERKES, A.: Production of unesterified fatty acids from isolated rat adipose tissue incubated *in vitro*. Proc. Soc. exp. Biol., N. Y. 97: 150–151, 1958.
76. GRAHAM, D. M., LYON, T. P., GOFMAN, J. W., JONES, H. B., YANKLEY, A., SIMONTON, J. AND WHITE, S.: Blood lipids and human atherosclerosis. II. The influence of heparin upon lipoprotein metabolism. Circulation 4: 666–673, 1951.
77. GROSSMAN, M. I., MOELLER, H. C. AND PALM, L.: Effect of lipemia and heparin on free fatty acid concentration of serum in humans. Proc. Soc. exp. Biol., N. Y. 90: 106–109, 1955.
78. GROSSMAN, M. I., PALM, L., BECKER, G. H. AND MOELLER, H. C.: Effect of lipemia and heparin on free fatty acid content of rat plasma. Proc. Soc. exp. Biol., N. Y. 87: 312–315, 1954.
79. GROSSMAN, M. I. AND STRUB, I. H.: Effect of heparin on fate of intravenously administered fat emulsions in rats. Proc. Soc. exp. Biol., N. Y. 85: 356–359, 1954.
80. HAHN, P. F.: Abolishment of alimentary lipemia following injection of heparin. Science 98: 19–20, 1943.
81. HAMPTON, J. C.: An electron microscope study of the hepatic uptake and excretion of submicroscopic particles injected into the blood stream and into the bile duct. Acta anat. 32: 262–291, 1958.
82. HARPER, P. V., JR., NEAL, W. B., JR. AND HLAVACEK, G. R.: Lipid synthesis and transport in the dog. Metabolism 2: 69–80, 1953.
83. HARRIS, L. V. D., ALBRINK, M. J., VAN ECK, W. F., MAN, E. B. AND PETERS, J. P.: Serum lipids in diabetic acidosis. Metabolism 2: 120–132, 1953.
84. HAVEL, R. J.: Evidence for the participation of lipoprotein lipase in the transport of chylomicrons. Third Internat. Conf. on Biochemical Problems of Lipids, pp. 265–273. Brussels, 1956.
85. HAVEL, R. J.: Early effects of fasting and of carbohydrate ingestion on lipids and lipoproteins of serum in man. J. clin. Invest. 36: 855–859, 1957.
86. HAVEL, R. J. AND BRAGDON, J. H.: Heparin-like activity of polymetaphosphate. Circulation 10: 591, 1954.
87. HAVEL, R. J. AND CLARKE, J. C.: Metabolism of chylomicron phospholipids. Clin. Res. 6: 264, 1958.
88. HAVEL, R. J. AND FREDRICKSON, D. S.: The metabolism of chylomicra. I. The removal of palmitic acid-1-C^{14} labelled chylomicra from dog plasma. J. clin. Invest. 35: 1025–1032, 1956.
89. HEWITT, J. E., HAYES, T. L., GOFMAN, J. W., JONES, H. B. AND PIERCE, F. T.: Effects of total body irradiation upon lipoprotein metabolism. Cardiologia 21: 353–365, 1952.
90. HILLER, A., LINDER, G. C., LUNDSGAARD, C. AND VAN SLYKE, D. D.: Fat metabolism in nephritis. J. exp. Med. 39: 931–955, 1924.
91. HILLYARD, L. A., CORNELIUS, C. E. AND CHAIKOFF, I. L.: Removal by the isolated rat liver of palmitate-1-C^{14} bound to albumin and of palmitate-1-C^{14} and cholesterol-4-C^{14} in chylomicrons from perfusion fluid. J. biol. Chem. 234: 2240–2245, 1959.
92. HIRSCH, E. F. AND CARBONARO, L.: The serum esterified fatty acids with fat tolerance tests in diabetes mellitus. J. Lab. clin. Med. 36: 835–836, 1950.
93. HOLLETT, C. AND MENG, H. C.: Purification and characterization of the lipemia-clearing factor of postheparin plasma. Biochim. biophys. Acta 20: 421–422, 1956.
94. HOLLETT, C. AND MENG, H. C.: Comparison of fat emulsion clearing activities of postheparin plasma and pancreatic lipase. Amer. J. Physiol. 184: 428–432, 1956.
95. HOLLETT, C. AND MENG, H. C.: Purification and characterization of a lipaemia clearing factor inhibitor from normal plasma. Third Internat. Conf. on Biochemical Problems of Lipids, pp. 190–199. Brussels, 1956.
