ered analogous to the gamma globulin fraction of mammals.

Additional work is required to assess the full phylogenetic significance of these results. It will be of interest to ascertain whether a similar electrophoretic distribution of antibodies is found in the serum of other lower vertebrates and in animals immunized to antigens other than those used here.

Summary. Adult specimens of *B. marinus* maintained at 25°C were immunized with a mixture of *S. typhosa* H antigen and rabbit gamma globulin (RGG). Each animal produced precipitins for the RGG and agglutinins for typhoid H after a short period of immunization. When the antiserums were separated electrophoretically, the agglutinins were found in the slowest-moving of the 4 major serum components. On the basis of electrophoretic mobility, this fraction is analogous to the gamma globulin of mammals.

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Endogenous Cathepsin Activation of Trypsinogen in Extracts of Dog Pancreas.* (26539)

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The pancreas and pancreatic juice normally contain negligible amounts of active trypsin (1). Trypsin, in the form of its zymogen trypsinogen, is secreted into the intestine where the activation of trypsinogen to trypsin is catalyzed by the enzyme enterokinase. Once formed, trypsin may also catalyze the formation of trypsin from trypsinogen. The mechanism by which the extracellular enzymes enterokinase and trypsin activate trypsinogen has been established as the hydrolytic cleavage of a hexapeptide from the amino terminal portion of the trypsinogen molecule(2,3). The only intracellular mammalian enzyme that has been demonstrated to activate trypsinogen is the partially purified enzyme cathepsin B(4,5). Our previous *in vitro* studies have shown that beef spleen cathepsin B purified 200 fold, catalyzes the activation of crystalline trypsinogen to trypsin (or a trypsin-like enzyme) as determined by the action of the active product against synthetic peptide substrates and its inhibition by trypsin-soy bean inhibitor(4,5). These studies demonstrated that optimum pH for this activation occurred at pH 3.6, a pH at which no trypsin activation of trypsinogen occurs. It was further demonstrated that addition of .001 M iodoacetic acid (a known inhibitor of cathepsin B) or omission of cysteine (a known activator of cathepsin B) inhibited the reaction.

In view of the intracellular location of cathepsin B(6), it was of interest to deter-

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mine whether there are present in the pancreas cathepsin B or enzymes similar in nature which can activate the endogenous trypsinogen. In the present study, extracts and concentrated extracts of dog pancreas were prepared which were demonstrated to contain materials which activate the endogenous trypsinogen in these extracts to trypsin (or a trypsin-like enzyme) under conditions similar to those in which cathepsin B activates trypsinogen. Activation of trypsinogen was enhanced by the presence of cysteine and inhibited by iodoacetic acid. These findings may be of interest in relation to diseases such as pancreatitis where the possibility of abnormal trypsin formation in the pancreas may occur, although this point has not been definitely established(7).

Methods. Extracts of dog pancreas were prepared as follows: All steps were carried out at 2°. 20-40 g of dog pancreas were homogenized in a Waring blender for 2 minutes with 2 to 4 volumes of 2% NaCl and .001 M versene in 0.015 N hydrochloric acid. The suspension was brought to pH 2.5 with 1 N HCl with stirring. 0.5 ml of toluene was added and the suspension placed in the refrigerator for 20 hours. The suspension was centrifuged at 16,000 rpm for 30 minutes in the cold and the supernatant fluid obtained was used in these experiments and is termed the "extract". In some experiments the supernatant fluid was saturated with ammonium sulfate (69 g ammonium sulfate/100 ml extract at 2°), the resulting suspension was centrifuged for 15′, and the precipitate suspended in a small amount of .001 M citrate solution at pH 3.6. Ammonium sulfate was removed by dialysis against citrate buffer at pH 3.6. The dialyzed solution is termed the "concentrated extract". Cathepsin B was prepared as previously described(8). Trypsinogen was the 1 × crystalline product of Worthington Biochemical Corp. purified as previously described(5). Trypsin soy bean inhibitor and pancreatic trypsin inhibitor complex were products of The Worthington Biochemical Corp. Trypsin assays were carried out by the spectrophotometric technic against benzoyl-l-arginine ethyl ester also previously described (5).

