Changes in the concentration of plasma lipoproteins and apoproteins following the administration of Triton WR 1339 to rats

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Abstract Changes in whole plasma and lipoprotein apoprotein concentrations were determined after a single injection of Triton WR 1339 into rats. Concentrations of apoproteins A-I (an activator of lecithin:cholesterol acyl transferase), arginine-rich apoprotein (ARP), and B apoprotein were measured by electroimmunoassay. The content of C-II apoprotein (an activator of lipoprotein lipase) was estimated by the ability of plasma and lipoprotein fractions to promote hydrolysis of triglyceride in the presence of cow's milk lipase and also by isoelectric focusing on polyacrylamide gels. Apoproteins C-II and A-I were rapidly removed from high density lipoprotein (HDL) after Triton treatment and were recovered in the d 1.21 g/ml infranate fraction. A-I was then totally cleared from the plasma within 10–20 hr after injection. Arginine-rich apoprotein was removed from HDL and also partially cleared from the plasma. The rise in very low density lipoprotein (VLDL) apoprotein that followed the removal of apoproteins from HDL was mostly attributed to the B apoprotein, although corresponding smaller increases were observed in VLDL ARP and C apoproteins. The triglyceride:cholesterol, triglyceride:protein, and B:C apoprotein ratios of VLDL more closely resembled nascent rather than plasma VLDL 10 hr after Triton injection. These studies suggest that the detergent may achieve its hyperlipidemic effect by disrupting HDL and thus removing the A-I and C-II proteins from a normal activating environment comprising VLDL, HDL, and the enzymes. The possible involvement of intact HDL in VLDL catabolism is discussed in relation to other recent reports which also suggest that abnormalities of the VLDL–LDL system may be due to the absence of normal HDL.—**Ishikawa, T., and N. Fidge.** Changes in the concentration of plasma lipoproteins and apoproteins following the administration of Triton WR 1339 to rats. *J. Lipid Res.* 1979. **20:** 254–264.

Supplementary key words electroimmunoassay · HDL disruption · activator protein

Since the discovery that Triton WR 1339 causes hyperlipidemia in experimental animals (1) the detergent has had widespread use as a tool for studying lipid metabolism (2, 3). Early investigators exploited its capacity to block lipid clearance for measuring rates of triglyceride and cholesterol synthesis (4–7) and, more recently, the inhibitory action of the detergent has been usefully employed for investigating metabolic interrelationships between plasma lipoproteins (8–10). We felt that Triton WR 1339 may also prove useful for studying the involvement of apoproteins in triglyceride catabolism since any changes in the composition or concentration of these proteins may be related to the delay in triglyceride catabolism that follows administration of the detergent. Some of the factors that are known to be important in the catabolism of very low density lipoprotein are the peptide components of high density lipoprotein, specifically the A-I and C-II apoproteins (11). The first of these (A-I) activates lecithin:cholesterol acyl transferase, an enzyme which is considered responsible for the formation of most of the serum cholesterol esters (12) and which, in cooperation with lipoprotein lipase, is thought to take part in the conversion of VLDL to the cholesterol ester-rich low density lipoprotein (LDL). C-II peptide is a powerful activator of lipoprotein lipase (13) which is responsible for the removal of the VLDL triglyceride during its transformation into LDL.

Because A-I is the major protein of HDL and most C-II in the plasma of rats is bound to this lipoprotein (14), it is possible that HDL plays a prominent role in the catabolism of rat VLDL. In a recent study, Portman et al. (10) reported disturbances in HDL structure after injecting Triton into squirrel monkeys and, in preliminary experiments with the detergent, we noted marked changes in HDL apoprotein composition following Triton administration to rats. In order to study the effect of the detergent on apo-

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Abbreviations: IEF, isoelectric focusing; TMU, tetramethylurea; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; ARP; arginine-rich protein; SDS, sodium dodecylsulfate; IDL, intermediate density lipoprotein.

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proteins in more detail and, specifically, to investigate the role of HDL apoproteins on VLDL catabolism, we have measured changes in apoprotein concentrations of whole plasma and lipoproteins at various times after injecting Triton WR 1339 into rats. Monovalent antisera to several key rat apoproteins were used to quantitate these changes.

METHODS AND MATERIALS

Male Sprague–Dawley rats, 220–250 g and fed commercial rat pellets containing 18% protein, 5% fat, 6% fiber, and the usual trace elements and vitamins were used in this study. Prior to Triton injection, they were fasted overnight and the experiments were started between 10 and 11 AM. Triton WR 1339 (Sterling Pharmaceuticals, NSW, Australia) dissolved in 0.15 M NaCl, pH 7.4, (phosphate-buffered saline) was injected into the tail veins at a dose of 250 mg per kg body weight. Rats were then bled from the abdominal aorta, under light ether anesthesia, at various times after injection. Four animals were exsanguinated at each time point (see Results) and in each experiment four animals were also injected with 1 ml of 0.15 M NaCl, providing a control group for comparison with the Triton group.

The plasma obtained from each rat was dialyzed for 72 hr against phosphate-buffered saline, pH 7.4, containing 1 mM EDTA with several changes of dialysate, to remove Triton WR 1339 (9). Samples of plasma were kept for lipid and apoprotein assays and the remainder was used to separate lipoproteins.

Isolation of lipoproteins

Plasma lipoproteins were isolated as described before (15). The lipoprotein fractions isolated in these experiments were very low density lipoprotein (VLDL, d < 1.006 g/ml), intermediate density lipoprotein (IDL, d 1.006–1.019 g/ml), low density lipoprotein (LDL, d 1.019–1.063 g/ml), high density lipoprotein (HDL, d 1.063–1.21 g/ml), and the infranate fraction (IF, d > 1.21). Lipoprotein fractions were dialyzed against phosphate-buffered saline, pH 7.4, and aliquots were removed for the quantitative determination of lipid and protein moieties as well as for electroimmunoassay. Portions of each lipoprotein fraction were also recentrifuged at their respective upper densities for compositional analysis and for separation of apoproteins on polyacrylamide gels.

Chemical analysis

Triglyceride and cholesterol concentrations were determined with a Technicon AA II Autoanalyzer using the fully enzymatic procedure for triglyceride (166448) and the enzymatic–colorimetric procedure for cholesterol (148393) of Boehringer Mannheim, Indianapolis, IN. The total protein content of washed lipoproteins was measured by the procedure of Lowry et al. (16) using bovine serum albumin as standard. B apoprotein was measured by differential solubility of the apoproteins in 50% TMU as described by Kane (17). Electrophoretic separation of apoproteins was performed on 15% polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS) according to the method of Weber and Osborn (18). The gels were prepared in a SDS (0.1%)–Tris–glycine system, with a stacking gel as described in Table III of the above publication (18). This system provided a better resolution of complex apoprotein mixtures more rapidly than the standard SDS–phosphate gel system (18). Samples were prepared in 1% SDS and 1% 2-mercaptoethanol and incubated at 80°C for 3–5 min before loading onto gels.

