might further impair this metabolic imbalance and thereby lead to death of tumor or host.

The protective action of glucogenic corticosteroids against tumor necrosis and lethal toxicity(5) may be related to similar metabolic mechanisms. In the present study, cortisol, given in dosage and time relationship adequate to protect adrenalectomized rats from large doses of Klebsiella polysaccharide, did not protect normal rats against the lethal-enhancing action of K, suggesting that K and glucocorticoids may act through separate mechanisms in producing their opposing effects upon the toxicity of bacterial polysaccharide.

Summary. Potassium injected intravenously increased the mild delayed lethal toxicity of Klebsiella polysaccharide in normal rats. In rats bearing relatively small lymphosarcomas the delayed lethal and tumor-necrotizing actions of the polysaccharide were markedly increased by minute quantities of potassium.

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Effect of Protamine on Activity of Hog Pancreatic Amylase.* (27266)

ROBERT L. MCGEACHIN AND WILLIAM C. PAVORD
Department of Biochemistry, University of Louisville School of Medicine, Louisville, Ky.

Krebs(1) and Madsen and Cori(2) have reported that the protamine, salmine, strongly inhibited rabbit muscle phosphorylase at low concentrations of salmine. At higher concentrations of protamine, there was partial reactivation of the phosphorylase probably by solubilization of the protamine-phosphorylase complex. In studying the ability of various proteins to reverse the beryllium inhibition of certain mammalian amylases(3), we have found that not only does protamine fail to reverse or prevent beryllium inhibition but also that protamine alone may be inhibitory. Observations of the effect of protamine at various concentrations and of the modifying effect of heparin are reported here.

Materials and methods. Crystalline hog pancreatic amylase used in these experiments was obtained from the Worthington Biochemical Corp.(4), heparin (U.S.P. grade) from Hynson, Westcott and Dunning, Inc.

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TABLE I. Effect of Protamine Sulfate on Activity of Hog Pancreatic Amylase.

Protamine added to 10 ml reaction mixture, mg	% Change from original activity			
	Reaction time in min.			
	0	10	30	60
.1	+18	+23	+20	+ 9
.3	+24	+22	+ 4	— 1
.4	+23	— 3	—19	—33
.5	+10	—19	—45	—55
.7	— 1	—47	—72	—80
.8	—66	—77	—79	—76
1.0	—82	—79	—77	—85

and protamine, in the form of protamine sulfate, from the Nutritional Biochemicals Corp.

Amylase activities were determined by the amyloclastic method of Van Loon *et al.*(5). The amylase contents of the final reaction mixtures (10 ml) were adjusted so that they contained a standard amount, 0.20-0.25 Van Loon units (0.10-0.12 μg) of hog pancreatic amylase. The reaction mixture was 0.01 N in KCl and contained 0.02 M pH 7.0 Tris-acetate buffer in place of the 0.02 M pH 7.0 phosphate buffer recommended in Van Loon's original procedure.

In the experiments testing the effect of protamine alone on amylase, varying amounts of protamine (Table I) were added to solutions of amylase containing 0.20-0.25 amylase units per ml. At zero, 10, 30 and 60 minutes following this addition, one ml aliquots were removed and their amylase activities determined. In other experiments (Table II), to a solution of protamine containing 2 mg/ml, was first added an equal amount (by weight) of heparin and the mixture agitated to insure solution of the heparin. One volume of this mixture was added to an equal volume of amylase solution containing 0.4-0.5 unit per ml. One ml aliquots for amylase analysis were removed at zero, 10, 30 and 60 minutes. To test the ability of heparin to reverse the protamine inhibition once it was established, in other experiments the heparin was added 30 minutes after mixing protamine with amylase.

TABLE II. Effect of Heparin on Protamine Inhibition of Amylase.

