strain ST that the synthesis of arginine dihydrolase, and consequently the utilization of arginine, is directly connected with growth. In spite of the fact that both cultures require arginine for growth, a highly active arginine dihydrolase enzyme system is not required by D10 for *maximum cell formation* to occur.

A possible explanation of the sharp drop in dihydrolase activity with ST is also evident from these data. The decrease did not begin until practically all the arginine had been removed. Tests for the presence of citrulline during the period of decreasing enzyme activity showed that sufficient citrulline (or a citrulline-like compound) was present to supply the requirement. The cells formed during this period (in the absence of arginine) did not contain arginine desimidase. Consequently, desimidase-active cells formed during the period of arginine fermentation were diluted with desimidase-inactive cells. The total desimidase content (E units) of the ST culture at the start of the phase of decline (6 hr) was the same as the quantity present at the end of the phase (12 hr). The decline in enzyme activity on high arginine media was also due to dilution of the active cells.

The opposite situation existed in strain D10. All cells produced during the culture cycle were formed in the presence of excess arginine. Accordingly, there was no opportunity for ureidase-positive, desimidase-negative cells to be formed.

The formation of arginine desimidase and citrulline ureidase in the presence of either arginine or citrulline as a substrate is in agreement with the concept of "simultaneous adaption" (6).

Summary. 1. Strains D10 and ST of *S. faecalis* were shown on a synthetic culture medium to require arginine for growth. Citrulline, the intermediate compound in the hydrolysis of arginine by these organisms, was found to replace arginine. Citrulline, however, produced only one-fourth as much growth. 2. Strain ST, in contrast to D10, possessed the ability to produce in very young cells a strong arginine dihydrolase enzyme system in the presence of small (10 μg/ml) quantities of arginine. Approximately 100 times as much arginine was required by D10 to produce a comparable enzyme activity. 3. Serial fermentation analyses of cultures of the 2 strains are presented in relation to the rate of synthesis of the arginine dihydrolase enzyme system by the cells. 4. No significant quantities of arginine desimidase were formed when citrulline was used as a substrate for growth. 5. It is concluded that the synthesis of arginine dihydrolase in strain ST is directly connected with growth. Although both ST and D10 require arginine for growth, a strong dihydrolase enzyme system is not required by D10 for maximum cell formation to occur.

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An Activator of Plasminogen in Normal Urine.* (19983)

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The presence of a potent fibrinolytic agent, similar to plasmin, in normal urine (1) has recently been confirmed (2). Further investigations have shown, however, that this agent is an activator of plasminogen and is not a fibrinolytic enzyme.

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Experiments and results. Activity of urine. A very high activity was observed when normal human urine, fresh or dialyzed against 0.9% NaCl, was tested by means of our standard fibrin plate method for the estimation of fibrinolytic activity(3). The effect obtained with undiluted urine was comparable to that produced by a solution of crystalline trypsin (Armour, bovine) containing about 15 mg per liter. However, no effect was obtained when the urine was applied to standard fibrin plates in which the adhering plasminogen had been previously destroyed by heating as described by *Lassen*(4). This result suggests that the fibrinolytic agent is an activator of the plasminogen contained in the fibrinogen solution and not an enzyme proper.

Effect of plasminogen. Plasminogen was prepared from human and bovine serum by precipitation with ammonium sulphate(5) followed by dialysis and lyophilization. Samples of plasminogen were then dissolved in the urine and the solutions were tested on heated fibrin plates. A considerable proteolysis was again produced, the magnitude of the effect depending upon the concentration of plasminogen present. A typical experiment with human urine and bovine plasminogen is presented in Fig. 1. Controls with plasminogen alone showed no effect. Confirmation of these results was found in other experiments, where casein was used as a substrate. Thus in one experiment, where 200 mg of a sample of human plasminogen was added to a mixture of 20 ml casein solution (Hammarsten; 4%; pH 7.5) with 10 ml human urine and placed at 39° (with toluene as preservative), the following results were obtained (micro-Kjeldahl):

Zero time:	.40 mg	N per	2.5 ml
After 4 hr:	1.80	" "	2.5 "
" 23 " :	4.17	" "	2.5 "

The controls (containing urine or plasminogen) were negative (<0.54 mg N after 23 hours).

