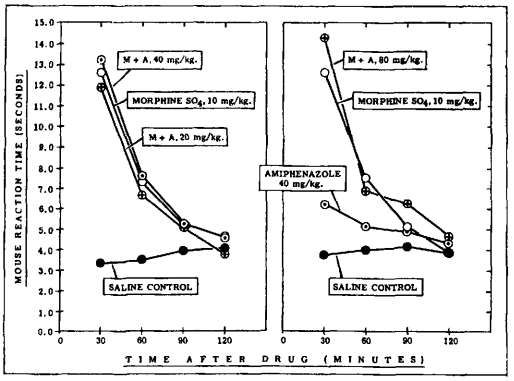

FIG. 1. Analgetic activity of morphine SO_4, amiphenazole and combinations of morphine SO_4, 10 mg/kg with various amounts of amiphenazole (M + A). All reaction times greater than 5.5 seconds were significantly greater ($P < .05$) than control. Each half of Figure represents independent experiment.

minute interval. This depression was not seen with morphine alone or with amiphenazole alone at 40 mg/kg. An unexpected and unexplained finding was that amiphenazole, 40 mg/kg, produced a significant ($P < .01$) analgetic effect itself at 30 minutes.

Summary. Amiphenazole was effective in antagonizing morphine depression in dogs; bromosulfolane was less effective even at toxic doses. Amiphenazole did not significantly alter the analgetic effect of morphine in mice, though this compound alone produced a mild but transient analgetic effect.

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Fibrinolysis II: Absence of Antibodies to Streptokinase Following Injection of Purified Human Plasmin in Rabbits.* (24609)

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Human plasmin (fibrinolysin) may be useful in control of thromboembolism. Thus it becomes important to establish its antigenic properties, since these could limit greatly its clinical use. This paper discusses the heteroantibody response in rabbits receiving purified human plasmin. Special reference is made to development of antibodies against streptokinase.

Materials and methods. (a) *Streptokinase,* a highly purified preparation, supplied by Dr. James Ruegsegger,‡ was stated to induce specific antigenic response when given intramuscularly to rabbits (Ablondi, personal communication). (b) *Preparation of human plasminogen (profibrinolysin).* Pooled citrated platelet-poor normal human plasma was stored at −20°C until used. After slow thawing in ice box, Fractions II and III of plasma were prepared by Cohn's method #6(1) (protein yield 1.8%), and Fraction III then obtained by

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Cohn's method #9(1) (protein yield 2.7%). Plasminogen was prepared from Fraction III by the method of Kline(2). The final material was dissolved in distilled water (acidified with a few drops of N HCl) in the ratio of 1 mg of weight to 1 ml of water. (c) *Activation of plasminogen* was carried out as described by Kline(2). Streptokinase was added in the ratio of 2.5 units/mg of plasminogen. The plasmin preparation contained 88 mg of protein/100 mg of dry powder (Kjeldahl's method). Its lytic activity, as determined with lysin-methyl-ester technic (Ablondi, to be published) was of 19.7 U/mg protein. The activated plasmin was lyophilized and stored at 4°C. (d) *Technic of antigenic stimulation.* Lyophilized plasmin was dissolved in 2 ml of 0.0025 M HCl and then mixed with 2 ml of alumina gel. Five mg/kg weight were given intramuscularly to each of 4 New Zealand rabbits. Animals received a total of 6 injections at 3 day interval (average total 90 mg plasmin). All animals were bled from central artery of ear 10 days after last immunizing injection. Antigenic response was evaluated by complement fixation and agar plate precipitation technics. (e) *Complement fixation test* was performed by 100% hemolysis technic (3). Reagents used were Bacto complement (2 units in Meyer's gelatin buffer); sheep hemolysin (4 units in Meyer's gelatin buffer); animal's serum at serial dilutions in Meyer's buffer; and plasmin (mother solution of 10 mg/ml diluted to 1/5, 1/50, and 1/500 in Meyer's buffer). (f) *Agar plate precipitation test* was performed as described by Ouchterlony(4), using plates of 1.5% Bacto agar in 10% merthiolate in saline. Solutions containing 5 mg/ml were prepared in Sorensen buffer at pH 7.0 of human plasmin; human plasminogen; human albumin fraction; human gamma globulin fraction; and highly purified streptokinase. Human citrated platelet-poor plasma was also used at dilution of 1/20 in Sorensen buffer. Undiluted immune rabbit serum represented source of antibodies. All reagents were used in volume of 0.2 ml.