Quantitative immunoassay of apoproteins

Purification of antigens. Purification of rat apoproteins was performed as follows. Rat B apoprotein was isolated from washed VLDL or LDL preparations with 50% TMU as described by Kane (17). VLDL or LDL, at concentrations of 0.7–1.0 mg/ml were divided into 10–20 small aliquots (generally 300–400 μl) and an equal volume of redistilled TMU was added to each tube. After a 30-min incubation at 37°C the B protein pellicle was washed once with 50% TMU, and once more with water, and the insoluble protein was dissolved in 0.1 M SDS in 0.05 M Tris buffer, pH 8.2. SDS gel electrophoresis showed only the presence of protein, which remained near the top of the main gel and had an amino acid composition identical to that reported previously for rat B apoprotein (19). The SDS content was reduced to about 5 mM by dialysis, which was sufficient to maintain B apoprotein in solution. A-I apoprotein was isolated from rat HDL by chromatographic techniques or by preparative gel electrophoresis. In the first procedure, A-I peaks from Sephadex G-200 gel filtration columns (20) were dialyzed and lyophilized and further purified on DEAE cellulose columns (0.9 × 17 cm) using a gradient of 0.01 M Tris-HCl (300 ml) to 0.15 M Tris-HCl, pH 8.2 (300 ml). In later experiments, we used SDS gel electrophoresis (described above) to prepare pure A-I. This method was as reliable and more rapid than column chromatographic techniques. The amino acid composition plus electrophoretic homeogeneity and mobility of the protein confirmed its identification as rat A-I (21). Arginine-rich protein was also prepared from rat HDL by preparative

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SDS gel electrophoresis. It was identified by its molecular weight characteristics on SDS gels and its mobility as compared to human ARP. The isolated rat ARP also had an amino acid composition similar to that reported elsewhere (21). The A-I and ARP apoproteins were both eluted into SDS buffers after slicing and crushing the appropriate segments with a glass rod. Approximately 95% of A-I and 75–85% of ARP was eluted from gels by this technique. Rat albumin was obtained from Sigma Chemical Co.

Preparation of antisera. Between 200 and 300 μg of each apoprotein in 2 ml of phosphate-buffered saline was mixed with 2 ml of Freund's adjuvant and thoroughly emulsified by the double syringe technique. The antigen mixture was injected into multiple intradermal and subcutaneous sites of rabbits (2–3 kg), using two or three animals for each preparation, since wide variations in antibody response were often noted between different rabbits. In the case of rat ARP antisera production, 1 ml of pertussis vaccine was also injected subcutaneously at the first injection. After 14 days, animals received booster injections of apoproteins, varying from 100 to 250 μg per animal, and thereafter every 2–3 weeks until the antisera reached sufficiently high titers to produce clearly visible precipitation lines by the "rocket" technique (see below). In most cases, this was achieved after two booster injections although occasionally further challenges were required.

Immunoassay was carried out by the rocket immunoelectrophoretic procedure described by Laurell (22) as modified for apoprotein quantitation by Curry, Alaupovic, and Suenram (23). For each apoprotein, the ability of the procedure to measure total apoprotein levels in whole plasma and lipoproteins was thoroughly tested by various procedures such as delipidation, heating, and the use of Triton X-100 to expose the full antigenicity of the apoproteins in plasma and lipoproteins. With A-I and ARP immunoassays, it was necessary to delipidate plasma with ethanol–ether 2:3 (v/v) and to dilute the delipidated plasma 1:50 (with electrophoresis buffer) for A-I and 1:5 for ARP determinations. In this respect, the need to remove lipid for complete unmasking of antigen reactivity is similar to that reported by Schonfeld and Pfleger (24). B apoprotein assays were performed in the presence of 0.1% Triton X-100. Serum albumin concentrations were estimated on 1:2000 dilutions of plasma. To enable accurate quantitation of apoprotein concentrations, samples that exceeded the linearity of standard curves were diluted appropriately or samples with low concentrations (such as HDL samples containing low amounts of A-I after Triton treatment) were diluted less than control samples. All antisera produced were monospecific and did not react with any apoproteins other than their corresponding antigens.

Measurement of plasma C-II levels. Rat C-II concentrations were estimated indirectly, making use of the total dependence of the enzyme lipoprotein lipase on C-II cofactor activity (25). To compare the relative cofactor activities of the control and Triton-treated plasma lipoproteins, we established, in preliminary experiments, the appropriate volume of each lipoprotein fraction required to promote lipolysis within a range extending from approximately 10% to 80% of the maximal reaction velocity of the enzyme system. These conditions were satisfied using the incubation system of 0.2 ml of 15% bovine serum albumin (Pentex, fatty acid-poor, Miles Laboratories) in NH_4Cl buffer, pH 8.2, 0.1 ml of substrate (sonicated ^3H-labeled triolein–lecithin mixture prepared according to Fielding, Lim, and Scanu (13) and containing 10 mg triolein per ml), and 0.1 ml of milk lipoprotein lipase (cow's milk lipase, 10% solution in 0.1 M Tris–HCl, pH 8.2, prepared according to Bier and Havel (25)). Aliquots of plasma or lipoprotein fractions were added to the incubation tubes (see Results) and the final volume was 0.5 ml. Each sample was tested in duplicate and, after incubation at 37°C for 2 hr, the free fatty acids were extracted by the method of Schotz et al. (26). In each experiment human C-II at various concentrations was also added to separate incubation tubes, which established that the relationship between the quantity of cofactor and lipoprotein lipase showed saturation kinetics when sufficient activator protein was added to the system. The assay was reproducible and within-sample coefficients of variation were approximately 3–4%. The proportion of C-II in VLDL sample was also determined directly by isoelectric focusing (IEF) of the soluble VLDL fractions. IEF was performed on 0.6 × 10 cm gels with carrier ampholytes added in the 4–6 pH range according to the method of Swaney and Gidez (14) and the distribution of peptides was estimated after scanning the stained gels.

