18. A NOTE ON THE POSSIBLE RELATIONSHIP OF THE CARBOHYDRATE COMPONENT OF PROTEINS TO THEIR ANTIGENIC PROPERTIES

BY A. NEUBERGER[1] AND M. E. YUILL

From the Department of Chemical Pathology, University College Hospital Medical School, London, W.C. 1

(*Received 29 November 1939*)

THE classical early studies of Avery & Heidelberger [1925] on pneumococcal antigens, followed by numerous investigations on other organisms by various workers, have shown that bacterial antigens in general owe their immunological properties not to their protein constituents alone but to protein-carbohydrate complexes, in which the carbohydrate component exercises a determinant influence with respect to immunological specificity. It is now generally recognized that most proteins contain carbohydrate groups as integral parts of their molecules, and the question therefore arises whether such carbohydrate groups may be of importance in determining the specificity of the immunological reactions to which they give rise.

It can scarcely be maintained that the presence of a carbohydrate group is indispensable to the development of antigenic activity, since there exist proteins, such as haemoglobin and pepsin, which contain no carbohydrate but nevertheless act as antigens. The possibility remains however that those proteins which do contain carbohydrate owe their immunological specificity, in part at least, to the presence of this constituent.

It has indeed already been claimed by Bierry [1931; 1934] that chemical differences between the carbohydrate groups of the serum proteins of different species are responsible for the species specificity of these proteins; no serological evidence however was offered in support of this conclusion. All attempts to demonstrate actual serological reactions between polysaccharides isolated from a protein and the antiserum against the same protein have in fact so far been unsuccessful [cf. e.g. Ferry & Levy, 1934], although Sevag & Seastone [1934] isolated a substance from egg white, containing 11·65% N and giving positive carbohydrate tests, which produced anaphylactic shock in guinea-pigs sensitized with egg white; this substance however was not chemically characterized, and in view of its high nitrogen content the presence of protein or peptide impurities cannot be excluded. The whole of the previous work on this subject is indeed open to the criticism that the so-called polysaccharides which have been employed have not been rigidly proved either to be free from protein or to be themselves other than artefacts produced in the process of their isolation.

One of us [Neuberger, 1938] has recently described the isolation of a polysaccharide from egg albumin which can reasonably be regarded as chemically unaltered and as representing an integral constituent of the original protein molecule; the possession of this and of a similar product derived from ovomucoid has made it possible to subject the hypothesis of the connexion between the carbohydrate groups and immunological properties of proteins to rigid experimental test.

[1] Beit Memorial Research Fellow.

Experimental

The egg albumin used for these experiments was a sample which had been recrystallized 5 times. The ovomucoid was prepared by heating egg white to 80° for 5 min., filtering off the denatured albumin and precipitating the solution with 5 vol. alcohol; the precipitate was again dissolved in water and heated to 80°, a small amount of insoluble material being filtered off, and the protein was precipitated from the filtrate with 3·5 vol. alcohol; the process of solution and precipitation was repeated once more. The polysaccharides were prepared according to Neuberger [1938], the method being slightly modified in the case of ovomucoid.

For each series of experiments groups of 3–6 rabbits were used. The animals immunized against egg albumin were given five courses of injections each consisting of 9 doses administered over a period of 3 weeks; the doses were increased in succeeding courses from an initial dose of 10 mg. in the first course to a final dose of 70 mg. in the fifth course. The rabbits immunized against ovomucoid were similarly treated except that they only had four courses of injections. In all cases an interval of 2–3 weeks was allowed between the successive courses.

At the end of the period of immunization ring precipitation tests were performed in the usual manner; bulk precipitation tests were carried out by mixing equal volumes of different dilutions of serum with varying dilutions of antigen. For complement fixation tests there were generally used 2 M.H.D. of complement and 2–3 M.H.D. of haemolysin, 1–2 hr. at 37° being allowed for fixation of the complement by the antigen-antibody mixture.

Results

Precipitation reactions carried out by the ring technique between the anti-egg albumin sera and egg albumin were strongly positive up to dilutions of 1 : 100,000. In the earlier series of experiments a faintly positive reaction was obtained between the anti-egg albumin sera and the egg albumin polysaccharide; this positive ring test only occurred however at high concentrations (antigen dilutions of 1 : 200–1 : 500) and in later experiments it was not observed at all. Attempts to inhibit the reaction between egg albumin and its antiserum by first incubating the latter with different dilutions of the polysaccharide were completely unsuccessful, as were also efforts to demonstrate bulk precipitation between the polysaccharide and the anti-egg albumin serum.

After the first course of injections complement fixation tests were uniformly negative; at the end of the third course slight fixation of complement was observed with all three sera at a dilution of 1 : 200 of the polysaccharide; this reaction however failed to persist in one out of the three sera after the fifth course of injections. In an earlier series of injections one out of three sera gave optimal fixation of complement with 50 % haemolysis at a dilution of 1 : 3200 of the polysaccharide.

In spite of the fact that ovomucoid has hitherto been regarded as a poor antigen [Lewis & Wells, 1927] the antisera which we obtained after five courses of injections reacted with the ovomucoid used as antigen to a dilution of 1 : 100,000. All attempts however to demonstrate direct reaction between the ovomucoid antisera and the polysaccharide derived from ovomucoid either by the ring or bulk precipitation tests or by complement fixation were unsuccessful, nor was it possible to demonstrate inhibition of the reaction between ovomucoid and its antiserum by means of the polysaccharide.

The results which we have obtained with the two proteins which we have investigated do not therefore afford any clear evidence that the polysaccharide component plays a part in the immunological reaction; the few positive results which were obtained with egg albumin were very feeble and moreover were not entirely reproducible, whilst our results make it clear that the polysaccharide component of ovomucoid is of no significance in the immunological reactions of the protein, in spite of the fact that the content of carbohydrate in ovomucoid is very high. It seems to us therefore to be unlikely in general that the immunological properties of proteins are significantly affected by the carbohydrate groups which the latter may contain.

Summary

The possible importance of the carbohydrate groups of egg albumin and ovomucoid in the serological reactions of these proteins has been studied by specific precipitation (ring and bulk techniques), complement fixation and serological inhibition reactions; no evidence has been obtained that the carbohydrate groups play any significant part.

REFERENCES

Avery & Heidelberger (1925). *J. exp. Med.* **42**, 372.
Bierry (1931). *C.R. Acad. Sci., Paris,* **192**, 1284.
—— (1934). *C.R. Soc. Biol., Paris,* **115**, 1168.
Ferry & Levy (1934). *J. biol. Chem.* **105**, xxvii, *Proc.*
Lewis & Wells (1927). *J. infect. Dis.* **40**, 317.
Neuberger (1938). *Biochem. J.* **32**, 1435.
Sevag & Seastone (1934). *Z. ImmunForsch.* **83**, 464.