Phosphorus Metabolism in the Dog Kidney. (20699)

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The experimental methods used to study metabolic processes of individual organs are of three general types: 1) use of tissue slices and homogenates *in vitro*; 2) perfusions of isolated organs *in vitro* or *in vivo*; and 3) tissue fractionations and analyses after the injection of metabolites or labelled materials into the intact animal. Although these methods have yielded valuable information, they have some serious shortcomings. Thus, the condition of tissue slices and homogenates is far from physiological and, in many cases, the amount of various metabolites which must be added in order to obtain a recognizable metabolic reaction is far greater than that existing *in vivo*. Furthermore, in most experiments of this type, no provisions are made for the removal of the products of metabolic reactions and the system works without the intervention of the diffusion and osmotic phenomena which play an important role in the kinetics of an intact biological system. The liquids used in perfusion experiments, in most cases, are not physiological since, on the one hand, they do not contain necessary metabolites and hormones while, on the other hand, a given metabolite may be added in amounts far in excess of the normal, bringing into play concentration effects and thus exaggerating reactions which normally may be negligible. Tracer experiments also have important limitations consisting, as they generally do, of the injection of a labelled material, sacrifice of the animal after a given period of time, and isolation of the tracer element. This method is adequate to study the overall metabolism or the metabolism of one organ within the framework of the general economy of the body, but may not be sufficient if one wishes to examine the individual contribution of a single organ to the metabolism of the organism as a whole.

In a normal animal, all organs are in a steady state not only within themselves, but also in respect to each other and when the metabolism of one organ becomes abnormal, other organs may be affected by the changes in the amounts of critical materials which are caused by the primary disturbance. The secondary phenomena may, indeed, overshadow the primary ones and tracer technics may not distinguish between the two. The interdependence of the endocrine glands offers many pertinent examples which are of practical as well as of theoretical interest. For these reasons, a more physiological method was sought.

Experimental. The method consists of injecting a tracer material into the intact artery of an organ while the blood emerging from the organ is collected in a beaker by means of a venous cannula. This technic prevents the blood leaving the organ under investigation from returning to it after having circulated elsewhere. It is, in effect, a "single pass" experiment which combines the advantages of a sensitive tracer technic with those of leaving an organ *in situ,* perfused by its own normal blood supply, and yet avoiding all contact of the tracer element with other organs of the body. In view of the fact that the blood vessels of the kidney are readily accessible, this organ was chosen for our studies. Healthy, medium-sized, mongrel dogs of both sexes were fasted for eighteen hours and anesthetized by means of an intraperitoneal injection of Na pentobarbital (35 mg/kg). One kidney was exposed and completely isolated except for the main renal vessels. After the dissection was completed, the dog was heparinized (2 mg/kg I.V.),† the renal vein cannulated and the blood allowed to flow into a beaker for about 1 minute. Fifty to 100 microcuries of P^{32} as sodium phosphate (10-20 μg of phosphorus)‡ dissolved in about 10 ml of physiological saline

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† Heparin was donated by Wilson Laboratories, Chicago, Ill.

‡ P^{32} was supplied by Abbott Laboratories on allocation from U. S. Atomic Energy Commission.

TABLE I. Concentration of P in TCA Soluble Fractions of Dog Kidney. (mg P/100 g wet tissue.)*

Dog No.	1		2		3		4		5	
Fraction	C	M	C	M	C	M	C	M	C	M
Inorganic	18.6	—	22.2	18.3	31.5	20.1	27.0	18.0	30.3	18.9
ADP-ATP	2.34	—	2.07	1.44	3.60	1.14	—	2.00	1.51	.84
G-1-P	.27	—	.94	1.44	.90	1.20	.75	.58	.70	1.26
G-6-P	—	—	—	—	1.48	2.46	2.10	2.66	.46	1.05
Coenzyme	1.14	—	—	4.6	6.75	1.83	1.65	5.66	1.08	3.78

* C = cortex, M = medulla, ADP-ATP = labile P of ADP-ATP, G-1-P and G-6-P = glucose-1 phosphate and -6 phosphate, respectively.

