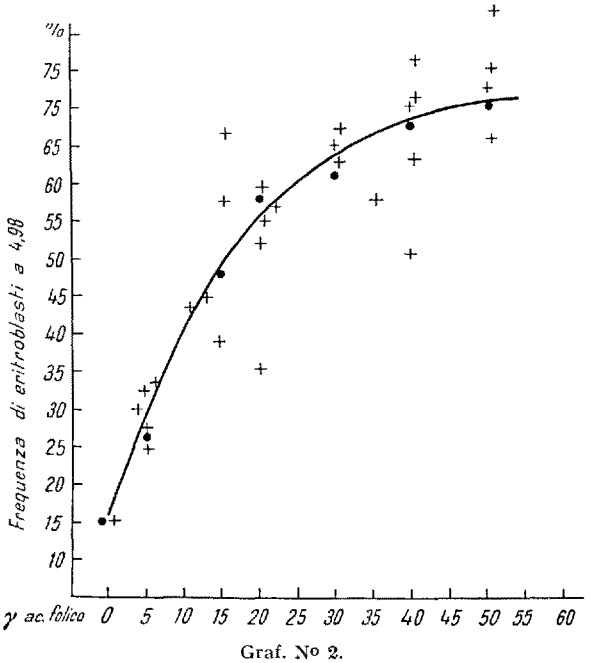
8,30 μ negli embrioni di controllo, in quelli trattati con acido folico è di 4,98 μ.

Tra le dimensioni trovate sperimentalmente in un notevole numero di cellule trattate con sostanze dotate di proprietà antianemica (in particolare tra diametro massimo e diametro minimo, tra media dei diametri citoplasmatici e nucleari, in funzione di varie concentrazioni di acido folico) abbiamo potuto mettere in evidenza un legame analitico algebrico che dal grado e modo di deformazione dei diametri cellulari e nucleari permette di stabilire con buona approssimazione la misura della attività antianemica della sostanza usata. Questa relazione ancora in corso di elaborazione per la trattazione matematica definitiva ci consente già di costruire una curva empirica di taratura usando come sostanza standard una soluzione di acido folico a titolo noto, iniettato sotto forma di folato sodico a p_H 7,6–7,7, a varie concentrazioni.



Graf. N° 2.

Variando le concentrazioni di acido folico, variano pure le percentuali di normoblasti che si trovano nel sangue circolante di embrione di pollo trattato alla 60ª ora di incubazione. Per normoblasti ho considerato le cellule aventi diametri nucleari compresi tra 3,32 e 4,98 μ. Dai dati di molti esperimenti, riportando le percentuali di cellule di questo tipo sulle ordinate e sulle ascisse le quantità di acido folico si ottiene una curva del tipo riportato nella graf. N° 2.

Analogo è l'andamento della curva se al posto della percentuale delle cellule aventi le suddette dimensioni si pone la percentuale delle cellule aventi caratteristiche morfologiche di normoblasti.

È possibile con questa curva empirica determinare con buona approssimazione; facendo la media di almeno cinque esperimenti, la attività antianemica di una sostanza in esame.

Ho limitato la taratura al tratto compreso fra 1 e 50 γ perché al di là di questi limiti i valori sono molto incostanti.

È in corso di elaborazione una modificazione di questo metodo basata sulla trasformazione normoblastica del megaloblasto di embrione di pollo coltivato *in vitro*.

Questo metodo è assai delicato e preciso perché titola frazioni di acido folico, ma presenta particolari difficoltà per la facile emolisi di queste cellule embrionali sensibilissime a qualsiasi variazione osmotica. G. C. PERRI

Istituto di chimica biologica dell'Università di Pavia, il 10 ottobre 1948.

Summary

In the early stages of development chick embryo's blood-cells are megaloblasts. As the author has demonstrated previously, folic acid and hepatic extracts, when injected into fecundated hen's eggs, are able, under certain experimental conditions, to cause a transformation of megaloblastic cells into normoblasts, the author proposes to use this phenomenon as a new test for antianæmic activity.

There is a mathematical-algebraic relation between percentage of normoblasts present in chick-embryo blood (at 60 h of incubation, treated with antianæmic substance) and the concentration of folic acid or hepatic extract, which allows the activity of antianæmic substance to be expressed numerically.

The empirical standard curve of the quantitative relations between percentage of normoblasts found and concentrations of folic acid is reported.