RESULTS

The effect of Triton on plasma lipids and lipoprotein concentrations is shown in **Table 1.** Triglyceride values increased continuously to 10 hr and at 20 hr remained approximately 30-fold higher (1300 mg/dl) than normal values (45 mg/dl). Cholesterol values also increased up to 20 hr but less markedly (7-fold) than triglyceride concentrations. Most of the

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TABLE 1. Changes in concentrations of plasma lipid and lipoproteins following Triton administration to rats

Fraction	Triglyceride[a]	Cholesterol	Protein[b]	Serum Albumin[a]
		mg per dl		*g per dl*
Whole plasma				
Control	45.3 ± 17.7	52.6 ± 12.5		2.6 ± 0.14
Triton				
5 min	81.2 ± 23.4	62.3 ± 8.8		2.6 ± 0.12
30	219.8 ± 34.0	74.6 ± 31.4		2.4 ± 0.05
2 hr	568.0 ± 97.3	88.3 ± 6.3		2.6 ± 0.18
10	1541.8 ± 267.4	213.5 ± 11.5		2.6 ± 0.11
20	1277.3 ± 191.5	364.1 ± 71.5		2.8 ± 0.18
VLDL d 1.006 g/ml				
Control	26.6 ± 11.5	5.2 ± 4.3	4.7 ± 1.3	
Triton				
5 min	56.4 ± 18.6	9.8 ± 2.9	5.8 ± 0.7	
30	214.5 ± 66.5	21.8 ± 4.7	12.3 ± 2.1	
2 hr	514.3 ± 34.6	52.0 ± 4.0	25.7 ± 2.1	
10	1533.3 ± 113.8	172.7 ± 2.3	74.4 ± 8.9	
20	1131.8 ± 198.0	316.9 ± 81.4	79.9 ± 5.4	
IDL d 1.006–1.019 g/ml				
Control	3.3 ± 1.7	1.4 ± 0.9	0.7 ± 0.3	
Triton				
5 min	5.2 ± 2.2	1.8 ± 1.0	0.6 ± 0.1	
30	6.3 ± 1.7	0.5 ± 0.9	0.7 ± 0.5	
2 hr	6.0 ± 0.0	not detected	0.4 ± 0.1	
10	18.3 ± 3.1	3.3 ± 0.6	2.3 ± 0.1	
20	51.6 ± 13.0	15.3 ± 5.0	12.7 ± 4.4	
LDL d 1.019–1.063 g/ml				
Control	5.3 ± 1.3	7.3 ± 2.8	2.2 ± 1.4	
Triton				
5 min	9.6 ± 4.8	15.7 ± 9.2	5.1 ± 3.7	
30	8.5 ± 2.7	15.6 ± 3.3	5.5 ± 0.2	
2 hr	8.7 ± 0.6	10.7 ± 2.1	7.5 ± 2.5	
10	11.3 ± 1.2	7.7 ± 1.2	4.4 ± 0.7	
20	24.6 ± 6.1	16.7 ± 5.8	12.0 ± 3.2	
HDL d 1.063–1.21 g/ml				
Control	5.9 ± 2.4	34.1 ± 7.6	52.7 ± 9.5	
Triton				
5 min	5.6 ± 1.8	29.4 ± 3.2	16.9 ± 2.3	
30	5.8 ± 1.7	27.4 ± 7.1	11.7 ± 3.8	
2 hr	3.3 ± 0.6	16.3 ± 2.5	6.5 ± 0.9	
10	3.3 ± 1.5	10.7 ± 1.5	5.5 ± 0.5	
20	6.7 ± 2.1	5.2 ± 4.1	5.0 ± 0.8	

[a] Mean ± SD of four rats.
[b] Protein concentration determined on washed lipoprotein fractions.

increased plasma lipid concentrations could be accounted for in the VLDL fraction but, although minor in absolute terms, relative increases in triglyceride and cholesterol concentrations of IDL and LDL fractions were high. The triglyceride to cholesterol ratio in VLDL increased from approximately 5 in the controls to approximately 10 within 2 hr and up to 10 hr following Triton administration. Although triglyceride concentrations of HDL were not markedly changed, the cholesterol concentration at 20 hr was reduced to 7-fold that of controls. Plasma albumin concentrations did not alter after Triton treatment (Table 1 and Fig. 1). However, HDL protein fell rapidly from 53 to 17 mg/dl 5 min after Triton injection and a sustained, marked increase in VLDL protein was observed up to 10 hr post Triton. Most of the increase in IDL and LDL occurred 10 and 20 hr after treatment.

Changes in total plasma apoprotein concentrations

The electroimmunoassay rockets in **Fig. 1** describe the relative changes in concentrations of albumin and B, A-I, and arginine-rich apoproteins of whole plasma after Triton injection into rats. B apoprotein levels at 10 and 20 hr were increased approximately 15 times those of controls whereas A-I concentrations fell rapidly to barely detectable levels at 10 and 20 hr after Triton treatment. The arginine-rich protein content of whole plasma fell rapidly to approximately

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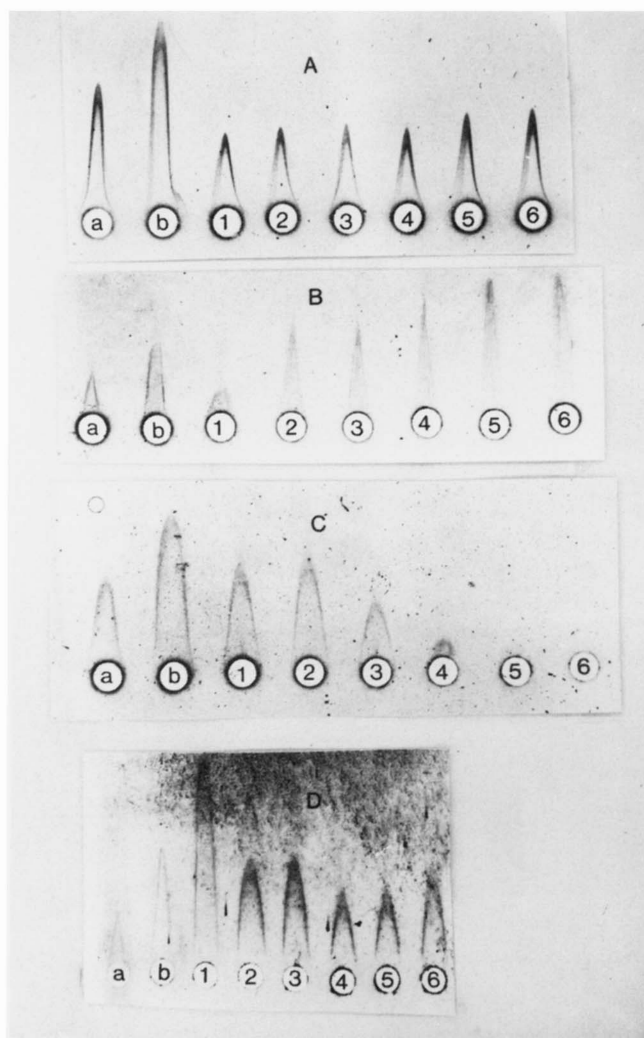

Fig. 1. Electroimmunoassay of rat plasma in the presence of antisera to *A*, rat albumin; *B*, apoprotein B; *C*, apoprotein A-1; and *D*, arginine-rich apoprotein. *a* and *b* are standards, approx. 150 and 300 ng, respectively; 1, control; 2 through 6, 5 min, 30 min, 2 hr, 10 and 20 hr post Triton injection, respectively.

half the control value at 5 min, reached lowest value at 2 hr, and then increased slightly from 2 to 20 hr post Triton.

