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Incorporation of $^{32}P_i$ and Other Radioactive Precursors into Lecithin of Brain Slices of Triton WR-1339 Injected Rats[1] (38139)

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Since the original findings by Kellner *et al.* (1) that the intravenous injection of the surface-active agents Tween 80 and Triton A20 into rabbits resulted in marked and sustained elevations of the cholesterol, phospholipid, and total lipid content of their blood, a number of investigators have demonstrated that lipid synthesis, notably cholesterol, increased significantly in the livers of Triton WR-1339 injected animals. This occurred both *in vivo* (2) and *in vitro* in which either perfused livers (3), liver slices (4) or liver homogenates (5) were used. However, in the latter studies the addition of the detergent (1–50 mg/ml) *in vitro* had no effect on lipid synthesis in liver homogenates. This suggests that the stimulatory action of Triton-WR-1339 on lipid synthesis occurs only when the detergent is administered into the animal. Paoletti and Fumagalli (4) reported an increase in the incorporation of [^{14}C] acetate into cholesterol, but not fatty acids of brain slices obtained from Triton-WR-1339 injected immature rats. In the present studies we investigated the effect of intraperitoneal administration of Triton WR-1339 on phospholipid synthesis and lactate formation in developing rat brain slices.

Materials and Methods. Sprague-Dawley rats, 14–20 days old were injected intraperitoneally with Triton WR-1339 (*p*-isooctyl polyoxy ethylenephenol polymer) in saline (500 mg/kg body wt.) and sacrificed at various time intervals. Controls were given saline. Brains were immediately removed, the cerebra dissected, cleaned separately in Krebs-Ringer bicarbonate buffer, pH 7.4 containing 11 m*M*-glucose, and any adhering material was removed. Slicing was performed by using a McIlwain chopper (Brinkman Instruments, Des Plaines, Ill.) at a setting of 0.35 mm. The pooled slices from each rat (about 0.5 g) were placed in 125 ml stoppered conical flask containing 7.5 ml of the same buffer. [$^{32}P_i$] Orthophosphate (50 μCi), [^{14}C]glycerol (2 μCi) or [^{14}C]choline (2μCi) was then added as indicated and following incubation at 37° for 1 hr in a shaking incubator the slices were washed twice with ice-cold Krebs–Ringer bicarbonate buffer, homogenized in chloroform–methanol (2:1) and their lipids extracted according to the procedure of Folch *et al.* (6). The individual phospholipids were isolated

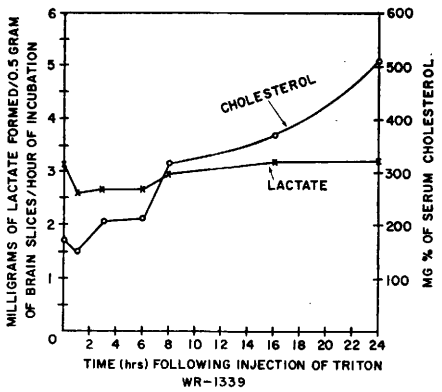

FIG. 1. Effect of intraperitoneal administration of Triton WR-1339 into immature rats at various time intervals on the serum cholesterol level and lactic acid formed upon incubation of control and Tritonized brain slices in Krebs–Ringer buffer. Conditions for incubation and methods for determination of lactic acid and cholesterol are described under "Methods."

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TABLE I. Incorporation of $^{32}P_i$ into Lecithin of Brain Slices of Control and Triton WR-1339 Injected Rats.

Time after Triton injection (hrs)	No. of experiments	Specific radioactivity of lecithin (cpm/μmole P_i)		
		Control	Triton injected	Stimulation as % of control
8	6	12,317 ± 4,789 (S.D.)	47,464[a] ± 33,118 (S.D.)	385
16	17	11,493 ± 6,217 (S.D.)	22,321[a] ± 19,143 (S.D.)	194

[a] Significantly different from control values at $P < 0.05$ level. In these experiments the rats were Tritonized, then sacrificed after 8 or 16 hrs, their brains removed, the slices prepared and incubated as described under "Methods". Lecithin was isolated from the lipid extracts by means of 2 dimensional TLC and its specific radioactivity determined as described previously (7–9).

from the extracts by means of 2 dimensional TLC, and their specific radioactivities determined as previously described (7–9). Serum cholesterol was analyzed by the method of Momose *et al.* (10). The supernatants, obtained after centrifugation of reaction mixtures, were used directly for lactic acid determinations. To determine the latter, the Huckabee modification (11) of the Barker and Summerson method (12) was used, with minor modifications to improve reproducibility for lactate determination as previously described (13).

Triton WR-1339 was purchased from Ruger Chem. Co., Irvington, N.J. All radioisotopes were obtained from New England Nuclear, Boston, Mass.

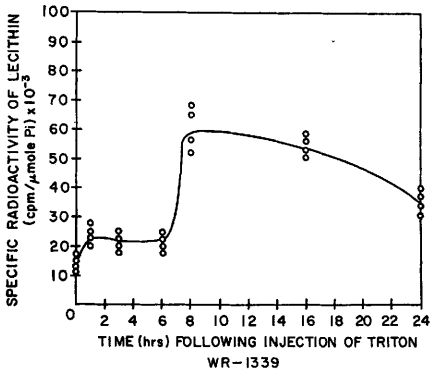

FIG. 2. Effect of intraperitoneal administration of Triton WR-1339 into immature rats at various time intervals on $^{32}P_i$ incorporation into lecithin of control and Tritonized rat brain slices. Conditions for incubation and methods for isolation and determination of the specific radioactivity of lecithin are described under "Methods." The values at each time interval correspond to four observations.