Results. Rabbits injected with purified human plasmin developed low complement fixation titers (1/32 or less) against the specific antigen. Results, however, were difficult to evaluate since plasmin is able to destroy complement activity. When the agar plate precipitation method was used, several identity bands developed against human plasmin, plasminogen and other components of plasma (Fig. 1). No reaction was evident against streptokinase, although we confirmed in rabbits the antigenicity of streptokinase injected in Freund's adjuvant.

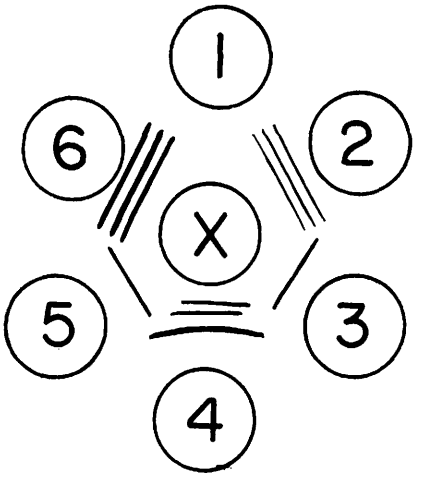

FIG. 1. Results of agar precipitation technic.*

* Central well (X): Serum of rabbit immunized against human plasmin. Peripheral wells: (1) purified streptokinase; (2) purified human plasmin; (3) human albumin; (4) purified human plasminogen; (5) human gamma globulin; (6) human plasma.

Conclusions. The results with the agar precipitation technic indicated the existence of multiple antigenic constituents of human plasmin, possibly due to contamination by other components of plasma. Our preparations of human plasmin, however, either contained no streptokinase or amounts of this agent insufficient to induce an antigenic response. Streptokinase represents a possible contaminant or constituent of plasmin. Thus, at least in our experimental condition, negative results are important as they eliminate one possible source of iso-antigenicity of human plasmin.

Summary. Agar precipitation technic detected no antibodies against purified streptokinase in rabbits immunized with streptokinase-activated, purified human plasmin.

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Effects of Surface-Active Agents on Carbohydrate Metabolism in Yeast.* (24610)

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Although inhibitory effects of surface-active agents on microbial metabolism are well known(1,2,3,4), several reports note that very small quantities of certain agents increase utilization of glucose, consumption of oxygen and production of CO_2(1,5,6,7). As far as is known, no detailed investigation of these stimulatory effects has been made.

Methods and materials. All experiments were performed on nonproliferating suspensions of commercial baker's yeast (Standard Brands) washed 4 times and aerated 4 hours prior to experiment. Gas exchange measurements were performed by standard Warburg technics. All suspensions were buffered with 0.03 M sodium succinate-tartrate adjusted to appropriate pH by addition of sodium hydroxide. With aid of commercially available glucose oxidase preparation· (Glucostat—Worthington Biochemical Corp.) glucose utilization was determined by measuring disappearance of glucose from the medium. The highest concentration of benzalkonium chloride in yeast suspensions had no significant effect on glucose determinations. Most studies were made with surfactant, benzalkonium chloride, but in some experiments cetylpyridinium chloride, dodecylammonium chloride or sodium dioctyl sulfosuccinate were used. All surfactant concentrations are recorded as mg of surfactant salt/mg yeast. All Figures and Tables represent averages of 2 experiments.

Results. Effects of benzalkonium ion on anaerobic metabolism of glucose. The effects of cationic surfactant, benzalkonium chloride, on anaerobic CO_2 production from glucose at pH 5.5 are shown in Fig. 1. At benzalkonium ion concentration below 0.003 (mg benzalkonium chloride/mg yeast) anaerobic CO_2 production was only slightly inhibited. Inhibition became more marked with higher surfactant concentrations until at 0.006 surfactant concentration anaerobic CO_2 production was inhibited approximately 70%. For comparison with later experiments it should be noted that, in the range of 0.003 to 0.004 of surfactant concentration, inhibition increased from 14 to 35%. It is concluded (Fig. 1) that benzalkonium chloride was either without effect or was inhibitory to anaerobic metabolism of glucose in the range of surfactant concentrations used.

Effects of benzalkonium ion on aerobic metabolism. Effects on metabolism of glucose. In Fig. 2 are plotted data for aerobic CO_2 production and oxygen consumption as functions of benzalkonium chloride concentration. Both CO_2 production and oxygen consumption at first rose and then declined as surfactant concentration was increased. In the range 0.003-0.004 rate of CO_2 production rose about 1 μM/mg yeast/hr to peak value approximately twice the control value. Rate of oxygen consumption in 0.003-0.004 range showed a smaller rise of about 0.24 μM/mg yeast/hr, a 35% increase over the control. If it is assumed that CO_2 produced by process involv-

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