• *Technical*

Hepatic Participation in Lipid Metabolism[1,2]

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PREVIOUS STUDIES have been demonstrated that the liver is the major organ involved in lipid metabolism. Although studies (1) have indicated that the cellular composition of liver is approximately 61% parenchymal cells, which are polygonal cells arranged in the form of plates, and 33% littoral or endothelial cells, the participation of specific hepatic cells in lipid metabolism has not been delineated. The endothelial cells which line the surface of liver sinusoids in their functionally active state are classified as Kupffer cells. Because of their phagocytic activity the Kupffer cells have been classified by Aschoff as an integral part of the reticuloendothelial system (RES).

Among the numerous functions that have been ascribed to the RES is its primary involvement in the normal removal of ingested fat (2) and cholesterol (3) from plasma. Since surgical removal of the RES as a means of producing a deficiency syndrome is impossible because of its highly diffuse location, the technique of RE blockade was established. This technique, based upon the physical loading of cells with particulate material, has been demonstrated to produce a temporary depression in RE function, followed by normal or hyperactivity (4). This questions the validity of previous studies that have employed RE blockade as an approach to the study of the participation of the RES in lipid metabolism.

In an attempt to ascertain the participation of the RES in the removal of chylomicra, an initial study was conducted on the effect of alimentary lipemia on the phagocytic activity of the RES to denote a possible competitive effect of chylomicra on the phagocytosis of other colloids.

With the improvement of a technique to isolate highly purified Kupffer and parenchymal cells, a more direct method was employed to evaluate the relative role of Kupffer and parenchymal cells in lipid metabolism. The lipid composition of Kupffer and parenchymal cells was determined in rats following the intravenous injection of a physiological triglyceride and a cholesterol emulsion.

Experimental Procedures

Phagocytic function was measured in 19 healthy male, mongrel dogs by means of the colloidal carbon technique (5). Nine dogs in a post-absorptive state served as controls. Marked alimentary lipemia was induced in seven other dogs by the oral intubation of liquefied lard in the amount of 4.5 g./Kg. of body weight. The optical density of plasma was determined prior to, and at frequent intervals following the administration of fat. When the lipemia was judged to be severe, the dogs were rapidly anesthetized with pentobarbital sodium, a control blood sample was obtained, and colloidal carbon [4] was administered intravenously in the amount of 200 mg./Kg. of body weight. Venous blood samples were obtained at various intervals, following injection of colloidal carbon, and the carbon content of blood was measured (5). The half-time (t/2)[5] of the injected material, an index of over-all phagocytic function, was determined.

The organ distribution of radioactive colloidal gold (Aurcoloid-Abbott) was determined in 10 normal dogs and 15 dogs manifesting marked alimentary lipemia due either to the ingestion of olive oil or lard in the amount of 4.5 g./Kg. The lipemia reached its maximum value 2.6 hrs. after lard and 4.6 hrs. after olive oil, and the plasma neutral fat concentration increased two to four-fold at the time of injection of the test colloid. The intravascular half-time and the organ uptake of Au^{198} was determined by the previously described techniques (7).

Male rats previously maintained on Purina chow and in a post-absorptive state were employed for studies on the cellular distribution of injected lipid. A cholesterol emulsion was employed, utilizing soya lecithin as a stabilizing agent. The mean cholesterol and phospholipid concentration of the emulsion was 1.50% and 0.6%, respectively. The triglyceride emulsion was prepared as an anhydrous base (8) employing glycerol, corn oil, and alcohol-soluble soybean phosphatide. The I^{131} Iodotriolein (Raolein-Abbott) was added to the corn oil. Appropriate amounts of the homogenized base were diluted with 5% glucose prior to injection. The rats were killed 15 min. after the injection of the triglyceride emulsion. The injection of carbonyl iron particles (Antara Chemicals, New York) in the amount of 0.12 g./100 g. of body weight was made 5 min. before the animals were killed. The rats which received the cholesterol emulsion were injected with the 3-micron iron particles at either 15, 30, or 60 min. after the injection of the cholesterol emulsion and were killed 5 min. later.

Liver Kupffer and parenchymal cells were isolated according to the previously described procedures (6). The cell suspensions were initially extracted with alcohol and ethyl ether and re-extracted with chloroform-methanol. The chloroform-methanol extracts of liver and plasma were purified according to the Folch Procedure (9). Lipid phosphorous was determined (10), and phospholipid concentration was obtained by multiplying the lipid phosphorous value by a factor of 25. Total and free cholesterol concentrations of the extract were determined by the Sperry-Webb procedure (11).

Data

The colloidal carbon disappearance rate was studied in nine normal and seven lipemic dogs. The normal

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[4] Gunther Wagner Company, Hannover, Germany: carbon suspension C 11/1431.

