brane, possibly by simple diffusion [3, 4], is not the rate-limiting step in the oxidation of these metabolites under the conditions used, or that the transport of these compounds by simple diffusion is not stimulated by dibucaine. These observations indicate that the properties of the transport systems for 3-hydroxybutyrate and acetoacetate differ in some respects from those of the system which transports pyruvate.

Acknowledgements—This work was conducted with the skilled technical assistance of Mr. Paul Spiel, and was supported by grant G1292/1120 from the National Heart Foundation of Australia.

Clinical Biochemistry Unit,
Flinders University School of Medicine,
Flinders Medical Centre,
Bedford Park,
South Australia 5042.

GREGORY J. BARRITT

REFERENCES

1. G. J. Barritt, *Biochem. Pharmac.* **28**, 1017 (1979).
2. A. P. Halestrap and R. M. Denton, *Biochem. J.* **138**, 313 (1974).
3. S. V. Pande and R. Parvin, *J. biol. Chem.* **253**, 1565 (1978).
4. A. P. Halestrap, *Biochem. J.* **172**, 377 (1978).
5. J. Mowbray, *Biochem. J.* **148**, 41 (1975).
6. G. Paradies and S. Papa, *Fedn. Eur. biochem. Soc. Lett.* **52**, 149 (1975).
7. G. J. Barritt, *Int. J. Biochem.* **9**, 317 (1978).
8. D. R. H. Fayle, G. J. Barritt and F. L. Bygrave, *Biochem. J.* **148**, 527 (1975).
9. M. Crompton, G. J. Barritt, J. H. Bradbury and F. L. Bygrave, *Biochem. Pharmac.* **25**, 2461 (1976).
10. M. B. Feinstein, M. Volpi, S. Perrie, A. Makriyannis and R. I. Sha'afi, *Molec. Pharmac.* **13**, 840 (1977).
11. J. M. Land and J. B. Clark, *Fedn. Eur. biochem. Soc. Lett.* **44**, 348 (1974).

Biochemical Pharmacology, Vol. 29, pp. 2879–2882.

0006-2952/80/1015–2879 $02.00/0

Triton WR-1339 induced changes in the fatty acid composition of serum lipids in rats

(*Received* 18 *March* 1980; *accepted* 11 *June* 1980)

Hyperlipidemia induced by Triton WR-1339 is a frequently used experimental model when studying hypolipidemic agents. According to Garattini *et al.* [1], hepatic cholesterol biosynthesis may be enhanced by activating hydroxymethylglutaryl-CoA-reductase with intravenously administered triton. Triton treatment results in considerably elevated serum triglyceride concentrations due to the protective coating developed on the surface of the very low density (VLD) lipoproteins. Accordingly, they are shielded from hydrolytic cleavage by lipoprotein lipase [2]. It has also been reported [3] that triton, in addition to increasing cholesterol biosynthesis, augments cell membrane permeability and inhibits the postheparin clearing factor. In Illingworth's [4] studies triton proved to inhibit the LCAT activity in squirrel monkeys. As the triton-induced hyperlipidemic model has been frequently used in our studies [5] we examined Triton WR-1339 for its effect on the esterification of cholesterol as well as on the fatty acid composition of different lipid fractions.

Methods. CFY rats of 180–200 g body wt were treated intravenously with single 200 mg/kg doses of Triton WR-1339. Ten animals per group were killed at 6-, 24-, 48- and 72-hr intervals. Blood samples were centrifuged and serum lipid extracted according to Carlson [6] and total serum cholesterol, free cholesterol [7], triglyceride [8] and phospholipid [9] levels assessed.