In order to further investigate the marked reduction in HDL protein (Table 1) following Triton treatment, the concentration of A-I apoprotein (the major protein component of rat HDL) was measured in all lipoprotein fractions and the infranate (d > 1.21 g/ml) from four animals at each time point. This investigation (**Table 2**) showed that the A-I concentration of the d > 1.21 g/ml fraction increased markedly while that of the HDL fraction decreased 5 min after injection of the Triton. After 30 min, the A-I concentration in the infranate decreased to normal levels while the HDL A-I level continued to fall to nondetectable levels 20 hr after Triton injection. There was no evidence for the appearance of A-I in either VLDL or LDL fractions (Table 2) after Triton injection.

Since it had previously been shown by others that Triton inhibits lipoprotein lipase and LCAT activity (26, 27) we examined the possibility that such inhibition could have been due to decreased availability of the activator protein, C-II. The rapid transfer of A-I from the HDL fraction to the d > 1.21 g/ml infranate from which A-I was also rapidly cleared from the plasma suggested the further possibility that the block in VLDL catabolism was due to failure of LCAT activation by A-I apoprotein.

The content of C-II apoprotein in lipoprotein fractions was estimated by measuring the ability of these fractions to promote lipolysis of ^{3}H-labeled triolein in the presence of cow's milk lipase, as described in Methods. As shown in **Fig. 2** the amount of C-II peptide present in 12 μl of normal rat VLDL (equivalent to the amount of VLDL in 12 μl of plasma) was very low compared to the amount of activator

TABLE 2. Changes in A-I and ARP apoprotein concentrations following Triton treatment

	Whole Plasma	VLDL d < 1.006 g/ml	LDL d < 1.063 g/ml	HDL d 1.063–1.21 g/ml	Infranate d > 1.21 g/ml	Recovery %
A-I (mg/dl)						
Control	58.9 ± 15.6	nd[a]	nd	44.0 ± 13.0	2.8 ± 0.8	79
Triton-treated						
5 min	53.2 ± 14.8	nd	nd	8.8 ± 2.3	28.8 ± 2.7	71
30	44.3 ± 8.6	nd	nd	4.2 ± 0.8	26.2 ± 1.4	69
2 hr	12.1 ± 1.0	nd	nd	1.8 ± 0.9	8.9 ± 1.9	88
10	7.5 ± 0.4	nd	nd	0.6 ± 0.2	5.4 ± 0.3	80
20	3.5 ± 0.8	nd	nd	nd	2.4 ± 0.3	69
ARP (mg/dl)						
Control	22.4 ± 3.1	3.9 ± 1.3	1.2 ± 0.9	6.9 ± 0.4	12.8 ± 1.3	111
Triton-treated						
5 min	16.3 ± 1.1	tr	0.3 ± 0.2	3.9 ± 0.4	13.1 ± 0.9	106
30	14.9 ± 0.9	1.4 ± 0.6	nd	2.1 ± 0.2	10.6 ± 1.1	95
2 hr	10.3 ± 1.4	2.1 ± 0.4	nd	1.5 ± 0.3	7.4 ± 0.8	106
10	11.9 ± 2.1	6.7 ± 1.1	nd	nd	4.3 ± 0.7	92
20	13.5 ± 1.7	8.4 ± 1.7	0.1 ± 0.1	0.4 ± 0.1	4.1 ± 0.8	96

[a] Not detectable.

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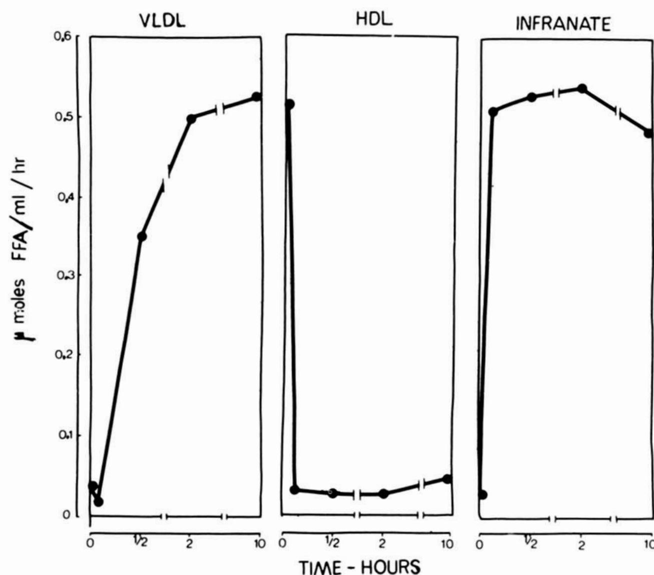

Fig. 2. Estimation of cofactor (C-II apoprotein) content of VLDL, HDL, and infranate (d > 1.21 g/ml) at various times after injection of Triton WR 1339 into rats. The estimation is based on the ability of an aliquot of lipoprotein sample from control or post-Triton samples equivalent to 12 μl of plasma to promote lipolysis of triglyceride (μmol FFA/ml/hr) in the presence of cow's milk lipase. FFA, free fatty acids.

protein in HDL from an equivalent amount (12 μl) of plasma. This is not surprising since the level of VLDL protein in fasting rats is also low compared to HDL (Table 1). Furthermore, we have found[2] as have others (14) that the proportion of C-II to C-III peptides is lower in rat VLDL compared to rat HDL and that rat HDL contains 15–20% of total C protein (unlike human HDL which contains only about 5% C protein). The activator protein content of rat VLDL increased after Triton treatment (Fig. 2, left panel) which roughly paralleled the rate of increase in total VLDL protein and specifically the C apoprotein fraction as shown in the gel patterns of **Fig. 3** (top panel). Furthermore, IEF of the soluble VLDL apoprotein exhibited an increase in all VLDL C apoproteins, including C-II apoprotein (labeled *a* in **Fig. 4**) after Triton injection. The stained bands from gels loaded with control VLDL apoprotein were barely visible (not shown) but 30 min after Triton (no. 1, Fig. 4) the accumulation of all VLDL C protein was apparent and increased up to 20 hr post Triton. These data support the evidence for the observed increase in activator protein in VLDL, determined indirectly by the cow's milk lipase assay. However, the content of cofactor protein in HDL (Fig. 2, center panel) decreased rapidly 5 min after Triton injection and remained low for up to 10 and 20 hr. The decrease in activator protein in HDL was accompanied by a rapid rise in cofactor activity in the d 1.21 g/ml infranate fraction which remained high at 10 and 20 hr after Triton injection. This reversal of C-II concentration in HDL and d 1.21 g/ml infranate fractions is similar to the reversal in the A-I content of HDL and d > 1.21 fractions.

