utes later. It has been reported that the convulsive and lethal effects of strychnine in mice are prevented by injection of Factor I(11).

In view of the above results it is apparent that there is present in brain more than one substance having some of the activities ascribed to Factor I.

Summary. Extracts of fresh mammalian brain contained substances which inhibited the "slow" closer and opener systems of the crayfish claw. Glutamic acid was one of the substances. Aspartic acid and γ-aminobutyric acid were also active. Glutamic acid had an inhibitory effect and aspartic acid had a stimulatory effect on the crayfish heart.

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Production of Hypercholesteremia in the Rabbit by Infusion of Phosphatide or Neutral Fat.* (22975)

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Recently we reported(1,2) that a rapid rise occurred in plasma cholesterol concentration of the rat, if a *sustained* rise in either its plasma phospholipid or triglyceride was induced initially by intravenous infusion of phosphatide or triglyceride. The mechanism responsible for the hypercholesteremic effect of a sustained hyperphospholipidemia or hypertriglyceridemia has not been determined but it has been observed(3) that a hypercholesteremia occurring after infusion of phosphatide takes place, surprisingly enough, in complete absence of the liver.

To determine whether this hypercholesteremic effect of experimentally induced and maintained hyperphospholipidemia or hypertriglyceridemia was applicable to other species, studies were made upon the rabbit.

Methods. Healthy male rabbits weighing approximately 1500-1800 g and 6 to 8 weeks of age were used. A sustained hyperphospholipidemia was induced in six rabbits for 12 and in 5 rabbits for 24 hours by rapid intravenous administration of 20 ml of 5% phosphatides,† w/v, in 5% dextrose solution

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† The phosphatide mixture was furnished by Chemurgy Division of Glidden Co., labeled Soya Lecithin, oil free, "RG" brand. Approximate composition as furnished by Glidden Co. as follows: Chemical lecithin, 29.5%; chemical cephalin, 29.5%; inositol phosphatides, 31.6%; sugar, sterol glucosides, etc., 5.3%; soybean oil, 3.1%; ether and insolubles and moisture, 1%. $CaCO_3$ is added in manufacture. Sterol content of this substance as measured by Liebermann-Burchard color reaction was 1.1%. Actual amount injected as 5% solution in our experiments therefore contained a negligible quantity of sterol (i.e. $<$ .6 mg/ml). Before use phosphatides were freed from $CaCO_3$.

TABLE I. Effect of Infusion of Phosphatide upon Plasma Cholesterol and Phospholipid.

	Rabbits infused with phosphatide 12 hr	24 hr	Control rabbits*
No.	6	5	5
Avg wt (g)	1760 (1400–1881)	1682 (1400–1845)	1500 (1340–1720)
Avg total amt phosphatide given (g)	3.9 (1.0–5.8)	2.6 (2.2–2.8)	—
Avg plasma cholesterol (mg/100 ml)			
Before inj.	45 (27– 63) ± 4.4†	69 (48– 96) ± 5.8	45 (33– 59) ± 4.8
12 hr after	189 (110–294) ± 26.4	—	54 (48– 59) ± 4.2
24 " "	—	153 (113–236) ± 19.2	—
Avg plasma phospholipid (mg/100 ml)			
Before inj.	119 (97–134) ± 5.8	129 (80–160) ± 6.4	110 (95–125) ± 6.3
12 hr after	361 (158–496) ± 51.0	—	135 (115–159) ± 7.1
24 " "	—	230 (171–394) ± 37.0	—

Fig. in parentheses give range of values.
* Infused 12 hr with dextrose (5%) solution. † S.E. of mean.

followed by continuous intravenous infusion of the same fluid at a rate of 4 ml/hour in rabbits infused for 12 hours and 1.5 to 2.0 ml/hour in rabbits infused for 24 hours. A sustained hypertriglyceridemia was induced in 8 rabbits by rapid intravenous injection of 20 ml of apricot oil (10%) emulsion‡ followed by continuous intravenous infusion of the same emulsion at 2 ml/hour for 10 hours. For control purposes 1) 5 rabbits were injected and infused with comparable volumes of dextrose solution alone; 2) 3 rabbits were injected and infused with dextrose (5%) and Tween (0.5%); 3) 3 rabbits were bled immediately after the initial injection of 20 ml of phosphatide and 4) 4 rabbits were bled immediately after the injection of 20 ml of the fat emulsion. Plasma samples were obtained before and at the end of the infusions. Those obtained from the rabbits infused with phosphatide were analyzed for cholesterol(4) and phospholipid(5). Total lipid analyses according to the method of Bragdon(6) were also done upon the plasma samples of the rabbits infused with fat. Neutral fat was calculated by difference, merely by subtracting total cholesterol and total phospholipid from total lipid.