The following conclusions can be drawn: Urine does not split plasminogen-free substrates (casein, heated fibrin). The addition of plasminogen produces a proteolytic activity which increases with increasing concentration of plasminogen. Urine apparently contains an activator of plasminogen.

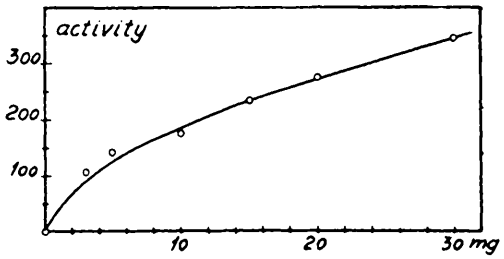

FIG. 1. Proteolytic effect of human urine in relation to concentration of bovine plasminogen. *Substrate:* Standard fibrin plates (.1% fibrinogen; pH 7.8; $\mu = .15$) heated at 85° for 30 min. *Abscissa:* mg plasminogen sample dissolved/ml urine. *Ordinate:* Activity recorded as product (in mm²) of 2 diameters of the lyzed zones after 20 hr at 37°C (avg of 3 determinations).

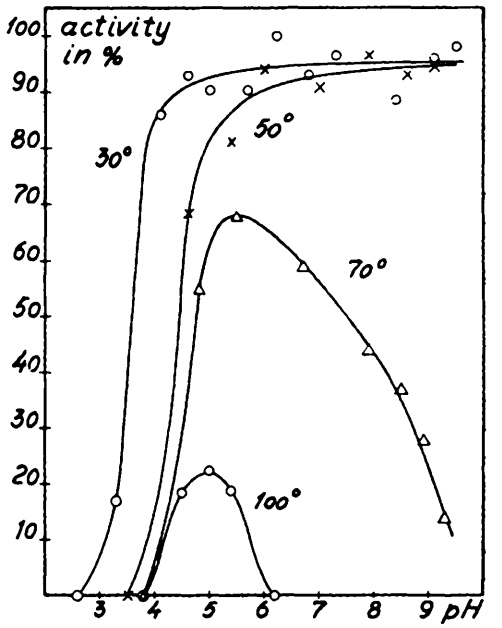

FIG. 2. Fibrinolytic activity of human urine after heating for 30 min. at different temperatures. *Abscissa:* pH of sample during heating. *Ordinate:* Activity in % of unheated control.

Stability of the urine activator. In order to characterize the urine activator we have in a number of experiments studied its heat stability. Fresh human urine was dialyzed against 0.9% NaCl and heated at different temperatures. Such an experiment is presented in Fig. 2, and it appears that the activator is rather stable at neutral and alkaline reaction but is very easily destroyed at acid reaction.

A dry preparation. Solutions of the urine activator are very labile and the activity often disappears after a few days (even at 0°C). It is therefore necessary to prepare the activator in the dried state. Considerable difficulties were encountered in accomplishing this. In most cases a partly insoluble and denatured product of low activity was obtained. This applies to the precipitation performed by dialysis against slightly acidified distilled water(1). The activator was precipitated by Zn salts at alkaline reaction but could not be recovered from the precipitate. It was not adsorbed or destroyed by $Mg(OH)_2$. Addition of alkali to pH 9 produced a precipitate in the urine, and did not influence the activity of the supernatant. Ammonium sulphate precipitation followed by dialysis against 0.9% NaCl and lyophilization gave soluble products containing about 50% of the original activity. The following procedure was adopted as the most convenient:

To fresh urine 6-N NaOH is added dropwise until pH 9.5 (phenolphthalein). After centrifugation the supernatant is neutralized (litmus), cooled in ice-water and precipitated with 3 vol. ice cold 96% ethanol. After 15 min. the precipitate is isolated by centrifugation, dissolved in a small volume of 0.9% NaCl and dialyzed overnight against 0.9% NaCl (0°C). Insoluble material is removed by centrifugation and the supernatant lyophilized. Yield is about 800 mg per liter urine, containing about 60% of the original activity.

