Purification of an Erythrocyte-coating Polysaccharide from *Staphylococcus aureus*

WHEN erythrocytes are exposed to culture filtrates of *Staphylococcus aureus* they can be agglutinated by certain antisera[1-6]. The erythrocytes are then said to be 'sensitized'. Crude extracts of *Staphylococci* have previously been used, and the sensitized erythrocytes have been agglutinated by antisera prepared in rabbits immunized with *Staphylococci*[5] and also by human sera[3,4,6,10]. In this communication, the isolation of a sensitizing substance from *Staphylococci* using gel-filtration with 'Sephadex' is described.

A method described by Oeding[5] was used to obtain a crude sensitizing extract from a concentrated culture filtrate of *Staphylococcus*; the organism (phage type 71) was isolated from an infected burn, and the crude extract, which was insoluble in 90 per cent phenol, was freeze-dried.

25 mg of the crude extract was dissolved in 0·5 ml. buffered saline (1 part isotonic phosphate, *p*H 7·0, and 9 parts physiological saline) and added to a column of 'Sephadex *G*-200'. The column (20 cm × 1·1 cm) was packed and prepared according to the makers' instructions[7]. 20 × 1 ml. fractions were eluted from the column using buffered saline.

Each fraction was examined for the sensitizing substance, by adding 0·02 ml. of packed, washed sheep erythrocytes to 0·2 ml. of fraction and incubating for 2 h in a 37° water bath. After washing the erythrocytes in four volumes of saline, 0·2 ml. of a 0·2 per cent suspension of sensitized erythrocytes was added to 0·2 ml. of heat-inactivated rabbit antiserum and the tubes were examined for hæmagglutination after 2 and 18 h[9].

The sensitizing substance was found in the first two fractions after the void volume had been collected. The purity of these two and all other fractions was further examined by double diffusion techniques[9]. The eluted fractions were concentrated to 1/3 of their volumes by ultrafiltration through 'Cellophane' and diffused against undiluted antiserum prepared by immunizing the rabbits[8] with the same strain of *Staphylococcus aureus* used for preparing the sensitizing substance.

For the gel-diffusion experiments, 0·6 per cent Davis New Zealand agar in barbiturate buffer, *p*H 8·6, was used[9].

A common precipitation line was formed by the first two fractions. The second fraction produced another precipitation line which was common to the next three fractions. The highest molecular weight substances, which were the first fractions collected from the column, formed their precipitation lines closer to the antigen holes than substances of smaller molecular weight collected in later fractions. As the sensitizing substance was found only in the first two fractions it seemed likely that the precipitation line shared by the first two fractions might be identified with the sensitizing substance.

In another gel-diffusion experiment in which the rabbit antiserum had been absorbed with sensitized sheep erythrocytes, the precipitation line common to the first two fractions was no longer formed, confirming that this line could probably be identified with the sensitizing substance. It was also found that when each of the first six fractions were added to the rabbit antiserum before the addition of sensitized erythrocytes, hæmagglutination did not occur. This would suggest that a close relationship exists between the sensitizing substance found in fractions 1 and 2 and some substance which inhibits hæmagglutination, found in fractions 3–6, yet the two substances have been shown to be antigenically different.

As the sensitizing substance was found in the two fractions immediately after the void volume, it seems that the sensitizing substance is probably a large molecule with a molecular weight of 200,000 or more, whereas the substance which inhibited hæmagglutination was partially retained by the 'Sephadex' and therefore would have a molecular weight of less than 200,000. Both the sensitizing and the inhibiting substances reacted with ninhydrin, but only the sensitizing substance gave a positive Molisch test. Thus it appears that the sensitizing substance is a carbohydrate-peptide compound and the inhibiting substance is an antigenically related peptide. It is possible that the inhibiting substance has been derived from the sensitizing substance by loss of the carbohydrate material, which itself is necessary for linking the sensitizing substance to the erythrocyte surface.

As the sensitizing substance can now be separated from other serologically reacting components in crude staphylococcal extracts, it should be possible to determine whether the sensitizing substances from different phage types of *Staphylococci* are similar both serologically and chemically to each other, and to sensitizing substances from other bacterial species.

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RADIOBIOLOGY

Strontium-90 Plasma Levels and Excretions in Young Adults on a Low Calcium Diet

A PREVIOUS report[1] summarized strontium-90 data obtained on ten young adult volunteers studied on a metabolic ward on a constant diet containing approximately 1·1 g of calcium and 13·4 μμc. strontium-90 a day, most of the calcium and strontium-90 being ingested with milk. This communication compares some of the results obtained in a later examination of ten young adults of a similar group on the same diet without the added milk. The average daily intake during 3 periods of 6 days each was 179 mg calcium and 3·3 μμc. strontium-90.

Urine and plasma values are given in Table 1, the urinary calcium and strontium-90 in the first two columns of data being determined analytically, while the values in the other column were calculated by methods previously reported[1-3]. Calculations from food were made on the assumption that 20 per cent of the dietary strontium-90 was absorbed.

Table 1. STRONTIUM-90 IN URINE AND IN PLASMA

Volunteer	Urinary calcium (mg/day)	Urinary ^{90}Sr (μμc./day)		Plasma ^{90}Sr (μμc./l.) calculated	
		Found	Predicted	from urine	from intake
1	68	0·46	0·24	0·15	0·08
2	38	0·33	0·16	0·18	0·09
3	63	0·35	0·31	0·12	0·11
4	43	0·32	0·24	0·15	0·11
5	91	0·39	0·28	0·10	0·07
6	114	0·41	0·32	0·09	0·07
7	58	0·40	0·22	0·15	0·08
8	66	0·55	0·24	0·18	0·08
9	28	0·31	0·13	0·21	0·09
10	87	0·72	0·29	0·19	0·08
Average	65.6	0·424	0·243	0·153	0·086

As can be seen, the average urinary excretion of strontium-90 calculated from the intake of strontium-90 was about 60 per cent of that found, and the average strontium-90 plasma level calculated from food was 60 per cent of the average levels calculated from the urine. This would suggest that approximately 40 per cent of the strontium-90 plasma levels and urinary excretions during