Results. 1. *Effect of sustained hyperphospholipidemia upon plasma cholesterol.* The immediate effect of the priming injection of 20 ml of phosphatide solution in the 3 control rabbits was an elevation of their average plasma phospholipid from 110 to 691 mg/100 ml as expected. No significant change was observed however in the plasma cholesterol concentration following this single injection. After 12 hours of continuous infusion of the same phosphatide solution into 6 rabbits, an increase of approximately 320% occurred in plasma cholesterol concentration. (Table I). Although the neutral fat content of plasma of these rabbits was not obtained, it was observed that no plasma sample obtained at end of infusion was lipemic. This suggests of course that no significant rise in neutral fat occurred(7). After 24 hours of continuous infusion, average plasma cholesterol of the 5 infused rabbits had increased approximately 120%. (Table I). The lesser rise in these rabbits as compared to that in the above rabbits was to be expected in view of a similar lesser elevation of the plasma phospholipid. Plasma samples obtained at end of infusion also were observed to be clear.

Injection and infusion of control rabbits receiving dextrose solution caused a slight rise in plasma phospholipid and no significant change in the plasma cholesterol level.

2. *Effect of sustained hypertriglyceridemia upon plasma cholesterol and phospholipid.* Immediately following the priming injection

‡ The apricot oil suspension was obtained from Abbott Laboratories. It consisted of 10% suspension of apricot oil containing 0.5% Tween and 5% dextrose.

TABLE II. Effect of Infusion of Emulsified Fat upon Plasma Cholesterol, Phospholipid and Triglyceride.

	Rabbits infused with fat emulsion	Control rabbits infused with Tween (0.5%) and dextrose (5%) solution
No.	8	3
Avg wt (g)	1793 (1496–2100)	1782 (1603–1904)
Avg total amt fat given (g)	5.2 (4.7–5.6)	—
Avg plasma cholesterol (100 mg/100 ml)		
Before inj.	62 (41– 96) ± 3.5*	43 (34– 50) ± 3.2
10 hr after	127 (82– 168) ± 13.8	49 (34– 56) ± 2.4
Avg plasma phospholipid (100 mg/100 ml)		
Before inj.	120 (82– 168) ± 13.8	112 (104–120) ± 12.4
10 hr after	199 (119– 341) ± 24.5	113 (104–122) ± 11.2
Avg total lipid (100 mg/100 ml)		
Before inj.	308 (173– 576) ± 38.4	272 (250–296) ± 36.8
10 hr after	1197 (552–2890) ±400	265 (245–300) ± 34.4
Avg plasma triglyceride (mg/100 ml)		
Before inj.	100 (7– 162) ± 35	117 (94–142) ± 38
10 hr after	842 (316–2332) ±250	111 (79–139) ± 32

* S.E. of mean. Fig. in parentheses give range of values.

of 20 ml of apricot oil into the 4 control rabbits, no change was observed in either plasma cholesterol or phospholipid concentration. Average plasma triglyceride concentration however rose from 80 to 513 mg/100 ml. After 10 hours of continuous infusion of the same fat emulsion into 8 rabbits, an increase of 105% occurred in the average plasma cholesterol concentration (Table II) and an increase of 66% in the phospholipid concentration. The 3 control rabbits injected and infused with the emulsifying medium alone exhibited no significant change in any plasma medium.

Discussion. The results obtained demonstrate that when the rabbit is injected and infused with either phosphatide or triglyceride in sufficient quantity to maintain a *continuous* state of hyperphospholipidemia or hypertriglyceridemia respectively, a hypercholesteremia quickly results. The rabbit thus resembles the rat(1) in the ease with which hypercholesteremia can be induced by this means.

