was shaken at 30° with 2.5 mg bacterial powder suspended in 2.5 ml of 0.2 m phosphate buffer at pH 6.0.

My sincere thanks are due to Dr. Rune Grubb for assistance in culturing the bacteria.

- Gale, E. T., and Epps. H. M. R. Biochem. J. 38 (1944) 232.
- Zittle, Z. D., and Eldred, N. R. J. Biol. Chem. 156 (1944) 401.
- Bergström, S., and Lindstedt, S. Arch. Biochem. 26 (1950) 323.
- Bergström, S., and Lindstedt, S. Acta Chem. Scand. 5 (1951) 157.
- 5. Touster, O. J. Am. Chem. Soc. 73 (1951) 491.

Received April 30, 1951.

## The Complex Nature of the "Labile Factor" in Chicken Plasma and the Characterization of the Individual Components

ØYVIND SØRBYE\*, INGERKRUSE and HENRIK DAM

Department of Biology, Polytechnic Institute, Copenhagen, Denmark

During the study of the coagulation anomaly produced in chicks by vitamin K-deficiency and dicumarol poisoning, it has been demonstrated, in this laboratory, that these two coagulation anomalies are not identical 1-3.

Dicumarol causes depression of both prothrombin and the  $\varkappa$ -factor  $^2$ , while in vitamin K-deficiency low levels of both prothrombin and the  $\delta$ -factor are found  $^3$ . A common property of these three coagulation factors — prothrombin,  $\varkappa$ -factor and  $\delta$ -factor — is their adsorbability by BaCO<sub>3</sub> and SrCO<sub>3</sub>.

During these studies a rather sensitive method for determination of the labile

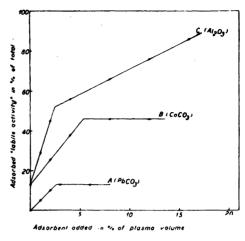


Fig. 1.

Curve A: effect of PbCO<sub>3</sub> on plasma treated with SrCO<sub>3</sub>.

Curve B: effect of CoCO<sub>3</sub> on plasma treated with SrCO<sub>3</sub> and PbCO<sub>3</sub>.

Curve C: effect of Al<sub>2</sub>O<sub>3</sub> on plasma treated with SrCO<sub>3</sub> and PbCO<sub>3</sub>.

activity of fresh plasma, viz. ability to restore the prothrombin time of stored oxalated chicken plasma, was developed using stored oxalated plasma as a substrate. It could be shown that adsorption with BaCO<sub>3</sub> or SrCO<sub>3</sub> sometimes, but not always, would reduce the labile activity of fresh plasma. The degree of reduction depends on the storage time of the plasma used as substrate and indicates a slow inactivation of the  $\varkappa$ -factor and possibly also of the  $\delta$ -factor during storage.

The determination of the labile activity — after total removal of prothrombin, κ-factor and δ-factor by SrCO<sub>3</sub>, has now made it possible to compare the labile activity of different plasmas and to investigate in detail the effect of various adsorbents on the labile activity of fresh plasma.

It is regularly found that the labile activity of different fresh chicken plasmas may show great differences — depending on the diet and the length of time during

<sup>\*</sup> Fellow 1951-52: Norwegian Research Council for Technical and Natural Sciences.

which the chickens have received a certain diet.

The introduction of adsorption procedures show clearly that the labile activity of fresh plasma cannot be explained by the assumption of only one labile factor.

CoCO<sub>3</sub> added to plasma in increasing quantities causes only partial reduction of the labile activity — and the adsorption curve (curve B) indicates the adsorption of one factor with labile activity — the CoCO<sub>3</sub>-factor.

Al<sub>2</sub>O<sub>3</sub> will give quite another type of adsorption curve, which seems to be composed of two straight lines (curve C). It indicates the adsorption of probably two factors with labile activity. Both Cr(OH)<sub>3</sub> and ZnCO<sub>3</sub> give the same type of adsorption curve as Al<sub>2</sub>O<sub>3</sub> does, indicating their similarity in adsorption properties.

Other experiments have shown that the two Al<sub>2</sub>O<sub>3</sub>-factors responsible for the broken-line adsorption curve are not adsorbed by CoCO<sub>3</sub> and that the CoCO<sub>3</sub>-factor is not adsorbed by Al<sub>2</sub>O<sub>3</sub>, Cr(OH)<sub>3</sub> or ZnCO<sub>3</sub> in the quantities studied so far.

The full details of the  $Al_2O_3$ -curve in the range approaching 100 % adsorption are still an object of further study.

By testing other adsorbents we became aware that PbCO<sub>3</sub> would remove a coagulation factor from SrCO<sub>3</sub>-treated plasma which seems to be of a more stable nature than the factors mentioned above and is not adsorbed by Al<sub>2</sub>O<sub>3</sub> or CoCO<sub>3</sub>. The adsorption of this PbCO<sub>3</sub>-factor can be studied by the same technique when stored plasma from chicks deficient in this factor is used as substrate (curve A).

It is not yet evident which of the labile factors mentioned above eventually corresponds to factor V of Owren, plasma Acglobulin of Seegers or labile factor of Quick, but selective adsorption analysis of the kind described seems to offer a convenient method for classification of the components of the chick coagulation mechanism not adsorbed by SrCO<sub>2</sub> or

BaCO<sub>3</sub>. The selective adsorption also offers a method for preparation of the different factors. It has been found possible to elute the adsorbed factors from CoCO<sub>3</sub> and ZnCO<sub>3</sub> by elution with phosphate buffers of suitable pH and molarity.

In the opinion of the authors this adsorption analysis offers a method for quantitative estimation of the different adsorbable factors. The minimum amount of adsorbent, necessary for full adsorption of each component has been interpreted as a measure of the amount present.

Thus we have been able to start a search for the dietary principles which seem to determine in what amount each factor will be present in the chicken plasma. Such studies are in progress.

- Dam, H., and Søndergaard, E. Biochim. et Biophys. Acta 2 (1948) 409.
- Sørbye, Ø., Kruse, I., and Dam, H. Acta Chem. Scand. 4 (1950) 549.
- Sørbye, Ø., Kruse, I., and Dam, H. Acta Chem. Scand. 4 (1950) 831.

Received May 5, 1951.

## A Note on the Food Sparing Effect of Liver Extracts on Adult Rats

GUNNAR ÅGREN

Institute of Medical Chemistry, University of Uppsala, Uppsala, Sweden

In a recent paper on the food sparing effect of liver extracts on growing rats <sup>1</sup> it was reported that a similar effect could not be observed on adult animals. A second series on nearly full-grown rats has now been carried out with the same negative result.

The animals were the same female rats as used in the last experiments on growing animals <sup>1</sup>, and the experimental procedures were the same as described for the second series in that paper. At the beginning of the present experiments the weight of the