were injected into the main renal artery and were thus carried into the kidney by the normally flowing arterial blood. The injection lasted for about 1 minute and was followed by a 1-3 minute period during which time the P^{32} remaining in the blood vessels of the kidney was flushed out by the supervening normal arterial blood. The venous blood, containing the excess P^{32}, was collected into a beaker and thus was not allowed to recirculate. The total time during which the renal blood was allowed to flow to the outside was 3 to 5 minutes; the total blood lost was 150 to 200 ml. This allowed the blood pressure to remain well above shock levels, as determined in a number of preliminary experiments. At the end of the perfusion period, the kidney was rapidly removed, chilled by injecting ice cold saline through the venous cannula (thus washing out most of the blood remaining in the organ), and cut longitudinally. Within 1 or 2 minutes, samples of cortical and medullary tissue were frozen in powdered dry ice. The parts which could not be well separated were discarded. The frozen tissue was weighed, dropped into 50 ml of ice cold 10% trichloracetic acid (TCA) and homogenized with a Waring blender in the cold room. The temperature of the room was about 1°C and that of the homogenate was kept below 5°C by the addition of small pieces of dry ice. The suspension was centrifuged in the cold room and the solids re-extracted 3 or 4 times with 25 ml of ice cold 5% TCA. This was sufficient to extract at least 95% of the total activity. The specific activity of the residue, containing phosphoprotein and phospholipid, was negligibly small and was not examined in detail. The volume of TCA extracts was measured and duplicate 40 ml aliquots were used to fractionate the phosphorus into the following fractions: inorganic-P, coenzyme-P, glucose-1-P (G-1-P), glucose-6-P (G-6-P), and the labile phosphorus of adenosine di- and triphosphate (ADP-ATP). The fractionation was done according to the method of Sacks (1). Phosphorus was determined according to Fiske and SubbaRow(2). Radioactivity was measured as follows: when necessary, each fraction was oxidized with nitric acid to inorganic phosphate and diluted to 10.0 ml. A suitable aliquot was used to determine phosphorus quantitatively. To another aliquot 1.0 mg of inorganic phosphate was added as carrier and the phosphorus was precipitated as ammonium phosphomolybdate. The precipitate was filtered through small circles of filter paper mounted on a stainless steel suction funnel,§ washed, dried, and its radioactivity measured by means of a thin window Geiger Muller tube. The error of counting was 3% or less.

Results. Table I shows that in all cases the concentrations of inorganic-P and of ADP-ATP were greater in the cortex than in the medulla. On the other hand, the concentrations of G-1-P, G-6-P and coenzyme-P tended to be greater in the medulla than in the cortex. The notable exception is animal No. 3 in which a larger amount of the coenzyme-P was found in the cortex. We are unable to explain this apparently aberrant result, as the only detectable difference between animal No. 3 and the others was the fact that this animal required a double dose of nembutal for anesthesia. The values obtained fall within the range of those reported by Dratz and Handler (3) for the whole cat kidney.

Table II shows that the specific activity of all fractions is greater in the cortex than in

§ Tracerlab, Inc., Boston, Mass.

TABLE II. Specific Activities of TCA Soluble Fractions of Dog Kidney. (Counts/min./mg P) $\times 10^{-3}$.

Dog No.	1		2		3		4		5	
Fraction	C	M	C	M	C	M	C	M	C	M
Inorganic	70.5	—	89.9	27.5	391	213	45.6	37.0	530	240
ADP-ATP	45.2	—	61.3	15.6	131	110	—	23.2	484	183
G-1-P	45.6	—	24.4	3.4	146	20	30.4	7.9	248	96.6
G-6-P	—	—	—	—	6.6	1.66	1.44	.69	11.4	14.4
Coenzyme	22.6	—	—	2.84	47	2.73	4.85	1.39	140	15.7

TABLE III. Specific Activities of TCA Soluble Fractions of Dog Kidney Divided by Specific Activity of Inorganic P.

$$\left(\frac{\text{S. A. bound P}}{\text{S. A. inorganic P}}\right)$$

Dog No.	1		2		3		4		5	
Fraction	C	M	C	M	C	M	C	M	C	M
ADP-ATP	.64	—	.68	.57	.33	.51	—	.62	.91	.76
G-1-P	.65	—	.26	.12	.37	.09	.67	.21	.47	.40
G-6-P	—	—	—	—	.02	.01	.03	.02	.02	.06
Coenzyme	.43	—	—	.10	.12	.01	.11	.04	.26	.06

the medulla. In both tissues, the specific activities decrease in the following order: inorganic-P, ADP-ATP, G-1-P, coenzyme-P, G-6-P.

