growth hormone partially restores this ratio to normal under the conditions employed. The plasma inorganic sulfate specific activities of hypophysectomized rats are several fold higher than those of normal rats.

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A Fibrinolytic System in Human Milk.*† (20727)

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The presence of an activator of plasminogen in urine suggested a search for fibrinolytic activity in other fluids secreted or excreted through narrow channels(1). Milk and casein are known to contain small amounts of a proteolytic enzyme(2). The experiments to be presented here show that there exists in human milk a fibrinolytic system resembling that demonstrated in human blood(3). This result is in accordance with the independent observation by Geiger(4), that streptokinase increases the proteolytic activity of mixtures of milk with rabbit plasma.

Materials and methods. Enzymic activity was estimated by the following 3 methods: I. Fibrinolytic effect was estimated on bovine fibrin containing plasminogen by the standard fibrin plate method(5) and recorded as the product (in mm^2) of 2 diameters of the lysed zones (average of 3 determinations). II. Effect on bovine fibrin, where plasminogen had been destroyed by heating, was estimated similarly on fibrin plates heated for 45 min. at 85°C(6). III. Proteolytic activity was estimated on casein (3% in borate or phosphate buffer) after precipitation with perchloric acid (final concentration 6%) by determination of the optical density of the supernatant at 270 mμ using a Hilger Uvispek spectrophotometer.

Human milk was obtained fresh from The Children's Hospital, Fuglebakken, Copenhagen. The samples were centrifuged, and the skimmed milk was used. Bovine plasminogen was prepared from ammonium sulfate precipitated fibrinogen(5), using 0.9% NaCl instead of the usual barbital buffer) by heating for 10 minutes at 54°C followed by dialysis of the supernatant against saturated ammonium sulfate. The precipitate was redissolved, dialysed against borate buffer, and lyophilized. Streptokinase ("Varidase") was obtained from the Lederle Laboratories. Buffers: Barbital: 0.1 M; pH 7.75; NaCl to $\mu = 0.15$. Borate: 0.05 M; pH 7.75; NaCl to $\mu = 0.15$. Phosphate: 0.06 M; pH 7.35; $\mu = 0.15$.

Fibrinolytic activity of human milk. A wide range of fibrinolytic activity was encountered when samples of human milk were applied to standard fibrin plates. Activity measurements of about 400 mm^2 were obtained maximally and a considerable number of samples showed no or only a slight activity. In all samples of milk the addition of streptokinase produced a high fibrinolytic activity. The streptokinase solution alone had only a very slight effect on the standard fibrin plates (Fig. 1). However, no or only a trace of activity was found when the effect

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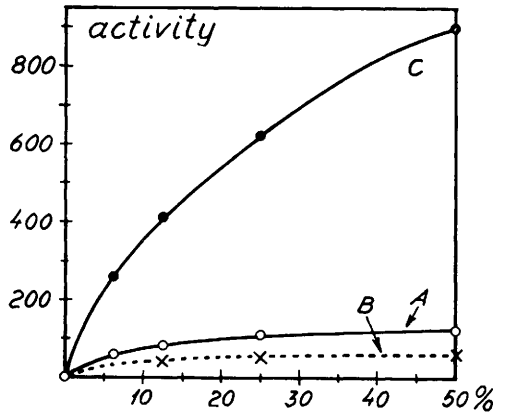

FIG. 1. Action of streptokinase on human milk. *Abscissa*: Final concentrations in % of stock solutions. *Ordinate*: Fibrinolytic activity on standard fibrin plates. *Curve A*: Serial dilutions of milk with barbital buffer. *Curve B*: Serial dilutions of streptokinase (stock solution: 1 mg in 64 ml barbital buffer). *Curve C*: Serial dilutions of human milk containing equal amounts of streptokinase (in conc. 50% of stock sol.).

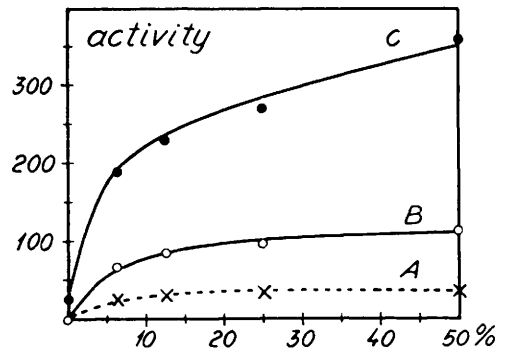

FIG. 2. Effect of plasminogen on the fibrinolytic activity of streptokinase activated human milk. *Abscissa*: Final concentration of plasminogen in % of stock solution (20 mg/ml in barbital buffer). *Ordinate*: Fibrinolytic activity on heated fibrin plates. *Curve A*: Serial dilutions (1 ml) of plasminogen with diluted (50%) streptokinase solution (1 ml; stock solution: 1 mg in 50 ml barbital buffer). *Curve B*: Serial dilutions (1 ml) of plasminogen with diluted (50%) human milk (1 ml). *Curve C*: Serial dilutions (1 ml) of plasminogen with 1 ml mixture prepared by heating human milk with equal vol of streptokinase stock solution for 30 min. at 37°C. *Controls*: Human milk, streptokinase or plasminogen alone: No activity. Mixture of milk and streptokinase: Trace of activity (25 mm^2).

