food consumption between the various groups are relatively constant. The utility of MSG in increasing food consumption was most evident during the first 7 days. After this time the food consumption of the unsupplemented group increased to a level comparable with the MSG groups. Although completely equivalent food consumption on the casein and amino acid rations has not been obtained by the use of MSG, it appears likely that the maximum effect obtainable with this substance and this particular amino acid ration has been reached since 2% MSG did not give significantly better results than 1%.

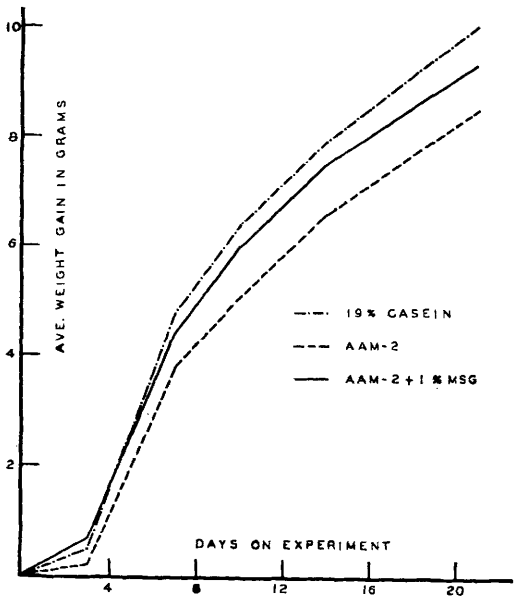


FIG. 1

Growth of mice fed rations containing casein, amino acids or amino acids plus MSG.

TABLE II. Food Consumption Data.

| Day | Avg food consumption per mouse per day, g | | | |
|---|---|---|---|---|
| | 19% casein | AAM-2 | AAM-2 + 1% MSG | AAM-2 + 2% MSG |
| 1 | 2.9 | 2.1 | 2.0 | 2.3 |
| 2 | 4.1 | 2.9 | 3.5 | 2.9 |
| 3 | 4.1 | 2.9 | 3.5 | 2.9 |
| Days 1-3 avg | 3.7 | 2.6 | 3.0 | 2.7 |
| 4 | 4.2 | 3.6 | 4.1 | 3.9 |
| 5 | 4.6 | 3.3 | 3.1 | 3.2 |
| 6 | 4.8 | 3.7 | 4.6 | 4.7 |
| 7 | 4.8 | 4.3 | 4.6 | 5.0 |
| Days 4-7 avg | 4.6 | 3.7 | 4.1 | 4.2 |
| 8 | 4.5 | 4.3 | 3.7 | 4.7 |
| 9 | 3.9 | 3.6 | 3.5 | 3.3 |
| 10 | 5.4 | 4.4 | 5.0 | 4.6 |
| Days 8-10 avg | 4.6 | 4.1 | 4.1 | 4.2 |
| Avg/day 10 days | 4.3 | 3.5 | 3.8 | 3.8 |

*Summary.* A synthetic ration in which all of the nitrogen is furnished as free amino acids is not as palatable to the mouse as one containing casein. The inferior palatability results in less food consumption during the first 3 to 7 days and consequently poorer growth. When the initial lag period is diminished or eliminated by the addition of 1% of monosodium glutamate to the ration, the final weights of the mice are more consistent and approach more closely those of the animals fed a ration containing casein.

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## Purification of the Egg-White Inhibitor of Influenza Virus Hemagglutination by Filtration.* (18115)

T. Z. CSÁKY, FRANK LANNI, AND J. W. BEARD

*From the Department of Surgery, Duke University School of Medicine, Durham, N. C.*

Egg-white (EW) is one of the richest and most readily available sources of a material capable of inhibiting hemagglutination by heated influenza viruses(1). This material has considerable interest as a substrate for the enzyme-like action of influenza viruses

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1. Lanni, F., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 312.

(2-5). One of the principal problems is the preparation of highly purified inhibitor in large quantity. The method usually employed in this laboratory(6) consists in the precipitation of the inhibitor from EW at pH 5.7 and extraction of the precipitate, after washing, with phosphate buffer of pH 7.2. The preparations obtained in this way are usually 40-60 times as active as EW on a nitrogen basis.

In this paper there is presented a new and very simple method, which yields inhibitor solutions about 80 times as active as EW. The method consists in filtration of diluted EW through rapid-flow filter papers and extraction of the inhibitor from the paper. Furthermore, the method is capable also of increasing the purity of preparations obtained by acid-precipitation.

