a slight enhancement of the rate of endogenous respiration. Once the endogenous rate of respiration of these cells (which was quite high compared with that for *E. coli*) began to decrease with time, the addition of an exogenous substrate suppressed the rate of endogenous respiration. The results of the various studies lead to the conclusion that addition of an exogenous substrate commonly results in a suppression of endogenous respiration but that the response does vary with the organism and with test conditions.

Summary. Pseudomonas aeruginosa, Escherichia coli or *Serratia marcescens* were uniformly labeled during growth on glucose in a buffered basal salt solution. The endogenous $C^{14}O_2$ production of these organisms in dilute suspensions of washed cells was suppressed by glucose under either growth or nongrowth conditions, to a somewhat greater extent under the former conditions. Increasing the concentration of bacteria tended to decrease the extent to which endogenous respiration was suppressed in the presence of glucose. These results suggest that the amount of substrate (or O_2) available per cell is an important factor in controlling the extent of endogenous respiration in the presence of an exogenous substrate.

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Received March 21, 1967. P.S.E.B.M., 1967, v125.

Activation of Trypsinogen by Plasmin.* (32199)

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The activation of pancreatic trypsinogen by trypsin is known to involve the hydrolysis of the peptide bond between lysine and isoleucine(1). Plasmin, the fibrinolytic enzyme of blood, is known to hydrolyze esters of the amino acids lysine and arginine as well as fibrin, fibrinogen, casein and other naturally occurring proteins(2,3,4). The experiments to be described demonstrate that plasmin can activate trypsinogen.

Materials. Trypsinogen and trypsin were obtained from the Worthington Biochemical Corp., Freehold, N. J. Trypsinogen was a once-crystallized powder that was 90% protein. According to the supplier, its intrinsic trypsin activity was 0.09% of that reached when it was activated. Lyophilized trypsin was once-crystallized and salt-free.

The lyophilized plasminogen was prepared from Fraction III by the method of Kline (5) and contained 4.35 Remmert & Cohen caseinolytic units per mg of powder. Plasmin was prepared from this plasminogen by activation with minimal amounts of streptokinase and was lyophilized from a solution containing 0.1 M lysine, added to stabilize plasmin. There was 0.70 Remmert & Cohen unit per mg of powder, 10% of which was protein.

Urokinase, prepared by Abbott Laboratories, contained 2100 units per vial.

p-Tosyl-L-arginine methylester, (TAMe), was obtained from Mann Research Laboratories.

Tris (hydroxymethyl) aminomethane

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* This study was supported in part by Research Grants HE- 03906 and HE-10830 from Nat. Inst. Health and NSF GB 5263.

[†] Recipient of a USPHS 2T5-GM-37-05 Summer Fellowship, 1966. The results are part of those being used to satisfy the thesis requirement for graduation Yale University School of Medicine.

(Tris), was the product of the Sigma Chemical Corp.

Assay of esterase. 0.5 ml of 0.05 M TAMe in 0.15 M KCl and 0.38 ml of 0.105 M $CaCl_2$ in 0.15 M KCl were added to a 5-ml vessel of the Radiometer pH-stat. The solution was kept under an atmosphere of nitrogen at 23 ± 1°C and it was stirred magnetically. 0.02 ml of 0.050 M NaOH in 0.15 M KCl were then added from the 0.25-ml buret of the pH-stat. Finally, 0.1 ml of the sample to be tested was added and the solution was maintained at pH 8.00 by the addition of NaOH in KCl by the pH-stat. The rate of hydrolysis of TAMe was determined from the average amount of base added per minute during the second and third minutes after starting the reaction. Activities were expressed as micromoles per minute, with the understanding that this referred to 1 ml of initial hydrolysis mixture, *i.e.*, to 0.1 ml of sample.

Activation of trypsinogen. 0.1 ml of a $CaCl_2$ solution at 10 times the final desired concentration was added to 0.5 ml of 0.05 M Tris buffer (pH 7.7) in 0.3 M KCl at 23 ± 1°C. One minute after the addition of 0.1 ml of trypsinogen (2 mg per ml in 0.001 M HCl), 0.3 ml of the material to be tested as activator was added. Samples were assayed for esterase activity at 1 and 61 minutes. The increase in 60 minutes was a measure of activator. Sometimes further samples were assayed at 121 minutes.

Activation of plasminogen by urokinase. 0.16 ml of plasminogen (7.0 Remmert and Cohen units) was added to 0.08 ml of 0.05 M Tris (pH 7.7). This was followed by the addition of 0.76 ml of urokinase (800 units in 0.05 M Tris). The solution was incubated at 32°C. One minute and 21 minutes after the urokinase had been added, 0.3 ml samples were tested as trypsinogen activator. In the control experiments Tris was used instead of urokinase and lysine (10 mg per ml) instead of plasminogen.

