exhibit a linear increase of luminosity as a function of concentration with up to 50 ppm of calcium ion in the presence of 5000 ppm of EDTA and 6000 ppm of KOH.

The applications of protective chelation with the use of internal standards for the flame-photometric determination of calcium and other cations in biological samples are under investigation.

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Evaluation of Chloroform Activation of Human Plasminogen

The presence of spontaneous proteolytic activity in human blood was first recognized 60 years ago (*1*). The observations were extended when it was discovered that chloroform treatment of human serum increased the proteolytic activity (*2*). Recently it was found that the production of proteolytic activity of human plasma or, in later experiments, of fraction III isolated from human plasma by cold ethanol fractionation (*3*) was more effectively achieved by reaction with streptokinase, a metabolic fermentation product of certain streptococci (*4*). It was of even more interest, however, that the reaction mixture of fraction III and streptokinase possessed considerable fibrinolytic activity.

In contrast, treatment of human fraction III with chloroform did not produce measurable fibrinolytic activity. The difference in behavior of the two activators has created considerable confusion; we have made an attempt to explain these divergences.

An aqueous acid extract of human fraction III was adjusted to a *p*H of 7.8, shaken with one-fifth its volume of chloroform, and left in contact at room temperature. At intervals, samples were analyzed for proteolytic activity by measurement of casein hydrolysis according to the method of Kunitz (*5*).

Proteolytic activity developed slowly but measurably over a period of several days. Addition of minute amounts of streptokinase-activated human fraction III (that is, human activator) increased the proteolytic activity during the first days of incubation, whereas after about 10 days of standing in contact with chloroform, no additional proteolytic activity was produced.

Concurrently, the determination of fibrinolytic activity of the reaction mixture was carried on according to our modification (*6*) of the procedure of Christensen (*4*). No measurable activity could be detected. However, addition of streptokinase brought forth rapid lysis of the standard fibrin clot.

These experiments may readily be explained in view of the recently proposed mechanism for the "activation of human plasminogen by streptokinase" (*7*). Only the proteolytic precursor is converted in contact with chloroform to the active proteolytic enzyme. The same conversion can be achieved by catalytic amounts of human activator which is produced by the reaction of streptokinase with human fraction III. This latter path of conversion is catalyzed by human activator to the extent of the presence of proteolytic precursor. After its exhaustive conversion in contact with chloroform, addition of human activator will not increase the proteolytic activity (Fig. 1).

The proteolytic component of human fraction III did not effect this conversion. Human fraction III that had been activated by streptokinase was heated at *p*H 2.0. The mixture contained 50 percent of the original proteolytic activity, but it did not possesss fibrinolytic activity or the ability to catalyze the conversion of the proteolytic precursor to the active proteolytic enzyme.

The proactivator component of human fraction III was not affected by treatment with chloroform and could be converted to the activator by reaction with streptokinase regardless of the length of time of prior incubation with chloroform. The resulting fibrinolytic activity is a direct measure of the activator activity and can be demonstrated only on a fibrin substrate that contains the proteolytic precursor (*8*). The therapeutic efficacy of streptokinase-activated human fraction III depends therefore on its activator content, since the clot-dissolving activity of the proteolytic enzyme is very small.

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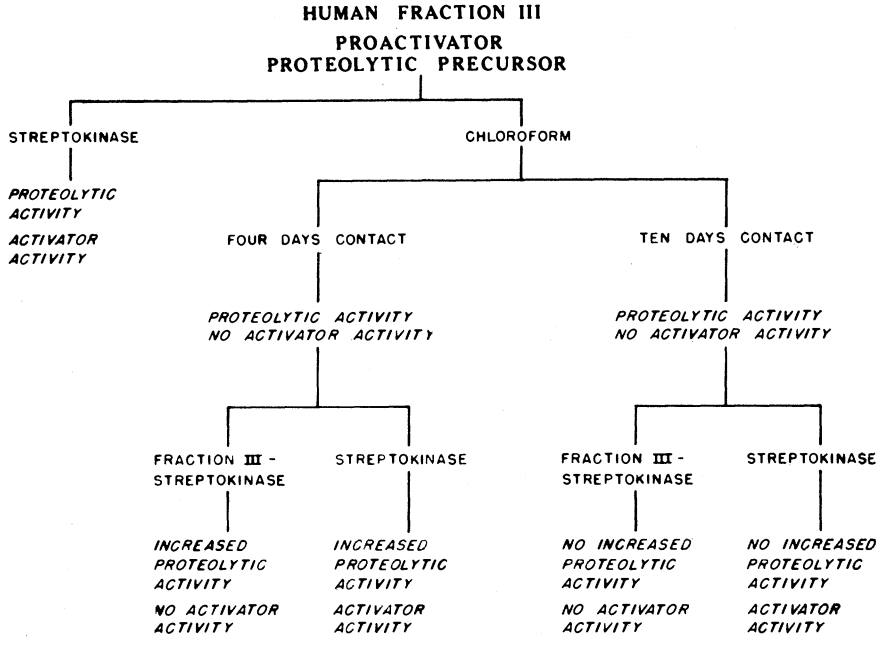

Fig. 1. Activation of human fraction III by chloroform or streptokinase.

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8. Normally, human fibrinogen contains appreciable amounts of proteolytic precursor found in fraction III.

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