*Materials and methods.* Thick EW for the filtration experiments was separated from fresh eggs with the aid of a screen, strained through cotton gauze, and poured into 9 volumes of 0.06 M phosphate buffer of pH 7.2; by gentle stirring for about 2 hours, a fairly homogenous solution was obtained. Semipurified EW inhibitor was prepared from undiluted thick EW by acid-precipitation as described before(6); some of the extracts were concentrated 4-5 times by prevaporation and were used as such in the filtration experiments. All materials were preserved with Merthiolate (Lilly), which was present in a concentration of 1:5,000 throughout the purification procedures. Filtrations were carried out in a wet chamber at 4°C. Inhibitory activity(6) and nitrogen(7) were determined† by the usual methods of this laboratory. The reported inhibition titers have been standardized by reference to a preserved solution of EW (EW Standard I) (8). The purification factor (PF) of a preparation is the ratio of the specific activity (titer/nitrogen) of the preparation to that of the standard EW.

TABLE I. Properties of Fractions Obtained by Filtration of Thick EW.

| Fraction | Inhibition titer | N, μg/ml | PF | Recovered from starting material: Activity, % | Recovered from starting material: N, % |
|---|---|---|---|---|---|
| Thick E W 1:10 | 7500 | 1750 | 1.1 | | |
| Filtrate | 190 | 1700 | 0.03 | 2.5 | 97.0 |
| Extract I | 280 | 44 | 1.6 | 3.7 | 2.5 |
| " II | 6000 | 18 | 83 | 80.0 | 1.0 |
| | | Total | | 86.2 | 100.5 |

*Experimental. Thick EW.* The results obtained by filtration of thick EW are illustrated in the findings of the following experiment: 500 ml of diluted (1:10) thick EW was poured onto a 32-cm folded filter paper (Reeve Angel No. 802).‡ A volume of 473 ml of filtrate was collected in 32 hours. The unfiltered portion of the solution was discarded, and the filter paper was unfolded and washed briefly with 470 ml of phosphate buffer (0.06 M, pH 7.2) by gentle swirling (Extract I). The paper was then put, together with 470 ml of fresh buffer, in a Waring Blendor and shredded by gentle blending for about 5 minutes. The mash was slowly stirred at room temperature for 24 hours and centrifuged; a viscous, opalescent supernatant was obtained (Extract II). Table I shows the relation between inhibitory activity and nitrogen at different steps.

*Semipurified Inhibitor.* The experiments were carried out as with thick EW. Illustrative results, obtained with 2 preparations

2. Lanni, F., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 442.

3. Eckert, E. A., Lanni, F., Beard, D., and Beard, J W., *Science,* 1949, v109, 463.

4. Gottschalk, A., and Lind, P. E., *Nature,* 1949, v164, 232.

5. Hirst, G. K., *J. Exp. Med.,* 1950, v91, 161.

6. Lanni, F., Sharp, D. G., Eckert, E. A., Dillon, E. S., Beard, D., and Beard, J. W., *J. Biol. Chem.,* 1949, v179, 1275

7. Lanni, F., Dillon, M. L., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 4.

† The authors are grateful to Mrs. Edith S. Dillon for the nitrogen analyses.

8. Lanni, F., Lanni, Y. T., and Beard, J. W., *J. Immunol.,* 1950, v65, 269.

‡ Other rapid filter papers (*e.g.,* Whatman No. 1 and No. 4) could be substituted in the purification procedure.

TABLE II. Properties of Fractions Obtained by Filtration of Acid-Purified Inhibitor.

| | Preparation | | | | | |
|---|---|---|---|---|---|---|
| | C20-PEIII | | | C21-PEIII-CII | | |
| | Titer | N, μg/ml | PF | Titer | N, μg/ml | PF |
| Starting material | 12000 | 168 | 18 | 33000 | 145 | 57 |
| Filtrate | 260 | 109 | 0.6 | 190 | 54 | 0.9 |
| Extract I | 4000 | 31 | 32 | 8900 | 31 | 72 |
| " II | 5200 | 18 | 72 | 5400 | 18 | 75 |
| Filter paper used | Reeve Angel #802 | | | Whatman #1 | | |

(C20-PEIII and C21-PEIII-CII) of acid-purified inhibitor, are shown in Table II. It is seen that the levels of PF of the 2 preparations, which differed widely before filtration, were increased to approximately the same value and one similar to that obtained by direct purification of the inhibitor by filtration of thick EW (Table I).

*Discussion.* Several alternative methods have previously been employed for purification of the EW inhibitor. The method(6) based on precipitation of the inhibitor at pH 5.7 and extraction at pH 7.2 has proved very useful for the processing of relatively small volumes (100-200) ml of EW. By this method, useful preparations, with PF in the range 40-60, can be obtained in 4-5 hours, since no prolonged period is needed for precipitation or extraction of the precipitate, and dialysis is not required. With the processing of larger volumes of EW, the method becomes cumbersome. Although acid-purified preparations have been shown to contain 3 related components, the properties of which are discussed elsewhere(9,10), electrophoretic analysis failed to reveal any lysozyme. However, a quantity of lysozyme amounting to less than 10% of the total protein would have been difficult to discern electrophoretically; no biological tests for lysozyme in these preparations have been carried out.

