IMMUNOLOGICAL AND CHEMICAL INVESTIGATIONS OF VACCINE VIRUS

II. Chemical Analysis of Elementary Bodies of Vaccinia

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Through the application of recently developed methods we have been able to obtain appreciable amounts of elementary bodies of vaccinia in a relatively pure state. In view of the current findings as concerns importance of the chemical nature of viruses (1–3), it has seemed advisable to analyze some preparations of elementary bodies and to record the results.

Materials and Methods

Elementary bodies of vaccinia were obtained according to the method of Craigie (4) and Parker and Rivers (5) which consists of differential sedimentation in horizontal and angle centrifuges. Suspensions of the bodies obtained in this manner (one rabbit yielded approximately 35 cc. of suspension containing about 2 mg. of dry bodies) were uniform in regard to the picture observed in stained preparations (5) and were infectious for rabbits in a dilution of 1×10^{-8}. Furthermore, the bacterial content of the suspensions, as determined by poured plate counts, was negligible inasmuch as there were less than 300 organisms per cc.

The suspensions of elementary bodies were frozen and dried *in vacuo*. The dried bodies were then subjected to standard qualitative tests for proteins, fats, sugars, and certain other substances. Samples of dry elementary bodies for quantitative analyses were weighed on a Kuhlmann microchemical balance sensitive to 0.001 mg. Residual moisture in the bodies was estimated by determining the loss of weight resulting from drying a sample of them in a platinum dish at 110°C. These figures were confirmed by means of drying other samples to constant weight *in vacuo* over dehydrating agents. Ash determinations were made on some of the samples by weighing the residue after ignition. The amount of protein present was estimated from the nitrogen content determined by the

Pregl (6) micro-Kjeldahl method and confirmed by a Pregl micro-Dumas combustion. The factor used to convert nitrogen to protein was 6.25. Fats were estimated by weighing the material extracted by ether from dried elementary bodies, centrifugation being used to eliminate the ether-insoluble portion.

EXPERIMENTAL

Elementary bodies of vaccinia washed 4 times in phosphate buffer and 3 times in distilled water (5) were dried and subjected to qualitative and quantitative chemical analyses in the manner described above. The following results were obtained.

TABLE I

Results of Chemical Analysis of Elementary Bodies of Vaccinia

Analysis	I	II and III	IV	Average
	per cent	*per cent*	*per cent*	*per cent*
Protein	83.38	82.30, 85.20	81.61	83.12
Fat	10.15	8.95	6.52	8.54
Ash	0.46	0.46	1.25	0.72
Residual moisture	5.37	5.11, 5.16	6.60	5.56
Undetermined, including a trace of carbohydrate	0.75	1.71	4.02	2.06

The figures for protein were calculated from the total amount of nitrogen found.

Qualitative Analysis

Positive biuret, xanthoproteic, Millon, and Ehrlich's para-dimethylamidobenzaldehyde tests indicated the presence of protein. The Liebermann, Adamkiewicz, and Acree-Rosenheim tests were negative. A positive acrolein test demonstrated the presence of fats. Some preparations yielded a strongly positive Molisch reaction for the presence of carbohydrates while others exhibited a very weak one. Tests for the presence of sulfur yielded negative results. Tests for phosphorus were not made, because the elementary bodies during one stage of their preparation had been washed in a phosphate buffer solution.

Quantitative Analysis

Four different batches of elementary bodies were prepared for quantitative analysis. Each lot contained approximately 20 mg.

of dry material. Two of the batches were examined separately, while the remaining two were pooled in order to obtain sufficient material for duplicate estimations of the nitrogen or protein content. The results of the analyses, summarized in Table I, indicate that elementary bodies of vaccinia contain fat, ash, carbohydrate, and nitrogen some of which is in the form of protein.

The protein in the elementary bodies was soluble in dilute alkali and to a certain extent in 70 per cent alcohol. It was only slightly soluble in water, insoluble in dilute acid, and coagulated when heated to a temperature of 65°C.

Since the concentration of sugar in the washed elementary bodies was low and relatively large amounts of material are required for quantitative determinations of carbohydrates, such determinations were not attempted. However, the results shown in Table I indicate that the carbohydrate fraction represents but a small part of the washed elementary bodies. We were surprised to find such a small amount. It occurred to us, however, that much of the sugar might have come away from the bodies during the process of purification by repeated washing and centrifugation. Consequently, we applied the Molisch test and the precipitin reaction to each of the seven wash waters obtained during the purification of a batch of the bodies. Each wash water consisted of about 50 cc. and was evaporated down to a volume of 4 cc. before the tests were made. The first wash water yielded a positive Molisch reaction in a dilution of 1:1600. The amount of sugar in the subsequent wash waters gradually decreased until a positive test was not obtained in the seventh in a dilution greater than 1:200. The results of precipitin reactions conducted with the different wash waters and antivaccinial serum roughly paralleled those of the Molisch tests in that less and less precipitinogen was found in the wash waters as purification proceeded.

DISCUSSION

The material analyzed by us was undoubtedly composed for the most part of elementary bodies. Furthermore, the elementary bodies either represent vaccine virus or are intimately associated with it (5). Therefore, the results of our analyses, which showed the presence of protein, fat, carbohydrate, and ash in the material examined, may

be construed as throwing light upon the chemical nature of vaccine virus or certain structures closely associated with it. It is also interesting to note that the elementary bodies were almost completely depleted of sugar by the repeated washings.

Our results do not support the idea of "protein-free" viruses, as set forth by Kligler (1), Kligler and Olitzki (2), and others, but agree closely with those of Schlesinger (3) who stated that he found in a purified coliphage, fats, an extractable carbohydrate, and nitrogen (13.2 per cent) presumably in the form of protein. Moreover, the work now described is in accord with that of Wilson Smith (7) and Ch'en (8) who have reported that they were able to obtain a specific carbohydrate from emulsions of tissues containing vaccine virus. Finally, the results of the chemical analyses of elementary bodies of vaccinia do not differ materially from those recorded for bacteria by Nencki and Schaffer (9).

CONCLUSION

Washed elementary bodies obtained from dermal vaccine virus contain ash, carbohydrate, fat, and nitrogen a part of which is undoubtedly in the form of protein. These components are similar to those found in bacteria and other substances of protoplasmic origin.

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