In the second stage of the experiment, lipids were separated by thin-layer chromatography in a mixture of *n*-hexane–diethylether–formic acid (80:20:2). Following spraying with Rhodamine 6G, cholesterol ester, triglyceride and phospholipid fractions were identified under a u.v. lamp, and subsequently removed together with the coating. The fatty acid composition of individual lipid fractions was measured after treatment with methanol–hydrochloric acid, with a Hewlett–Packard gas chromatograph, type 5830A, at 195° (glass column 1.6 m × 4 mm, stationary phase Sp 2340 Chromosorb W, AW 80/100). The fatty acids were identified using a set of standard fatty acid methyl esters (KD Applied Science Laboratories, State College, Pennsylvania).

Statistical evaluation was performed by applying Student's *t*-test. In cases of *F*-test positivity, the *d*-test was used.

Results. Maximum concentration of total serum cholesterol was observed in the sixth hour after Triton WR-1339 administration, thereafter it decreased reaching normal level by the 72nd hour. Cholesterol esterification characterized by the cholesterol ester/free cholesterol ratio showed, however, opposite changes (Table 1). This ratio fell to minimum in the first 6 hr after triton injection, thereafter increased progressively and by the 72nd hour became identical with that of the control. Both serum triglyceride and phospholipid levels showed an abrupt rise during the first 6 hr and were found to have returned to the normal level by the 48th hour.

The fatty acid composition of the cholesterol ester fraction is represented in Table 2. The 6th hour and even more the 24th hour after triton administration were characterized by decreased proportions of both linoleic acid (18:2) and arachidonic acid (20:4), in contrast to the rise observed in the proportion of oleic acid (18:1). A moderate rise could also be noted in the percentage of stearic acid (18:0). After 72 hr, however, the above values proved to be identical with those of the controls. In these experiments no change could be detected in the proportions of palmitic (16:0) and palmitoleic (16:1) acids.

Table 1. Changes induced in serum lipid data by acute Triton WR-1339 treatment

	No. of animals	Blood withdrawal (hr)	Serum cholesterol (mmoles/l ± S.D.)	Ratio of cholesterol ester/free cholesterol in serum (mmoles/l ± S.D.)	Serum triglyceride (mmoles/l ± S.D.)	Serum phospholipid (mmoles/l ± S.D.)
Control	10	0	2.10 ± 0.29	2.84 ± 0.18	0.93 ± 0.38	1.13 ± 0.19
Triton WR-1339 200 mg/kg i.v.	10	6	4.52 ± 0.69‡	0.95 ± 0.13‡	6.52 ± 1.70‡	2.53 ± 0.55‡
	10	24	2.89 ± 0.49‡	2.42 ± 0.30*	1.63 ± 0.61†	1.53 ± 0.23‡
	10	48	2.78 ± 0.45†	2.76 ± 0.23	0.95 ± 0.27	1.32 ± 0.30
	10	72	2.36 ± 0.38	2.85 ± 0.21	0.96 ± 0.25	1.31 ± 0.27

* $P < 0.05$.
† $P < 0.01$.
‡ $P < 0.001$.

Table 2. Fatty acid composition of serum cholesterol esters*

Fatty acid	Control serum	Serum following Triton WR-1339 treatment			
		6 hr	24 hr	48 hr	72 hr
16:0	18.4 ± 3.3	19.6 ± 1.5	18.9 ± 2.9	19.2 ± 2.7	17.8 ± 1.7
16:1	5.1 ± 2.2	6.3 ± 0.4	5.2 ± 2.4	5.4 ± 0.4	4.6 ± 0.8
18:0	3.3 ± 1.9	5.9 ± 0.7†	5.4 ± 2.1	3.6 ± 1.0	2.8 ± 0.7
18:1	16.0 ± 2.9	23.8 ± 3.5‡	29.5 ± 4.3§	14.8 ± 1.4	15.5 ± 1.2
18:2	31.2 ± 4.6	23.0 ± 1.8‡	21.6 ± 1.6‡	27.9 ± 2.7	31.3 ± 3.0
20:4	26.0 ± 3.4	21.4 ± 3.0	19.4 ± 4.1	29.1 ± 2.4	28.0 ± 3.3

* Results expressed as percentages.
† $P < 0.05$.
‡ $P < 0.01$.
§ $P < 0.001$.