Discussion. The presence of a plasminogen activator in urine is of considerable interest. The urine activator seems to be completely soluble, while the activator present in the tissues (fibrinokinase) appears to be bound preferably to the structural proteins(6-8). The activator present in urine is therefore a physiological plasminogen activator chemically different from the fibrinokinase present in tissues.

Several authors have observed a tryptic agent in urine. *Bendersky*(9) found that some of its properties were different from those of trypsin. Other investigators, however, were not able to find a tryptic enzyme in urine(10) and the problem was finally left unsettled. Macfarlane(1) recently observed a tryptic agent, which digested fibrinogen and fibrin as well as albumin and casein. The agent resembled plasmin though some differences were observed. The similar properties of the urine factor and plasmin were recently confirmed by our group(2). They find their explanation in the experiments here presented. The effect measured is caused by plasmin formed by an interaction between plasminogen and the urine activator during the estimation. The controversies between the older authors may be explained in the same way though it is not always possible from their descriptions to reproduce accurately the substrates used in their experiments.

It is probable that the presence in urine of a powerful activator of the fibrinolytic system has a definite physiological significance. In view of the importance of a normal excretion of urine, the formation of blood clots in the urinary passages may be just as harmful to the life of the mammalian organism as is a thrombus formation in the blood vessels. The presence of a plasminogen activator in the urine makes possible a rapid resolution of clotted blood in the urinary system. It is not probable therefore that this agent is a simple excretory product. Investigations on normal and pathological cases concerning this problem are under way.

The urine activator must be considered also in an evaluation of the urinary proteases of *Abderhalden* ("Abwehrproteinases"; "Abwehrfermente")(11), which are said to be activated by serum(12). So far no agreement exists concerning the nature—or even existence—of these substances.

In addition to the plasminogen activator we have found small and varying amounts of a trypsin inhibitor in urine. Occasionally traces of a tryptic enzyme were also observed. The potencies of these agents were insignificant in comparison with the activity of the plasminogen activator, and the active substances are probably accidental excretory products.

Summary. 1. The fibrinolytic agent present in normal urine is a plasminogen activator. 2. This activator is very sensitive to acid reaction but rather stable at neutral and alkaline reaction. 3. It is suggested that the signifi-

cance of this physiological activator of plasminogen is to keep the urinary system free from the harmful effects of clotted blood.

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Effect in Man of a New Indandione Anticoagulant.* (19984)

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Since anticoagulant drugs have become an accepted part of the physician's armamentarium there has been considerable stimulus to develop new drugs which give a better anticoagulant effect with less care and expense (1-4). The new anticoagulant drug which is the subject of this study, is an effort in this direction.

Derivatives of 1, 3 indandione were reported to induce hypoprothrombinemia in animals by Kabat, Stohlmann and Smith(5) and a number of clinical studies(6,7) have dealt with the potential usefulness of phenylindandione. A recent report(8) described the development of diphenylacetyl-1,3-indandione (Dipaxin)† in a survey of new indandiones. Correll *et al.* (8) indicate that Dipaxin possessed the unique ability to induce hypoprothrombinemia in rabbits in a quantity 1/200 the amount of Dicumarol necessary to induce an identical hypoprothrombinemia. This would suggest that Dipaxin is the most potent hypoprothrombinemic drug available. In order to evaluate the basic properties of this new agent in man and to determine if its potency offered any advantages for anticoagulant therapy the present investigation was undertaken.

Experimental. Prothrombin estimations were made by a diluted plasma technic involving a special heparin-oxalate collection fluid (9). The results are reported in per cent of a dried stable plasma standard which was adjusted to the mean of 20 normal individuals. Ac-Globulin was determined by a one-stage method using Ac-Globulin free plasma as substrate(10). About 75 subjects from the wards of the Los Angeles County General Hospital were used in the control studies in which they were given single doses of the drug; they were unselected as to age and sex. The acutely ill and those suffering from hepatic and renal disease or bleeding dyscrasias were omitted. An additional 35 patients who had indication for anticoagulant therapy received Dipaxin in therapeutic doses. Dipaxin (diphenylacetyl-1,3-indandione) was given orally in the form of 1 mg tablets.

Results. Effect of single doses. Plasma

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† A trademarked product furnished by Dr. H. F. Hailman, the Upjohn Co.