red or green, while vitamin K<sub>1</sub> and 2-amino-1,4-naphthoquinone gave different shades of violet. The Dam et al. test was not found to be quantitative.

The sodium diethyl dithiocarbamate test is sensitive to 0.01 mg vitamin K<sub>1</sub> per 2 cc of 95 per cent. alcohol of 5 gamma per cc. With the use of a Klett-Summerson photoelectric colorimeter and No. 54 green filter this reaction is practically quantitative for vitamin  $K_1$ in pure solution, from a range of 0.01 mg per 2 cc to 1.0 mg per 2 cc. Since the color is stable only for a few minutes the colorimetric readings must be taken every minute for 10 minutes immediately following the addition of the last reagent. The highest reading is then used. Example: 0.1 mg  $K_1$  gives the highest reading of 60 in 5 minutes, while 0.05 mg reads 30, the highest in 5 minutes. The reading of 30 is just half of 60, while 0.05 is half of 0.1. The stability of the color changes with respect to concentration. At lower concentration the color is more stable than at the higher ones. This reaction of sodium diethyl dithiocarbamate and alcoholic alkali with vitamin  $K_1$  gives a color five-fold that of the Dam et al. The use of absolute alcohol as solvent for standards and reagents in both tests has practically no advantage over 95 per cent. ethyl alcohol.

The reaction of vitamin K<sub>1</sub> with sodium diethyl dithiocarbamate and alcoholic alkali is far more sensitive than that of Dam et al. and has the additional advantage in that it can be used quantitatively.

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## CONCENTRATION OF ENZYMES AND OTHER BIOLOGICAL COLLOIDS BY DIALYSIS

It is sometimes desirable to reduce the volume of a tissue extract or biological fluid containing desired enzymes or proteins, yet to avoid the denaturation which occurs more or less during concentration processes such as evaporation by use of mild heat, distillation in vacuo, long standing in desiccators with dehydrating agents, etc. By dialyzing against a concentrated solution of dextrin it is usually rather easy and simple to concentrate many such solutions to one tenth to one fiftieth of their volumes. There is no disturbing physical or chemical treatment; the process is relatively rapid, 4 to 18 hours of dialysis is in all probability sufficient for any requirement; and (in some cases the greatest advantage) the dialysis can be done under a low temperature in a refrigerator or cold room. Stirring will, of course, further hasten the process when speed is of very considerable importance, although at room temperature there will usually be no need for this.

The writer has used the process particularly for purifying and concentrating phosphatase extracts from kidneys. The work involved mostly dialysis of 10 or 20 cc only, although larger set-ups can be used, and there seems to be no reason why the process can not be of use on a commercial scale.

Cellophane tubing 1.9 cm in diameter with 0.00183 em wall (Fisher Scientific Company 3 inch diameter by 0.00072 inch wall) and about 13 cm in length was used most. If the lower half of a No. 2 rubber stopper is holed to contain a glass tube 1 cm outside diameter by about 5 cm long, the Cellophane may be readily slipped over the stopper and held by means of a rubber band or cord. The lower end of the Cellophane tube becomes completely impermeable when tied close with polished cord. If, however, a cord tie is not wanted in the solution the tubing may be cut twice as long, doubled back, and the cord end tied to the glass tube above the solution. The large inside diameter of the glass tube (about 8 mm) is best because it allows the free introduction of a pipette for adding or removing liquid from the inside of the dialysis tube.

Dialysis is best made against 45–50 per cent. of dextrin in water. Seventy-five per cent. dextrin solution is not difficult to prepare and can be used, but the viscosity is great and there is much precipitation on standing in a refrigerator. When 10 or 20 cc is to be concentrated the writer has found 200 cc 45-50 per cent. dextrin as the "outside solution" to be desirable. In a tall beaker or glass this conveniently submerges the Cellophane tube contents suspended from above and allows diffusion to take place readily.

The properties of dextrin make it very suitable, in fact outstandingly so, for the above process. Other colloids, such as albumin, gelatin, gum ghatti, acacia, starch, agar, pectin, etc., are not suitable.

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