not demonstrated to possess any antienteroviral substances.

Two beneficial characteristics of albumin were evident during its use in applied, or diagnostic, virology. First, the tendency toward earlier appearance of cytopathic changes observed when EAS was used, as compared with M150, may well be attributable to the relatively high pH (about 8.5) which characterizes EA and which the latter imparts to EAS. That acid reaction in tissue culture medium exerted an adverse effect on cytopathogenicity of certain viruses ("pH effect") and that this could be overcome by neutralization of the acid was pointed out by Barron and Karzon (5). Secondly, the antibacterial factor in EA is a particular advantage since bacterial contaminants occasionally escape preliminary centrifugation and antibiotics to which fecal specimens are subjected. It was obvious that the EAS medium was able at least to suppress multiplication of 2 bacterial agents in isolation cultures, as described, although 2 commonly used antibiotics were unable to do so. The active agent in albumin may be lysozyme (6).

Ability of EAS to serve as substitute for calf serum-balanced salt solution in agar overlay used for plaque formation was advantageous; so also was survival of its attributes following lyophilization and reconstitution.

Summary. Albumin from domestic hen's egg can be simply adapted for use as tissue culture medium for certain purposes. Combined with physiological saline it supports outgrowth of secondary cultures of monkey and rabbit kidney cells; it also sustains grown out cultures of fresh tissue and established cell lines for 2 weeks. Albumin possesses antibacterial properties, but no demonstrable antienteroviral activity. It can be lyophilized and reconstituted without loss of attributes.

1. Reeves, W. C., Sturgeon, J. M., French, E. M., Brookman, B., *J. Inf. Dis.*, 1954, v95, 168.

2. Kissling, R. E., Eidson, M. E., Stamm, D. D., *ibid.*, 1954, v95, 179.

3. Jukes, T. H., Fraser, D. T., Orr, M. D., *J. Immunol.*, 1934, v26, 353.

4. Morgan, J. F., Campbell, M. E., Morton, H. J., *J. Nat. Cancer Inst.*, 1955, v16, 557.

5. Barron, A. L., Karzon, D. T., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 393.

6. Gottschalk, A. Lind, P. E., *Brit. J. Exp. Path.*, 1949, v30, 85.

Received May 9, 1960. P.S.E.B.M., 1960, v104.

Clearance of Blood Coagulation Product I in Rabbits.* (25888)

THEODORE H. SPAET AND JOSE CINTRON
Dept. of Hematology, Montefiore Hospital, N. Y. City

The hypothesis has been proposed that blood fluidity is preserved *in vivo* largely by cellular clearance of clotting intermediates (1). Data are reported which suggest that blood thromboplastin is removed from circulation by reticulo-endothelial cells(2). In the present study evidence is presented indicating that coagulation intermediate product I, a precursor of blood thromboplastin(3), disappears from blood of intact rabbits more rapidly than could be explained by action of circulating anticoagulants.

* Study supported by grants from Nat. Heart Inst., NIH.

Materials and methods. Experimental animals were male New Zealand rabbits weighing about 3 kg. Blood for preparation of product I was collected into uncoated glass tubes from central artery of ear. Plasma was obtained from blood collected into 1/10th volume of 3.8% sodium citrate, and aliquots were adsorbed with $Al(OH)_3$ as described by Pool and Robinson(4). Serum was prepared from blood incubated 1 hour after clotting at 37°C. Crude "cephalin" was made from human brain by method of Bell and Alton(5). Product I was prepared by mixing equal volumes of 0.025 M $CaCl_2$, undiluted serum, and

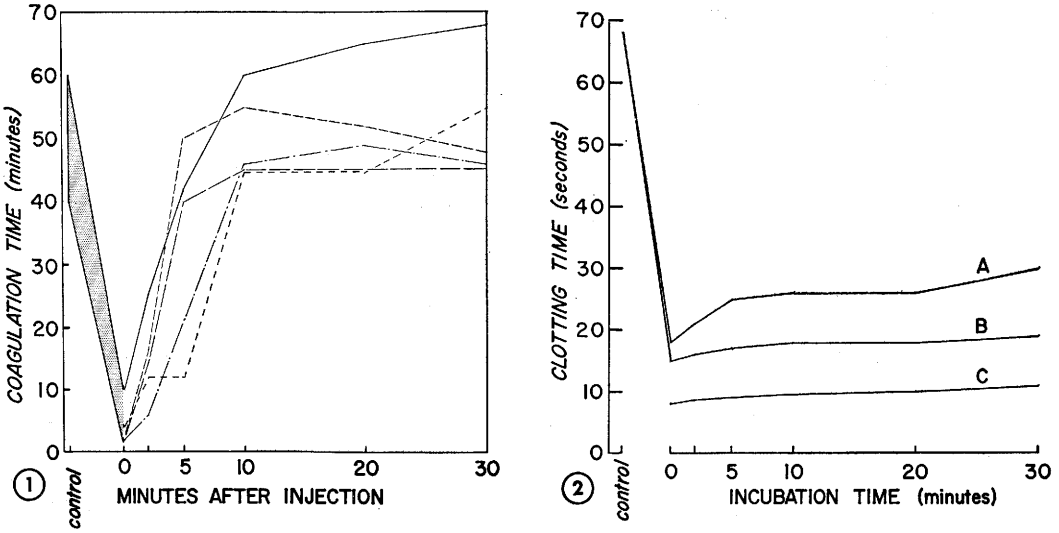

FIG. 1. Plastic tube clotting times following intrav. product I.
FIG. 2. *In vitro* inactivation of product I. (A) Diluted with 9 vol of plasma. (B) Diluted with 4 vol of plasma. (C) Undiluted. Control is recalcified clotting time plasma with optimal cephalin.