Discussion. Individual results vary from experiment to experiment. This is probably due to differences between individual experiments such as the rate of injection of the isotope, the time elapsed before freezing the tissues or the amount of blood flowing through the kidney, as affected by anesthesia, visceral manipulation and variable blood loss. The fact that the specific activities of all fractions are higher in the cortex than in the medulla indicates a greater turnover in the former than in the latter, which is in agreement with the fact that the cortex has a more active metabolism than the medulla(4,5). When the data are calculated in terms of specific activity (SA) of inorganic-P, some of the differences between cortex and medulla are not as large (Table III).

The high relative specific activity for G-1-P in the cortex indicates a very high turnover rate, in some cases as high or higher than that of ADP-ATP. G-1-P can be formed in three ways(6): 1) from glucose-6-phosphate; 2) directly from glucose through a reaction similar to that described for galactose; and 3) from glycogen and inorganic-P via the phosphorylase reaction. The first two possibilities do not appear likely because the specific activity of G-1-P is much higher than that of the would-be precursors, G-6-P and ADP-ATP. G-1-P must, therefore, be formed predominantly via the third reaction.

When a steady state exists, the rate of formation of a substance must be equal to its rate of disappearance. Three possible manners of G-1-P disappearance have been suggested (7): 1) conversion to glycogen through a reversal of the phosphorylase reaction; 2) conversion to G-6-P; or 3) conversion to glucose through a reversal of the glucose-phosphokinase reaction. The first alternative is possible despite the low glycogen content of the kidney (8). The second possibility appears unlikely because the difference between the activity of G-1-P and that of G-6-P is too great. Sacks (9) has shown that also in the liver G-1-P is not a likely precursor of G-6-P. The third possibility has not been investigated as yet.

The high concentration and low specific activity of the G-6-P in renal tissue seems to indicate that its synthesis in the kidney is insignificant and that a large part of it is synthesized elsewhere and carried by the arterial blood to the kidney to be stored and used there. This seems possible since the blood cells contain 50 to 100 mg of acid soluble phosphorus per 100 ml(10), 2.72 mg of which appears to be G-6-P(11), both values being

similar to those found in kidney tissue. In view of the high overall metabolic activity of kidney tissue, the low S.A. of G-6-P would indicate that, in the kidney, this compound may not occupy a central position in the metabolism of carbohydrate.

Conclusions. 1. A "single pass" technic is described for studying the intrinsic metabolism of the kidney. 2. It is shown that P uptake by ADP-ATP and G-1-P is very rapid, more so in the cortex than in the medulla. G-1-P probably is derived from glycogen via the phosphorylase reaction. 3. The very low relative S.A. of the G-6-P seems to indicate that most, if not all, of the G-6-P found in the kidney is formed elsewhere in the body and carried to this organ by the blood.

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Antagonism of Chemotherapeutic Activity of p-Aminosalicylic Acid in Experimental Tuberculosis by p-Aminobenzoic Acid.* (20700)

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In vitro antagonism of the tuberculostatic properties of para-aminosalicylic acid (PAS) by para-aminobenzoic acid (PABA) has been established(1-3). The *in vivo* relationship of PAS to PABA has not been reported. The purpose of this communication is to present the results of an investigation of the effect of dietary PABA on the chemotherapeutic activity of PAS in experimental tuberculosis.

Methods. The H37Rv strain of *M. tuberculosis* was utilized as the infectious agent in this study. Male CF_1 mice weighing 14 to 16 g each were employed as test animals. The basal ration represented a modification of a synthetic ration devised by Pearce *et al.*(4). Its composition was as follows: vitamin-free casein, 20%; Wesson's salt mixture(5), 4%; Ruffex, 10%; corn starch, 55.68%; sucrose, 5%; lard, 5%; and supplement, 0.313%. The following components of the supplement are expressed in terms of mg per 100 g of basal ration: pyridoxine hydrochloride, 1.0; nicotinic acid, 1.0; thiamine chloride, 0.6; riboflavin, 0.6; calcium pantothenate, 2.0; folic acid, 0.05; biotin, 0.05; PABA, 30; inositol, 50; choline chloride, 100; cystine, 100; alpha tocopherol, 3.5; and menadione, 2.5. Concentrations of 1,500 I.U. of vit. A and 200 I.U. of vit. D were added per 100 g basal ration. Adjustment of the concentrations of PABA and PAS in the rations was accomplished by the addition or omission of equal weights of starch. Five rations were studied in Exp. A and were as follows: 1) basal ration; 2) basal ration, PABA-free;‡ 3) basal ration containing 2% PABA; 4) basal ration containing

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‡ The term, "PABA-free" refers only to omission of PABA from the supplement.