[2] Ishikawa, T., and N. Fidge. Unpublished results.

Effect of Triton WR 1339 on the composition of VLDL

The triglyceride:cholesterol ratio rose from 5:1 to about 10:1 30 min after Triton treatment and the triglyceride to protein ratios increased from approximately 5.7:1 to 20:1 during the same time period (Table 1). The changes in apoprotein composition are illustrated in the gel patterns (Fig. 3, top) in which the amount of apo VLDL equivalent to that present in 320 μl of serum was loaded on SDS gels. Intensity of

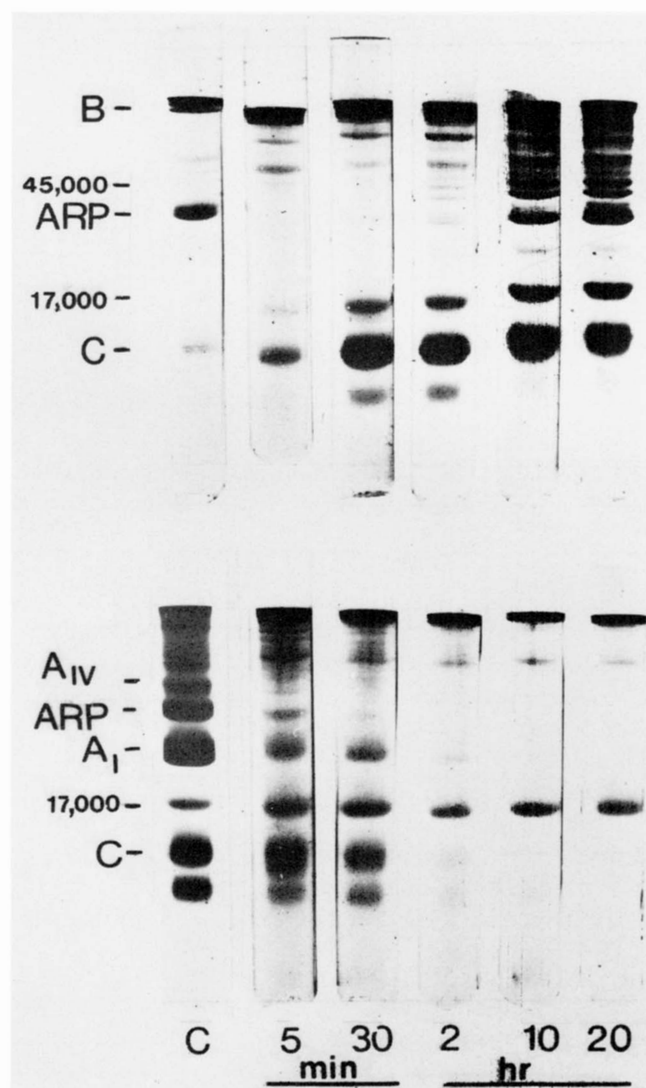

Fig. 3. SDS polyacrylamide gel (15%) electrophoresis of VLDL (top) and HDL (bottom) apoproteins. Samples loaded corresponded to lipoproteins from equivalent amounts of plasma as described in the text.

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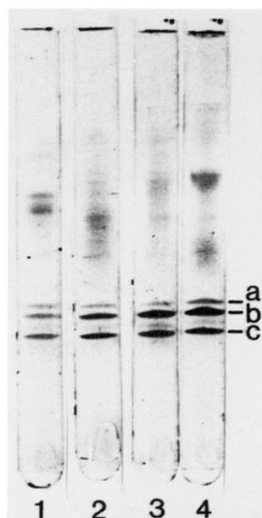

Fig. 4. IEF of rat VLDL soluble apoprotein. Each sample loaded corresponded to the same equivalent volume of plasma. *a*, *b*, and *c* correspond to C-II, C-III-0, and C-III-3 as described by Swaney and Gidez (14). *1*, *2*, *3*, and *4* are VLDL from 30 min, 2, 10, and 20 hr post-Triton plasma samples, respectively.

stained bands corresponding to the B protein and C proteins increased up to 20 hr, whereas 5 min after Triton injection, the proportion of arginine-rich protein was markedly decreased, although it reappeared in the 10 and 20 hr VLDL samples. Of particular interest was the appearance, post Triton, of proteins in addition to those normally detected in VLDL from fasted control rats. The most prominent of these bands was in the 17,000 dalton region while other bands appeared in the 40–45,000 dalton region of the gels.

The proportion of insoluble to soluble apoproteins in VLDL, which was determined by TMU extraction, showed that most of the increase in VLDL protein that followed Triton injection was due to B apoprotein content. Concentrations of the soluble proteins comprising mainly ARP and C apoproteins were estimated by scanning the stained gels (Fig. 3) and relating the proportions of areas obtained for each band with the TMU-soluble and total VLDL protein concentrations. These calculations demonstrated a change in B:C ratio (protein mass) from approximately 1:1 (control) to about 8:1 at 20 hr after Triton administration.

Changes in the ARP content of VLDL, shown visually in the gel patterns (Fig. 3, top), were confirmed and quantitated by electroimmunoassay (Table 2). Five minutes after Triton, ARP was not detectable in VLDL, but rose to 6.7 and 8.4 mg/dl after 10 and 20 hr, respectively, which represented approximately 7% of the total VLDL protein. In VLDL from fasting control rats, the ARP content was quite high and varied from 25 to 30% of the total protein content. Thus, although increasing approximately 2- to 3-fold in concentration in the VLDL fraction after Triton treatment, the actual proportion of ARP of total VLDL protein fell from about 25–30% to 7%.

Effect of Triton WR 1339 on composition of IDL and LDL

The rapid changes of lipid and protein concentrations in VLDL and HDL were not observed in IDL. Most of the marked increases occurred 10 and 20 hr after Triton injection (Table 1). Both the lipid and protein concentrations of the LDL fraction increased 2–3 times over the control value 5 min post Triton and were most highly elevated 20 hr after treatment. Most of the changes in LDL protein concentration were due to increases in B protein content (not shown) and no A-I protein was detected in LDL by gel electrophoresis or electroimmunoassay. ARP present in control LDL disappeared from this fraction 5–30 min after Triton treatment (Table 2).

Effect of Triton WR 1339 on the composition of HDL

Whereas the concentration of lipid and protein components of VLDL and LDL increased markedly after Triton, the lipid and protein concentrations in HDL decreased (Table 1). The rapid fall in HDL protein preceded the observed decrease of its lipid components; the rate of decrease in total HDL protein also preceded the increase observed for VLDL and LDL protein. Whereas HDL triglyceride concentrations only altered slightly post Triton, the HDL cholesterol concentration fell from 34 to 5 mg/dl, 20 hr after injection.

