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The Components of Prothrombin.

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The author[1] presented evidence that prothrombin appears to be composed of calcium and 2 components which he designated as A and B. It was postulated that the 2 components were linked through calcium and that removal of the latter element caused the complex to split with the liberation of the 2 components. Thus, component A in undecalcified or native plasma disappears very slowly when stored, in contrast to its instability in oxalated plasma. Furthermore, component B which is promptly removed with aluminum hydroxide from oxalated plasma, is not effectively adsorbed by this agent in heparinized plasma which has not been decalcified.

In this study the stability of component A in native (undecalcified) hemophilic plasma was demonstrated. (Table I). The keeping quality of component A does not appear, however, to be as good in human as in avian blood.

TABLE I.
Effect of Storage* on the Prothrombin Time of Native and Oxalated Hemophilic Plasma.

Age of plasma, hrs	Prothrombin time in seconds	
	Native plasma†	Oxalated plasma
0	11½	11½
24	12½	15
48	12½	18

* The centrifuged blood was kept in celluloid test tubes and stored in a refrigerator.

† The prothrombin time was determined by adding 0.1 cc saline solution and 0.1 cc thromboplastin to 0.1 cc of native plasma.

Since native hemophilic plasma is not coagulated when aluminum hydroxide is added, as occurs in normal plasma, the adsorption of component B by this agent could be studied without the use of heparin, which was used in the original investigation. On mixing 2 cc of hemophilic plasma (without the removal of the calcium) with 0.1 cc of aluminum hydroxide cream, and incubating for 10 minutes at 37°C, component B was completely removed as indicated by the fact that on treating 0.1 cc of this plasma with 0.1 cc of a highly potent thromboplastin, no coagulation occurred. It may be concluded that component B can be adsorbed by aluminum hydroxide from native plasma, and that the inability of aluminum hydroxide to absorb it from heparinized plasma is apparently caused by an alteration of component B by heparin and is not due to a firm union of the component in the prothrombin complex as the writer formerly believed.

In order to test whether the fibrinogen is affected in stored plasma as Lavergne and Lavergne-Poindessault[2] have claimed, the following experiment was carried out:

Three cc of oxalated rabbit plasma was mixed with 0.2 cc of aluminum hydroxide cream, incubated at 37°C for 10 minutes, and then centrifuged. The clear plasma was treated with 0.01 cc of thrombin prepared according to the author's method.[3] After 10 minutes the fibrin was removed. The complete removal of the fibrinogen was shown by the fact that on mixing 0.1 cc of the plasma with 0.1 cc of thrombin, no further coagulation occurred. To permit neutralization of the small amount of added thrombin by the natural antithrombin, the plasma was allowed to stand 30 minutes. This plasma is designated as defibrinated alumina plasma. It contains neither fibrinogen nor component B.

Human oxalated plasma was stored in a

1 Quick, A. J., *Am. J. Physiol.*, 1943, **140**, 212.

2 Lavergne, G. H., and Lavergne-Poindessault, B., *C. R. Soc. de biol.*, 1942, **136**, 445.

3 Quick, A. J., *Am. J. Physiol.*, 1936, **115**, 317.

refrigerator for 7 days. During that period the prothrombin time increased from 12 to 28 seconds. On adding 1 part of defibrinated alumina plasma to 3 parts of this stored human plasma, a mixture was obtained which had a prothrombin time of 10 seconds. Since the only reacting fibrinogen was supplied by the stored plasma, the striking decrease of the prothrombin time brought about by the addition of alumina plasma to stored plasma must have been due to a specific factor which is not related to fibrinogen and which for the sake of simplicity has been named component A.

In this study the procedures which were employed have been previously described.[1]

Summary. 1. Undecalcified hemophilic plasma shows markedly less loss of prothrombin activity on storage than does oxalated plasma. 2. Aluminum hydroxide removes component B from native hemophilic plasma. 3. The prolonged prothrombin time in stored human plasma is strikingly shortened by the addition of defibrinated plasma treated with aluminum hydroxide. This proves that the delayed prothrombin time in preserved plasma is not due to an alteration of the fibrinogen.

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Effects of Injections of Equine Gonadotrophin upon the Gonads and Adrenals of Fetal Rats.*

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Using a method recently described,[1] fetuses of the Sprague-Dawley strain of rats were transferred to the abdominal cavity of the mother and subjected to subcutaneous injections of equine gonadotrophin. Thirty-two fetuses received injections twice daily and about 12 hours apart. Near term, the 16 that had survived were secured by severing the umbilical cord. Fourteen and certain littermate controls were immediately autopsied, while 2 were placed in the nest of a foster mother. Although one was promptly destroyed by the mother, the other one received 3 additional injections before it was sacrificed by decapitation (Fetus 20, Table I). Litter 112 (Fetuses 35 and 36) differs from others in that the mother received subcutaneous injections of progesterone for the purpose of delaying parturition. Injections, each containing 1.0 mg, were made twice daily on days 20, 21 and 22 of pregnancy. In all cases, the procedure at autopsy was to open the abdominal cavity before placing the specimen in Bouin's fixing fluid. After fixation, the lumbar and pelvic regions of the body were sectioned serially and the sections stained with hematoxylin and eosin. In some instances, certain sections of the series were segregated for special staining (Dominici's, Mallory's triple and Heidenhain's iron hematoxylin).

When sections of testes were examined with the aid of a micro-comparator, an instrument which brings half the field of each of 2 microscopes into view, it was found that the interstitial cells of Leydig of each injected male were larger and more abundant than those of litter-mate controls (Fig. 1 to 4). They were most abundant in the 2 fetuses which received the most hormone (3 and 5, Table I).

Although it is reasonable to suppose that the interstitial cells secreted androgen more rapidly than normally (?), evidence that they actually did could not be detected by inspecting serial sections of such developing

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1 Wells, L. J., *Anat. Rec.*, 1946, **94**, 530.