P:O ratios of mouse and rat liver mitochondrial controls were not affected by thyroxine.

In conclusion it may be stated that thyroxine has ready access to certain tumour mitochondria *in vitro*.

Department of Biochemistry,
Antoni van Leeuwenhoek-Huis, The Netherlands Cancer Institute,
Amsterdam (The Netherlands)

P. EMMELOT
P. J. BROMBACHER

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The exclusive assay of the pro-activator of plasminogen by lysis of bovine fibrin clot

The pro-activator of plasminogen is a factor in blood plasma that forms a stoichiometric complex with streptokinase, which in turn acts catalytically in plasminogen activation[1].

A now obsolete method of "plasmin" assay depended on determining the rate at which standard clots of bovine fibrin were lysed in the presence of the plasmin preparation[2]. However, the discovery firstly that the bovine fibrinogen employed contained plasminogen[3] and secondly that the plasma activator was involved in the reaction[4], indicated that the lytic powers of the preparation might be determined by the content of pro-activator, plasmin or plasminogen. In fact, it was demonstrated[5] that under the conditions of assay then employed, the pro-activator predominated in determining potency.

It appeared that the lytic system might be modified to form a sensitive and rapid method of measuring pro-activator. In subjecting the assay to an elementary kinetic analysis, it has been assumed that the interaction of streptokinase and pro-activator is so rapid that it does not determine the rate of lysis[6,1].

Let

a = concentration of pro-activator in the system
p = concentration of plasmin formed by time t
F = the quantity of fibrin that must be digested in order that the clot may lyse
D = extent of fibrin digestion at time t
T = clot life.

If now streptokinase and plasminogen are present in excess

$$\mathrm{d}p/\mathrm{d}t = k_1 a$$

and

$$p = k_1 a t$$

Hence the rate of proteolysis

$$\mathrm{d}D/\mathrm{d}t = k_2 p = k_1 k_2 a t$$

and

$$D = (k_1 k_2/2)\, a \cdot t^2$$

where k_1 and k_2 are rate constants.

At the time T, D will be equal to F, which is a constant,

$$\therefore a = k\, 1/T^2$$

where

$$k = 2F/k_1 k_2.$$

Hence

$$\log a = \log k + 2 \log 1/T$$

If then the conditions of assay employed provide a true measure of pro-activator, a graph of log reciprocal clot life (T) against log concentration of pro-activator fraction employed will have a slope of 0.5. However, if the assay is in fact measuring the plasmin content of the fraction a slope of 1.0 will be obtained. Consequently, in a mixed assay (*i.e.* an assay of a mixture of pro-activator and plasmin) the slope would lie between these values.

CHRISTENSEN[2] employed clots of 0.2% bovine "fibrinogen" and took as a unit of activity that quantity of plasma fraction that induced lysis in 30 min. When the result with a Christensen system was subjected to the above analysis, the slopes were of the order of 0.6–0.7, *i.e.* the assay is mixed.

An assay for pro-activator alone must be designed to avoid errors arising from the lability of the activator and must provide an excess of plasminogen. We have therefore reduced the standard time of lysis from 30 to 15 min, and we have used a 0.5% solution of "plasminogen-fibrinogen" for making the clot. Under these conditions, a slope of 0.5 is obtained, but with lower concentrations of pro-activator and the consequently longer lysis times the slope increases (Fig. 1).

In the proposed assay system, the materials are: "Fibrinogen", Ox fraction I – Armour Laboratories; Thrombin, Thrombin – surgical (S. Maw & Sons); Streptokinase, streptokinase streptodornase varidase (Lederle Laboratories). All solutions are prepared in phosphate-saline, pH 7.2 ($Na_2HPO_4 \cdot 2H_2O$, 2.89 g/l; KH_2PO_4, 0.856 g/l; NaCl, 8.76 g/l). Three-tenths of a ml of the solution for assay is incubated at 37° C in a 2 × $^3/_8$″ test tube with 0.1 ml thrombin; 0.6 ml of a mixture of 5 volumes of 1% "fibrinogen" and 1 volume of streptokinase (2,000 units/ml) are added. The time of clot lysis is noted, taking the release of air bubbles within the clot as the end-point. The assay is performed at several concentrations of the preparation of pro-activator and the concentration necessary to give a 15 min clot life found by interpolation. Fig. 2 shows the assay of four different serum globulin preparations by this method.

The reproducibility of a single reading is high; thus with six separate readings at one concentration of activator, where the mean $\log \frac{100}{\text{clot life}} = 0.99$, the standard deviation was 0.01.

It must be noted that dilute solutions of pro-activator rapidly lose activity even at 0° C. For this reason it is better to compare the activity of different fractions directly in a simultaneous assay.

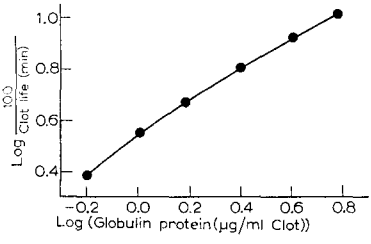

Fig. 1

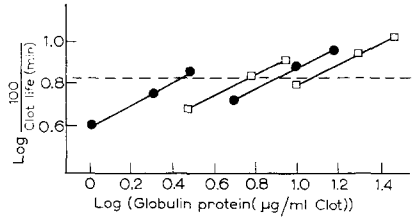

Fig. 2

Fig. 2. Assay of four serum globulin fractions of respective potencies 372, 166, 123, 83 units/mg protein. The broken line lies at a clot life of 15 min.

P. J. MILL*

The Lister Institute of Preventive Medicine, London (England)

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* Jenner Memorial Student.