# OBSERVATIONS ON THE COAGULATION ANOMALY IN VITAMIN K-DEFICIENCY AND DICUMAROL POISONING

by

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QUICK[1] (1947) has suggested that the coagulation factor lacking in the plasma of animals poisoned by dicumarol* might be different from that lacking in cases of vitamin K-deficiency. However, mixtures of the two abnormal plasmas did not exhibit normal coagulation (DAM[2], 1948). We have continued the investigation of this problem by the simple means of mixing plasmas in various proportions and determining the clotting time (according to LARSEN AND PLUM[3], 1941) after addition of chicken brain thromboplastin.

The results indicate that in addition to the lack of a common factor in both kinds of plasma there also seems to be involved to some extent the lack of second factor in vitamin K deficiency and of a third in dicumarol poisoning.

## EXPERIMENTAL

The experiments were carried out with chicks.

Normal controls were given a commercial chicken ration from the day of hatching.

Vitamin K-deficiency was developed by means of the following diet.

### Diet 67 B

| | |
|---|---|
| Dried pancreas (waste product from insulin manufacture) extracted with gasoline | 200 |
| Dried brewer's yeast, ether extracted | 70 |
| Sucrose | 670 |
| Salt mixture** | 40 |
| Cod liver oil | 20 |
| | 1000 |

+ 25 mg *d, l*-α-tocopherol acetate (Ephynal acetate, "Roche") per 1000 g diet.

Dicumarol poisoning was produced by giving tablets of the substance (Synparin, "Ferrosan") orally to chicks receiving the commercial diet. An amount, varying from 0.3 to 0.6 mg daily per g body weight, was given for several days in succession.

Several samples of 0.2 ml blood were taken from the carotid artery by means of needle and syringe, the latter containing 0.4 ml of a 0.3% solution of sodium citrate

* 3.3′-Methylene-bis(4-hydroxycoumarin).

** MCCOLLUM's Salt mixture no. 185 supplemented with 13.5 mg KI, 139 mg $CuSO_4$, $5H_2O$, 556 mg $MnSO_4 \cdot 4H_2O$ per 100 g.

*References p. 413.*

($2H_2O$). The citrated blood was chilled in icewater, centrifuged, and the plasma (kept on ice) used for preparing mixtures with other plasmas obtained in the same way.

Concentrated thromboplastin was prepared from brains of vitamin K-deficient chicks according to the technique described by DAM AND GLAVIND[4] (1938) and stored in frozen state. Before use the concentrated thromboplastin was liquefied by gentle heating and dilutions 1 : 10 were made with 0.9% NaCl. To 4 ml of this diluted thromboplastin 1.25 ml 1.94% $CaCl_2$, $2H_2O$ were added. For the determinations 0.6 ml of the plasma citrate mixture was placed in a centrifuge tube in a waterbath at 37° C and 0.21 ml of the preheated thromboplastin-calcium chloride mixture added. The tube was agitated gently once every 2 seconds until clotting was observed.

## RESULTS

The results are presented in Figs 1–7.

Each curve represents the clotting times for mixtures of plasmas obtained from two chicks.

Ordinate: Clotting time in seconds

Abscissa: Ratio between the two components of the mixtures

K = Plasma of vitamin K-deficient chick

D = Plasma of chick poisoned by dicumarol

N = Plasma of normal chick

KD = Plasma of vitamin K-deficient chick poisoned by dicumarol

Indices 1, 2, 3 etc. refer to individual chicks.

## DISCUSSION

It is apparent that in mixtures of 2 plasmas of the same kind (normal — normal; K-deficient — K-deficient; dicumarol — dicumarol) the clotting times never decline below that of the most rapidly clotting plasma. The same applies to mixtures of vitamin K-deficient and normal plasma and to mixtures of dicumarol plasma and normal plasma. Contrary to this, mixtures of dicumarol plasma and plasma of vitamin K-deficient animals show a decline of clotting time to a point somewhat lower than the clotting time of the most rapidly clotting component, provided that the difference in clotting time of the two plasmas is not too great. In no case the clotting time of the mixtures will become normal. These observations are most easily explained by the assumption that both plasmas (dicumarol plasma and plasma of the vitamin K-deficient animal) are primarily lacking in one and the same component, whereas in addition to this, dicumarol plasma is lacking in another component and vitamin K-deficient plasma in a third.

