ESTIMATION OF HEPARIN AND FIBRINOLYSIS IN THE BLOOD SAMPLES OF A DOG SUBJECTED TO PEPTONE SHOCK

Amounts of protamine added (γ/0·5 ml.)		Blood samples after several intervals following the injection of 300 mgm. of peptone per kilo of body-weight:							
		Before	1st injection				2nd injection		3rd injection
		I	II	III	IV	VI	VII	VIII	IX
100	clot. time (min.)	10	12	13	10	8	20	15	10
	fibrinolysis	0	++++	++++	++++	0	++++	0	0
50	clot. time (min.)	8	11	12	8	7	14	9	10
	fibrinolysis	0	+++	++++	+++	0	++	0	0
25	clot. time (min.)	5	8	10	7	9	13	7	8
	fibrinolysis	0	+	+++	+++	0	0	0	0
10	clot. time (min.)	5	6	11	9	10	12	5	5
	fibrinolysis	0	++	+++	0	0	0	0	0
0	clot. time (min.)	4	∞	∞	∞	∞	∞	∞	∞
	fibrinolysis	0	—	—	—	—	—	—	—

++++, Total fibrinolysis in less than 1 hr.; +++, total fibrinolysis in less than 2 hr.; ++, total fibrinolysis in less than 3 hr.; +, partial fibrinolysis; 0, no fibrinolysis for at least 24 hours. The minimum clotting time indicates the optimum amount of protamine and therefore the amount of heparin present in the blood samples.

engaged in testing those two possibilities, since they certainly will have a bearing upon the problem of counteracting anaphylactic and allergic manifestations. Another conclusion can be drawn from those experiments: since fibrinolysis strongly suggests the interaction of a proteolytic ferment[6,7], it is quite probable that activation of plasma trypsin takes part in the production of those modalities of shock.

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Histochemical Demonstration of Acid Polysaccharides in Animal Tissues

RECENT work on the acid polysaccharides occurring in the animal body and on enzymes, such as hyaluronidase, which hydrolyse them, has emphasized the need for more specific methods for their histological demonstration.

Unsuccessful attempts were made to modify the usual methods of fixation and staining with basic dyes such as toluidine blue. Aqueous fixatives, such as formaldehyde, which act chiefly as protein fixatives, do not fix hyaluronic acid, which dissolves out into the solution and into the subsequent washing water. It is necessary to use a dehydrating fixative such as Carnoy's fluid in order to preserve the polysaccharide *in situ*. Although toluidine blue is satisfactory for sulphated polysaccharides, such as chondroitin sulphate, which stain metachromatically, it is of less value for hyaluronic acid and similar polysaccharides, which probably contain no sulphate and do not stain metachromatically. It is difficult to differentiate a polysaccharide which stains purple-blue from other tissues which stain similarly.

Preliminary experiments have indicated that the method described below may be of value; a thorough investigation has not been practicable, but it is hoped that other workers may find the description useful. In principle, the method depends upon the treatment of the tissue with a suitable fixative; after dehydration and the preparation of a paraffin block, sections are cut, mounted and then treated with an acid solution of ferric hydroxide. The iron combines with acid polysaccharides but not with neutral polysaccharides or proteins. The combined iron is then demonstrated as prussian blue by treatment with hydrochloric acid and potassium ferrocyanide. If the sections are counterstained with a red dye such as fuchsin, the acid polysaccharides are stained blue against a red background of other tissue structures.

The detailed method is as follows: (1) Pieces of tissue not more than 3·4 mm. thick in Carnoy's fluid (absolute alcohol, 6 vol.; chloroform, 3 vol.; glacial acetic acid, 1 vol.) for half an hour. (2) Dehydrate in absolute alcohol, clear and mount in paraffin block. Sections are cut and mounted on clean slides without albumen. (3) Sections brought rapidly to water and flooded with dialysed iron (B.D.H.), 1 vol.; acetic acid (2*M*), 1 vol. for 10 min. (4) Wash well with distilled water. (5) Flood with a solution containing potassium ferrocyanide (0·02 *M*), hydrochloric acid (0·14 *M*), for 10 min. (6) Wash with water and counter-stain with any convenient contrasting dye. (7) Dehydrate rapidly, clear in xylene and mount in Canada balsam.

