damage by intravenously injected immune complex requires further study.

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Flavokinase Activity of Rat Tissues and Masking Effect of Phosphatases.* (26754)

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Riboflavin-5'-phosphate or flavin mononucleotide (FMN) is an essential coenzyme form of the vitamin, riboflavin, and occurs widespread in plant and animal organisms (1). However, scanty information is available on the ability of animal tissues to synthesize FMN from riboflavin. It was suggested that intestinal mucosa is the site of this conversion within the mammal(2) by either a transphosphorylation catalyzed by non-specific alkaline phosphomonoesterase(3) or a phosphorylation requiring adenosine-5'-triphosphate (ATP) and the specific flavokinase(4). With the recent partial purification and characterization of flavokinase from rat liver(5), the ability of a mammalian tissue other than intestine to form the coenzyme *in situ* has been demonstrated. In this work, the recognition of interfering phosphatases which hydrolyze FMN, thereby decreasing formation of FMN with flavokinase *in vitro*, has led to the current assessment of these enzymes in animal tissues. This report demonstrates that several rat tissues possess flavokinase, and the level of apparent activity is markedly suppressed by phosphatases which are maximally active at pH 5.

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Methods. Female, 200 g Sprague-Dawley rats were sacrificed by rapid decapitation and exsanguination. Tissues were excised, rinsed and homogenized in 4 volumes of cold, 0.05 M potassium phosphate buffer, pH 7. Homogenates were centrifuged in the cold at 18.500 $\times$ g for 30 min to obtain the supernatant solutions. Protein was determined by the method of Lowry *et al.*(6).

Mixtures for determining flavokinase activity contained: 10^{-4} M riboflavin, 10^{-3} M ATP, 10^{-4} M Zn^{++}, 0.075 M potassium phosphate buffer, pH 8, and 1 mg of protein in 5 ml total volume. Trichloroacetic acid was added to stop the reaction before and after incubation was carried out in the dark at 37° C for 1 hr. The product, FMN, was determined by Kearny's adaptation(7) of the differential extraction method of Burch *et al.* (8). In addition, the product remaining after removal of excess riboflavin with benzyl alcohol was applied to Whatman No. 1 paper and the spots detected under U.V. light following development in ascending *n*-butyl alcohol/acetic acid/water (4/1/5, upper phase). Rf values agreed with those reported

TABLE I. Distribution of Flavokinase and the Masking Effect Due to Phosphatases.

Tissue supernatant	Δ mμmoles FMN* Apparent formation: Extraction(8)	Chromatography(9)	Maximum hydrolysis†
Liver	14	++++	88
Kidney	9	++++	90
Brain	4	+++	76
Spleen	2	++	74
Heart	1	+	51
Intestine (small)	<1	—	40

* Values represent avg of 3 to 5 animals with S.D. <25%.

† 10^{-4}M FMN replaced riboflavin and ATP under conditions used for kinase assays with 10^{-4}M Zn^{++} and 0.075 M potassium phosphate buffer, pH 8.

for FMN(9), and no other flavin products could be detected.

In determining phosphatase activity under conditions optimal for flavokinase, incubation mixtures contained 10^{-4} M FMN in place of riboflavin and ATP. Under optimal conditions for the phosphatase, 0.5 mg of protein in 10^{-4} M FMN and 0.075 M potassium acetate buffer, pH 5, was incubated for 30 min. Hydrolysis of FMN was measured by the differential extraction procedure (8) and by liberation of inorganic phosphate using the method of Fiske and Subbarow (10).

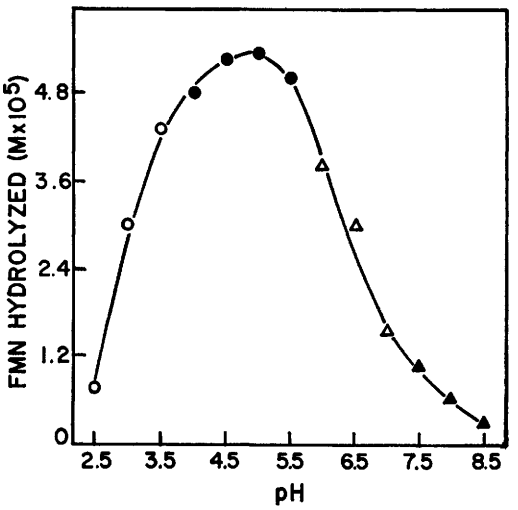

FIG. 1. pH optimum for hydrolysis of FMN by phosphatase from rat liver. 5 ml incubation mixtures contained: 10^{-4}M FMN, 0.5 mg protein, and 0.075 M buffers of glycine-HCl (○), potassium acetate (●), tris-maleate (△), or tris-HCl (▲).