of the milk on heated fibrin plates was studied. This was true before as well as after the addition of streptokinase to the samples. Though a denaturation occurs during the heat treatment of the fibrin this does not significantly interfere with the lytic effect of a number of proteolytic enzymes(6). Bovine fibrin normally contains large amounts of plasminogen, but in the heated fibrin plates the plasminogen is destroyed. The fibrinolytic activity observed on standard fibrin plates is therefore probably caused by an activation of the plasminogen contained in the fibrin. This means that the fibrinolytic agent present in milk acts as an activator of plasminogen, and that the action of streptokinase might consist in the transformation of a proactivator into an activator of plasminogen. This is similar to the system found in human blood(3).

Effects of plasminogen. If a plasminogen activator is involved, the addition of plasminogen should result in the formation of plasmin. Appropriate mixtures of human milk, plasminogen and streptokinase were therefore prepared and the resulting activities measured on heated fibrin plates (Fig. 2). It is seen that streptokinase alone produced a very low activity in the bovine plasminogen. The effect produced by a mixture of human milk and bovine plasminogen was small in this experiment. This effect was found in other experiments to depend upon the fibrinolytic activity of the sample of human milk. Very large activities were always found when a mixture of human milk and streptokinase was added to plasminogen. The effects increased with increasing concentrations of plasminogen. These results therefore prove the assumption made above concerning a plasminogen activator and proactivator.

A dry preparation. An effort to control these results by estimating the proteolytic activity on casein was complicated by high blank values in the estimation of proteolytic activity by light absorption at 270 mμ. This difficulty was overcome by treating with chloroform (1 ml per 10 ml milk; 45 minutes slow shaking at room temperature), followed by isoelectric precipitation at pH 4.5 of the resulting (undiluted) solution, resolution of the precipitate in borate buffer, and lyophilization. Less than half the activity remained after these procedures.

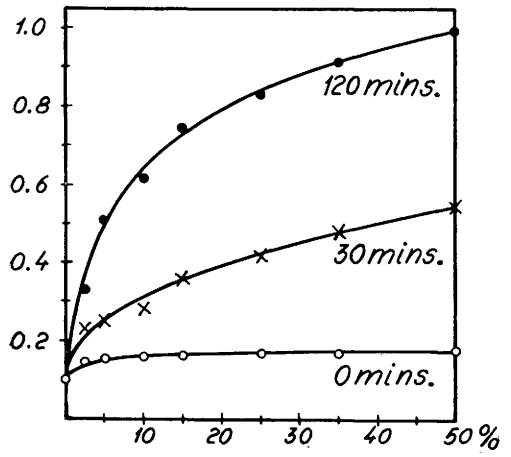

FIG. 3. Digestion of casein with plasminogen in presence of streptokinase activated human milk. *Stock solutions*: Bovine plasminogen: 30 mg/ml H_2O. Milk preparation: 25 mg/ml H_2O. Streptokinase: 1 mg in 25 ml phosphate buffer. Dilutions of plasminogen prepared in phosphate buffer: 100, 70, 50, 30, 20, 10, 5, 0%, respectively. *A*: Dilution of milk preparation: 16.5 ml stock sol. + 5.5 ml phosphate buffer. *B*: Dilution of streptokinase: 5.5 ml stock solution + 16.5 ml phosphate buffer. *C*: Mixture of milk preparation (16.5 ml) with streptokinase solution (5.5 ml). Samples (2.5) of plasminogen solutions mixed with 2.5 ml of A, B, and C. After 30 min. at 37° 4.5 ml were added to 4.5 ml casein solution. Samples of 2 ml removed after 0, 30 and 120 min. and added to 3 ml 10% perchloric acid. Samples were left overnight, centrifuged, and optical density at 270 mμ estimated. *Abscissa*: Final concentration of plasminogen in % of stock sol. *Ordinate*: Optical density at 270 mμ. Curves for casein digestion at zero time, after 30 and 120 min. (Series C). Controls (optical density): Casein alone (max 0.060). Streptokinase + plasminogen + casein (max after 2 hr: 0.092) (Series B). Milk preparation + plasminogen + casein (max after 2 hr: 0.116) (Series A).