96. HOOD, B. AND ANGERVALL, G.: The relation between inhibition towards clearing, total cholesterol and the distribution of cholesterol in plasma. Acta med. scand. 158: 13–20, 1957.
97. HORIUCHI, Y.: Studies on blood fat. II. Lipemia in acute anemia. J. biol. Chem. 44: 363–371, 1920.
98. HORLICK, L.: Effect of acute fat loads on serum lipids in atherosclerosis. Circulation Res. 5: 368–374, 1957.
99. ISELIN, B. VON AND SCHULER, W.: Über die Einwirkung von Heparin auf Lipoprotein-Lipase (clearing factor) aus Gewebe. Helv. physiol. acta 15: 14–24, 1957.
100. JAFFÉ, R. H. AND BERMAN, S. L.: The relations between Kupffer cells and liver cells. Arch. Path. (Lab. Med.) 5: 1020–1027, 1928.
101. JEFFRIES, G. H.: The effect of fat absorption on the interaction of chyle and plasma in the rat. Quart J. exp. Physiol. 39: 77–81, 1954.

101a. JEFFRIES, G. H.: The sites at which plasma clearing activity is produced and destroyed in the rat. Quart. J. exp. Physiol. **39**: 261-270, 1954.
102. JEFFRIES, G. H.: Factors influencing fat transport and metabolism. Thesis, D. Phil., Oxford, 1955.
103. JONES, H. B., GOFMAN, J. W., LINDGREN, F. T., LYON, T. P., GRAHAM, D. M., STRISOWER, B. AND NICHOLS, A. V.: Lipoproteins in atherosclerosis. Amer. J. Med. **11**: 358-380, 1951.
104. KAPLAN, A., JACQUES, S. AND GANT, M.: Effect of long-lasting epinephrine on serum lipid levels. Amer. J. Physiol. **191**: 8-12, 1957.
105. KELLNER, A., CORRELL, J. W. AND LADD, A. T.: Sustained hyperlipaemia induced in rabbits by means of intravenously injected surface-active agents. J. exp. Med. **93**: 373-383, 1951.
105a. KNOBIL, E.: Direct evidence for fatty acid mobilization in response to growth hormone administration in the rat. Proc. Soc. exp. Biol., N. Y. **101**: 288-289, 1959.
106. KORN, E. D.: Clearing factor, a heparin-activated lipoprotein lipase. I. Isolation and characterization of the enzyme from normal rat heart. J. biol. Chem. **215**: 1-14, 1955.
107. KORN, E. D.: Clearing factor, a heparin-activated lipoprotein lipase. II. Substrate specificity and activation of coconut oil. J. biol. Chem. **215**: 15-26, 1955.
108. KORN, E. D.: Inactivation of lipoprotein lipase by heparinase. J. biol. Chem. **226**: 827-832, 1957.
108a. KORN, E. D. AND QUIGLEY, T. W.: Studies on lipoprotein lipase of rat heart and adipose tissue. Biochim. biophys. Acta **18**: 143-145, 1955.
109. KORN, E. D. AND QUIGLEY, T. W.: Lipoprotein lipase of chicken adipose tissue. J. biol. Chem. **226**: 833-839, 1957.
110. LAURELL, C. B.: Composition of chylomicrons isolated from rat lymph. Acta physiol. scand. **30**: 289-294, 1954.
111. LAURELL, S.: The effect of free fatty acids on the migration rates of lipoproteins in paper electrophoresis. Scand. J. clin. Lab. Invest. **7**: 28-31, 1955.
112. LAURELL, S.: Plasma free fatty acids in diabetic acidosis and starvation. Scand. J. clin. Lab. Invest. **8**: 81-82, 1956.
113. LAURELL, S.: Turnover rate of unesterified fatty acids in human plasma. Acta physiol. scand. **41**: 158-167, 1957.
114. LAURELL, S.: Distribution of C^{14} in rats after intravenous injection of non-esterified palmitic acid-1-C^{14}. Acta physiol. scand. **46**: 97-106, 1959.
115. LAURELL, S.: Recycling of intravenously injected palmitic acid-1-C^{14} as esterified fatty acid in the plasma of rats and turnover rate of plasma triglycerides. Acta physiol. scand. **47**: 218-232, 1960.
116. LE QUIRE, V. S., GRAY, M. E. AND COBB, C. A.: The alteration of the lipemia-clearing effect of heparin following the intravenous injection of thorium dioxide (thorotrast). Circulation Res. **1**: 523-529, 1953.