The mechanism responsible for the hypercholesteremia induced by *sustained* infusion of either phosphatide or triglyceride remains to be elucidated. It appears probable however that the infusions do not lead to hypercholesteremia by interference with hepatic R-E cells by their possible colloidal contents. This is believed because hypercholesteremia does not occur with interference of R-E cell activity by particle administration unless large amounts of exogenously derived cholesterol are given at the same time(8). Then too, in the case of phosphatide injection at least, hypercholesteremia led to an excess of cholesterol deposited in the liver(3),—a phenomenon incompatible with the concept of possible severe interference with the function of the hepatic R-E cell.

Summary. Continuous elevation of plasma phospholipid in the rabbit by continuous infusion of a suitable phosphatide quickly leads to hypercholesteremia. A similar phenomenon was observed following elevation of plasma triglycerides by infusion of a fat emulsion. These results are in agreement with those observed in rats infused with similar substances.

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Role of Vitamin B_{12} in Nucleic Acid Metabolism. V. Liver Deoxyriboaldolase Activity.* (22976)

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Vit. B_{12} deficiency has been associated with decreased nucleic acid content in rat liver (1,2), and microorganisms(3,4). This decreased nucleic acid content may be related to defective carbohydrate metabolism in view of reported reduction in ability of the deficient animal to utilize carbohydrate(5). Liver contains an enzyme system for synthesis of ribose and deoxyribose by condensation of C_2 and C_3 compounds(6,7). Lowered rate of formation of C_5 sugars, and hence of nucleic acids in liver, of vit. B_{12} deficient animals could, therefore, be due to lowered substrate concentrations of these C_2 and C_3 compounds, and/or to decreased activity of condensing enzymes to form the C_5 sugars. Accordingly, deoxyriboaldolase (DR-aldolase), the enzyme catalyzing the reaction: Glyceraldehyde-3-phosphate + acetaldehyde = Deoxyriboside-5-phosphate, was chosen for study.

Materials and methods. Vit. B_{12} deficient rats of Sprague-Dawley strain were raised as previously described(2). Livers were removed from exsanguinated animals with or without previous ether anesthesia, and 1 g portions were immediately homogenized in 10 ml 0.2 M Tris buffer (pH 7.4) in Potter-Elvehjem glass homogenizer. The 10% homogenate was centrifuged at room temperature for 10 minutes, and the centrifugate used as enzyme source. Livers from deficient and supplemented paired littermates were used for each enzyme experiment. In initial experiments, each incubation tube contained: 0.2 ml 0.1 M Fructose-1, 6-diphosphate, 0.2 ml 0.06 M Sodium-iodoacetate, 0.1 ml 10% (v/v) Acetaldehyde, Enzyme preparation (0.2-1.4 ml), 0.2 M Tris buffer, pH 7.4, total volume of mixture = 2 ml. Fructose 1,6-diphosphate solutions were prepared fresh from Barium salt (Mann) by adding equivalent amounts of Na_2SO_4 and removing resulting $BaSO_4$ precipitate by centrifugation. After 30 minutes incubation at 37°C, with tubes stoppered, 2 ml 10% trichloracetic acid was added. The mixture was rapidly stirred, centrifuged, and aliquots of supernatant assayed for deoxyribose by the colorimetric method of Dische: 2 ml acetic acid-H_2SO_4 reagent containing 1% diphenylamine was added to 1 ml sample. Color was developed by heating mixture for 10 minutes at 100°C. Optical densities at 610 mu and 650 mu were determined in Beckman DU spectrophotometer, and the Δ O. D. ($O.D._{610} - O.D._{650}$) compared to that of a standard DNA preparation. The findings are shown in Table I. The following modifications of above conditions for measuring enzyme activity were made in later experiments. An increased amount of sodium-iodoacetate (0.3 ml of solution not more than 6 weeks old) was used, and the solution of 10% acetaldehyde was made in 0.008M Tris buffer, pH 7.4. Better stoichiometry in the colorimetric assay was observed when 0.1 ml 10% acetaldehyde was added to every 30 ml acetic acid-H_2SO_4-diphenylamine reagent.

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