Fibrinogenolysis in Joints

THE following observations were made in the course of an investigation into the reasons for the apparent failure of blood to clot when shed into synovial joints.

Growing rabbits, 3–4 months old, were used. Plasma was prepared from citrated blood (two volumes 3·8 per cent sodium citrate to eight of blood). Veterinary nembutal was used as anæsthetic. The rabbit's knee joint was injected with plasma until full (2·0–2·5 ml.). A control specimen was placed in a siliconed tube in a water-bath at 37° C. At pre-set intervals 0·25-ml. samples of fluid were aspirated for fibrinogen estimations. At the end of the experiment the animal was killed by excess nembutal or bleeding. The knee joint was then opened, free fluid collected and clots thoroughly searched for. Any clot found was retained for estimation of fibrin content.

Fibrinogen estimations were carried out in duplicate using King's Nesslerization method[1] modified only by the use of thrombin (Maw's, 10–20 units) to precipitate the fibrin instead of simple recalcification. The duplicate results were averaged. Plasma clots recovered from the joints were washed in saline once, and in distilled water three times, with firm expression of the contained fluid between glass slides at each stage. The fibrin content was estimated by Nesslerization.

The findings in fifteen experiments are exemplified in Fig. 1. The fibrinogen-level falls to zero in 1–1¼ hr. Plasma incubated *in vitro* shows no fall or only a relatively slight drop in fibrinogen-level over the same period.

Recovery of the full volume of plasma injected was not achieved, about half leaking out of the joint during the experiment. In three experiments no clot was found in exploring the joint. Analysis of the residual clot when present in no instance revealed enough fibrin to account for the observed fall of fibrinogen-levels in the volume of fluid recovered, let alone in that injected. In the experiment illustrated 0·33 mgm. fibrin was recovered from the joint as clot. The loss of fibrinogen observed in the volume of fluid recovered was equivalent to 1·29 mgm. fibrin. As fibrinogen was lost almost certainly from a greater volume of plasma than that recovered by aspiration, the total fibrinogen to be accounted for is greater than this figure.

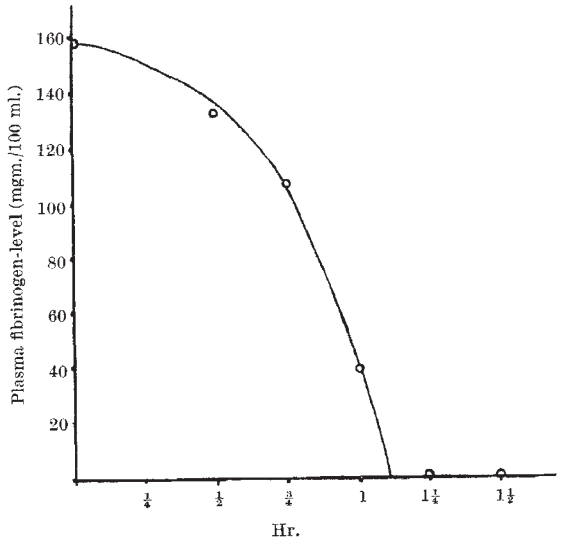

Fig. 1. Fibrinogen-levels of citrated plasma in the knee joint of the rabbit

Although it was difficult to avoid the entry of thrombin and the formation of small clots, it seemed that the greater part of the fibrinogen in the joint was destroyed by lysis.

In further experiments it was found that fibrinogenolysis does not occur in the dead animal; it is much slower in fully grown animals than in the young; it is completely inhibited by heparin (5 units/ml.) and is inhibited by crystalline soya bean trypsin inhibitor at a concentration of 1 mgm./ml., but not at 0·13 mgm./ml.

These experiments suggest that, in contrast to most *in vitro* experiments, *in vivo* conditions may exist in which fibrinogenolysis occurs quite rapidly while, at the same time, fibrinolysis occurs but slowly, if at all. The possibility that fibrinogenolysis is caused by the activation of plasmin is supported by the observed inhibitory effects of heparin and soya bean inhibitor. The presence of plasmin activators in joint tissues has already been reported[2,3].

Intra-articular fibrinogenolysis could be one factor responsible for the partial failure of blood to clot when shed into synovial joints.

A. J. HARROLD

Institute of Orthopaedics,
Royal National Orthopaedic Hospital,
Brockley Hill, Stanmore,
Middlesex.

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HISTOLOGY

Localization of Amino-acids for Histochemical Estimation

SERRA[1] attempted to determine total amino-acids in frozen tissue sections with alloxan and ninhydrin. However, the coloured products diffused rapidly from their original sites so that localization was not possible. Other methods have also been found to be unsatisfactory for the same reason[2,3]. Certain amino-acids have been successfully fixed *in situ*[1,4,5], but these probably cannot be used as a reliable indication of total amino-acids in the tissues.

To circumvent the above difficulties, the following method was developed which prevents diffusion of the acids and their ninhydrin products from their original sites. Kill, dehydrate and fix the tissue with anhydrous, peroxide-free dioxane (1,4-diethylene dioxide). Be sure that the dioxane is entirely peroxide-free because even traces of peroxides depress colour development. Fix the tissue in three changes of dioxane under partial vacuum over a period of 24 hr. or longer, depending upon the size of the piece of tissue. Clear in steps with mixtures of dioxane and chloroform to pure chloroform. Infiltrate with 'Tissuemat'. Section to desired thickness and mount adjacent sections on duplicate slides covered with a thin film of Haupt's adhesive[6,7]. Duplicate slides containing adjacent