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Action of Choline on Lipid Phosphorylation in the Kidney, Heart and Aorta.* (26657)

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Earlier studies with the aid of radioactive phosphorus showed that administration of a single dose of choline stimulated the turnover of liver phospholipids in animals maintained on low protein-high fat diet(1) or low protein-low fat diet(2). These low protein diets produced a choline deficiency involving the liver, kidney(3) and aorta(4,5). The hemorrhagic kidney lesion was observed only in choline deficient weanling rats(3) and a single dose of choline stimulated P^{32} uptake in this tissue(6). In the present investigation an attempt has been made to study the action of single dose of choline on formation of phospholipids in the heart, aorta and kidney of rats maintained on high fat-low protein diets and in acute choline deficiency by supplementation of the diet with 1% guanidoacetic acid, methyl group acceptor.

Methods. Male albino rats of Sprague-Dawley strain, weighing 100-150 g were divided into 2 series. The animals in Series I were maintained on a low protein-high fat (7) diet containing vitamin-free casein 5 parts, dextrin 28, sucrose 29, crisco 30, cod liver oil 2, salt mixture 4, Ruffex 2 plus the following vitamin mixture in mg per kilo of ration; dextrin 9639, thiamin 8, riboflavin 8, pyridoxine 8, nicotinic acid 241, calcium pantothenate 32, inositol 32, paraaminoben-

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zoic acid 32. This purified diet containing 5% vitamin free casein (Nutritional Biochemicals Corp.) would represent approximately 0.16 g of methionine and 0.015 μg of Vit. B_{12}. In Series II rats were maintained on similar diet supplemented with 1% guanidoacetic acid for 2 weeks. Animals were fed *ad libitum*. In each series, 4 to 6 rats were housed in individual cages, with raised screen bottoms.

At the end of the dietary regime, the rats were stomach-tubed with a single dose of choline (100 mg in 1 ml of water, control animals received water). One minute later all animals were injected intraperitoneally with 1 ml of physiological saline containing 100 μc as $NaH_2P^{32}O_4$. Six hours later the animals were killed by decapitation and kidney, heart and aorta were removed and acid-soluble phosphorus and lipid phosphorus fractionated. The acid-soluble phosphorus was removed by extracting with 10% trichloroacetic acid (TCA) containing 0.4 M $MgCl_2$(8) and radioactivity(9) and phosphorus(10) were determined. The TCA residue was covered with alcohol, and the lipids extracted with ethanol in Soxhlet extractor for 8 hours, purified with chloroform (9). On aliquots of the chloroform solution radioactivity(9) and phosphorus were determined(10). Radioactivity was determined with an ultra-thin window, gas flow geiger tube (Tracerlab TGC-14). From these results and the radioactivity measurements, the relative specific activities of the phospholipids were calculated. The relative specific activity is the ratio of the specific activities of the phospholipid P to that of the acid soluble P. The specific activity is defined as counts per minute per aliquot (% of dose injected)/mg of phosphorus per aliquot.

A control group of rats were injected with P^{32} and sacrificed at 3, 6, and 10 hours later to determine the time uptake of the isotope by the liver, heart, kidney and aorta. The acid-soluble and phospholipid P were extracted and radioactivity(9) and phosphorus (10) were determined on each fraction.

Results. The time course of incorporation of $NaH_2P^{32}O_4$ into liver, kidney, heart and aorta phospholipids is shown in Table I. It

TABLE I. Time Course of Incorporation of $NaH_2P^{32}O_4$ into Liver, Kidney, Heart and Aorta Phospholipids.

Time after adm. P^{32}, hr	No. of rats	Liver		Kidney		Heart		Aorta	
		Spec. activity	Rel. spec. activity	Spec. activity	Rel. spec. activity	Spec. activity	Rel. spec. activity	Spec. activity	Rel. spec. activity
3	3	11.85 ± 1.9	6.22 ± .9	10.07 ± 2.4	10.7 ± .3	2.19 ± .1	2.84 ± .08	4.2 ± .9	6.58 ± .8
6	4	32.80 ± 1.2	19.00 ± 1.3	17.30 ± 1.4	20.5 ± .9	3.28 ± .1	3.55 ± .08	4.73 ± .3	9.30 ± .7
10	4	25.6 ± 1.0	24.20 ± 2.3	16.40 ± .7	24.6 ±2.2	4.20 ± .1	6.42 ± .59	5.33 ± .8	14.60 ±2.5

Figures preceded by ± sign indicate stand. dev.

is apparent that 6 hour time interval after administration of $NaH_2P^{32}O_4$ corresponds to the ascending part of the specific activity time curve where synthesis of phospholipid in the liver, kidney, heart and aorta occurs to a degree greater than their breakdown. The relative specific activity(11) expresses the relationship between isotopic concentration in a compound and in its precursor(12). Under the conditions of our experiments an increase in formation of phospholipids may be reasonably assumed when higher values for relative specific activities are found. Various factors, such as difference in the role of absorption and in the equilibrium between intra- and extra-cellular inorganic phosphorus with different specific activities may conceivably produce changes in the specific activity of the inorganic fraction. Comparison of the relative specific activity should provide a means of reducing their cause of error. Marinetti *et al.*(13) have demonstrated that the specific activity of the individual phospholipids of liver, heart and kidney at the 6 hour interval corresponds to the ascending part of specific activity time curve for lecithin, phosphatidylethanolamine, sphingomyelin and phosphatidylserine.