Table 3. Fatty acid composition of serum phospholipids*

Fatty acid	Control serum	Serum following Triton WR-1339 treatment			
		6 hr	24 hr	48 hr	72 hr
16:0	41.1 ± 2.7	29.3 ± 7.4†	27.3 ± 0.7§	35.1 ± 3.8	42.1 ± 2.8
16:1	0.9 ± 0.5	1.2 ± 0.2	1.1 ± 0.6	0.6 ± 0.2	0.5 ± 0.1
18:0	33.0 ± 2.4	34.1 ± 4.6	24.2 ± 1.6§	35.4 ± 6.7	33.6 ± 8.3
18:1	9.2 ± 0.6	10.5 ± 2.4	13.4 ± 2.0‡	11.1 ± 3.9	9.0 ± 4.4
18:2	12.6 ± 1.8	14.5 ± 4.0	24.3 ± 1.0§	14.4 ± 3.8	12.0 ± 4.5
20:4	3.2 ± 1.3	10.4 ± 4.3†	9.7 ± 1.9§	3.4 ± 0.9	2.8 ± 1.3

* Results expressed as percentages.
† $P < 0.05$.
‡ $P < 0.01$.
§ $P < 0.001$.

Table 4. Fatty acid composition of serum triglycerides*

Fatty acid	Control serum	Serum following Triton WR-1339 treatment			
		6 hr	24 hr	48 hr	72 hr
16:0	39.0 ± 3.1	27.4 ± 4.7‡	34.2 ± 3.8	32.9 ± 5.3†	37.2 ± 2.7
16:1	4.4 ± 1.1	3.5 ± 0.5	3.1 ± 0.8	4.5 ± 1.4	4.0 ± 0.9
18:0	7.5 ± 1.8	7.3 ± 1.5	7.0 ± 0.4	6.6 ± 1.5	7.0 ± 1.0
18:1	35.0 ± 3.1	38.5 ± 1.1	35.4 ± 3.7	37.0 ± 5.2	37.3 ± 2.5
18:2	14.0 ± 3.1	21.8 ± 4.7†	19.2 ± 1.9†	18.8 ± 1.6†	14.3 ± 0.3
20:4	0.1 ± 0.05	1.5 ± 0.5§	1.1 ± 0.4‡	0.2 ± 0.05	0.2 ± 0.06

* Results expressed as percentages.
† $P < 0.05$.
‡ $P < 0.01$.
§ $P < 0.001$.

The fatty acid composition of phospholipids compared to that of cholesterol esters exhibited opposite changes Table 3. The linoleic and arachidonic acid contents decreased in the cholesterol ester but were elevated in the phospholipid fraction. The most pronounced changes could be observed after 24 hr. At this time there was a fall in the percentage of palmitic and stearic acids and a rise in that of the oleic acid. Percentage values of fatty acids measured in the 48th and 72nd hours were identical with those of the controls.

Table 4 demonstrates the fatty acid composition in the triglyceride fraction. As early as 6 hr after triton administration a significant rise could be observed in the proportion of both linoleic and arachidonic acids and a decrease in that of palmitic acid. Control values were reached by the 72nd hour only.

Discussion. A single i.v. injection of Triton WR-1339 resulted, in agreement with literature findings [1, 10], in significantly elevated levels of serum cholesterol, triglyceride and phospholipid. There is also a shift in the fatty acid composition of the lipid fractions isolated, and a decrease in the cholesterol ester/free cholesterol ratio, suggesting that the esterification process of cholesterol had been affected.

The conversion of free cholesterol into cholesterol ester is of physiological importance for the significant difference between the turnover times of free and esterified cholesterol [11]. In addition, the saturated/unsaturated cholesterol ester ratio has also to be considered since the deposit formation in the intima is mainly affected by the degree of unsaturation. These two types of cholesterol esters form different gradients in the membrane [12].