Results. Activation of trypsinogen by trypsin and by plasmin in the presence of varying amounts of $CaCl_2$ is shown in Fig. 1. Controls with trypsinogen or plasmin alone revealed no activation. Plasmin had a maximum effect at 0.02 M $CaCl_2$ and there was almost complete suppression of activation by plasmin at 0.5 M $CaCl_2$.

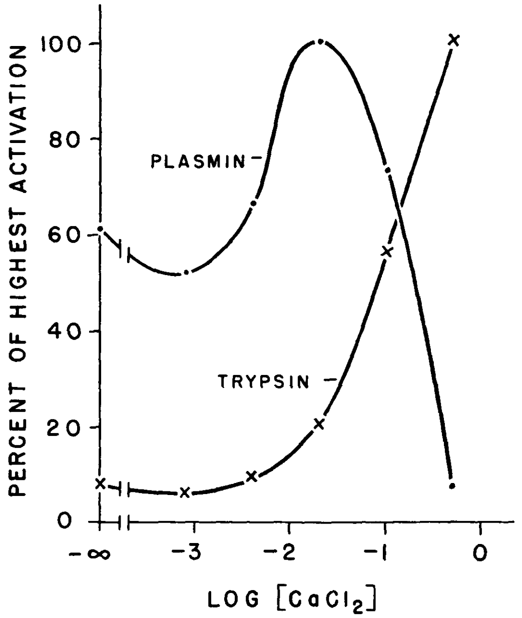

FIG. 1. Comparison of plasmin and trypsin as activators of trypsinogen in the presence of 0, 0.0008, 0.004, 0.02, 0.1 and 0.5 M added $CaCl_2$. Trypsinogen, 0.2 mg per ml. For each curve, the values are plotted as percent of the highest activity reached in 1 hour, which was 0.44 micromoles per minute with 1 mg streptokinase-activated plasmin per ml as activator, and 1.92 micromoles per minute with 0.025 mg trypsin per ml as activator.

Plasmin itself was able to hydrolyze 0.48 micromole of TAMe per minute per mg of powder. When plasmin was incubated with trypsin, instead of trypsinogen, the esterase activity at 61 minutes was no higher than the combined esterase activities measured at 1 minute; and these were small compared to the activity usually developed in the experiments with trypsinogen. The amounts of trypsin used were 2 and 4 μg per ml, which were 10 and 20 times the amount of trypsin that contaminated the usual portion of trypsinogen.

The combination of urokinase with plasminogen produced a trypsinogen activator while either protein alone had no effect (Table I). This activator was stronger at 1 minute than at 21 minutes after addition of urokinase, which suggests that activation and deterioration were rapid.

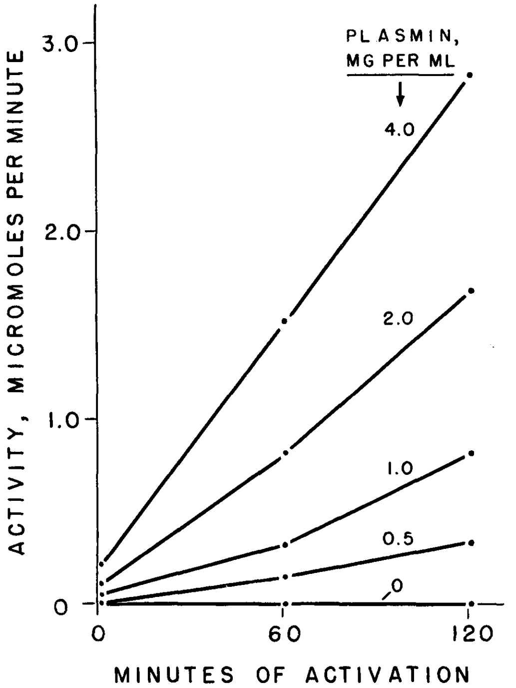

FIG. 2. Activation of trypsinogen by plasmin in the presence of 0.02 M $CaCl_2$. Each mixture contained 0.2 mg trypsinogen per ml and the amount of streptokinase-activated plasmin shown on chart. Samples were assayed at 1, 61 and 121 minutes.

The activation of trypsinogen by various amounts of streptokinase-activated plasmin is shown in Fig. 2. The average rise over the first hour was 0.44 micromoles per minute per unit of plasmin; whereas it was 0.57 over the second hour.