The fall in A-I concentration in the HDL and rise in the d 1.21 g/ml infranatant fractions have already been discussed. However, the ARP content of HDL also fell to low (nondetectable) levels 10 hr post Triton (Table 2). A small increase in the concentration of ARP in the d $>$ 1.21 g/ml fraction was observed in the first 5 min after injection and the ARP levels then fell to about one third of the control values. The high concentrations of ARP noted in the d $>$ 1.21 g/ml fraction of normal rat serum have also been reported elsewhere (29) and it is possible that ARP therefore exists in free form as well as in lipoprotein complexes or dissociates during ultracentrifugation. The total plasma concentration of ARP fell from 22 to approximately 12 mg/dl 10 hr after Triton injection.

Changes observed for total HDL protein concentrations (Table 1) or individually (A-I and ARP concentrations, Table 2) were confirmed in the gel patterns shown in Fig. 3. In this experiment the volume of HDL from an equivalent of 150 μl of plasma was

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loaded on each gel. This gel pattern visually describes the disappearance of A-I, ARP, A-IV, and C proteins from the HDL fraction after Triton treatment. IEF of HDL apoprotein also demonstrated the loss of C apoproteins after Triton. As noted in the VLDL fraction, there was a corresponding increase in a protein in the 17,000 dalton region. This is unlikely to represent the A-II protein because, in the rat, A-II exists as a monomer of molecular weight 8000–9000 (unlike A-II of human plasma which exists as a dimer with a molecular weight around 17,500). After 10–20 hr, this protein was the major peptide present in the running gel. It was also noted (after scanning the gels) that the amount of material at the top of the main gel increased when post Triton samples were applied. This may possibly have represented an increase in aggregated material, suggesting that the delipidation effect of Triton may have increased the protein–protein association for A-I and possibly for other HDL protein components as well.

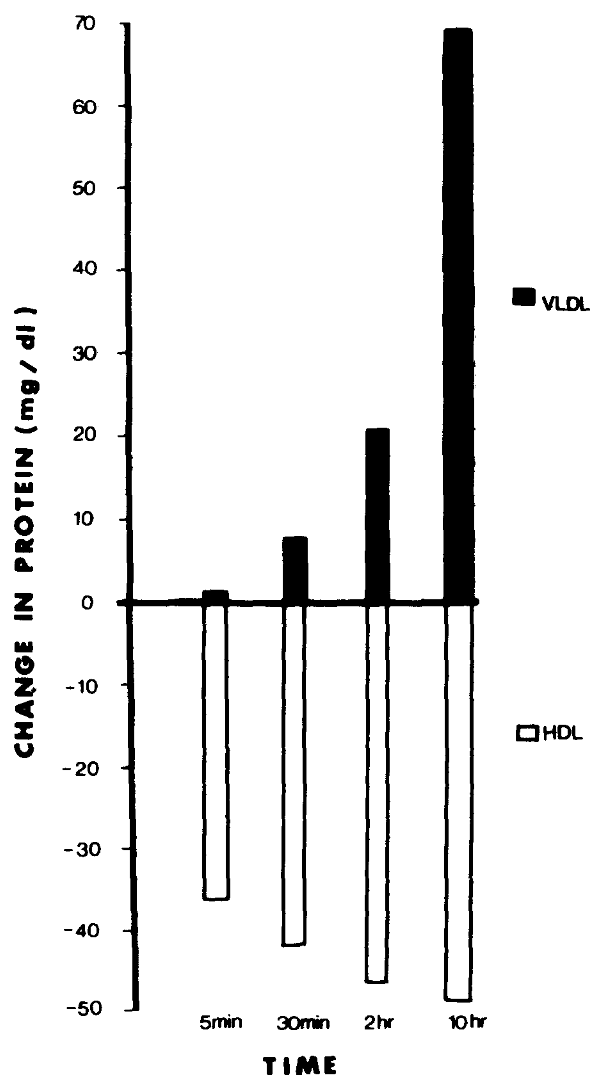

Fig. 5. Relative changes in HDL and VLDL protein after Triton injection into rats. SD are shown in Table 1. The changes in HDL protein were negative and those of VLDL were positive.

DISCUSSION

This investigation has found that several important apolipoproteins are affected by administration of Triton WR 1339 to rats. The large increase in plasma triglyceride and cholesterol (mostly confined to the VLDL fraction) found in this study and previously reported by many other laboratories (1–10) was preceded by a rapid fall in the concentration of HDL and, more particularly, in the reduction of HDL apoprotein. Other groups have also shown that Triton produces changes in HDL composition. Scanu and Oriente (30) established that the detergent, in vitro, produced a dissociation of HDL lipid and protein while Scanu et al. (31) found in vivo in dogs that prolonged Triton treatment caused a reduction in HDL which was followed by its total disappearance. Portman et al. (10) confirmed these findings in squirrel monkeys and provided the first demonstration of the rapidity with which HDL concentrations were reduced following Triton injection.

Our own findings have confirmed the early disappearance of HDL post Triton and, in addition, provided new information about the sequential changes in concentrations of apoproteins in whole plasma and in lipoprotein fractions. Assessment of these changes by immunological assay has enabled us to propose a different hypothesis for the mechanism of Triton-induced hyperlipemia. As shown in **Fig. 5** the rapid removal of protein from the HDL fraction rapidly preceded the increase in VLDL protein concentrations. The fall in HDL protein was due to the removal of A-I and ARP apoproteins (shown by electroimmunoassay, Fig. 1 and Table 2) and C proteins (shown by gel electrophoresis and measurement of C-II activator content) all of which appeared in the d > 1.21 g/ml fraction, presumably as lipid-free or lipid-poor complexes. These events are consistent with the suggestion (2, 10) that Triton may dissociate the protein from HDL forming Triton–lipid complexes. In contrast, it was noted (Fig. 1) that the concentration of serum albumin remained constant in rats treated with Triton. This reinforces the argument that HDL apoprotein is more severely affected by the detergent and that synthesis of proteins in general is probably not altered. The A-I and ARP proteins, which were recovered in the d 1.21 g/ml fraction 5 min after Triton, were then rapidly removed from the plasma (at a faster rate than the t½ of 10–12 hr reported for

