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Received March 1, 1954. P.S.E.B.M., 1954, v85.

A Practical Laboratory Preparation of Avidin Concentrates for Biological Investigation. (20936)

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The production of a biotin deficiency in laboratory animals or in patients requires the ingestion of large quantities of egg white. For example, one investigator(1) gave each patient 36-42 egg whites per day for 6 months. Inasmuch as the anti-biotin activity of egg white is due to avidin(2) which constitutes less than 0.05% of egg white proteins(3), a convenient method of concentrating this activity is desirable.

The objective of this work was a simple method of preparing substantial amounts of avidin concentrate, adequate in activity for animal experimentation and clinical trial, rather than a method for obtaining a small amount of "pure" avidin for chemical and physical analysis.

Method. Four hundred grams of dried egg white* was dissolved in 4 liters of distilled water and adjusted to *pH 5.1* with one normal hydrochloric acid. Then 4½ liters of acetone were added and after stirring for 15 minutes more, the batch was filtered on a 24 cm diameter Buchner funnel using Whatman No. 52 paper. The acetone filtrate was discarded and the filter cake suspended in 2 liters of distilled water, stirred 15 min. and filtered. The wash water filtrate was discarded and the precipitate was extracted in 4 liters of 1% sodium chloride for one hour (the batch was usually allowed to stand overnight at this point). The batch was filtered and the saline filtrate used to extract a second 400-g portion of egg white treated in the same manner as the first. The batch was filtered and a fresh 4-liter portion of saline was used to extract each cake in turn. The saline filtrates (representing 800 g of dried egg white), were poured into 15-inch lengths of dialysis tubing,† 3 inches in diameter. These "sausages" were then put into tall (6 x 18 in.) pyrex battery jars filled with distilled water. The dialysis was carried out in a refrigerator at about +5°C, and is continued for 2 days with twice daily changes of distilled water. The active fraction precipitates at this time. The contents of the dialysis bags were rinsed out into a 2-gallon pyrex battery jar. However, most of the precipitate was quite sticky and clung to the tubing, but it could be removed by rinsing the bags with 3 successive 100 ml portions of 1% NaCl in which the precipitate is completely soluble. The saline rinsings were kept separate from the water rinsings. About 50 g of filter aid‡ was then stirred into the water rinsings from the dialysis bags to collect the suspended avidin. The Celite was filtered out and suspended in the saline rinsings, stirred for about one-half hour and filtered. The salt solution which contains the avidin activity was mixed with an equal volume of cold ethyl alcohol which caused precipitation of the protein but not of the salt. The precipitate was removed by centrifugation in a refrigerated centrifuge and after washing once with 50% alcohol. The precipitate freeze-dried to a powder.

* Product of Wilson Co., Chicago, Ill.

† Cellulose Sausage Casings-Visking Corp., Chicago, Ill.

‡ Celite analytical filter aid—Johns-Manville Co. A Diatomaceous silica filter aid.

TABLE I. Distribution of Avidin Activity during Fractionation.

Fraction	Conc. of avidin activity, units/g or /ml	Total wt or vol of fraction	Total activity of fraction	No. of fractions tested	Variation in total activity of fraction
1. Dried egg white*	4.0 /g	800 g	3200	5†	3.6-6.8/g
2. Acetone filtrate	0 /ml	9000 ml	0	5	0-0
3. Wash water filtrate	.02 /ml	4000 "	80	5	80-320
4. 1% NaCl extract prior to dialysis	.24 /ml	8000 "	1920	5	1600-2400
5. Filter cake residue‡ (discarded after saline extraction)	.20 /ml	5000 "	1000	3	450-1500
6. Filtrate from solutions in bags after dialysis	.005/ml	8400 "	42	6	42-67
7. 1% saline extract of precipitate in dialysis bags	2.5 /ml	600 "	1500	10	1080-1800
8. Final dried product (yield from dried egg white—38%)	1500 /g	.80 g	1200	24	900-2000

* One unit of avidin activity is that amount required to completely neutralize the yeast growth supported by one μg of free crystalline biotin.

† Five different lots of egg white from same and different suppliers were tested.

‡ Filter cake residue (after regular saline extraction) was suspended in 5000 ml of 1/10 molar dibasic potassium phosphate solution—stirred for 1 hr to set overnight and filtered next morning. Figures shown are for assay of this filtrate.

Microbiological assay. The anti-biotin activity of the fractions was followed by a microbiological assay of the inhibition, by avidin, of the biotin induced growth of yeast§ on the medium of Hertz(4). Suitable aliquots of sample (ca. 0.05 μg of solids with most active fractions) were added in graded amounts to test tubes containing the complete medium and 1 x 10^{-4} μg of biotin. The biotin binding capacity was calculated from the difference between the known biotin added and that found by assay after addition of avidin.

