1.5 M sodium sulfate by a technique similar to that of Howe[3] for blood serum proteins. Non-protein nitrogen was determined by using Alman's tannic acid reagent or 2.12 sodium sulfate for protein precipitation. The ovomucoid content was determined by heat coagulation of the other proteins in the presence of acetate buffer approximately pH 4.7 and 0.2 M sodium sulfate. No attempt was made to separate the ovoglobulins or ovalbumins. Nitrogen was determined by macro-Kjeldahl.

TABLE II.
Average Protein Distribution in Ten Eggs.

	Ovomucin N mg.	Ovoglobulin N mg.	Ovalbumin N mg.	Ovomucoid N mg.	Non-protein N mg.
Mature eggs	42.1*	45.38.	350.19	61.55	17.48
Immature eggs	40.4*	15.44	342.58	53.02	16.64

* Average of 5 eggs only.

The ovoglobulin fraction shows the only significant difference and in this case, in each of the 10 pairs, the mature egg contains the larger amount. The material added through the membrane is a dilute solution of ovoglobulin with perhaps the salts necessary to keep it dispersed. The passage of the ovoglobulin through the shell membrane shows a behavior similar, in some respects, to that noted by Howe[4] in the rapid passage of globulins into the blood stream of young animals.

7387 P

Immunological Specificity of Carbohydrates Derived from Staphylococci.*

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During the course of studies concerning hypersensitiveness in patients with trachoma, it became necessary to determine the possibility of the existence of different immunological types among Staphylococci. Preliminary experiments undertaken with the agglutination reaction revealed that strains isolated from different sources and

[3] Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 93.

[4] Howe, P. E., *J. Biol. Chem.*, 1921, **49**, 115.

* Conducted under a grant from the Commonwealth Fund of New York City.

conditions are not differentiated satisfactorily by this technique. Consequently an effort was made to fractionate carbohydrates from this organism, and this was accomplished successfully.

The method finally adopted for the separation of carbohydrates from Staphylococci may be outlined as follows: (1) concentration of broth cultures (18-24 hours) by centrifugation in a Sharples; (2) heating the thick suspension in N/16 HCl for 20 minutes in a boiling water bath; (3) after centrifuging, the supernatant is treated with 40% NaOH until flocculent precipitation occurs, which is at a slightly acid reaction; (4) to the supernatant N acetic acid and Na acetate, and 2 volumes of 95% alcohol are added; (5) after standing in refrigerator over night, the residue following centrifugation is taken up in H_2O, and the supernatant is reserved for further separation; (6) the water solution is treated with N acetic acid until precipitation occurs and to the supernatant after centrifugation are added 2 volumes of 95% alcohol and sufficient Na acetate to cause precipitation. The residue is re-extracted until all the soluble material is obtained; (7) up to 6 precipitations with acetic acid are sometimes required to cause complete removal of precipitable matter. The end product never precipitates in the usual protein precipitants; it is always ninhydrin negative, although from time to time biuret positive. The total nitrogen has varied up to 5%. The Molisch reaction is always strongly positive.

In testing the reactivity of this material in rabbit antisera, it was found that while all sera contain a high titre of agglutinins following immunization to Staphylococcus, only about one-third possess precipitins for this material. Where precipitation occurs, the titres are high reaching one to 6 or 8 million. On the basis of precipitation in antisera, then, it was shown that at least 2 serologically type-distinct substances occur in Staphylococci. The one has been found always and only in strains isolated from pathologic conditions, and the other, always and only in saprophytic strains, regardless of source. Thus far 15 strains have been studied by this method. Of these, 9 isolated from different human infections have fallen into one type, while the remaining, isolated from non-pyogenic conditions have fallen into a second type. It is, therefore, proposed to designate the virulent, pyogenic strains as Type A and the avirulent, non-pathogenic strains as Type B.

Both materials appear to be carbohydrates and should be termed soluble specific substances as originally described in Avery's laboratory. While the chemical characteristics are still in the course of study, sufficient data are available to indicate that the 2 substances

are carbohydrates as chemically distinct as they are serologically. Because of the preliminary nature of the data, however, they are being reserved for more complete study.

In contrast to the type-specificity of the carbohydrate, is the species-specificity of the "nucleoproteins" derived from the organisms. While the proteins are obviously mixtures, it is nevertheless clear that these solutions react in common in homologous and heterologous antisera.

It is interesting to point out a recent observation in 2 patients, one ill with a Staphylococcus septicemia, the other with osteomyelitis due to the same organism. With convalescence both patients exhibited a marked skin reactivity to 0.1 cc. of a 1-50,000 dilution of the carbohydrate of Type A, but not Type B. Coincident with the development of heightened skin reactivity, the blood serum of the patients showed a high titre of precipitins for the homologous carbohydrate.

7388 P

Effect of Irradiation of Hypophysis on Experimental Diabetes.

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Various investigators have presented evidence to show that the hypophysis plays an important rôle in carbohydrate metabolism. The first conclusive evidence is that of Houssay and coworkers, who found (1) that extracts of the anterior lobe of the hypophysis injected into normal dogs produce hyperglycemia, glycosuria and ketonuria, and when injected into depancreatized dogs, an increase in severity of the diabetes;[1, 2] (2) that removal of the hypophysis in normal dogs leads to a hypersensitivity to insulin,[3] hypoglycemic convulsions during fasting, and an increased tolerance to glucose;[4, 5] (3) that in depancreatized dogs, removal of the hypophysis restores the carbohydrate metabolism to an approximately normal condition,

[1] Houssay, B. A., and Biasotti, A., *Rev. Soc. Argent. Biol.*, 1931, **7**, 3.

[2] Houssay, B. A., *Klin. Wchnschr.*, 1933, **12**, 773.

[3] Houssay, B. A., and Magenta, M. A., *C. R. Soc. Biol.*, Paris, 1925, **92**, 822.

[4] Braier, B., *Rev. Soc. Argent. Biol.*, 1931, **7**, 140.

[5] Houssay, B. A., and Biasotti, A., *Endocrinology*, 1931, **15**, 511.