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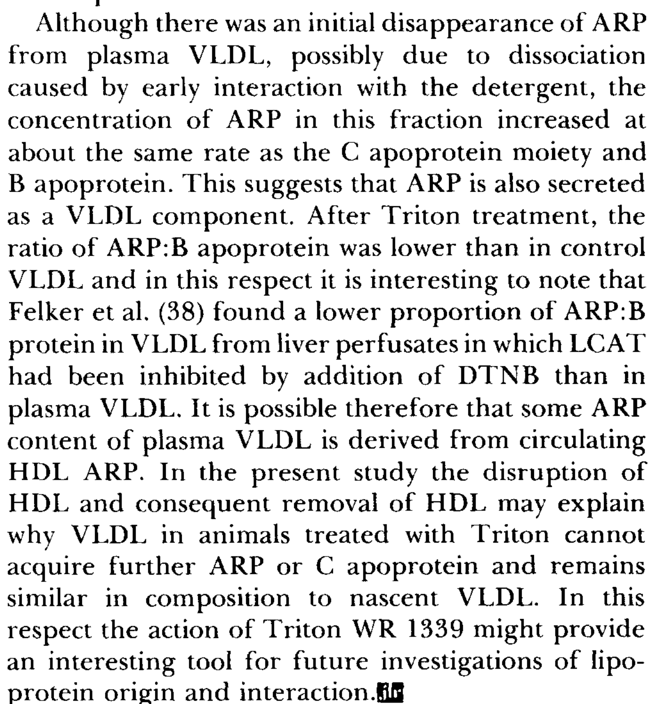
A-I clearance in normal rats (32)) presumably because they formed aggregates or were changed in other ways so that they were removed by processes other than those responsible for that of A-I in intact HDL. Catabolism of VLDL triglyceride normally proceeds very rapidly in the fasted state and is regulated by lipoprotein lipase (C-II activated) and LCAT (A-I activated). In the Triton-treated fasted animal, triglyceride was not cleared and reached concentrations that were several-fold higher than that of normal plasma. Thus, lack of A-I, which is essential for normal functioning of LCAT and hence involved in VLDL catabolism, possibly contributed towards the triglyceride that accumulated at later times. The same argument applies to C-II. Experiments with cow's milk lipase showed that cofactor activity was rapidly transferred from the d 1.063–1.21 g/ml fraction to the infranatant and that C-II activator increased in VLDL post Triton. However, the presence of C-II in VLDL, although sufficient to promote hydrolysis of some triglyceride in the artificial in vitro system, was apparently not capable of promoting hydrolysis in vivo. Presumably then, activator protein was required in higher concentration or in another form. Since C-II and A-I were transferred to, and at early times, concentrated in the lipid-free infranate, we suggest that they are most active when present as part of intact HDL. The necessity for HDL to remain intact in order for it to act as a donor of activator protein is further supported by Havel, Kane, and Kashyap (33) who investigated the interchange of C-II between HDL and triglyceride-rich particles and more recently by Heinen et al. (34) who suggested that the absence of normal HDL in Tangier disease, with a consequent loss of C apoprotein reservoir, may contribute to the abnormal metabolism of the VLDL–LDL system in this disease.

Although it has been suggested that Triton forms a surface coat around lipoproteins rendering them inaccessible as substrates to lipoprotein lipase and LCAT enzymes (2, 30, 31), there are other reports, apparently conflicting, which support the argument that the hyperlipemia is due to a direct action of Triton on the enzymes rather than due to a physical modification of the substrate (27, 28). We suggest that the apparent contradiction between some studies that claim that there are physical alterations of the lipoprotein substrates and other studies that support inhibition of lipolytic enzymes as causes of the hyperlipidemia can alternatively be explained by an inactivation of cofactor proteins as a result of their displacement from HDL. As suggested above (33, 34) presence of intact HDL may be essential for VLDL catabolism and thus the absence of intact HDL cofactor complex is the primary cause of the massive increase in plasma triglyceride that follows Triton injection.

Other interesting changes which were seen to accompany Triton administration were the alterations in proportions of VLDL lipid and protein complexes. The B:C apoprotein and triglyceride:cholesterol ratios increased markedly 10 hr post Triton so that VLDL more closely resembled nascent VLDL rather than plasma VLDL. Such a phenomenon has also been described in the rabbit by Klauda and Zilversmit (7) and by Portman et al. (10) in the squirrel monkey. The composition of VLDL apoprotein before and after Triton treatment may also provide some clues to the origin of lipoprotein peptides. The rise in C apoprotein concentration that accompanied the increased secretion of B apoprotein suggests that at least some C proteins are secreted as part of the VLDL complex. This observation is consistent with the findings of other laboratories although there is little agreement about the proportion of C protein in nascent VLDL. Thus results have varied depending on whether VLDL was isolated from perfused livers (35), isolated liver cells (36), or Golgi apparatus (37) from the liver. There is little doubt however that VLDL acquires more C apoproteins from HDL after entering the circulation (33). In the present studies, since this source of C proteins has apparently been blocked, the proportion of C:B protein (1:8) may more closely resemble that of nascent VLDL produced in the liver.



Although there was an initial disappearance of ARP from plasma VLDL, possibly due to dissociation caused by early interaction with the detergent, the concentration of ARP in this fraction increased at about the same rate as the C apoprotein moiety and B apoprotein. This suggests that ARP is also secreted as a VLDL component. After Triton treatment, the ratio of ARP:B apoprotein was lower than in control VLDL and in this respect it is interesting to note that Felker et al. (38) found a lower proportion of ARP:B protein in VLDL from liver perfusates in which LCAT had been inhibited by addition of DTNB than in plasma VLDL. It is possible therefore that some ARP content of plasma VLDL is derived from circulating HDL ARP. In the present study the disruption of HDL and consequent removal of HDL may explain why VLDL in animals treated with Triton cannot acquire further ARP or C apoprotein and remains similar in composition to nascent VLDL. In this respect the action of Triton WR 1339 might provide an interesting tool for future investigations of lipoprotein origin and interaction.