Heparin added to protamine	% Change from original activity			
	Reaction time in min.			
	0	10	30	60
Prior to addition of protamine to amylase	+11	+ 2	+ 5	+ 5
30 min. after addition of protamine to amylase	—29	—76	—82	—96
Control (only heparin added)	+19	+14	+10	+10

Results and discussion. The effect of protamine on amylase activity varied both with its concentration and time. At the lowest concentrations used it actually increased the activity of hog pancreatic amylase, an effect similar to that noted with plasma proteins(6). At intermediate concentrations, there was an initial increase in activity, followed by inhibition. At concentrations above .05 mg/ml of reaction mixture, only inhibition was noted, increasing to a maximum of 75-80% at 60 minutes. No reactivation of the type obtained with phosphorylase(1,2) was found with amylase at higher concentrations of protamine.

When protamine was allowed to react with heparin before being added to the amylase, the protamine inhibition was prevented (Table II). This perhaps is partially due to precipitation of the protamine by heparin. Where the same quantity of heparin was added 30 minutes *after* adding protamine to the amylase, however, the inhibition was not reversed. In fact, at 60 minutes the inhibition was 96%, even greater than with the protamine alone. In control experiments with heparin alone added to the amylase, enzyme activity was enhanced about 10%.

Hog pancreatic amylase contains large amounts of glutamic and aspartic acids(7) and would therefore have many free carboxyl groups in its side chains. It is postulated that the inhibitory effect of protamine, a polycation at pH 7, may be partially explained on the basis of its combination with carboxylate side chains of the amylase which at pH 7 would be a poly-anion since its isoelectric pH is 5.2-5.6(8). Krebs(1, personal communication) believes that the effects of protamine on phosphorylase are likewise "due to combination with negatively charged side-chains on the enzyme." However, one must remember that lower concentrations of protamine pro-

duce an increase of amylase activity and intermediate concentrations cause first an increase in activity with time and then a decrease. This indicates that the process is more complicated than just that of the ionic binding of protamine to amylase with a masking of the active site or sites. Caldwell and her co-workers(9) have shown that the free amino groups of hog pancreatic amylase are essential to its activity but no reference in the literature to the role of carboxyl groups can be found.

The observation that heparin added to protamine before amylase prevents the inhibition by protamine, but will not reverse the inhibition if added after protamine and amylase have been allowed to react, could perhaps be explained in several ways. The most likely explanation is that some irreversible change takes place in the amylase in the presence of, or after combining with protamine.

Summary. Protamine, which at low concentrations caused slight enhancement of the activity of hog pancreatic amylase, was inhibiting at higher concentrations. This inhibition could be prevented by heparin but heparin would not reverse the inhibition once established. Heparin alone produced some enhancement of amylase activity. It is postulated that part of the explanation for the inhibiting effect of protamine is the combination with negatively charged side chains on the amylase followed by some irreversible change in the amylase.

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Fat Digestion and Absorption in the Proximal Intestine of the Dog.* (27267)

LEON K. KNOEBEL (Introduced by E. E. Selkurt)
Department of Physiology, Indiana University School of Medicine, Indianapolis

Most investigations which deal with the digestive processes of the gastrointestinal tract utilize animals in acute experiments in which a test meal is fed, the animal is killed after a certain time, and the gastrointestinal contents are analyzed for the products of digestion under consideration. This approach simply supplies information regarding digestion at one particular time following feeding. Furthermore, a quantitative study of absorption cannot be made unless unabsorbed material from the entire gastrointestinal tract and feces is recovered. Another approach to the problem of digestion and absorption is the use in chronic experiments of animals with fistulas established in various regions of the intestine. Intestinal contents can be quantitatively collected from a fistula during the entire passage of a test meal through the intestine and analyses of the material so collected give data regarding the overall result of the activity of the digestive enzymes. In addition, absorption can be quantitatively evaluated, since the material recovered from a fistula represents that which was not absorbed to the point of collection. Jejunal fistula dogs were used in this study to investigate the proximal intestinal digestion and absorption of a common dietary triglyceride, cottonseed oil. Data of general interest concerning how the gut functions as chyme passes through the intestinal tract were also obtained.

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Methods. Maydl jejunostomies(1) were prepared in 2 dogs by transecting the jejunum