In cases where a vitamin K-deficient animal was poisoned with dicumarol the mixtures of such a plasma with plasma from a vitamin K-deficient animal or an animal poisoned by dicumarol showed no depression of the clotting time below the lower endpoint. This is in agreement with the assumption set forth above, since in these cases the component which is assumed to be present in vitamin K-deficient plasma but not in dicumarol plasma and vice versa is now eliminated.

From previous investigations it seems most likely that the component lacking in both of the plasmas is the "classical prothrombin", whereas the possible identity of the

*References p. 413.*

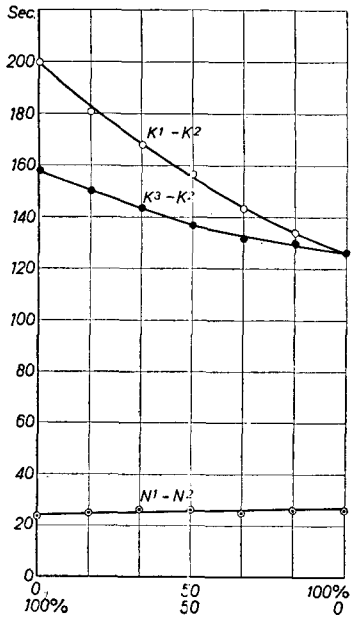



Fig. 1. Pairs of plasmas of identical types with no or only moderate difference between the endpoints

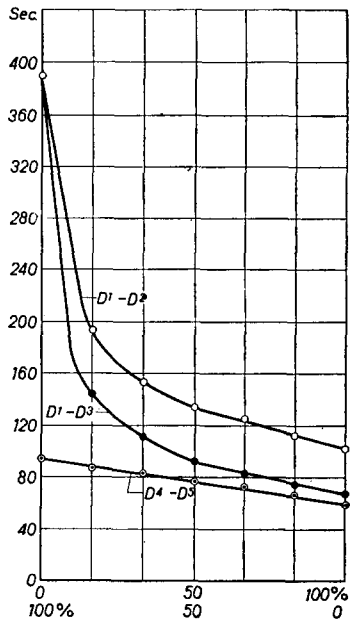



Fig. 2. Pairs of dicumarol plasmas with varying degree of difference between the endpoints

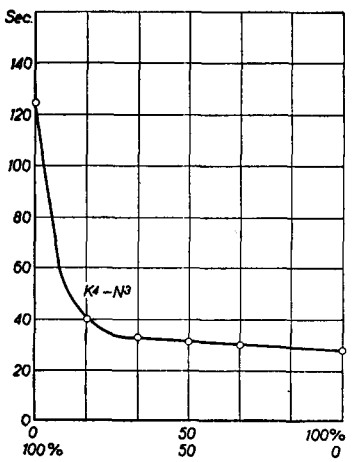



Fig. 3. Mixtures of vitamin K-deficient and normal plasma

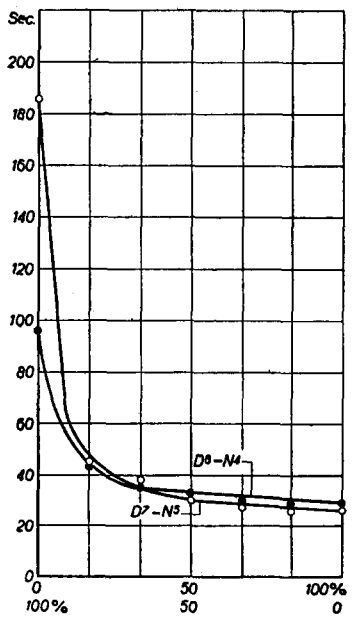
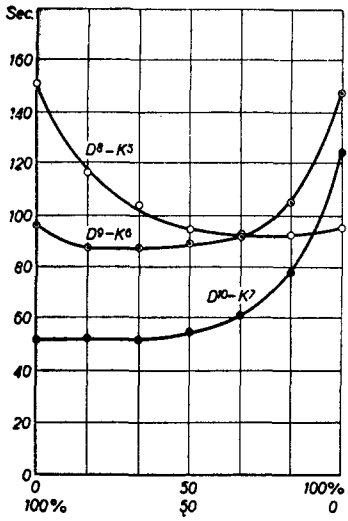



