

compared with 104 days for haemoglobin F). Haemoglobin A₂ was found to follow a pattern similar to that of haemoglobin A.

These findings suggest that in this patient the haemoglobins A and F tend to be segregated in different populations of red cells. They further indicate that the synthetic rates of these haemoglobins may not be proportional to their concentrations in the peripheral blood.

This study was supported by grants from the U.S. Public Health Service, the John A. Hartford Foundation, and the Medical Foundation, Inc.

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Adsorption of Human and Bovine Thrombin

ALTHOUGH certain metallic compounds are known and used to adsorb prothrombin and factors VII, IX and X from blood, hitherto the effect of these adsorbents on thrombin has not been reported.

The following observations are derived from the experiments presented in Table 1.

Table 1. ADSORPTION OF BOVINE AND HUMAN THROMBIN

Compound used*	Clotting-time of substrate (sec)†		Species adsorbed
	Human thrombin	Bovine thrombin	
Saline (control)	22	22	—
Aluminium hydroxide	99	> 120	both
Aluminium phosphate	43	22	human slightly affected
Aluminium silicate (kaolin)	69	> 120	bovine more than human
Bismuth carbonate	36	120	bovine
Calcium phosphate	21	> 120	bovine
Barium carbonate	22	86	bovine
Barium sulphate	22	22	neither
Magnesium hydroxide	24	26	neither
Magnesium oxide	29	25	neither
Magnesium trisilicate	22	21½	neither
Magnesium carbonate	57	40	both slightly affected
Polyaminostyrene	22	22	neither

* To 10 volumes of thrombin solution (clotting substrate in 20 sec) was added 1 volume of the suspension to be tested. After incubating the mixture for 5 min (with frequent shaking) the compound was precipitated by centrifugation and the thrombin activity of the supernatant tested as follows.

† To 0.4 ml. of the substrate (fibrinogen 200 mg/100 ml.) at 37° C was added 0.1 ml. of the solution tested for thrombin activity, recording the clotting time.

(a) A compound which adsorbs prothrombin is not necessarily a thrombin adsorbent. Barium sulphate is an example of this.

(b) Thrombin exhibits species specificity; the adsorption of the bovine material is different from that of the human reagent.

Further investigations demonstrated that intrinsic thrombin formed in recalcified citrated plasma or blood was similarly affected as shown by the deposition of fibrin on the suspended adsorbent particles and fragmentation of the clot. Accordingly, care should be exercised in choosing the alkali and the species of thrombin used in the control of upper gastrointestinal bleeding.

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PATHOLOGY

Production of Hyaluronic Acid in Tissue Culture of Rous Sarcoma

Rous chicken sarcoma has been known for a long time to produce large quantities of hyaluronic acid¹⁻³. Rous sarcoma homogenates were shown to contain all the enzymes necessary to synthesize hyaluronic acid *in vitro*⁴. No information, however, was available as to whether or not Rous sarcoma cells produce hyaluronic acid in tissue culture as they do in the tumour. Such information may have a bearing on the question of identification of the Rous sarcoma cell. Also it was hoped to find a link between the enormous increase of hyaluronic acid synthesis in cells of a virus-induced tumour and certain aspects of virus-cell interaction, such as the increased synthesis of non-viral RNA in cells of a RNA-virus tumour.

1-2 tenths of a ml. of various dilutions of a standard virus (10^{-1} – 10^{-8}) were injected subcutaneously in the wing web of 5- to 7-day old White Leghorn chicks. At various times after injection chicks were killed and the tumours were removed aseptically, washed, minced with scissors and suspended in an equal volume of saline. After 24 h at 4° C, and after centrifugation a more or less sticky supernatant fluid was obtained, and was tested for the presence of hyaluronic acid. From parts of the tumours cultures were prepared. The glass floor of the culture vessel was coated with a thin layer of heparinized plasma just enough to make the explants stick to the glass and about five tissue fragments per cm² were explanted. After several hours at 37° C the fluid medium was added consisting of Eagle's minimum essential medium with 10 per cent horse serum, 1 per cent glutamine and 5-10 per cent half-diluted embryo extract. One hundred units potassium penicillin and 100 µg dihydrostreptomycin sulphate per ml. were added. Renewal of the medium was carried out three times a week and the used medium of each culture was separately tested for the presence of hyaluronic acid. In most cultures the explants were detached after several feedings, leaving behind continuous monolayer cultures. The mucin clot test, which is the method of choice for testing small samples of native non-purified fluids containing hyaluronic acid, was used⁵⁻⁷. The concentration of hyaluronic acid was estimated by a sample dilution method. Assuming that the lowest concentration of high-polymer hyaluronic acid required for formation of mucin clot is about 30 µg per ml. (ref. 5), multiplication by thirty of the minimal amount of fluid in ml., which prevents formation of mucin clot, allows a rough estimation of the concentration of hyaluronic acid in the original undiluted sample. The supernatant fluids obtained from centrifugation of the minced tumours were mostly sticky and on addition of a few drops of 1 N acetic acid to about 1 ml. sample yielded a tight mucin clot. The formation of the clot could be prevented by hyaluronidase which was obtained from rat testes. The endpoint of dilution which gave a mucin clot on acidification was 1:72 in two tumours; 1:68 in four tumours; 1:40 in four tumours; and 1:23 in one tumour. Since an equal volume of saline was added to the minced tumour for extraction, the highest dilution which gave a positive mucin clot test in the first two sarcomas was 1:144. The concentration of hyaluronic acid in two tumours was thus roughly as high