117. LEVY, S. W.: Heparin and blood lipids. Rev. canad. Biol. **17**: 1-61, 1958.
118. LEVY, S. W. AND SWANK, R. L.: The effects of *in vivo* heparin on plasma esterase activity and lipaemia clearing. J. Physiol. **123**: 301-314, 1954.
119. LINDGREN, F. T., FREEMAN, N. K., NICHOLS, A. V. AND GOFMAN, J. W.: The physical chemistry of lipoprotein transformation. Third Internat. Conf. on Biochemical Problems of Lipids, pp. 224-242. Brussels, 1956.
120. LINDGREN, F. T., NICHOLS, A. V. AND FREEMAN, N. K.: Physical and chemical composition studies on the lipoproteins of fasting and heparinized human sera. J. phys. Chem. **59**: 930-938, 1955.
121. LIPSKY, S. R., HAAVICK, A., HOOPER, C. L. AND MCDIVITT, R. W.: The biosynthesis of the fatty acids of the plasma of man. I. The formation of certain chromatographically separated higher fatty acids of the major lipid complexes from acetate-1-C^{14}. J. clin. Invest. **36**: 233-244, 1957.
122. LIPSKY, S. R., MCGUIRE, J. S., BONDY, P. K. AND MAN, E. B.: The rates of synthesis and the transport of plasma fatty acid fractions in man. J. clin. Invest. **34**: 1760-1765, 1955.
123. MAN, E. B. AND GILDEA, E. F.: The effect of the ingestion of a large amount of fat and of a balanced meal on the blood lipids of normal man. J. biol. Chem. **99**: 61-69, 1932.
124. MAN, E. B. AND PETERS, J. P.: Lipoids of serum in diabetic acidosis. J. clin. Invest. **13**: 237-261, 1934.
125. MARDER, L., BECKER, G. H., MAIZEL, B. AND NECHELES, H.: Fat absorption and chylomicronemia. Gastroenterology **20**: 43-59, 1952.
126. MCCANDLESS, E. L. AND ZILVERSMIT, D. B.: Fate of triglycerides and phospholipids of lymph and artificial fat emulsions: Disappearance from the circulation. Amer. J. Physiol. **193**: 294-300, 1958.
127. MEAD, F. J. AND FILLERUP, D. L.: The transport of fatty acids in the blood. J. biol. Chem. **227**: 1009-1023, 1957.
128. MENG, H. C., HOLLETT, C. AND COLE, W. E.: Some factors affecting the clearing of neutral fat emulsion by post-heparin plasma. Amer. J. Physiol. **179**: 314-318, 1954.
129. MITCHELL, J. R. A.: Inhibition of heparin clearing by platelets. Lancet **1**: 169-172, 1959.
130. MORA, R., REBEYROTTE, P. AND POLONOVSKI, J.: Influence des substances tensioactives sur la mobilité électrophorétique des lipoprotéides plasmatiques. Bull. Soc. Chim. biol., Paris **37**: 957-968, 1955.
131. MORRIS, B. AND COURTICE, F. C.: The origin of chylomicrons in the cervical and hepatic lymph. Quart. J. exp. Physiol. **41**: 341-348, 1956.
132. MORRIS, B. AND FRENCH, J. E.: The uptake and metabolism of ^{14}C labelled chylomicron fat by the isolated perfused liver of the rat. Quart. J. exp. Physiol. **43**: 180-188, 1958.
132a. MORRIS, B. AND FRENCH, J. E.: The metabolism of chylomicron fat in experimental nephrosis. Quart. J. exp. Physiol. **44**: 190-199, 1959.
133. MURRAY, R. G. AND FREEMAN, S.: The morphologic distribution of intravenously injected fatty chyle and artificial fat emulsions in rats and dogs. J. Lab. clin. Med. **38**: 56-69, 1951.
134. NAPOLITANO, L. AND FAWCETT, D.: The fine structure of brown adipose tissue in the newborn mouse and rat. J. biophys. biochem. Cytol. **4**: 685-692, 1958.

135. NIKKILA, E. A.: Partial purification of clearing factor of post-heparin human plasma. Biochim. biophys. Acta 27: 612–217, 1958.