The esterification of serum cholesterol is induced by lecithin-cholesterol-acetyltransferase (LCAT) which, when bound to the apoprotein of high density lipoproteins (HDL), catalyses mainly the transfer of polyunsaturated (18:2, 20:4) fatty acids from the C-2 position of lecithin to the free hydroxy group of cholesterol. In this process lecithin is transformed into lysolecithin [13]. Consequently, the presence of phospholipids, mainly lecithin, is needed for cholesterol esterification. The lipids of VLDL also play an important role in the process of cholesterol esterification as LCAT esterifies mainly the cholesterol moiety in VLDL, while in the case of HDL and LDL origin, the process takes place at a reduced rate [14].

The changes induced by triton in the fatty acid composition of cholesterol ester, phospholipid and triglyceride fractions involved mainly the polyunsaturated fatty acids, linoleic and arachidonic acids. Up to 24 hr following injection there was a decrease in the proportion of linoleic and arachidonic acids of cholesterol esters, and an increase in that of both triglycerides and phospholipids. Altered fatty acid composition induced by triton treatment is presumably due to inhibited LCAT activity. The amount of VLDL, the best substrate for LCAT is, however, significantly increased by triton [14]. Thus, our results suggest that in the cholesterol esterification process of triton-induced hyperlipemic state the enzyme inhibition is the prevailing factor compared to the effect of the substrate [15].

Institute for Drug Research, Budapest, Hungary.

EVA ORBAN*
ANDREA MADERSPACH†
EVA TOMORI

REFERENCES

1. S. Garattini, L. Paoletti, E. Bizzi and R. Vertus, in *Drugs Affecting Lipid Metabolism* (Eds. S. Garattini and R. Paoletti), p. 144. Elsevier Publishing Company, Amsterdam (1960).
2. A. M. Scanu and P. Oriente, *J. exp. Med.* **113**, 735 (1961).
3. K. Reber and A. Studer, in *Drugs Affecting Lipid Metabolism* (Eds. S. Garattini and R. Paoletti), p. 181. Elsevier Publishing Company, Amsterdam (1960).
4. D. R. Illingworth, O. W. Portman and L. E. Whipple, *Biochim. biophys. Acta* **369**, 304 (1974).
5. A. Maderspach, J. Borsy, I. Elekes *et al.*, in *Symposium on Drug Induced Metabolic Changes* (Ed. J. Knoll), p. 161. Akadémiai Kiadó, Budapest (1974).
6. L. A. Carlson, *J. Atheroscler. Res.* **3**, 334 (1963).
7. M. Kates, in *Techniques of Lipidology* (Eds. T. S. Work and E. Work), p. 360. American Elsevier, New York (1972).
8. E. Van Handel and D. B. Zilversmit, *J. Lab. clin. Med.* **50**, 152 (1957).
9. R. K. Raheja, C. Kaur, A. Singh and I. S. Bathia, J. Lipid Res. **14**, 695 (1973).
10. T. Kremmer and L. Holczinger, *Biochem. Pharmac.* **23**, 3317 (1974).
11. D. S. Goodman, *Physiol. Rev.* **45**, 747 (1965).
12. J. S. Schweppe and R. A. Jungmann, *Proc. Soc. exp. Biol. Med.* **136**, 449 (1971).
13. J. A. Glomset, *J. Lipid Res.* **9**, 155 (1968).
14. J. S. K. David, L. A. Soloff and A. G. Lacko, *Life Sci.* **18**, 701 (1976).
15. C. Soler- Argilaga, R. L. Russel and M. Heimberg, *Archs Biochem. Biophys.* **178**, 135 (1977).

* Present address: Research Institute of Oncopathology, Budapest, Hungary.

† Present address: Chemical Works of Gedeon Richter Ltd., 1035 Budapest, Vörösvari ut 13, Hungary.