to infect mice in order to bring about a fatal infection in guinea pigs. Assuming—and we agree that it is a large assumption—that the pathogenesis of the infection as it occurs in guinea pigs more closely resembles the human than does the mouse infection, it would necessarily follow that the mouse test cannot measure all of those factors which may be of potential importance in establishing the disease in humans. If these factors happen to be antigens, their presence or absence might not be detected or evaluated in a mouse protection test. Yet it is this type of test which is now generally used to measure the potency of cholera vaccines. Thus, in this regard, the implications of the present work are obvious.

Summary. Mouse virulence of a purine-requiring strain of *Vibrio comma* is significantly increased if purines are injected at the same time as the challenge suspension. A purine-independent mutant isolated from this strain was significantly more virulent for mice than the parent culture. A concomitant increase in ability to fatally infect guinea pigs by the oral route was not noted in the mutant strain.

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TAMe Esterase Activity of Blood Thrombokinase after Repeated Electrophoretic Fractionations.* (25519)

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In recent studies thrombokinase was prepared from bovine plasma(1) and purified further by continuous electrophoresis which separated most of the contaminating thrombin. TAMe esterase appeared in 2 peaks, corresponding respectively to thrombin and thrombokinase peaks(2). Material from the thrombokinase peak has now been subjected to repeated electrophoretic fractionations; and there has continued to be a close correspondence between esterase and kinase activities.

Methods and materials. Continuous paper electrophoresis was performed in a refrigerated Spinco Model CP cell. Buffer: veronal, pH 8.6; ionic strength 0.02. Current, 50 ma. Protein was estimated by the method of Lowry *et al.*(3), with crystallized bovine albumin as standard; and values are subject to limitations noted by them. Thrombokinase was assayed by its capacity to activate prothrombin in presence of cephalin, calcium and bovine barium carbonate serum(1). Activity was expressed in terms of a working standard which has been stored at −23°C and used at intervals for 6 years. A value of 10 indicated that the fraction showed 10 times as much activity/ml as did the standard kinase solution. Esterase activity on tosylarginine methyl ester (TAMe) was determined by the method of Sherry and Troll(4). Thrombin was estimated as described(1), with a dry sample of NIH thrombin as the ultimate standard. Beginning with thrombokinase obtained in a yield of 1.2 mg/liter of plasma, a series of

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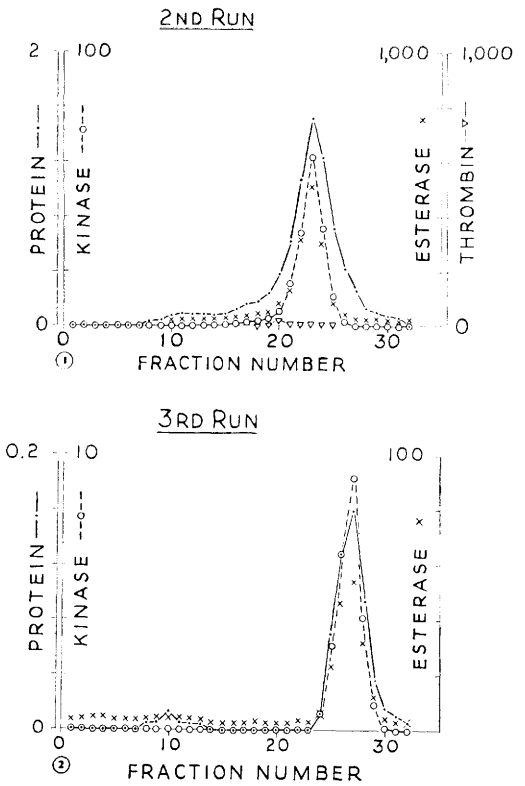

FIG. 1. Continuous flow paper electrophoresis of thrombokinase prepared from bovine plasma. 2nd run. Protein, mg/ml. Kinase, activity/ml, relative to working standard. Esterase, TAMe units/ml. Thrombin, NIH units/ml.

FIG. 2. Continuous flow paper electrophoresis of thrombokinase. 3rd run. Units of protein, kinase and esterase same as for Fig. 1. Input and fractions were less concentrated than those of 2nd run; and scale has been adjusted accordingly.

first electrophoretic runs was made, essentially as described(2). The best thrombokinase fractions were stored at –23°C until the product from 738 liters of plasma had been collected. When these were thawed and pooled, the volume was 455 ml. After addition of 455 ml cold distilled water and 228 ml 0.1 M acetic acid, the pH was 4.8. The resulting precipitate was collected by centrifugation at 4°C and dissolved in cold veronal buffer to make 11.4 ml. This solution was then subjected to the 2nd electrophoretic run.