Casein digestion. By means of this partially purified dry preparation it was possible now to confirm on a casein substrate the existence of the plasminogen proactivator demonstrated in the fibrin plate experiments. Appropriate mixtures of the partially purified milk agent with streptokinase were incubated with plasminogen for 30 minutes and the solutions then added to the casein solution. After digestion at 37°C for either 30 minutes or 2 hours, samples were precipitated with perchloric acid solution; and the optical density of the supernatant was read at 270 mμ. For details see Fig. 3. The results compare exactly with those obtained with streptokinase on the heated fibrin plates.

Discussion. The results presented here show that human milk contains: 1. An agent which acts as an activator of plasminogen, and which is present in varying and usually slight amounts. This agent was also recently found in bovine milk. 2. An agent which in the presence of streptokinase is transformed into an activator of plasminogen. This agent is present in all samples in considerable amounts.

The existence of a fibrinolytic system in milk is of interest because it supports the idea(1) that fluids occurring in narrow channels in the organism are provided with mechanisms by which the obstruction of the channels with clotted fibrin can be counteracted. The similarity of this system with that found in blood(3) suggests that the origin of these substances in milk is probably to be found in the blood. The observations are therefore also of significance in the discussion of the blood proteins as a source of some of the proteins contained in milk. However, a simple nonselective contamination of the milk with blood proteins appears less probable because other experiments showed, that human milk contains very small amounts of the trypsin inhibitor which is present in high concentration in blood. In this respect it is of less significance that the amount of plasminogen in human milk was found to be so small that it could hardly be observed by the methods applied. Since the plasminogen content of human blood is low(3), dilution might make it difficult to detect the small amounts present.

It is worth noticing that the chloroform treatment which is used in producing fibrinolytic activity in blood(7), does not produce fibrinolytic activity in milk. However, the development of activity in blood after short treatments was very slow(8).

The relation between the activities observed here and the slight proteolytic activity found in milk and casein by previous authors (2) is not known.

Summary. 1. Human milk contains varying amounts of an agent, which activates plasminogen to plasmin. 2. Human milk contains considerable amounts of a proactivator, which

by addition of streptokinase is transformed into an activator of plasminogen.

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Observations on the Nature of Radioactive Contaminants in Biosynthetic Cholesterol-C^{14}.* (20728)

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Schwenk and co-workers have reported that the digitonide of biosynthetic cholesterol-C^{14} is contaminated in certain instances by unidentified compounds of high specific radioactivity(1-3). This phenomenon has been of particular interest because of the possibility that the contaminating substances might represent biological precursors of cholesterol.

It has been found in this laboratory that up to 80% of the radioactivity of the sterol digitonide obtained from the livers of rats sacrificed 3 minutes after injection of sodium acetate-1-C^{14} was due to non-cholesterol contaminants. A number of substances related either chemically or biologically to cholesterol have been examined as possible sources of radioactive contamination.

Experimental. Each experiment employed cholesterol digitonide obtained from the pooled livers of several animals. Normal adult male Wistar rats were anesthetized by intraperitoneal injection of sodium pentobarbital, 6 mg per 100 g body weight. After exposure of the femoral vein, each animal was given 4.0-6.7 mg of sodium acetate-1-C^{14} intravenously (total radioactivity 5.8-13.4 x 10^6 counts/min). Three minutes later the liver was rapidly excised and immersed into 10 volumes of hot 95% ethanol containing 12% KOH. The mixture was boiled for 3 hours under reflux and filtered through sintered glass. Sufficient water was added to the filtrate to reduce the ethanol concentration to 50%, and the non-saponifiable material was obtained by 3 extractions with one-half volume of petroleum ether (B.P. 60-68°). The petroleum ether extracts were combined, washed once with 5% sodium acetate and twice with water, then dried over Na_2SO_4 and evaporated to dryness under N_2. The residue was taken up in 1:1 acetone-ethanol, and cholesterol digitonide was precipitated by the addition of a 1% solution of digitonin in 80% ethanol. The digitonide was washed twice with 80% ethanol and once with ether.

In a preliminary experiment sodium acetate-1-C^{14} containing 2.5 x 10^5 counts/min was added to the liver of a normal rat, and cholesterol digitonide isolated as described above. The digitonide was free of radioactivity. Radioactivity assay was carried out with a windowless flow gas counter. Cholesterol, 3β-cholestanol and 7-ketocholesterol were counted as the digitonides, mounted on sintered alundum discs. Other compounds were subjected to wet combustion by a modification of the method of Lindenbaum *et al.*(4) and counted as $BaCO_3$ mounted on alundum discs.

Exp. 1. The digitonide obtained in one ex-

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