22 days after hatching. While these experiments do not furnish evidence for the congenital transmission of the virus in the chicken, it is possible that in a more susceptible species the virus could be carried more effectively through the developmental and hatching period after either experimental or congenital infection.

17003 P

Studies of the "Thrombin" Effect of Fresh Serum.*

RALPH F. JACOX AND ROBERT P. BAYS. (Introduced by W. S. McCann.)

From the University of Rochester School of Medicine and Dentistry, and the Clinic of the Strong Memorial and Rochester Municipal Hospitals, Rochester, N. Y.

A factor capable of producing prothrombin conversion has been described in a previous report.[1] This factor can be easily demonstrated after thromboplastin is added to serum which contains no thrombin and only traces of prothrombin. The resultant mixture of serum and thromboplastin causes rapid coagulation of a 0.01 M oxalated plasma.† Suitable control studies revealed that this factor (designated "prothrombin-converting factor"), is not thrombin, but a substance which requires prothrombin to mediate a coagulation effect on fibrinogen. Furthermore, the coagulation of 0.01 M oxalated plasma by the serum-thromboplastin mixture could not be explained as a separate action of either component.

A study has been made of the coagulation effect of *fresh serum alone* on 0.01 M oxalated plasma. It has been assumed by other workers,[2,3] that the residual clotting action of fresh serum is related to thrombin. After conversion of fibrinogen to fibrin, thrombin was believed to combine with albumin to form inactive metathrombin,[2] or that thrombin was destroyed by an enzyme.[3] Our results reveal that the residual coagulating action of fresh serum is almost entirely due to "prothrombin-converting factor". This data confirms and extends the observations of Bordet and Gengou[4] who showed that serum coagulated whole oxalated plasma much more rapidly than it did oxalated plasma from which prothrombin was removed by adsorption with tricalcium phosphate.

The coagulation effect of fresh serum was studied by the following methods. Blood was withdrawn from human donors and placed in glass tubes with internal dimensions of 1.0 x 10.0 cm. Ordinary care was used in collecting the specimens to avoid contamination with tissue thromboplastic substances. Within 15 minutes of collection, the tubes containing the blood were placed in a 26°C water bath. Approximately 50 minutes later the serum was separated from the clot. One-tenth cubic centimeter of serum (kept at 26°C) was pipetted into 0.1 cc of a 0.01 M oxalated plasma and the coagulation time determined.

It was observed that serum, which coagulated plasma rapidly when tested within 70 minutes of the time of collection from the donor, was incapable of coagulating a buffered‡ fibrinogen solution (200-300 mg %

* Research was carried out under a grant for the study of Rheumatic Fever made by the Masonic Foundation for Medical Research and Human Welfare.

1 Jacox, Ralph F., to be published in *J. Clin. Invest.*

† All plasma used in these experiments was prepared by adding 9.0 cc of whole blood to 1.0 cc of 0.1 M potassium oxalate solution.

2 Quick, A. J., *Am. J. Physiol.*, 1938, **123**, 712.

3 Glazko, A. J., and Ferguson, J. H., *J. Gen. Physiol.*, 1940, **24**, 169.

4 Bordet, J., and Gengou, O., *Ann. Inst. Pasteur*, 1904, **18**, 98.

‡ Collidine buffer (pH 7.3) was used in all experiments.[5]

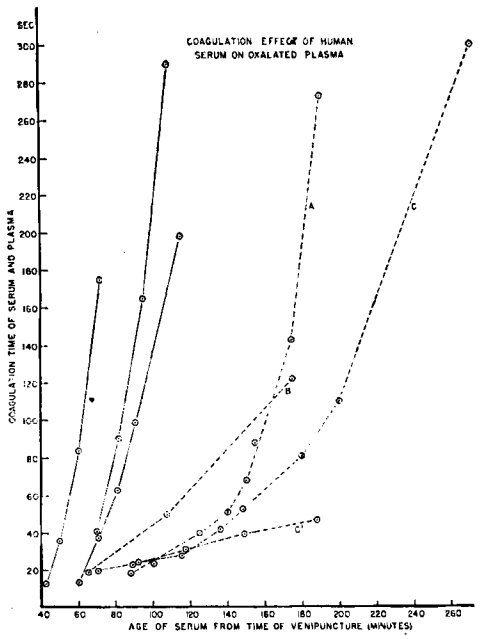

FIG. 1.

Human serum was added to 0.01 M oxalated plasma at designated intervals (abscissa). The coagulation time of serum and plasma is recorded on the ordinate. Normal serum is depicted by unbroken lines; serum from patients receiving dicumarol by broken lines. C1 serum (obtained from a patient receiving dicumarol with prothrombin less than 20 per cent of normal) had the most prolonged coagulation effect for oxalated plasma.

concentration). The fibrinogen solution§ and the plasma were equally reactive to thrombin since each coagulated with the same speed when bovine thrombin (Upjohn Co.) was added. Fig. 1 demonstrates the coagulating effect of serum when it is added to oxalated plasma. Immediately after separation of serum from the clot, coagulation of oxalated plasma was prompt (12-40 sec.). The coagulation effect of serum from normal individuals rapidly disappeared until no clotting activity remained 100-120 minutes after the time of venipuncture. Serum obtained from patients receiving dicumarol (Fig. 1—broken line) had a significantly decreased rate of degradation of the coagulating substance. As the plasma prothrombin decreased, the "prothrombin-converting factor" rate of degradation was proportionately decreased. These results are in accord with a previous observation[1] that thromboplastin activation of serum from patients receiving dicumarol, produced a slowly decaying action of the freed "prothrombin-converting factor".