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REFERENCES

1. Kellner, A., J. W. Correll, and A. T. Ladd. 1951. Sustained hyperlipemia induced in rabbits by means of intravenously injected surface active agents. *J. Exp. Med.* **93:** 373–385.
2. Scanu, A. 1965. Factors affecting lipoprotein metabolism. *Adv. Lipid Res.* **3:** 63–138.
3. Recknagel, R. O. 1967. Carbon tetrachloride hepatotoxicity. *Pharm. Rev.* **19:** 145–208.
4. Otway, S., and D. S. Robinson. 1967. The use of a nonionic detergent (Triton WR 1339) to determine rates of triglyceride entry into the circulation of the rat under different physiological conditions. *J. Physiol.* **190:** 321–332.
5. Schurr, P. E., J. R. Schultz, and T. M. Parkinson. 1972. Triton induced hyperlipidemia in rats as an animal model for screening hyperlipidemic drugs. *Lipids.* **7:** 68–74.
6. Zilversmit, D. B., L. B. Hughes, and M. Remington. 1972. Hypolipidemic effect of pregnancy in the rabbit. *J. Lipid Res.* **13:** 750–756.
7. Klauda, H. C., and D. B. Zilversmit. 1974. Influx of cholesterol into plasma in rabbits with fasting hyperbetalipoproteinemia. *J. Lipid Res.* **15:** 593–601.
8. Illingworth, D. R., L. E. Whipple, and O. W. Portman. 1975. Metabolism of lipoproteins in nonhuman primates. Reduced secretion of very low density lipoproteins in squirrel monkeys with diet induced hypercholesterolemia. *Atherosclerosis.* **22:** 325–334.
9. Illingworth, D. R. 1975. Metabolism of lipoproteins in nonhuman primates. Studies on the origin of low density lipoprotein apoprotein in the plasma of the squirrel monkey. *Biochim. Biophys. Acta.* **388:** 38–51.
10. Portman, O. W., M. Alexander, M. Tanaka, and D. R. Illingworth. 1977. Triacylglycerol and very low density lipoprotein secretion into plasma of squirrel monkeys. *Biochim. Biophys. Acta.* **486:** 470–482.
11. Eisenberg, S., and R. I. Levy. 1975. Lipoprotein metabolism. *Adv. Lipid Res.* **13:** 1–89.
12. Fielding, C. J., V. G. Shore, and P. E. Fielding. 1972. A protein cofactor of lecithin:cholesterol acyltransferase. *Biochem. Biophys. Res. Commun.* **46:**1493–1498.
13. Fielding, C. J., C. T. Lim, and A. M. Scanu. 1970. A protein component of serum high density lipoprotein with cofactor activity against purified lipoprotein lipase. *Biochem. Biophys. Res. Commun.* **39:**889–894.
14. Swaney, J. B., and L. I. Gidez. 1977. Analysis of rat serum apolipoproteins by isoelectric focusing. II. Studies on the low molecular weight subunits. *J. Lipid Res.* **18:** 69–76.
15. Fidge, N. H., and P. Poulis. 1975. Studies on the metabolism of rat serum very low density apolipoprotein. *J. Lipid Res.* **16:** 367–378.
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
17. Kane, J. P., T. Sata, R. L. Hamilton, and R. J. Havel. 1975. Apoprotein composition of very low density lipoproteins of human serum. *J. Clin. Invest.* **56:** 1622–1634.
18. Weber, K., and M. Osborn. 1975. Proteins and sodium dodecyl sulphate: Molecular weight determination on polyacrylamide gels and related procedures. *In* The Proteins. H. Neurath and R. L. Hill, editors. Academic Press, N.Y. 179–223.
19. Faergeman, O., T. Sata, J. P. Kane, and R. J. Havel. 1975. Metabolism of apoprotein B of plasma very low density lipoproteins in the rat. *J. Clin. Invest.* **56:** 1396–1403.
20. Fainaru, M., R. J. Havel, and T. E. Felker. 1976. Radioimmunoassay of apolipoprotein AI of rat serum. *Biochim. Biophys. Acta.* **446:**56–68.
21. Swaney, J. B., H. Reese, and H. A. Eder. 1974. Polypeptide composition of rat high density lipoprotein: characterisation by SDS-gel electrophoresis. *Biochem. Biophys. Res. Commun.* **59:** 513–519.
22. Laurell, C. B. 1966. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal. Biochem.* **15:** 45–52.
23. Curry, M. D., P. Alaupovic, and C. A. Suenram. 1976. Determination of apolipoprotein A and its constitutive A-I and A-II polypeptides by separate electroimmunoassays. *Clin. Chem.* **22:** 315–322.
24. Schonfeld, G., and B. Pfleger. 1974. The structure of human high density lipoprotein and the levels of apolipoprotein A-I in plasma as determined by radioimmunoassay. *J. Clin. Invest.* **54:** 236–246.
25. Bier, D. M., and R. J. Havel. 1971. Activation of lipoprotein lipase by lipoprotein fractions of human serum. *J. Lipid Res.* **11:** 565–570.
26. Schotz, M. C., A. S. Garfinkel, R. J. Heubotter, and J. E. Stewart. 1970. A rapid assay for lipoprotein lipase. *J. Lipid Res.* **11:** 68–69.
27. Soler-Argila, C., R. L. Russell, and M. Heimberg. 1977. Effect of Triton WR-1339 on lecithin–cholesterol acyltransferase in the rat. *Arch. Biochem. Biophys.* **178:** 135–139.
28. Borensztajn, J., M. S. Rone, and T. J. Kotlar. 1976. The inhibition in vivo of lipoprotein lipase (clearing-factor lipase) activity by Triton WR-1339. *Biochem. J.* **156:** 539–543.
29. Fainaru, M., R. J. Havel, and K. Imaizumi. 1977. Radioimmunoassay of arginine rich apolipoprotein of rat serum. *Biochim. Biophys. Acta.* **490:**144–155.
30. Scanu, A., and P. Oriente. 1961. Triton hyperlipemia in dogs. I. In vitro effects of the detergent on serum lipoproteins and chylomicrons. *J. Exp. Med.* **113:** 735–757.
31. Scanu, A., P. Oriente, J. M. Szajewski, L. J. McCormack, and I. H. Page. 1961. Triton hyperlipemia in dogs. II. Atherosclerosis, diffuse lipidosis and depletion of fat stores produced by prolonged administration of the non-ionic surface-active agent. *J. Exp. Med.* **114:** 279–294.
32. Roheim, P. S., H. Hirsch, D. Edelstein, and D. Rachmilewitz. 1972. Metabolism of iodinated high density lipoprotein subunits in the rat. III. Comparison of the removal rate of different subunits from the circulation. *Biochim. Biophys. Acta.* **278:** 517–529.
33. Havel, R. J., J. P. Kane, and M. L. Kashyap. 1973. Interchange of apolipoproteins between chylomicrons

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and high density lipoproteins during alimentary lipemia in man. *J. Clin. Invest.* **52:** 32–38.
34. Heinen, R. J., P. N. Herbert, D. S. Fredrickson, T. Forte, and F. T. Lindgren. 1978. Properties of the plasma very low and low density lipoproteins in Tangier disease. *J. Clin. Invest.* **61:** 120–132.
35. Windmueller, H. G., P. N. Herbert, and R. I. Levy. 1973. Biosynthesis of lymph and plasma lipoprotein apoproteins by isolated perfused rat liver and intestine. *J. Lipid Res.* **14:** 215–223.
36. Jeejeebhoy, K. N., J. Ho, C. Breckenridge, A. Bruce-Robertson, G. Steiner, and J. Jeejeebhoy. 1975. *Biochem. Biophys. Res. Commun.* **66:** 1147–1153.
37. Nestruck, A. C., and D. Rubinstein. 1976. The synthesis of apoproteins of very low density lipoprotein isolated from the Golgi apparatus of rat liver. *Can. J. Biochem.* **54:** 617–628.
38. Felker, T. E., M. Fainaru, R. L. Hamilton, and R. J. Havel. 1977. Secretion of the arginine-rich and A-I apolipoproteins by the isolated perfused rat liver. *J. Lipid Res.* **18:** 465–473.

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