Results. The distribution of avidin activity in the fractionation process is shown in Table I. The yield of final active fraction in a typical batch was 1200 units (in 800 mg) from dried egg white containing 3200 units or 38% recovery. The accountable losses, due to *water soluble* and *saline-insoluble* avidin fractions were 80 + 1000 + 42 = 1122 units. Subtracting (1200 + 1122) units from the original 3200 leaves 898 units of unaccountable losses, due to inactivation of the avidin and general losses in processing.

The solubility in 1% saline (25 mg/ml) and characteristic stickiness of the dried product answer to the description of the avidin "XA" fraction reported by Fraenkel-Conrat(3,5) who described "avidin" as being composed of 3 different fractions, "Avidin A", "Avidin NA", and "Avidin XA."

Our method is a modification of the method of Eakin, Snell, and Williams(2). These authors coagulated fresh egg white with acetone. The coagulum was washed and filtered and the filter cake extracted with salt solution. The volume of this salt solution was reduced by precipitating the protein containing the active fraction, with 2 volumes of acetone. The precipitate (with filter aid) was filtered out and suspended in 2% ammonium sulfate, and filtered again. The active fraction was separated from the filtrate by ammonium sulfate fractionation, collected on a filter aid and the active precipitate filtered out and suspended in a small volume of water. The filter aid was removed and the solution dialyzed. The active protein was precipitated, from the solution in the dialysis bag, with acetone. The precipitate was then centrifuged out and dried in a vacuum.

The process described in this paper differs from that of E.S.W. in that dried egg white is used instead of fresh egg white. The second acetone precipitation and the ammonium sulfate fractionation steps are omitted. Furthermore, an initial adjustment of the egg white solution to pH 5.1 is made. This pH 5.1 value was determined empirically and is a

§ The yeast was Saccharomyces Cerevisia ATCC 9896, from American Type Culture Collection, Washington, D. C.

critical step in the process. The final yield of avidin from the original egg white is approximately the same by both methods (*i.e.*, 38% shown in Table I and 35% for E.S.W.).

Although distilled water was used in the dialysis, it would be more convenient to use *cold* running tap water if water of good quality and low in iron is available.

Summary. 1. Preparation of avidin concentrates by precipitation of the active fraction during a 2-day static dialysis (in distilled water) of a 1% saline extract of an acetone coagulation of dried egg white solution at pH 5.1 has been described. 2. The activity of these preparations (1.5 units/mg) averages 20% that of "pure" avidin‖ (8.0 units/mg) and the yield is about one g of such material from 800 g of dried egg white. 3. About 100 lb of dried egg white has been processed by the method described.

‖ "Pure" avidin—considered as 8 units/mg (Ref. 3) and having electrophoretic characteristics of a single species of molecule.

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Received March 2, 1954. P.S.E.B.M., 1954, v85.

Pantothenic Acid Deficiency and Loss of Natural Resistance to a Bacterial Infection in the Rat.* (20937)

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This report deals with a consequence of pantothenic acid deficiency which, while alluded to by others, has not been subjected to detailed study. Many sudden deaths were encountered due, apparently, to an acute respiratory infection of a type we had not previously encountered in any rats. Dr. Nelson† (personal communication) obtained from some typical lesions pure cultures of a corynebacterium which reproduced a similar disease picture in mice. He called attention to the fact that this type of disease ("pseudotuberculosis"—for description see Dingle(1)) is known to occur spontaneously in mice, and that the corynebacterium responsible for it is generally held to be non-pathogenic for rats. This indicated that pantothenate deficiency abolished the species-determined natural resistance of the rat to this organism. The data given below were obtained some time ago, but publication was delayed awaiting the findings of Seronde(2) which verify the observations on the spontaneous disease by means of inoculation studies.

Methods and materials. The basal diet had the following composition, in g/100 g of diet: vitamin "free" casein (Labco) 18.0; glucose (Cerelose) 71.85; salt mixture (modification of Wesson salt mixture with 1.0% Ca and 0.55% P in the resulting diet) 6.1; cottonseed oil 2.0; cellulose (Celluflour) 2.0; l-cysteine hydrochloride 0.05.‡ Incorporated

* This investigation was supported in part by a grant from the National Vitamin Foundation. Vitamins were generously supplied by Hoffmann-La Roche.

† We take this opportunity to express our thanks to Dr. John B. Nelson of the Rockefeller Institute for his kind cooperation. Out of his wide experience with respiratory infections in laboratory animals he has been of frequent assistance in our struggles with the knotty problem of lung infections in the rat.

‡ As an antioxidant. By this addition, and by the use of ferrous iron ($FeSO_4$) and cuprous copper (Cu_2O) in the salt mixture, keeping quality of the diets is much improved. An unpleasant odor fails to develop, and carotene color persists.