[5] t/2 or half-time is equal to the time at which the amount of injected material present in blood is reduced to one-half its initial or zero-time concentration. This is determined by plotting, semilogarithmically, the concentration of the injected material present in blood against time.

intravascular half-time of colloidal carbon was 12.2 min. in contrast to 9.5 min. in the lipemic group. The mean 25% increase observed in the lard-fed animals appears to be caused by post-prandial changes in liver blood flow as three additional dogs fed Kam dog food in the amount of 4.5 g./Kg. showed a mean half-time of 9.2 min. even though no lipemia was manifested. These data indicate that it is highly unlikely that colloidal carbon and chylomicra are handled by the same removal mechanism.

The intravascular half-life and organ distribution of colloidal gold in normal and lipemic dogs are presented in Table I. Removal rate of colloidal gold was

TABLE I

Colloidal Gold Distribution [a] in Normal and Lipemic Dogs

Group	Number animals	t/2 Seconds	Liver	Lung	Spleen
Normal	10	123±6.6	90.5±3.0	0.38±0.11	1.34±0.22
Lipemic, olive oil fed	7	99.4±5.9	86.5±8.7	0.19±0.07	1.38±0.26
Lipemic, lard-fed	8	86.8±4.9	86.6±4.8	0.17±0.09	1.02±0.19

[a] Values are expressed as percentage of the injected dose per total organ ± standard error.

significantly increased in the lard fed ($.02<P<.05$) and in the olive oil groups ($P<0.001$). Since colloidal gold removal is dependent on liver blood flow, these changes appear in part to result from enhanced liver blood flow. Thus a lipemic state did not depress RE phagocytic function, and a slight enhancement in phagocytosis may well have occurred.

The mean hepatic distribution of an I^{131} triolein emulsion 15 min. after its intravenous administration in rats indicated that approximately 49% of the injected dose was localized in liver. The cellular distribution was 47% in parenchymal cells and 2% in the isolated Kupffer cells.

TABLE II

Hepatic Fate of Intravenously Injected I^{131} Triolein [a]

Cell	Number rats	% Injected triolein	% Total liver uptake
Kupffer	7	1.7±0.41	4.8±1.30
Parenchymal	7	47.2±6.3	95.1±1.37

[a] Mean value ± standard error.

The plasma lipid alterations after administration of the cholesterol-phospholipid emulsion are presented in Table III. The amount of cholesterol injected was sufficient to produce a mean 10-fold elevation in the plasma free cholesterol level at the 15-minute period. No change was observed in the ester cholesterol fraction. A mean 72% increase occurred in the phospholipid fraction. An approximation of the intravascular half-life indicated a value of 35 min. for phospholipid and 50 min. for free cholesterol. The disappearance rate of the phospholipid component of the emulsion was similar to that reported employing P^{32} labelled homologous plasma phospholipid (12).

TABLE III

Plasma Lipid Levels [a] Following Intravenous Cholesterol Administration

Time, min.	Treatment	Number rats	Mean body weight (g.)	Phospholipid	Cholesterol	
					Free	Ester
0	.....	6	333	1.15±0.14	0.15±0.01	0.31±0.05
15	Cholesterol-phospholipid emulsion	6	327	1.98±0.11	1.50±0.31	0.28±0.09
30	Cholesterol-phospholipid emulsion	6	325	1.71±0.10	0.94±0.17	0.45±0.09
60	Cholesterol-phospholipid emulsion	6	371	1.58±0.07	0.70±0.11	0.32±0.08

[a] Plasma lipid values are expressed as mg./cc. ± standard error.

The cellular distribution of free and ester cholesterol is presented in Table IV. The injection of cholesterol resulted in an initial and sustained elevation in the parenchymal cell free cholesterol. The Kupffer cell cholesterol content remained unchanged until 60 min. after the administration of cholesterol, at which time a 105% elevation was observed.

Table IV

Hepatic Cholesterol Levels [a] Following Intravenous Cholesterol Emulsion

Time, min.	Treatment	Number animals	Free cholesterol		Ester cholesterol	
			Reticulo-endothelial	Parenchymal	Reticulo-endothelial	Parenchymal
0	.....	6	9.1±1.2	7.4±2.1	4.1±0.7	2.4±0.5
15	Cholesterol-phospholipid emulsion	6	11.7±1.9	13.3±2.3	1.9±0.6	1.2±0.3
30	Cholesterol-phospholipid emulsion	6	12.4±1.6	14.9±0.14	4.4±0.9	2.4±0.6
60	Cholesterol-phospholipid emulsion	6	18.7±2.7	14.7±1.7	3.8±1.2	1.5±0.5

[a] Values are expressed as mg. of lipid per g. of dry defatted tissue, ± standard error.