Results. The distribution of flavokinase within rat tissues and the significant masking effect of phosphatases under conditions used to assay the kinase are shown in Table I. Liver appears to contain relatively high kinase activity which is present in decreasing amount in extracts from kidney, brain, spleen, heart, and intestine, respectively. All of the measurable kinase activities may be increased several fold when account is taken of the extensive hydrolysis of FMN which is possible under these assay conditions.

The optimum for hydrolysis of FMN under conditions where rate is approximately zero-order (excess substrate) is near pH 5, as shown in Fig. 1 for the liver phosphatase.

TABLE II. Hydrolysis of FMN by Phosphatases at pH 5.

Tissue supernatant*	mμmoles FMN hydrolyzed†
Kidney	345
Liver	252
Spleen	232
Brain	210
Heart	152
Intestine (small)	135

* Preparations containing 0.5 mg protein were those used in Table I.

† The same relative order of activities was found using whole homogenates.

p-Nitrophenylphosphate behaves similarly as substrate with the same pH optimum and approximately the same rate; however, FMN is much more readily hydrolyzed than adenosine-5′-monophosphate > α- or β-glycerophosphate > fructose-6-phosphate. Other tissues examined (Table II) possess the FMN phosphatase activity in varying amounts with kidney extracts showing the greatest specific activity.

Discussion. The occurrence of flavokinase in several rat tissues, *e.g.*, liver, kidney, brain, spleen, and heart, in addition to its previously reported presence in intestine(4), would support the belief that this enzyme may play an important role in maintaining adequate flavin coenzyme levels in this mammal(5). Intestinal synthesis of FMN is probably not the sole means for supply to the other organs. Moreover, the presence of masking phosphatases may account for

the low activities of kinase measured in extracts from animal tissues. Hydrolysis of FMN by spleen homogenates has been noted previously(11). The physiological significance of the pH 5 phosphatase may be to maintain a check on the intracellular level of FMN. This phosphatase is relatively specific as little activity is found with several organic monophosphates tested as substrates. More extensive examinations of the partially purified enzyme are being made.

Summary. Flavokinase is present in extracts from several rat tissues with activity in liver > kidney > brain > spleen > heart. Phosphatases, maximally active at pH 5, partially mask the kinase activity in these extracts. It is concluded that synthesis of FMN occurs in several organs rather than intestine alone.

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Distribution of Blood Flow to the Canine Heart.* (26755)

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Total blood flow to an organ can be measured by several methods. The distribution of blood or regional flow within an organ is more difficult to quantitate. In the present investigation radioactive microspheres were used to measure regional distribution of blood in the myocardium of the dog and to estimate the size and volume flow through intercoronary anastomoses and arteriovenous shunts.

Methods. The method used is a modification of the technic of Prinzmetal *et al.*(1) developed by Grim and Lindseth(2). In these experiments glass microspheres, 20 μ in diameter, rendered radioactive by the conversion of some of the Na^{23} content of glass to Na^{24} by neutron bombardment, were injected into the coronary arteries of the dog. With the reasonable assumption that the spheres are distributed at arterial bifurcations in the same proportion as is the blood, the fraction of the injected spheres which passed through arteriovenous anastomoses could be quantitated by collecting all venous drainage and counting it in a scintillation counter. Likewise, since those spheres that remained behind in any particular tissue presumably did so because they lodged in vessels smaller than the diameter of the spheres, the "sphere count" recovered from this tissue would provide a measure of flow through vessels smaller than the spheres. If the spheres were small enough to pass through all arteriovenous shunts except capillaries, the radioactivity of the tissue would be a measure of tissue capillary flow. For the purposes of the present study tissue blood flow refers to flow through vessels 20 μ or smaller. This includes all true capillaries (less than 12 μ) and capillary-like vessels(16-20 μ).

The fraction of spheres which entered the myocardium supplied by the anterior de-

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