Since the degradation rate of "prothrombin-converting factor" in sera of dicumarolized subjects proceeds slowly, it has been possible to obtain sera which had little loss of coagulation effect for 0.01 M oxalated plasma, 30-60 minutes from the time of separation from clotted whole blood. Such a serum was prepared by withdrawing blood from rabbits who had been given large amounts of dicumarol 24-28 hours before collection of the serum. This serum was utilized to produce the results shown in Fig. 2. The active, slowly degrading rabbit serum was added to 0.01 M oxalated

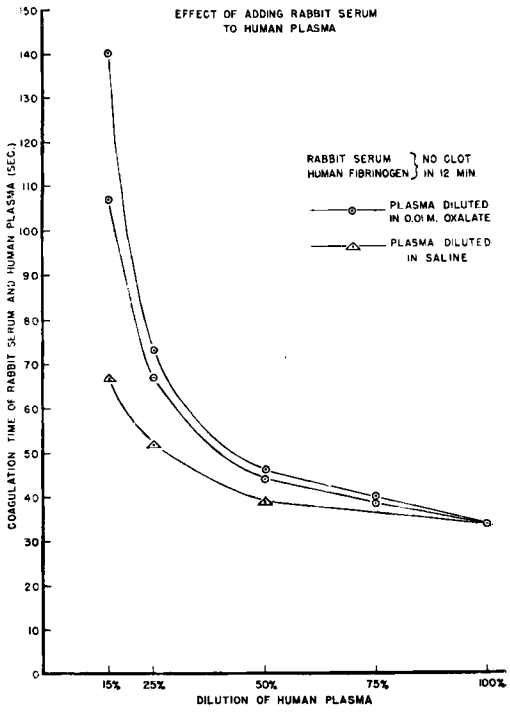

FIG. 2.

Serum obtained from a dicumarolized rabbit was added to varying dilutions of two normal human plasmas (upper 2 curves). The coagulating effect of rabbit serum is dependent upon prothrombin (fibrinogen did not clot in 12 minutes). The lower curve represents dilution of one of the two plasmas in saline rather than 0.01 M potassium oxalate, following which rabbit serum was added as described.

[5] Gomori, G., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 33.

§ Fibrinogen was supplied through the generosity of Armour and Company.

human plasma and to dilutions of plasma made in 0.01 M oxalated 0.9% sodium chloride solution. The coagulation time for each dilution of plasma was then determined after addition of active rabbit serum. It will be observed (Fig. 2) that the activity of the "prothrombin-converting factor" in rabbit serum is dependent upon prothrombin concentration of the plasma. A curve can therefore be constructed (Fig. 2) which resembles the reaction curve obtained in the one stage prothrobin test of Quick.[6] A similar curve can be obtained by substitution of rabbit for human plasma. This suggests that rabbit and human plasma contain nearly identical amounts of prothrombin.

It will be observed furthermore (Fig. 2), that plasma diluted in 0.9% sodium chloride solution rather than 0.01 M oxalated sodium chloride solution, produced faster coagulation in the diluted fractions when the active serum was added. This suggests that the "prothrombin-converting factor" may be partially inhibited by the oxalate ion (calcium effect?) or that "prothrombin-converting factor" may be auto-catalytically activated from plasma.

Discussion. The residual coagulating power of serum, after complete coagulation of whole blood has taken place, is not related to thrombin but rather to a "prothrombin converting factor". It is assumed that this factor is initially activated through the action of plasma thromboplastin and platelets. The "prothrombin-converting factor" then causes thrombin production by reacting with prothrombin. The thrombin quickly disappears after fibrin is formed, whereas the "prothrombin-converting factor" can be easily measured in the serum for at least 100 minutes from the time of venipuncture. In sera of patients who receive dicumarol, the "prothrombin-converting factor" effect is greatly prolonged over that observed in normal serum.

An accurate analysis of plasma prothrombin concentration can be made by use of a relatively stable "prothrombin-converting factor" obtained from rabbits receiving dicumarol. This technic of assay reproduced results obtained with the one stage determination of prothrombin by Quick's method.[6]

Owren,[7] who described factor VI (which we believe to be the same as "prothrombin-converting factor"), has reasonably credited Bordet and Gengou[4] with the first demonstration of this forgotten concept in blood coagulation.

[6] Quick, A. J., *The Hemorrhagic Diseases and Physiology of Hemostasis* (Thomas, 1942).

[7] Owren, P., *Acta Med. Scand.*, 1947, Supp. 194.

17004

Evaluation of Dubos' Solid Medium Containing Penicillin in the Isolation of Tubercle Bacilli.

J. W. SMITH, J. HUMISTON, W. P. CREGER, AND W. M. M. KIRBY.
(Introduced by A. L. Bloomfield.)
From the Department of Medicine, Stanford University School of Medicine, San Francisco, California.

Following the observation by Dubos and Davis that a liquid medium containing Tween 80 would allow rapid, submerged growth of mammalian tubercle bacilli,[1] the diagnostic potentialities of this medium have been explored in many laboratories.[2,3] The results have in general been favorable, but two disadvantages have become apparent. First, no

[1] Dubos, R. J., and Davis, B. D., *J. Exp. Med.*, 1946, **83**, 409.

[2] Foley, G. E., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 298.

[3] Foley, G. E., *J. Lab. and Clin. Med.*, 1947, **32**, 842.