The author finds that it is preferable to calculate the intensity of antianæmic activity from reduction in diameter of the cellular nucleus of chick-embryo blood-cells rather than from their morphological aspect.

Under these experimental conditions many cells are present whose morphological aspect is intermediate between normo and megaloblasts, and therefore classification is uncertain and difficult, while the citometric values of nuclear diameters are clearly reduced to normoblast values.

The standard curve is not substantially modified if the values of cells having a 4·98 micron nuclear diameter are substituted to the percentage of cells having normoblasts' morphological caracter.

Comparative Studies on the Coagulation Process with Heparin and Sea-Urchin Fertilizin

In the course of studies on the jelly coat of the sea-urchin egg, attention was drawn to the analogies in chemical composition of this structure and heparin, the natural inhibitor of blood coagulation. The jelly coat contains fertilizin[1], which is considered essential for the fertilization of the sea-urchin egg. According to TYLER[2] the jelly coat substance and fertilizin are even identical. RUNNSTRÖM[3] endows fertilizin with antienzymatic properties in the process of fertilization membrane development, which he compares with blood coagulation.

A comparison was therefore made between the action of dissolved jelly coat substance and of heparin on the coagulation of pig-blood plasma[4].

A diluted solution of heparin (Vitrum) (0·250 mg p. ml) and jelly coat solution of *Echinus esculentus* L. (1·050 mg p. ml) were subjected to (1) dialysis in a cellophane tube against distilled water, (2) heating in a water-bath for 90 minutes at 100°C, (3) oxidation with about

[1] E. VASSEUR and B. HAGSTRÖM, Ark. Zool. *37 A*, No. 17 (1946).
[2] A. TYLER, Western J. Surg., Obstet., Gynecol. *50*, 126 (1942).
[3] J. RUNNSTRÖM, Proc. VI. Int. Congr. Exptl. Cytol., in press (Stockholm 1947).
[4] J. H. FERGUSON, Ann. N.Y. Acad. Sci. *49*, 486 (1948).

0·04 M $NaIO_4$ in a water-bath for 1,000 minutes at 50°C, (4) hydrolysis with about 0·5 N HCl for 90 minutes at 100°C, (5) and (6) digestion with 0·01 p.c. crystalline trypsin solution for 60 minutes at 25°C, f_H 8·50, (6) followed by heating in a water-bath for 60 minutes at 100°C. After this treatment the samples (2–6) were dialysed against distilled water. The effect of these preparations and of the untreated solutions (0) on the coagulation process is summarized in the figures.

Dialysis had a somewhat diminishing effect, heating and tryptic digestion had no effect, whereas periodate oxidation and acid hydrolysis decreased or destroyed the impeding effect on blood clotting by both heparin and the jelly-coat substance. These studies demonstrate that the jelly-coat substance exerts an antithrombic action which, however, is about twenty times weaker than that of heparin. An inhibitory effect on blood coagulation is also obtained with jelly-coat solutions from other sea-urchin species.

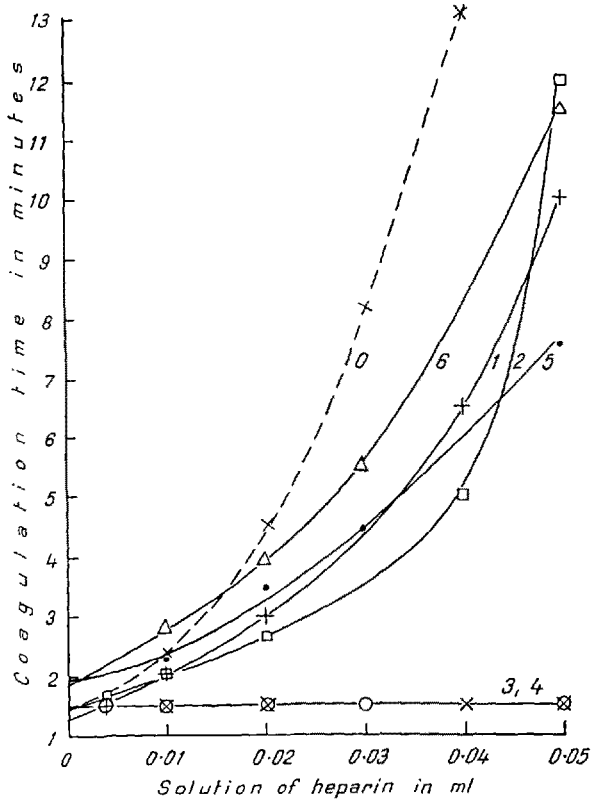


Fig. 1 a. – Influence of heparin (Vitrum) solution (0·250 mg p. ml) on the coagulation of citrated pig plasma.