TABLE I. Increase in Trypsin Units* from Zero Time.†

Incubation mixture‡	△ 20 min.	△ 40 min.	△ 120 min.
Extract alone	—	—	36
Extract + .001 M iodoacetic (pH 3.6)	—	—	0
Extract + trypsinogen§	—	—	53
Concentrated extract	53	103	
Idem + iodoacetic	18	34	
" − cysteine	29	53	
" + soy bean inhibitor‖	10	26	

* A trypsin unit is defined as that amount of trypsin that produces an increase in .001 absorbance units/min. when assayed against benzoyl-l-arginine ethyl ester at 253 mμ under the conditions set forth previously(5). Values expressed as trypsin units/ml incubation mixture. A trypsin unit is approximately equal to .001 mg crystalline trypsin when assayed against benzoyl-l-arginine ethyl ester(5).

† All extracts had zero time activity, attributed to the fact that extraction procedures were carried out at pH 3.6. Zero time activity was subtracted from final activity to give values in the table.

‡ Incubation mixture consisted of 0.6 ml "extract" or 0.25 ml "concentrated extract" in .04 M acetate, .04 M cysteine, .08 M $CaCl_2$ to a total of 1 ml pH 3.6 at 25°.

§ 4.0 mg of crystalline trypsinogen in 1.0 ml of incubation mixture.

‖ 3× the weight (in mg) of trypsin present in incubation mixture at 40′.

Results and discussion. As can be seen from a typical experiment in Table I, when pancreatic extracts are allowed to incubate at pH 3.6 for 2 hours there is an increase in endogenous trypsin activity with time. In the presence of iodoacetic acid no increase in activity was observed. The inhibition of trypsin formation by iodoacetic acid demonstrates that activation of endogenous trypsinogen is mediated by materials sensitive to iodoacetic acid and not by any trypsin present in these extracts since the latter enzyme is not affected by iodoacetic acid. When crystalline trypsinogen is added to these extracts, a further increase in trypsin activity occurs with time over that of the extract alone. This is further proof of the presence of trypsinogen activating material in these extracts. Control

experiments with crystalline trypsinogen alone under these conditions demonstrated no increase in trypsin activity. Incubation mixtures of crystalline trypsin and trypsinogen under these conditions also produced no increase in trypsin over that of the trypsin added. Control experiments with pancreatic trypsin inhibitor complex(9) demonstrated that this complex does not dissociate under the conditions of the experiments.

To reduce incubation times, concentrated extracts were prepared as described above. Measurable quantities of trypsin were formed by these extracts in 40 minutes. When iodoacetic acid is added to these extracts or when cysteine is omitted there is again a reduction in trypsin formation. Addition of crystalline trypsin soy bean inhibitor to these extracts reduced the trypsin activity formed, demonstrating that trypsin activity is actually being measured in these experiments. Addition of cathepsin B to extracts similar to those shown produced an increase in trypsin activity over that of the extract alone. This is further proof of the presence of trypsinogen in these extracts and that these extracts contain no materials particularly inhibitory to the action of enzymes like cathepsin B towards trypsinogen.

On the basis of the cysteine and iodoacetic acid sensitivity and formation of trypsin at pH 3.6, it is reasonable to assume that activation of trypsinogen in these extracts is mediated at least in part by cathepsin-like enzymes, the same or similar to cathepsin B. The complex enzymatic activity of these extracts, however, makes necessary further investigation with large quantities of these extracts to define precisely the cathepsin activity. Nevertheless, it is of interest that materials other than trypsin are present in the pancreas which under proper conditions activate endogenous trypsinogen to trypsin. Such reactions may be important in certain diseases of the pancreas in which the structural integrity of the pancreas is destroyed so that the zymogen granules containing trypsinogen, normally protected from the action of intracellular proteases, may be brought in contact with these proteases causing activation. This would lead to proteolytic destruction of the pancreas and release of the proteases into the blood stream as is found in cases of pancreatitis(10).

Summary. Evidence is presented that endogenous trypsinogen in extracts of dog pancreas is activated under the same conditions that cathepsin B activates trypsinogen *in vitro*. The activation occurs at pH 3.6 and is enhanced by cysteine and is inhibited by iodoacetic acid. The tentative conclusion reached is that cathepsin B-like enzymes in pancreas are catalyzing the reaction. The possible clinical implications of such reactions is discussed.

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