Some acid-purified inhibitor preparations precipitate in the Tiselius cell during electrophoresis. The precipitate contains about 60% of the original activity with considerable increased PF(11). The use of this method in routine purification procedures is limited by the capacity of the Tiselius cell.

A second method, employed by Gottschalk and Lind(12) and based on the method described by Young(13) for the purification of ovomucin, consists in the precipitation of the inhibitor by dilution of EW with distilled water at 0°C. The precipitate is washed in the cold, dispersed in 5% NaCl solution, and dialyzed for 48 hours at 4°C to bring the salt concentration to 1%. The method is thus rather gentle, but a disadvantage consists in that it requires laborious handling of the material in the centrifuge. The final product contains up to about 15% of lysozyme, part of which may be removed by crystallization. No values for the yield or PF have been reported; however, in a limited experience of the authors (unpublished) the method yielded preparations with PF 30-40, with the lysozyme not specifically removed.§ The preparations are reported to contain at least one inert component in addition to lysozyme(4).

A third method, based on centrifugation of the inhibitor from diluted EW at high speed(6), has yielded products with PF in the range 40-50. The method has not been extensively explored, because of the need for specialized apparatus and the availability of other methods.

Finally, it has recently proved possible to obtain considerable further purification of

9. Lanni, F., Sharp, D. G., Csáky, T. Z., and Beard, J. W., *Arch. Biochem.*, 1950, v28, 313.

10. Sharp, D. G., Lanni, F., Lanni, Y. T., Csáky, T. Z., and Beard, J. W., submitted for publication.

11. Sharp, D. G., Lanni, F., Dillon, E. S., and Beard, J. W., PROC. EXP. BIOL. AND MED., 1949, v71, 244.

12. Gottschalk, A., and Lind, P. E., *Brit. J. Exp. Path.*, 1949, v30, 85.

13. Young, E. G., *J. Biol. Chem.*, 1937, v120, 1.

§ In a personal communication, Dr. Gottschalk has informed the authors that the PF of his preparations is approximately 40-50.

semipurified inhibitor by isolation of the fast-moving, active component in the electrophoresis apparatus(10). A preparation with PF 190 was obtained from a starting material with PF 44. The yield of such highly purified inhibitor by this method is still smaller than that obtained through the use of electrophoretic precipitation (cited above).

In relation to these methods, the method based on filtration possesses the distinct advantages that (a) the procedure is extremely simple, (b) the conditions are mild, (c) no special apparatus is required, (d) the capacity is large, and (e) the products are superior in PF to all except those obtained by electrophoretic isolation; disadvantages are (a) the products are dilute and, for some uses, must be concentrated by pervaporation, with subsequent dialysis to reduce the salt concentration, and (b) the preparation of a batch of inhibitor requires at least 3 days, 2 for filtration and 1 for extraction. Thus far, attempts to reduce the time of filtration or extraction have proved unsuccessful.

The conditions affecting the filtration properties of the inhibitor have been studied extensively in experiments which will be reported separately.

*Summary.* A simple method is described for the purification of the egg-white inhibitor of influenza virus hemagglutination by filtration of diluted egg-white through rapid-flow filter papers and extraction of the unfilterable residue. The inhibitor has been purified about 80-fold in this way. The method has proved useful also for the further purification of acid-purified inhibitor. The advantages and disadvantages of the new method are discussed in relation to those of other methods.

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## Failure to Find C-Reactive Protein in Viral Hepatitis.* (18116)

W. PAUL HAVENS, JR., HAZEL L. EICHMAN. AND MARJORIE KNOWLTON
(Introduced by Abraham Cantarow)

*From the Jefferson Medical College, Philadelphia, Pa., and the 98th General Hospital, Munich, Germany.*

The presence of C-reactive protein in the blood of patients with a wide variety of diseases has been described(1-4). Although the exact mechanism which potentiates the appearance of this substance is not clearly defined, it has been suggested that several different and apparently non-specific stimuli producing damage to tissues may be operative. Thus, C-reactive protein has been found in the blood of patients with infarction of the myocardium, acute and chronic infectious diseases, and following the intramuscular injection of such materials as vaccines and colloidal sulphur(5). Although frequent observations have been made of the appearance of C-reactive protein in the blood of patients with bacterial infections, only a limited amount of data is available concerning the presence of this substance in the blood of patients with viral diseases. It has been described(4) as occurring in occasional patients with influenza, viral hepatitis, infectious mononucleosis, poliomyelitis, mumps, and following vaccination for small-pox, but Löfström(3) reported that C-reactive protein was uncommonly present in the blood of patients

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1. Tillett, W. S., and Francis, T., Jr., *J. Exp. Med.*, 1930, v52, 561.

2. Ash, R., *J. Infect. Dis.*, 1933, v53, 89.

3. Löfström, G., *Acta med. Scandinav., Supplement 141*, 1943.

4. Hedlund, P., *Acta med. Scandinav., Supplement 196*, 579, 1947.

5. Hedlund, P., *Acta med. Scandinav.*, 1944, v118, 329.