

the original compounds or the degradation products in the Pichat synthesis<sup>1</sup>.

J. H. M. HENDERSON\*

George Washington Carver Foundation,  
Tuskegee Institute,  
Alabama.

\* Present address: Le Phytotron, CNRS, Gif-sur-Yvette, Seine-et-Oise.

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### Distribution of Trypsin Inhibitors in the Sera of Various Animals

TRYPSIN inhibitors have been found in pancreas, serum, colostrum, egg-white, soya bean, and lima bean<sup>1</sup>. They have all been crystallized except from egg-white<sup>2</sup>. Thus, examination of the distribution of trypsin inhibitors in proteins of them appears to be out of the question. Recently, Nakamura *et al.*<sup>3</sup> have demonstrated, by two-dimensional paper electrophoresis, that two trypsin inhibitors were contained in the extract of soya-bean. Hence the distribution of trypsin inhibitors in other biological fluids deserves to be re-investigated to see whether there are also two inhibitors in them or not.

In Fig. 1 is shown one of the 'crossing diagrams' of human serum against trypsin. The electropherogram shown was obtained by two-dimensional electrophoresis as follows: Human serum was applied on line AB and the first electrophoresis was carried out in direction 1. A solution of trypsin was then applied on line XY and the second electrophoresis was carried out in direction 2. Under the given experimental conditions, trypsin migrates towards

the cathode and serum proteins towards the anode. Thus the former migrates over the lines of the latter. As can be seen from Fig. 1, the lines of serum proteins were partially digested by trypsin. In this way the front of the trypsin line, which is named the 'crossing diagram' of the serum against trypsin, appeared across the zones of serum proteins. It shows two grooves (or peaks, if viewed conversely), one in the zone of  $\alpha_1$ -globulin and the other in that of  $\alpha_2$ -globulin. At the grooves in the line, trypsin is retarded. The retardation of the migration of trypsin must have been caused by the reaction with substances in serum, which combined with it and inhibited its activity. Therefore from the 'crossing diagram' of serum proteins against trypsin the distribution of trypsin inhibitors in serum can be investigated easily. Fig. 1 shows that human serum contains two trypsin inhibitors, one in  $\alpha_1$ - and the other in  $\alpha_2$ -globulin.

Table 1 shows some of the results obtained by the same method with sera of various animals. From the results obtained so far, it can be inferred that sera of many animals contain two trypsin inhibitors (although some contain only one) and the distribution of them in the serum proteins is characteristic of the species. The circumstances are the same as for the 'crossing diagrams' of serum against protein J of jack beans<sup>4</sup>.

Table 1. DISTRIBUTION OF TRYPSIN INHIBITORS IN SERA OF VARIOUS ANIMALS

| Animal     | Albumin | $\alpha$ | $\beta$ $\gamma$ |
|------------|---------|----------|------------------|
| Human      | +++     | +        |                  |
| Dog        |         |          | ++               |
| Cat        | ++      |          | ++               |
| Horse      |         | +++      |                  |
| Cattle     |         | +++      |                  |
| Sheep      |         | +++      | +                |
| Pig        |         | +++      |                  |
| Rabbit     | +++     |          | +                |
| Guinea pig | +++     |          | +                |

The number of (+) denotes rough estimation.

The method of two-dimensional electrophoresis to obtain the 'crossing diagram' is not strictly quantitative, but from the height of the peak, if other conditions were the same, a rough comparison can be made between the sera of different animals. In human sera particularly, the changes of distribution of trypsin inhibitors in diseases deserve further investigation.

S. NAKAMURA  
T. WAKAYAMA

Institute for Medical Chemistry,  
Yamaguchi Medical School,  
Ube,  
Japan.

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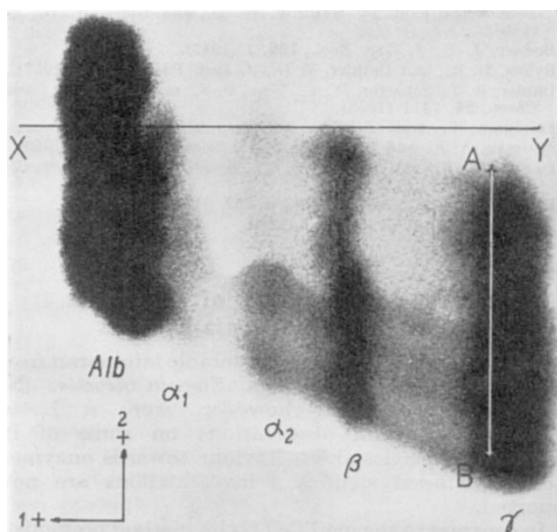


Fig. 1. 'Crossing diagram' of human serum against trypsin. First electrophoresis, 0.04 ml./6 cm. of human serum were applied on line AB and run at 100 V. for 16 hr. Second electrophoresis, after the first one, 0.1 ml./14 cm. of 1.5 per cent crystallized trypsin solution was applied on line XY, and run at 100 V. for 8 hr. Barbiturate buffer, pH 8.6 and ionic strength 0.05. Stained with bromophenol blue

### Trehalose in the Cellular Slime Mould *Dictyostelium mucoroides*

IN 1925 Iwanoff<sup>1</sup> demonstrated the presence of the non-reducing disaccharide, trehalose, in isolated spores of the acellular slime mould, *Reticularia lycoperdon* (Myxomycetes). Recent work<sup>2</sup> on soluble carbohydrates in the cellular slime moulds have not mentioned the presence or absence of this sugar. The present report concerns the occurrence of