136. OLSON, R. E. AND PIATNEK, D. A.: Conservation of energy in cardiac muscle. Ann. N. Y. Acad. Sci. 72: 466–479, 1959.
137. ONCLEY, J. L., WALTON, K. W. AND CORNWELL, D. G.: A rapid method for the bulk isolation of β-lipoproteins from human plasma. J. Amer. chem. Soc. 79: 4666–4671, 1957.
138. OTT, M.: Austausch von ^{32}P zwischen markierten und unmarkierten Serumlipoproteiden in vitro. Z. Naturf. 136: 365–366, 1958.
139. PAGE, I. H., LEWIS, L. A. AND PLAHL, G.: The lipoprotein composition of dog lymph. Circulation Res. 1: 87–93, 1953.
140. PETERS, J. P. AND VAN SLYKE, D. D.: Quantitative Clinical Chemistry. Interpretations. Vol. 1, pp. 503, 508. Williams & Wilkins, Baltimore, 1946.
141. PIERCE, F. T.: The interconversion of serum lipoproteins in vivo. Metabolism 3: 142–153, 1954.
142. PIHL, A. AND BLOCK, K.: The relative rates of metabolism of neutral fat and phospholipides in various tissues of the rat. J. biol. Chem. 183: 431–439, 1950.
143. POMERANZE, J., BEINFIELD, W. H. AND CHESSIN, M.: Serum lipid and fat tolerance studies in normal, obese and atherosclerotic subjects. Circulation 10: 742–746, 1954.
144. QUAGLIARIELLO, G. AND SCOZ, G.: The existence of a lipase in adipose tissue. Arch. Sci. biol., Bologna 17: 513–529, 1932.
144a. RABEN, M. S. AND HOLLENBERG, C. H.: Effect of growth hormone on plasma fatty acids. J. clin. Invest. 38: 484–488, 1959.
145. RENOLD, A. E. AND MARBLE, A.: Lipolytic activity of adipose tissue in man and rat. J. biol. Chem. 185: 367–375, 1950.
146. RESHEF, L., SHAFRIR, E. AND SHAPIRO, B.: In vitro release of unesterified fatty acids by adipose tissue. Metabolism 7: 723–730, 1958.
147. ROBINSON, D. S.: The chemical composition of chylomicra in the rat. Quart. J. exp. Physiol. 40: 112–126, 1955.
148. ROBINSON, D. S.: Further studies on the lipolytic system induced in plasma by heparin injection. Quart. J. exp. Physiol. 41: 195–204, 1956.
149. ROBINSON, D. S.: Unpublished observations.
150. ROBINSON, D. S. AND FRENCH, J. E.: The role of albumin in the interaction of chyle and plasma in the rat. Quart. J. exp. Physiol. 38: 233–239, 1953.
151. ROBINSON, D. S. AND FRENCH, J. E.: Review article. The heparin clearing reaction and fat transport. Quart. J. exp. Physiol. 42: 151–163, 1957.
152. ROBINSON, D. S. AND HARRIS, P. M.: The effect of heparin injection on the lipaemia induced in the rabbit by excessive bleeding. Biochem. J. 66: 18P, 1957.
153. ROBINSON, D. S. AND HARRIS, P. M.: The production of lipolytic activity in the circulation of the hind limb in response to heparin. Quart. J. exp. Physiol. 44: 80–90, 1959.
154. ROBINSON, D. S., HARRIS, P. M. AND FRENCH, J. E.: Changes in the solubility of cholesterol during the interaction of chyle and plasma. Quart. J. exp. Physiol. 41: 309–317, 1956.
155. ROBINSON, D. S., HARRIS, P. M., POOLE, J. C. F. AND JEFFRIES, G. H.: The effect of a fat meal on the concentration of free fatty acids in human plasma. Biochem. J. 60: 37P, 1955.
156. ROBINSON, D. S., HARRIS, P. M. AND RICKETTS, C. R.: The production of lipolytic activity in rat plasma after the intravenous injection of dextran sulphate. Biochem. J. 71: 286–292, 1959.
157. ROBINSON, D. S., JEFFRIES, G. H. AND FRENCH, J. E.: Studies on the interaction of chyle and plasma in the rat. Quart. J. exp. Physiol. 39: 165–176, 1954.
158. ROBINSON, D. S., JEFFRIES, G. H. AND POOLE, J. C. F.: Further studies on the interaction of chyle and plasma in the rat. Quart. J. exp. Physiol. 40: 297–308, 1955.
159. RODBELL, M. AND FREDRICKSON, D. S.: The nature of the proteins associated with dog and human chylomicrons. J. biol. Chem. 234: 562–566, 1959.