Results. While the serum cholesterol level increased by 88% and 200% in 8 hr and 24 hr respectively following the time of administration of Triton, lactate formed from the brain slices during the same intervals was almost constant (Fig. 1). The results of a number of experiments on the effect of Tritonization on $^{32}P_i$ incorporation into lecithin are shown in Table I. Although the specific radioactivities of lecithin varied considerably from one experiment to another, which resulted in the large standard deviations, we observed significant stimulation ($P < 0.05$) of lecithin synthesis in the brain slices of the Triton WR-1339 injected rats. Furthermore the deviations within the same experiment were within experimental error as can be seen from Fig. 2. In the latter experiment the level of Triton stimulation increased slowly with time until it reached its maximum after 8 hr from the time of administering the detergent, then declined gradually from 378% of that of the control at this time interval to 222% at 24 hr (Fig. 2).

The intraperitoneal administration of Triton also increased markedly the incorporation of [^{14}C]choline and [^{14}C]glycerol, in addition to $^{32}P_i$, into lecithin (Table II). However, the addition of Triton WR-1339 (0.05–1 mg/ml), to brain slices incubated in Krebs–Ringer and $^{32}P_i$ had no effect on the labelling of lecithin. This suggests to us that the Triton effect occurs only *in vivo*, and it is in accord with the observations of Scarselli and Fossati (14) who

TABLE II. Incorporation of $^{32}P_i$, [^{14}C] Choline and [^{14}C] Glycerol into Lecithin of Brain Slices of Control and Triton WR-1339 Injected Rats.

Precursor	Specific radioactivity of lecithin (cpm/μmole P_i)		
	Control	Triton Injected	Stimulation as % of control
$^{32}P_i$	7,310	11,857	162
[^{14}C] Choline	28,356	37,106	131
[^{14}C] Glycerol	3,090	6,276	203

[a] In the above experiment the conditions for incubation, extraction and separation of the individual lipids were the same as described under Table I. The amounts (in μCi) of $^{32}P_i$, [^{14}C] choline and [^{14}C] glycerol added to each flask were 50, 2 and 2 respectively. Rats were sacrificed 16 hr following the time of the Triton WR-1339 administration. The above experiment was run in duplicate and the values are averages of 2 different experiments. The deviation from the mean averaged about 10%.

observed that this detergent has no effect on cholesterol synthesis in liver homogenates.

Discussion. It is well established from the work of several investigators that lipid synthesis in liver increases significantly following the administration of Triton WR-1339. In the present studies we have shown that this hyperlipemic agent, when administered intraperitoneally, also stimulates phospholipid synthesis in the central nervous system at least *in vitro*. Thus when various percursors, including $^{32}P_i$, [^{14}C]choline or [^{14}C]glycerol were added to brain slices from control and Tritonized rats the rate of their incorporation into the lecithin of the latter ranged from 131%–385% of the control (Tables I and II). The fact that the stimulatory action of the detergent on lecithin synthesis in brain slices is dependent on the time of Tritonization (Fig. 2) could be due to formation of the precursor macromolecules, or loss of feedback inhibition, in the liver and their transport through the blood-brain barrier into the brain.

Although Triton WR-1339 was shown to be a hyperlipemic agent several years ago (1) the molecular mechanism underlying this action is still not clear at the present time. In this respect the following mechanisms have been put forward: (a) Molecular alterations in the circulating lipoproteins (15, 16) which in turn might be responsible for inhibiting the lipoprotein lipase (17, 18). (b) A decrease in the removal of lipoproteins from the blood or an increase in lipid synthesis in the liver (2, 16, 19, 20). (c) Mobilization of the body cholesterol pool (20). An increase in serum cholesterol concentration was observed by several investigators including the present work (Fig. 1). Paoletti *et al.* (4) demonstrated an increase in the rate of cholesterol but not fatty acid synthesis from [^{14}C] acetate in brain slices of Triton WR-1339 injected immature rats. Whether the effect of Triton WR-1339 on brain phospholipids observed in our work is primary or secondary cannot be deduced from the present studies, however the data presented in this report demonstrate, to our knowledge hitherto unreported, that the intraperitoneal administration of this hyperlipemic agent stimulates phospholipid synthesis in brain.

Summary. Young rats (14–20 days old) were injected intraperitoneally with Triton WR-1339 (500 mg/kg body wt.) and at various time intervals they were sacrificed, brain slices prepared then incubated in Krebs–Ringer buffer containing either $^{32}P_i$, [^{14}C] choline or [^{14}C]glyceral. The specific radioactivities of lecithin in brain slices from the Triton injected rats were found to be 131%–385% as high as those of the controls. Maximal $^{32}P_i$-incorporation into lecithin occurred 8 hr following the administration of the detergent. Formation of lactate was the same in both of the control and Tritonized slices, and no effect of Triton WR-1339 on $^{32}P_i$-incorporation into lecithin of brain slices was observed *in vitro*. The level of serum cholesterol in-

creased by more than 200% in the 24 hr Tritonized rats. It was concluded that the brain, in addition to the liver, is an organ which responds to Triton administration.

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