THE ISOLATION OF A CRYSTALLINE COMPOUND WITH VITAMIN K ACTIVITY

AN oil, very active in Vitamin K properties, has been obtained by the fractionation of a petroleum ether extract of alfalfa leaf meal. This oil, containing crystals in suspension, was dissolved in a mixture of benzene and absolute alcohol and allowed to stand at −5° C. The crystalline material which separated was recrystallized from a mixture of benzene and absolute alcohol, then from absolute alcohol, then from acetone and finally from absolute alcohol. The crystals, M.P. 69° C., were small, well-formed, transparent, colorless plates. The chick unit as determined by the procedure developed in this laboratory[1] is approximately 0.6 microgram.

Although it is possible that the activity of these crystals may be due to (1) formation of mixed crystals of the vitamin and an inert substance or (2) adsorption of the vitamin on the crystals, we believe that the crystals are really Vitamin K for the following reasons. These crystals have a greater potency than any other fraction that we have obtained, including the oil from which the crystals were separated. Moreover, the crystals were recrystallized from a variety of solvents without loss of potency.

We have also obtained certain indications that more than one substance possessing Vitamin K activity may be present in our extracts.

Our work is being continued, and the detailed data will be published in a subsequent contribution.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

CENTRIFUGING OF LIQUIDS

IN a previous article[1] a tubular vacuum-type centrifuge was described which has proven to be a very useful tool for the separation of comparatively large quantities of substances. Hence it may be of interest to mention briefly a rather simple change in the design of the spinning tube which has considerably increased its efficiency. The apparatus consists of a tube that is spun to a high rotational speed inside an evacuated chamber. The vacuum around the rotor avoids heating due to air friction and insures absence of troublesome temperature gradients that cause stirring. Fig. 1a shows a schematic diagram of the tubular rotor. For the method of mounting and spinning this rotor, reference should be made to the previous article.[1] The material to be centrifuged entered the spinning hollow tube at A at a continuous rate and the lighter and heavier fractions were collected at C and B, respectively. The separation took place as the material flowed down the length L of the spinning tube. It can easily be seen that for a given material and rotational speed the amount of separation increases with increasing radius of the tube, increasing L, and decreasing rate of flow of the material; *i.e.*, the radius of the tube and the time the material is in the centrifuge. By definition[2] of the sedimentation constant s, the rate of settling of the heavier substance toward the periphery $\frac{dx}{dt} = w^2 s x$ where w is the angular velocity of the centrifuge and x the distance of the material from the axis of the tube. Hence $\frac{dx}{dt} = o$ when $x = o$, and in the absence of diffusion the separation would be very small near the center. To overcome this we have been using for some time a solid cylinder in the center of the tube as shown shaded in Fig. 1b. R_1–R_2 is made comparatively small and D is a wire cloth filter to prevent possible remixing at the exits. With this arrangement the material is always in a strong centrifugal field and the

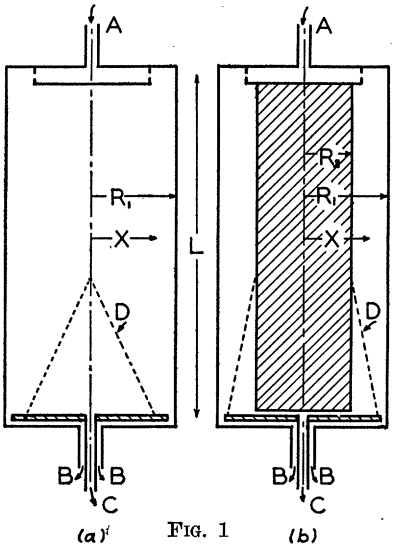

FIG. 1

[1] S. A. Thayer, D. W. MacCorquodale, R. W. McKee and E. A. Doisy, *Jour. Biol. Chem.*, 123: cxx, 1938.

[1] Beams, Linke and Skarstrom, SCIENCE, 86: 293, 1937; Beams, *Phys. Rev.*, 53: 850, 1938.

[2] Svedberg, "Colloid Chemistry."