0 × — — — × untreated solution
1 + ——— + dialysed
2 □ ——— □ heated and dialysed
3 × ——— × oxidized and dialysed
4 o ——— o hydrolysed and dialysed
5 • ——— • digested and dialysed
6 △ ——— △ digested, heated, and dialysed

The chemical composition of the jelly-coat substance is indeed rather similar to heparin. With a refined modification of the orcinol reaction of SÖRENSEN and HAUGAARD the polysaccharide nature[1] has been confirmed. Sulphate determinations bear evidence that the acid groups[1] depend on ester-linked sulphuric acid (cf. VASSEUR[2]). The jelly coat *in situ* presents a typical metachromatic staining with toluidine blue. It is interesting to note that the agglutinating power of the jelly-coat solution of *Echinus esculentus* is only feebly affected by heating and by tryptic digestion, but is not resistant against periodate oxidation and acid hydrolysis.

J. IMMERS and E. VASSEUR

Wenner-Gren's Institute for Experimental Biology, University of Stockholm, Sweden, August 16, 1948.

[1] J. RUNNSTRÖM, A. TISELIUS, and E. VASSEUR, Ark. Kemi Mineral., Geol. *15 A*, No. 16 (1942).

[2] E. VASSEUR, Ark. Kemi, Mineral. Geol. *25 B*, No. 6 (1947).

Zusammenfassung

Es wird gezeigt, daß die Gallerthüllensubstanz des Seeigeleies, die vielleicht mit Fertilizin identisch ist, jedenfalls aber dieses enthält, wie Heparin auf die Koagulation des Blutes hemmend wirkt. Diese Wirkung wird durch Perjodatoxydation sowie durch saure Hydrolyse zerstört, nicht aber durch Erhitzung oder tryptische Spaltung.

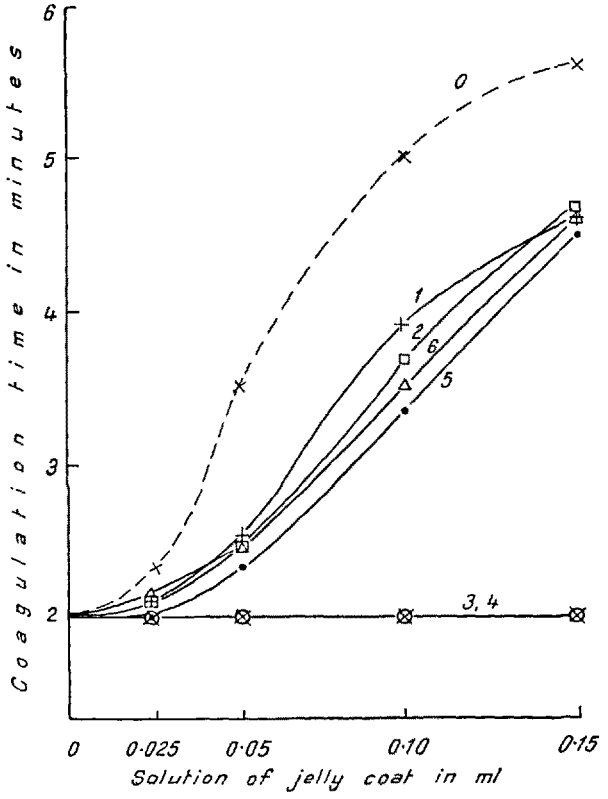


Fig. 1 b. – Influence of jelly-coat solution of *Echinus esculentus* L. (1·050 mg p. ml) on the coagulation of citrated pig plasma.

Über die Nachweisbarkeit des Äthylesters der 3,3'-Dicumarinylessigsäure und des Dicumarols im menschlichen Blut

Eine Erschwerung der Dicumarolbehandlung ist die lange Wirkungsdauer einer einmal gegebenen Dosis. Versuche, die Gründe dieser längeren Wirkungsweise näher zu analysieren, sind uns bisher nicht bekanntgeworden.

Die Einführung von Dicumarolderivaten mit wesentlich kürzerer Wirkungsdauer in die Therapie hat diese Frage in den Vordergrund gestellt. Insbesondere wäre es interessant, die Ursachen dieses unterschiedlichen Verhaltens der Derivate zu erfahren.