# Evaluation of Synthetic Substrate Methods for Clinical Determination of Fibrinolysis. (22898)

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During the course of a clinical study on fibrinolysis, the question arose as to desirable methods for the quantitative estimation of fibrinolysis. The method of fibrin determination(1) had been in use at this laboratory. It was hoped that a more rapid method could be found since this method requires a fairly long incubation period. An investigation was begun on the utilization of synthetic substrate methods which have been reported to measure the activity of purified plasmin(2,3). Although the number of determinations in this study is not large, it is felt that it is sufficient to demonstrate the clinical inapplicability of the synthetic substrate methods as a measure of spontaneous fibrinolysis in plasma.

*Materials and methods.* Citrated venous blood was obtained from healthy personnel of this laboratory and from patients in whom marked fibrinolysis was induced by intravenous injection of pyrogens.* The cells were separated from the plasma immediately after drawing by centrifugation at 2000 rpm for 10 minutes. An aliquot of the plasma was diluted 1:4 for the lysine ethyl ester and tosyl arginine methyl ester assays. These assays were performed as described by Troll and Sherry(2,3), measuring both the spontaneous (SP) and streptokinase† (SK)-activated activity at 0, 40 and 60 minutes of incubation. The results are expressed as mean increase in micromoles of acid liberated. A second aliquot of plasma, diluted 1:25 in a 0.1 M $CaCl_2$ solution, was used for the determination of fibrin following a 45-minute and 24-hour incubation. The amount of fibrin was estimated by its tyrosine content(1).

*Results.* Blood was drawn at random from 8 normal individuals, 4 of whom had blood drawn on 4 successive days. These bloods were run only in the ester assays. Extreme variation was shown with these assays, both from individual to individual (Table I) and in one individual from day to day (Table II). Only the data for the 60-minute incubations are shown since a statistical analysis showed a similar variation in the 40- and 60-minute values.

It was thought that possibly diet might be a factor in this variation. Therefore, fasting samples of blood were taken from 8 normal individuals, 5 of whom had been in the random group. On these 5 normals, blood was drawn on the same day one hour after coffee and 1½ hours after lunch. The series was repeated for 3 days. The ester assays and the fibrin method were performed on all the plasma samples. The results from the fasting samples showed as extreme a variation in the ester assays as in the random group. There were variations in the enzyme activities following coffee and lunch but no consistent trends (Tables I and II). Comparing the 24-hour with the 45-minute incubations in the fibrin method, a mean increase of 2.3% of fibrin in the fasting group, an increase of 1.9% in the post coffee group, and a decrease of 10.9% in the post lunch group was observed. No lysis was shown in any of the normals.

The plasmas from the 6 patients treated with pyrogens showed complete spontaneous lysis in 30 to 120 minutes with the fibrin method but both the spontaneous and SK-activated specimens fell within the normal range in the ester assays (Table I). Pretreatment bloods were drawn on 3 of the patients, one of which lysed within 45 minutes and can

* These samples were kindly donated by Dr. K. N. von Kaulla, Univ. of Colorado, and analyzed one hour after drawing. Pyrogen administered was 300 μg of preparation No. 1083, Wander Co., Chicago, Ill.

† Varidase (10,000 units/cc.), Lederle Laboratories Division, Am. Cyanamid Co., N. Y. L-Lysine Ethylester Dihydrochloride and p-Toluene Sulfonyl L-Arginine Methylester Hydrochloride, Mann Research Laboratories, N. Y.

TABLE I. Group Comparison of Results from Ester Assays. (Expressed as mean increase μmoles of acid liberated per 60 min. incubation.)

| | Mean | | Range ($2\sigma$) | | % in normal range | |
|---|---|---|---|---|---|---|
| | SK | SP | SK | SP | SK | SP |
| *Lysine:* | | | | | | |
| Normals: | | | | | | |
| Random (18)* | .59 | .09 | .37- .81 | .0 -.19 | 100 | 100 |
| Fasting (14) | .69 | .08 | .33-1.05 | .0 -.34 | 100 | 92.9 |
| Post coffee (11) | .62 | .12 | .16-1.08 | .0 -.46 | 100 | 100 |
| " lunch (6) | .71 | .15 | .35-1.07 | .0 -.35 | 100 | 100 |
| Patients (fibrinolytic) (7) | .44 | .05 | .20- .68 | .0 -.13 | 100 | 100 |
| *Tosyl arginine:* | | | | | | |
| Normals: | | | | | | |
| Random (19) | .55 | .19 | .39- .71 | .09-.29 | 100 | 94.7 |
| Fasting (15) | .66 | .20 | .32-1.00 | .0 -.44 | 100 | 100 |
| Post coffee (11) | .60 | .17 | .24- .96 | .0 -.55 | 100 | 100 |
| " lunch (8) | .57 | .15 | .19- .95 | .0 -.31 | 87.5 | 100 |
| Patients (fibrinolytic) (7) | .67 | .25 | .49- .85 | .13-.37 | 100 | 85.7 |

* No. of plasma samples.

be included with the lytic plasmas. The other 2 controls showed no lysis with the fibrin method while the ester assay values were similar to those of the respective lytic plasmas. Although only the spontaneous enzyme activities can be compared with the fibrin method, it is of interest to note that there is also no difference between the activated lytic and non-lytic plasmas.