Discussion. The relative activities of trypsin and plasmin in the presence of various concentrations of $CaCl_2$ demonstrate that plasmin behaves differently from trypsin as an activator of trypsinogen. This evidence suggests that plasmin does not function merely as a cofactor for trypsin. The finding that plasminogen does not activate trypsinogen also favors a separate activator effect for plasmin that can be attributed to its enzymatic activity rather than to a cofactor function.

TABLE I. Urokinase-Activated Plasmin as Activator of Trypsinogen.

Material tested as trypsinogen activator	Increase in esterase over 1 hr, µmoles/min
Urokinase	.00
Plasminogen	.00
Urokinase + plasminogen at 1 min	.90
" " " at 21 min	.65

See text for technical details.

The relationship between the rate of trypsinogen activation and the concentration of plasmin is evidence that plasmin is acting as an enzyme. The data of Fig. 2, during the first hour, demonstrate the type of kinetics that would be predicted for the early stages of a first-order enzymatic reaction. More activation occurred during the second hour than the first because of the formation of trypsin and its contribution to the activation of trypsinogen. The lack of activation in the absence of plasmin supports the conclusion that most of the initial activation is due to plasmin.

The failure of an increase in TAMe esterase activity to occur upon the addition of trypsin to plasmin above the values obtained with each enzyme separately, shows that no potential TAMe esterase activity (plasminogen) was revealed to be present in the plasmin for possible activation by trypsin, a contaminant of the trypsinogen used.

The possibility that the activation of trypsinogen depends on the presence of an enzyme other than plasmin in the plasmin preparation is unlikely. The streptokinase-activated plasmin was prepared from the same preparation of plasminogen that was activated by urokinase. Since neither urokinase nor plasminogen activated trypsinogen, and since the product of the reaction of urokinase with plasminogen, plasmin, activated trypsinogen, the existence of an active contaminant seems to be ruled out.

Summary. Trypsinogen was activated by streptokinase-activated plasmin; and the optimal concentration of $CaCl_2$ was 0.02 M. By avoiding higher concentrations of calcium, and by using a dilute solution of trypsinogen which contained little trypsin, it was possible to limit interference from autocatalysis. Under these conditions, the rate of activation depended on the amount of plasmin. An incubated mixture of urokinase and plasminogen was able to activate trypsinogen,

whereas neither urokinase nor plasminogen did so alone.

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Received March 21, 1967. P.S.E.B.M., 1967, v125.

Chronic Rubella Virus Infection in the Ferret *(Mustela putorius fero)* Puppy. (32200)

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Recent advances in the knowledge of the natural history of rubella virus infections have shown that intrauterine infection of rubella virus in the first trimester of pregnancy frequently results in a series of characteristic malformations. These inclube abnormalities of the eyes and heart, deafness, and mental retardation. In addition, children born with congenital rubella have been found to remain infected beyond the newborn period. Many also have thrombocytopenia, neumonitis, hepatosplenomegaly, and radiolucent long bone lesions. It would be very helpful to have experimental animal models for the study of congenital and acquired rubella. A suitable animal study system could be of particular value for evaluation of rubella vaccines and studies of the pathogenesis of chronic rubella infection.

Schiff *et al*(1) and Cusumano *et al*(2) have reported that acute limited infection could be induced in adult ferrets. Other investigators(3,4,5) have reported similar results in experimentally infected monkeys. Oxford and Schild(5) have also shown that rubella virus could propagate in the golden hamster.

The present report is from a study initiated to determine the experimental susceptibility of ferret puppies to experimental infection with rubella virus. The results presented indicate that prolonged infection can be established by inoculation of these animals during neonatal life and that the infection persists despite significant levels of antibody to rubella virus.

Materials and methods. The RV strain (7) rubella virus grown in primary African green monkey kidney (AGMK) tissue culture was used. The virus had been passed serially 14 times in primary AGMK tissue culture cells obtained from commercial sources.* Control tissue culture fluid free of virus had the same number of serial tissue culture passages in AGMK tissue culture. The virus and control tissue culture fluids were tested and determined to be free of detectable contaminating viruses, bacteria, fungi, and mycoplasma. Rubella virus was identified by neutralization tests employing paired human sera from confirmed cases of rubella virus infection. The infectivity titer of the virus was 4.5 $TCID_{50}$/ml ($\log_{10}$).

Virus isolation. 0.2 ml of each suspension was inoculated into each of 5 primary AGMK tissue culture tubes. After incubation at 37°C for 24 hours the maintenance medium in each tube was replaced with fresh medium and the tubes were reincubated at 37°C. Eight to ten days later, 3 tubes were challenged with 100 $TCID_{50}$ of Coxsackie A-9 virus. The tubes were examined microscopically 4 days later for cytopathic effects of Coxsackie virus. Two weeks post-inocu-

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