Discussion

A primary role of the RES in lipid metabolism has been one of the numerous metabolic functions that have been ascribed to RE cells. So much importance has been attached to the RES in previous studies that it was considered an intermediary apparatus in cholesterol metabolism (13). The diffuse location and functional complexity of the RES as well as the inability to produce a significant functional depression by the technique of RE blockade have handicapped studies of RE physiology.

Biozzi *et al.* (5) have demonstrated that the rate of clearance of intravenously injected colloidal or particulate material is inversely proportional to the initial mass of the injected material. From this observation, if colloidal carbon and gold were handled in a manner similar to chylomicra, one would anticipate that during alimentary lipemia a decrease would occur in the removal rate of the test colloid. The results of our studies indicate that alimentary lipemia, induced in dogs by feeding either saturated or unsaturated fats, does not modify the disappearance rate of colloidal carbon or gold. Thus it appears that there is a different removal mechanism for chylomicron and for colloidal carbon and gold which are known to localize exclusively in the Kupffer cell. This finding as well as observations on the hepatic distribution of triglyceride indicates that both parenchymal and Kupffer cells participate in the removal of chylomicra although the major site of removal is the parenchymal cell. These findings are in agreement with the observations of Woerner (14), Murray and Freeman (15), Waddell *et al.* (16), and Morris and French (17).

The present findings support the concept of Waddell *et al.* (16) that free permeation of fat into the hepatic parenchymal cell is the main method of passage of fat into the liver from the blood stream. This permeation could well occur through the pores which Fawcett has demonstrated to exist between the sinusoidal walls (18).

The cellular distribution of a free cholesterol emulsion indicates an initial uptake by the parenchymal cell and later by the Kupffer cell. Our studies agree with those of Bailey *et al.* (19) that the uptake of cholesterol as well as of various lipids is a general cellular phenomenon and that it is not an exclusive function of the RES (2, 3).

The late rise in Kupffer cell free cholesterol is suggestive of a role of these cells in cholesterol excretion. Although little direct evidence is available, the concept of Kupffer cell participation in the metabolism or biliary excretion of cholesterol has been suggested by Stambul (20). If the excretory function of Kupffer cells in cholesterol metabolism is correct, then the elevated lipid levels in Kupffer cells in the various lipid storage diseases, as well as atherosclerosis may reflect more a failure in metabolism or excretion of cholesterol than of enhanced phagocytosis. Studies on the quantitation of hepatic cell function in cholesterol metabolism are in progress.

Summary and Conclusions

During alimentary lipemia induced in dogs by the feeding of saturated or unsaturated fats no significant alteration occurred in phagocytic function as indicated by colloidal carbon removal and colloidal gold tissue distribution studies. The cellular distribution of intravenously administered triglyceride indicated that most of the injected triglyceride was found in the isolated hepatic parenchymal cell. The distribution of an intravenously administered free cholesterol emulsion indicated initial localization in the parenchymal cell and a later elevation in the Kupffer cell. These findings suggest that both hepatic parenchymal and Kupffer cells participate in the removal of chylomicra; the parenchymal cell has the greatest role. The late elevation in cholesterol content of the Kupffer cell is indicative of a metabolic or excretory function of these cells in cholesterol metabolism.

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Determination of Water-Dispersible Protein in Soybean Oil Meals and Flours[1]

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INVESTIGATION of the water-soluble or dispersible-protein portion of soya products began in the ADM laboratories in the early 1930's. The original studies were conducted in an effort to find a laboratory method that would measure the comparative fertilization value of various types of soybean oil meals for use in the tobacco industry. In these experiments an adaptation of the A.O.A.C. Nitrogen Activity method (1) was used. This consisted of taking 0.7 g. of soya product, blending with 10 or 15 ml. of water, and allowing to stand 2 hrs. The blend was then filtered through a filter paper, the residue was washed, and a protein analysis was made on the residue. Although this method lacked in accuracy and precision and was highly empirical, it did show that there was a definite correlation between the water-soluble fraction of the soya protein and the amount of heat treatment given to the meals during processing. J. W. Hayward (2, 3) subsequently noted a correlation between heat treatment and the nutritional value of soybean oil meals. Therefore this method was established as a processing control tool in the ADM laboratories and was used successfully for many years.

Continued research work on soya flours and meals showed a definite correlation between the water solubility or dispersibility of these products and their use in other industrial applications. As a result, terms such as "Water-Soluble Protein," "Protein-Solubility Index," and "Water-Dispersible Protein" began to appear in many customer and product specifications. It was soon found however that the original methods used for determining protein solubility or dispersibility did not have the necessary accuracy or precision

[1] Technical Paper 178, Archer-Daniels-Midland Company.