A longer period of incubation of 120 and 180 minutes was done with the ester assays on the lytic blood and normals. The increased period of time brought out no difference between the values for the lytic and non-lytic. Also, undiluted samples were used in the ester assays, again showing no difference between the two.

Therefore, this study demonstrates that the synthetic substrate methods as presently outlined are not suitable clinical methods for the detection of lysing or potential lysing blood. These results agree with the observations of Ratnoff(4) who found that the plasma proteolytic enzyme concentration, as measured by the casein method, did not differ between normal and fibrinolytic bloods.

Bastian *et al.*(5) have shown with the ester substrates that the esterase activity of trypsin and chymotrypsin are inhibited by rabbit plasma. The variation in demonstrable esterase activity in this study could be due to variations in the amount of inhibitor.

TABLE II. Individual Variations with Ester Assays. (Values represent mean increase in μmoles of acid liberated in 60 min. incubation.)

| | Fasting | | Post coffee | | Post lunch | | Random | |
|---|---|---|---|---|---|---|---|---|
| | SK | SP | SK | SP | SK | SP | SK | SP |
| *Lysine:* | | | | | | | | |
| LM | .98 | .016 | .83 | .15 | .88 | .14 | .76 | .15 |
| JC | .58 | .045 | .53 | .14 | .56 | .14 | .47 | .06 |
| RS | .59 | .26 | .53 | .13 | .70 | .16 | .57 | .11 |
| EJ | .49 | .005 | .45 | .02 | .32 | .05 | .57 | .07 |
| JM | .62 | .21 | .66 | .15 | .90 | .32 | .60 | .11 |
| Group | .69 | .08 | .62 | .12 | .71 | .15 | .59 | .09 |
| *Tosyl arginine:* | | | | | | | | |
| LM | .79 | .19 | .71 | .13 | .61 | .13 | .63 | .10 |
| JC | .44 | .14 | .52 | .13 | .51 | .11 | .53 | .25 |
| RS | .68 | .26 | .55 | .25 | .70 | .23 | .54 | .27 |
| EJ | .67 | .13 | .39 | .16 | .43 | .20 | .48 | .16 |
| JM | .59 | .21 | .64 | .19 | — | — | .58 | .19 |
| Group | .66 | .20 | .60 | .17 | .57 | .15 | .55 | .19 |

*Summary.* 1. An attempt was made to determine a normal level of enzyme activity in human plasma as measured by the lysine ethyl ester and tosyl arginine methyl ester assays. There was not only a variation in activity from one individual to another, but also variation in one individual from day to day and within one day. 2. When normal blood and blood with marked fibrinolytic activity were compared, no difference in enzyme activity was noted. Therefore, these methods are not suitable clinically for the

demonstration of fibrinolytic activity.

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## Evidence for Function of Aberrant Thyroid Tissue in Thymus of Rats.* (22899)

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In the course of a study on the long-term effects of massive doses of $I^{131}$ in rats, aberrant thyroid tissue in the thymus was found in unexpectedly high incidence in the untreated controls(1,2). In 21 normal female rats of the Sprague-Dawley strain whose ages ranged from 260 to 427 days, one or more thyroid follicles were found in routine sections of thymus of 8 rats. Cords or sheets of epithelium resembling that of the thyroid were found in an additional 4 rats; in these latter there was no follicular arrangement or association with colloid. An attempt has been made to demonstrate whether the thyroid follicles in the thymus take up radioiodine and incorporate it in colloid.

*Methods.* Female rats of the Sprague-Dawley strain received 50 $\mu$c/g body weight of $I^{131}$ when 55 days old and were autopsied 24 hours later. Histological sections were made of the thymus, and autoradiographs were prepared on Eastman Kodak 10 $\mu$ NTA stripping film.

*Results.* Fig. 1 illustrates the thymus of one of the rats at low magnification; thyroid follicles may be seen in the medulla. The tissue surrounding these follicles is characteristic of the thymic medulla. At higher magnification (Fig. 2) it is seen that these follicles possess a cuboidal epithelium and are filled with colloid. The colloid variably appears deeply eosinophilic and homogeneous or pale staining and vacuolated—the latter suggesting resorption. The follicles also vary in autoradiographic density (Fig. 3), but all show some radioiodine concentration. Thus, in structure and in iodine concentration, these follicles are like those found in the thyroid gland proper.

*Discussion.* The possibility that accessory thyroids and aberrant thyroid tissue may occur, especially in the mediastinum, has long been recognized. Adequate embryological explanations may be derived by study of the development of the branchial pouches which are the anlagen of both thyroid and thymus (3). It has been shown that administration of thiourea to Wistar strain rats resulted in a high incidence of thyroid adenomata in the thymus(4). The objective of the present communication is to point out a relatively high incidence of accessory thyroid tissue in an animal commonly employed in endocrinological experiments, and to show that this aberrant tissue may be functional rather than merely rudimentary. The findings have a bearing on experiments where total thyroid ablation is critical; surgical thyroidectomy may not reach all the functional thyroid tis-

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