Animals were fed a 32% fat-5% casein diet to investigate the effects of a single dose of choline on lipid phosphorylation in the heart, aorta and kidney. It has previously been shown that phospholipid turnover is greater following a single dose of choline in rats maintained on 32% fat-5% casein diet than 5% fat-5% casein diet(14). The stimulating effect of lipid phosphorylation by a single dose of choline is somewhat related to the previous diet, for it does not occur in experimental animals maintained on stock diet(2). Apparently phospholipid turnover in the liver of animals maintained on a stock diet of 25% casein-5% fat diet is at a maximum value and administration of additional lipotropic agent will not further stimulate lipid phosphorylation(15).

The effect of a single dose of choline on lipid phosphorylation in the kidney, heart and aorta is shown in Table II. To evaluate the statistical significance of the results,

TABLE II. Relative Specific Activity of Tissue Phospholipids Following Single Dose of Choline.

Material admin.	No. of rats	Kidney	Heart	Aorta
Water	12	13.7 ± 5.1	5.1 ±1.0	10.9 ±1.3
Choline	15	41.8 ±12.6*	5.9 ±1.3	14.4 ±4.1†
+ 1% guanidoacetic acid in diet				
Water	7	20.9 ± 2.3	4.5 ± .6	5.6 ±1.4
Choline	8	38.5 ±10.9*	6.4 ±1.2*	8.3 ±2.0†

Test of significance was applied to difference between means of control and experimental values. Probability for chance occurrence of this difference was: * <0.01; † <0.02.

the *t* test of significance(16) was applied to the difference between mean for control receiving water and experimental values. It is apparent that administration of a single dose of choline stimulates lipid phosphorylation in the heart and kidney.

This increase in lipid phosphorylation in these tissues is probably a direct reflection of phospholipid synthesis and not uptake of plasma phospholipids. Liver is the principal if not the sole tissue concerned not only with synthesis of plasma phospholipids but also with their removal(17). When radioactive phospholipids are injected into a hepatectomized animal very little is taken up by other tissues(18). The phospholipids synthesized in the kidney are not available to the plasma in a hepatectomized animal.

This stimulation of phospholipid synthesis by a single dose of choline becomes much more exaggerated with a statistical significant increase when a choline deficiency was produced by administration in the diet of a methyl group acceptor, guanidoacetic acid. The kidneys of the animals fed the guanidoacetic acid for 2 weeks developed a hemorrhagic appearance which is seen only in dietary choline deficiency of weanling rats (3). It has been demonstrated that a single dose of choline stimulates lipid phosphorylation in the liver of rats fed a high fat-low protein(1,6) and low fat-low protein(2) and in cellular fractions of the liver, mitochondria and nuclei of rats fed a low protein-low fat diet(19). The results of our present experiments on the kidney, heart and aorta give further evidence that choline deficiency

effects the phospholipids in these tissues.

Summary. 1. The lipid phosphorylation affects of a single dose of choline were compared in rats maintained on low protein-high fat diet or similar diet supplemented with methyl acceptor, guanidoacetic acid. 2. Administration of a single dose of choline stimulated lipid phosphorylation in the kidney, heart and aorta.

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Release of a Histamine-Destroying Factor During Anaphylactic Shock in Guinea Pigs.* (26658)

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Rose and Leger(1) reported that a substance capable of destroying histamine was released in rabbits during anaphylactic shock. Code and associates(2) demonstrated such a substance, probably histaminase, in the blood of white rats during anaphylactic shock. The present study was done to determine whether a similar histamine-destroying factor is released during anaphylaxis in guinea pigs.

Methods and materials. A group of 22 guinea pigs (700 to 1100 g) of both sexes of our mongrel laboratory strain was sensitized by 4 subcutaneous injections of crystalline egg albumin given at intervals of 24 to 48 hours. The animals were used 3 weeks to 3 months after these sensitizing injections.

During the studies of anaphylaxis, the animals were anesthetized by intraperitoneal administration of pentobarbital sodium in a dose of 3 mg per 100 g of body weight. A catheter of No. 60 polyvinyl tubing was inserted into the right atrium *via* the right jugular vein for withdrawal of samples of blood. After a control sample had been taken, 2 mg of crystalline egg albumin in 0.3 ml of an isotonic solution of sodium chloride was given *via* the catheter. Three to 5 minutes later, a specimen of blood was drawn while the animal was in shock. All samples of blood were drawn in a syringe that had

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