160. RODBELL, M., FREDRICKSON, D. S. AND ONO, K.: Metabolism of chylomicron proteins in the dog. J. biol. Chem. 234: 567–571, 1959.
161. RONY, H. R., MORTIMER, B. AND IVY, A. C.: Fat transport through the lymph system in fasting and phlorhizin poisoning. J. biol. Chem. 96: 737–743, 1932.
162. ROUILLER, C.: Les canalicules biliaires. Acta anat. 26: 94–109, 1956.
163. RUBIN, L. AND ALADJEM, F.: Serum lipoprotein changes during fasting in man. Amer. J. Physiol. 178: 263–266, 1954.
164. RUDMAN, D. AND SEIDMAN, F.: Lipemia in the rabbit following injection of pituitary extract. Proc. Soc. exp. Biol., N. Y. 99: 146–150, 1958.
165. SAMUELS, P. B. AND WEBSTER, D. R.: The role of venous endothelium in the inception of thrombosis. Ann. Surg. 136: 422–438, 1952.
166. SCANU, A. AND PAGE, I. H.: Separation and characterization of human serum chylomicrons. J. exp. Med. 109: 239–256, 1959.
167. SCHOENHEIMER, R.: The dynamic state of body constituents. Harvard University Press, Cambridge, Mass., 1942.
168. SHAPIRO, B.: Lipid dynamics in adipose tissue. Progr. in Chem. Fats Lipids 4: 177–196, 1957.
169. SHAPIRO, B., CHOWERS, I. AND ROSE, G.: Fatty acid uptake and esterification in adipose tissue. Biochim. biophys. Acta 23: 115–120, 1957.
170. SHAPIRO, B. AND WERTHEIMER, E.: The metabolic activity of adipose tissue. A review. Metabolism 5: 79–86, 1956.

171. SHORE, B., NICHOLS, A. V. AND FREEMAN, N. K.: Evidence of lipolytic action by human plasma obtained after intravenous administration of heparin. Proc. Soc. exp. Biol., N. Y. **83**: 216–220, 1953.
172. SIEFTER, J. AND BAEDER, D. H.: Lipid mobilizer (LM) from posterior pituitary of hogs. Proc. Soc. exp. Biol., N. Y. **95**: 318–320, 1957.
173. SPITZER, J. J.: Influence of protamine on alimentary lipemia. Amer. J. Physiol. **174**: 43–45, 1953.
174. SPITZER, J. J.: Discussion. Third Internat. Conf. on Biochemical Problems of Lipids, p. 308. Brussels, 1956.
175. SPITZER, J. J. AND MILLER, H. I.: Unesterified fatty acids and lipid transport in dogs. Proc. Soc. exp. Biol., N.Y. **92**: 124–126, 1956.
176. SPITZER, J. J. AND SPITZER, J. A.: Hemorrhagic lipemia: A derangement of fat metabolism. J. Lab. clin. Med. **46**: 461–470, 1955.
177. SWANK, R. L. AND LEVY, S. W.: Chylomicron dissolution. Dosage and site of action of heparin. Amer. J. Physiol. **171**: 208–217, 1952.
178. TULLER, E. F., MANN, G. V., SCHERTENLEIB, F., ROEHRIG, C. B. AND ROOT, H. F.: The effect of diabetic acidosis and coma upon the serum lipoproteins and cholesterol. Diabetes **3**: 279–286, 1954.
179. WADSTRÖM, L. B.: Lipolytic effect of the injection of adrenaline on fat depots. Nature, Lond. **179**: 259–260, 1957.
180. WELD, C. B.: Alimentary lipaemia and heparin. Canad. med. Ass. J. **51**: 578, 1944.
181. WERTHEIMER, E. AND SHAPIRO, B.: The physiology of adipose tissue. Physiol. Rev. **28**: 451–464, 1948.
182. WOLDOW, A., CHAPMAN, J. E. AND EVANS, J. M.: Fat tolerance in subjects with atherosclerosis: heparin effects upon lipaemia, lipoproteins and gamma globulin. Amer. Heart J. **47**: 568–579, 1954.
183. YOFFEY, J. M. AND COURTICE, F. C.: Lymphatics, lymph and lymphoid tissue. Edward Arnold Ltd., London, 1956.
184. ZEMPLÉNYI, T. AND GRAFNETTER, D.: Species and sex differences in fatty acid release by tissues incubated with lipaemic human serum. Brit. J. exp. Path. **39**: 99–108, 1958.