Fig. 4. Mixtures of dicumarol plasma and normal plasma

*References p. 413.*



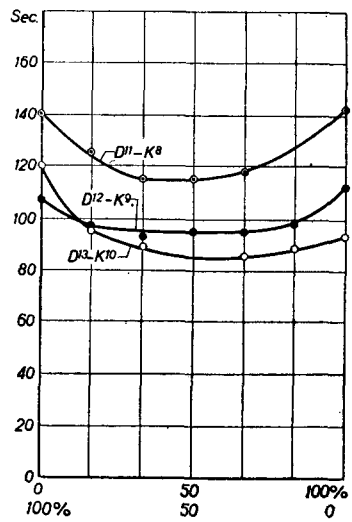




Fig. 5 and 6. Mixtures of dicumarol plasma and vitamin K-deficient plasmas

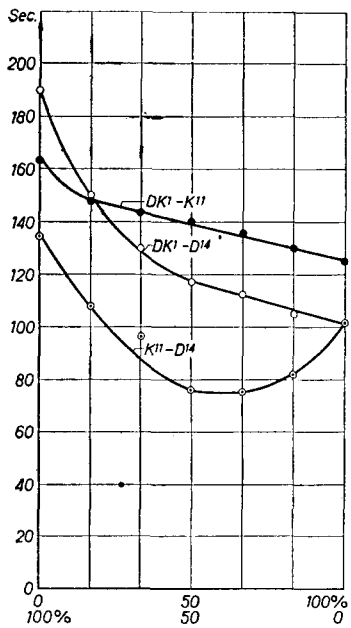



Fig. 7. Mixtures of plasma of vitamin K-deficient chicks poisoned by dicumarol with vitamin K-deficient and dicumarol plasma respectively, compared with mixtures of vitamin K-deficient and dicumarol plasma

2 other factors with any of those recently announced (OWREN[5], 1947, QUICK[1], 1947, WARE *et al.*[6], 1947) remains to be established.

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*References p. 413.*

## SUMMARY

Clotting times of mixtures in varying proportions of plasmas of vitamin K-deficient chicks and chicks poisoned by dicumarol have been determined by the technique of LARSEN AND PLUM. Provided the difference between the clotting times of the 2 separate plasmas is not too great, mixtures in certain proportions will clot somewhat faster than does the most rapidly clotting of the 2 plasmas, but never as rapidly as normal plasma. This is explained by assuming that the two plasmas are primarily lacking in one and the same component, whereas in addition to this vitamin K-deficient plasma is lacking in another component and dicumarol-plasma in a third.

## RÉSUMÉ

Détermination, par la méthode de LARSEN ET PLUM, de la vitesse de coagulation de mélanges en proportion variable de plasmas de poulets carencés en vitamine K, et de poulets intoxiqués par le dicumarol. A condition que la différence entre les vitesses de coagulation de chacun des deux plasmas ne soit pas trop élevée, leurs mélanges dans certaines proportions coagulent un peu plus vite que celui des deux plasmas qui coagule le plus rapidement; mais ces mélanges ne coagulent jamais aussi vite que le plasma normal. Pour expliquer ce fait, on peut supposer que les deux plasmas manquent fondamentalement d'un seul et même constituant, et que en plus, le plasma carencé en vitamine K manque d'un second constituant, et le plasma des animaux intoxiqués par le dicumarol manque d'un troisième.

## ZUSAMMENFASSUNG

Plasma von Hühnern mit Vitamin K-Mangel und Plasma von mit Dicumarol vergifteten Hühnern wurden in verschiedenen Verhältnissen gemischt, und die Gerinnungszeiten nach der Methode von LARSEN UND PLUM bestimmt. Unter der Voraussetzung, dass die Gerinnungszeiten der beiden reinen Plasmen sich nicht zu sehr unterscheiden, werden gewisse Gemische von ihnen etwas schneller gerinnen als das schnellstgerinnende der beiden Plasmen, jedoch niemals so schnell wie normales Plasma. Dies wird durch die Annahme erklärt, dass beiden Plasmen primär eine und dieselbe Komponente fehlt, während Plasma mit Vitamin K-Mangel noch eine weitere, und Dicumarolplasma eine dritte Komponente abgeht.


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