undiluted adsorbed plasma. This mixture was incubated at 37°C until full activity had developed and stabilized for at least 5 minutes. Product I activity was estimated by adding 0.1 cc of mixture to 0.1 cc of 0.025 M $CaCl_2$. To this was then added 0.1 cc of unadsorbed substrate plasma enriched with optimal concentration of cephalin, and clotting time determined. Optimal product I activity developed in about 10-15 minutes, causing substrate plasma to clot in 7-10 seconds. Times obtained with cephalin-free substrate plasma were 40 seconds or longer. Product I generating mixture clotted in about 7-10 minutes, and this clot was removed. Plastic tube clotting times were accomplished on blood drawn from right ventricle catheterized *via* right jugular vein as previously described(6). The effect of intravenous product I on coagulation time of rabbits was determined as follows: 20 cc of product I were given into marginal vein of left ear in about one minute. Clotting times were obtained before infusion, during administration of final few cc, and at intervals after completion of injection as indicated below. Additional blood was obtained through tubing for further clotting studies prior to injection and upon completion of experiment. *In vitro* inactivation of product I was estimated by diluting this reagent with various volumes of untreated plasma as indicated below. These mixtures were incubated at 37°C and aliquots repeatedly tested for product I activity as described above.

Results. The effect of intravenous product I on plastic tube clotting times in rabbits is shown in Fig. 1. There is striking acceleration of clotting during infusion, but this "hypercoagulable" state is rapidly reversed. Within 5-10 minutes after completion of infusion, the original clotting time is almost completely restored. Rapid administration of these large volumes of highly potent coagulant failed to cause detectable changes in prothrombin time, fibrinogen or thromboplastin generation, and reduction of platelets was modest: no *in vivo* defibrination was evident (Table I).

In vitro dilution of product I with plasma,

TABLE I. Coagulation Changes Following Product I Infusion (Mean Values).

	Before	After
Prothrombin (Quick) (sec.)	8.3	8.8
" (Ware & Stragnell) (sec.)	25	27
Fibrinogen (mg/100 cc)	240	225
Thromboplastin generation test (substrate time in sec. at 4 min. incubation)	8	8

comparable to that achieved *in vivo,* was followed by considerably less product I inactivation (Fig. 2). Loss of product I activity was slow, and even after 30 minutes considerable activity remained. These findings are in conformity with relative stability of product I reported by others(7).

Discussion. Formation of blood thromboplastin is evidently a multi-step reaction. Although the term "product I" is not entirely apt, and its nature is poorly defined, this intermediate can be partially characterized. Its formation depends upon previous activation of a plasma "contact factor" (Hageman factor) and presence of at least antihemophilic factor, Stuart factor, plasma thromboplastin component and Ca^{++}(7). Product I subsequently reacts with phosphatide and factor V to form blood thromboplastin(8). Unpublished studies by Dr. H. Horowitz of this laboratory indicate that reactions leading to formation of product I are slow, but that product I reacts with phosphatide and factor V almost instantaneously to form blood thromboplastin. The slow *in vitro* inactivation of product I here demonstrated is inadequate to account for the rapid restoration of plastic tube clotting time and it may be presumed that its removal from circulation of experimental animals represents a process involving clearance during passage through various organs. Although it appears unlikely that product I rapidly equilibrates with the entire extracellular fluid space, even this degree of dilution would not account for the loss of *in vivo* activity. The present data are not sufficient to suggest which cells or organs are responsible for product I clearance. In previous studies(1) intravenous administration of product I was not followed by increase in circulating anticoagulants. It therefore seems likely that product I is removed rather than neutralized. Rapid removal of product I from circulating blood appears an important component of hemostatic homeostasis, since its prolonged presence would lead to generalized clotting.

Summary. Removal of blood coagulation product I was studied in rabbits. Intravenous injection of this reagent markedly shortened plastic tube clotting time, but restoration to normal occurred in a few minutes. There was no evidence of defibrination. Mixed with plasma *in vitro,* product I was inactivated slowly. It is suggested that product I is removed from the circulation as blood passes through various organs.

1. Spaet, T. H., Kropatkin, M., *Am. J. Physiol.,* 1958, v195, 77.

2. Spaet, T. H., Horowitz, H. I., Zucker-Franklin, D. *J. Clin. Invest.,* 1960, v39, 1029.

3. Bergsagel, D. E., Hougie, C., *Brit. J. Haematol.,* 1956, v2, 113.

4. Pool, J., Robinson, J., *ibid.,* 1959, v5, 17.

5. Bell, W. N., Alton, H. G., *Nature,* 1954, v174, 880.

6. Spaet, T. H., Cintron, J., Kropatkin, M., *J. Lab. & Clin. Med.,* 1959, v54, 467.

7. Fisch, U., Duckert, F., *Thromb. Diath. Haem.,* 1959, v3, 98.

8. Hougie, C., *J. Lab. and Clin. Med.,* 1957, v50, 61.

Received May 17, 1960. P.S.E.B.M., 1960, v104.

Experimental Epilepsy in the Mouse.* (25889)

LENORE M. KOPELOFF (Introduced by Warren M. Sperry)
Dept. of Bacteriology, N. Y. State Psychiatric Inst., N. Y. City

Previous studies demonstrated that a chronic epileptic state may be induced in the monkey (*Macaca mulatta*) by topical or intracerebral application of alumina cream to precentral motor cortex(1,2). Recently, certain pure metals implanted as pellets(3) or powders have also shown epileptogenic properties. Features of monkey epilepsy include spontaneous and induced clinical seizures, lowered thresholds to convulsant drugs(4,5), characteristic electroencephalographic abnor-

* Aided in part by Grant from NINDB, USPHS.