Results. Thrombokinase appeared in a single peak (Fig. 1) and closely accompanied by esterase activity. The small amount of thrombin reached its highest level at fraction 20, and was quite inadequate to account for the esterase. Protein was spread out more broadly than kinase, and this revealed the presence of a significant amount of protein impurity.

For the 5 best fractions of Fig. 1, average specific activity for esterase was 246 TAMe units/mg protein; average ratio of kinase activity to protein was 28.2. Average ratio of esterase to kinase was 8.7.

However, the best fraction, No. 23, had 339 TAMe units/mg protein, and a kinase to protein ratio of 40.4. Fraction 23 was then subjected to isoelectric precipitation. The precipitate was dissolved in buffer to make 8.0 ml, used as the input for the 3rd run.

The 3rd run (Fig. 2) showed improvement, but a small amount of protein impurity was still evident in Fractions 8-13 and 30-32. No thrombin was detected by usual tests. Fraction 27 was assayed for kinase at protein concentration of 0.0016 μg/ml and a dilution of 1/100,000. This dilution was approximately equivalent to diluting fraction 27 to the volume of parent plasma, 738 liters.

For the 5 best fractions of Fig. 2, average specific activity for esterase was 337 TAMe units/mg protein; average ratio of kinase to protein was 44.7. Average ratio of esterase to kinase was 7.9, the same as before, within limits of error.

Capacity of purified thrombokinase to activate prothrombin in presence of 0.01 M oxalate was verified for the 3 best fractions of the 3rd run.

Discussion. These results add further weight to the hypothesis that thrombokinase is a trypsin-like enzyme. TAMe is a good substrate for trypsin, and it now appears to be a substrate for thrombokinase. Sherry *et al.*(5) anticipated that TAMe might be a substrate for the natural activator of prothrombin, and concluded that more purified concentrates would be necessary to test the hypothesis. Arscott *et al.*(6) found that thromboplastin generating systems prepared with plasma and serum components exhibited TAMe esterase characteristics. The present thrombokinase may well represent the prime, specific factor in their systems.

The defining property of thrombokinase is

its capacity to activate prothrombin. In further characterization, other properties have been described for thrombokinase prepared from plasma(7). It was not sedimented appreciably in 2 hours at 85,000 g, and thus differed from certain easily sedimentable thromboplastins. It converted prothrombin to thrombin in presence of excess oxalate, but was much more effective when accompanied by platelets (or cephalin) plus calcium. Activation of prothrombin by thrombokinase was suppressed by soy bean trypsin inhibitor.

It is now added that purified thrombokinase has shown esterase activity of the order of 337 TAMe units/mg protein. This is more than half that reported for highly purified thrombin and several times that reported for highly purified plasmin(4). No conclusions are drawn regarding relative purity of these preparations, since degree of TAMe esterase activity characteristically varies from one enzyme to another.

These properties of thrombokinase may facilitate comparison with other preparations of clotting factors, purified by electrophoresis (8,9).

Summary. Thrombokinase, purified from 738 liters of bovine plasma, was subjected to repeated fractionations by continuous flow paper electrophoresis. This increased the specific activity of kinase and led to a 3rd electrophoretic run which placed almost all the protein in a single peak. Kinase activity was closely accompanied by TAMe esterase activity through successive stages of electrophoretic fractionation. The kinase fractions of the 3rd run showed esterase activity of the order of 337 TAMe units/mg protein. The results are in accord with the view that TAMe is a substrate for thrombokinase and that thrombokinase is a trypsin-like enzyme.

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Effect of Glucose and Insulin on Magnesium Metabolism in Rabbits. A Study with Mg^{28}.*† (25520)

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The factors regulating metabolism of magnesium are not well understood, although previous studies have suggested that it may be related to the metabolism of carbohydrate and insulin(1,2). Tissue distribution of radioactive magnesium (Mg^{28}) following its intravenous administration into normal rabbits has been described(3). Our purpose was to determine whether administration of glucose and insulin would alter the exchange of Mg^{28} in rabbit tissues.

Material and methods. Normal domestic adult rabbits of both sexes, with initial body weights between 2 and 4 kg were kept in individual stainless steel metabolism cages and fed a stock diet of compressed pellets. Water was given without restriction. Commercial zinc *insulin* crystals (USP), 40 units/ml, and a sterile 5% solution of *dextrose* in distilled water were used. Mg^{28} was received as $MgCl_2$

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