By treating selected pieces of tissue with a preparation of hyaluronidase from streptococcus prior to stage (2), hyaluronic acid may be distinguished from other blue-staining structures. This hyaluronidase preparation hydrolyses the hyaluronic acid and prevents the combination of the polysaccharide with the iron: the enzyme appears to be specific since it has no similar action on other acid polysaccharides tested. Umbilical cord, muscle and skin have been examined by this method.

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An Agglutinable Factor in Red Blood Cells

IN 1938, Levine and Katzin[1] described a phenomenon in which the red blood cells of a patient suffering from a severe infection with pneumococcus Type 1 were agglutinated by the sera of about 15 per cent of normal persons. The agglutination occurred at room and lower temperatures but not at 37° C. The authors considered that a latent agglutinogen in the cells was activated by some unidentified mechanism and that this agglutinogen was rendered susceptible to the action of agglutinins which were normally present in the sera. This property of the red cells lasted only for four months. Gaffney and Sachs[2] have described a similar occurrence in two subjects. The first, a boy aged 11 years, had been treated for congenital syphilis for four years. His red cells were agglutinated at room temperature but not at 37° C. Of the sera used the percentage which produced agglutination varied at different times, 89 per cent being the highest. The second was a healthy woman student whose cells were agglutinated at room temperature by 73 per cent of the sera against which they were tested. The 'poly-agglutinability', as the authors term the phenomenon, disappeared in both patients after some months.

A somewhat similar phenomenon has been observed recently with the red cells of a woman whose first pregnancy was terminated at six months because of an increasing hypertension. After delivery, hæmorrhage was severe and one litre of group *O* blood was administered. Three days later she was admitted to hospital with intense jaundice and anæmia (the red cell count was 1,760,000 cells per c.mm.) and died seven days after the fœtus was delivered. Autopsy revealed gross sepsis of the uterus and para-metrial tissue with numerous pyæmic abscesses in the liver. Unfortunately, cultures were not successful due to spreading organisms, but Gram-positive rods were observed both in the uterine tissues and in the liver abscesses. 860 c.c. of group *O* blood were transfused shortly before the patient's death.

It was found that although the patient was group *O*, her cells were strongly agglutinated by all of forty-five sera which were obtained from normal individuals of all groups. The reactions were marked at room temperature (20°–22° C.) and in the ice box (2°–4° C.) but did not occur at 37° C. Repeated saline washing of the red cells and preliminary inactivation of the sera in no way affected the agglutination reaction. It was, however, noted that agglutination of the washed red cells occurred spontaneously when the cells suspended in saline were left overnight in the refrigerator or at room temperature. Unlike the agglutination produced by the sera, this spontaneous agglutination was readily dispersed by agitation. The patient's serum produced no agglutination at room temperature or at 37° C. with numerous suspensions of group *O* red cells from *Rh* positive, *Rh* negative, *M*, *N* and *MN* individuals. Non-specific cold agglutinins and both α- and β-agglutinins were, however, present.

It seems probable, as the other workers have concluded, that some change occurred in the red cells rendering them susceptible to an agglutinin present in normal human sera. The nature of this change could not be determined. Presumably the cells encountered by Levine and Katzin were not as sensitized by the change and thus were agglutinated with only those sera (about 15 per cent) which possessed the agglutinin in highest concentration. The agglutinin to which the cells were sensitized appears to be the non-specific cold agglutinin. The patient's cells after agglutination with normal serum were washed with cold saline and heated to 37° C. The supernatant saline obtained by centrifuging this suspension at 37° C. was found to possess cold agglutinins for normal group *O* cells. Complete absorption of the agglutinin in normal serum could not be effected with the patient's cells because of shortage of the cells. There was, however, some positive correlation between the titre of the cold agglutinins in ten sera tested against normal group *O* cells and the titre of the same sera against the patient's cells.

The abnormal sensitivity of the patient's cells to testing sera could have resulted in an incorrect grouping and transfusion of incompatible blood if the cross-matching test had not been performed.

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