GEL FILTRATION APPLIED TO SOME CLINICAL PROBLEMS

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In the present communication the preliminary results obtained by applying gel filtration to the study of some urinary enzymes and serum proteins, in clinical problems, will be briefly summarized.

URINARY ENZYMES

Gel filtration was used to study the possible occurrence of amylase isoenzymes, and it was shown that two amylolytic enzymes which could be separated by Sephadex G-100 gel filtration (Fig. 1)[1] occur in human urine. This was in apparent contrast with the data of other workers[2]. Peak II, on the basis of its catalytic properties, appeared to be an α-amylase[3]. Peak I, on the other hand, exhibited, in addition, maltase activity, and could be further fractionated into two enzymes by means of Sephadex G-200, as shown in Fig. 2. These two enzymes differ from α-amylase in several catalytic properties[4,5]. By means of gel filtration it was thus possible to find in human urine two polysaccharide-splitting enzymes, which can be regarded as gluco-amylases (E.C. 3.2.1.3) or α-glucosidases (E.C. 3.2.1.20). They probably bear some relationship

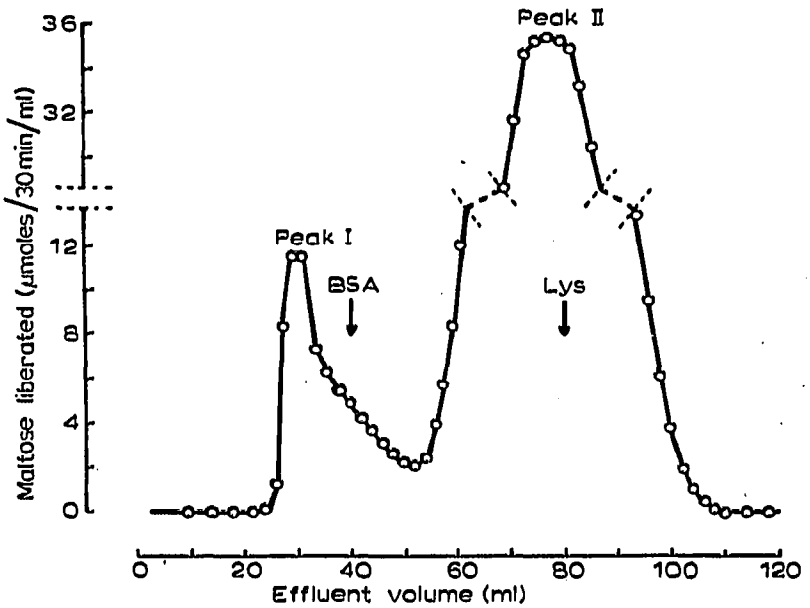

Fig. 1. Elution pattern of amylolytic activity from a Sephadex G-100 column. 1 ml of 1000-fold concentrated urine was applied to the column, and amylolytic activity (substrate: starch) was determined in the eluted fractions. The elution volumes of beef serum albumin (BSA) and lysozyme (Lys) are marked by arrows, for comparison.

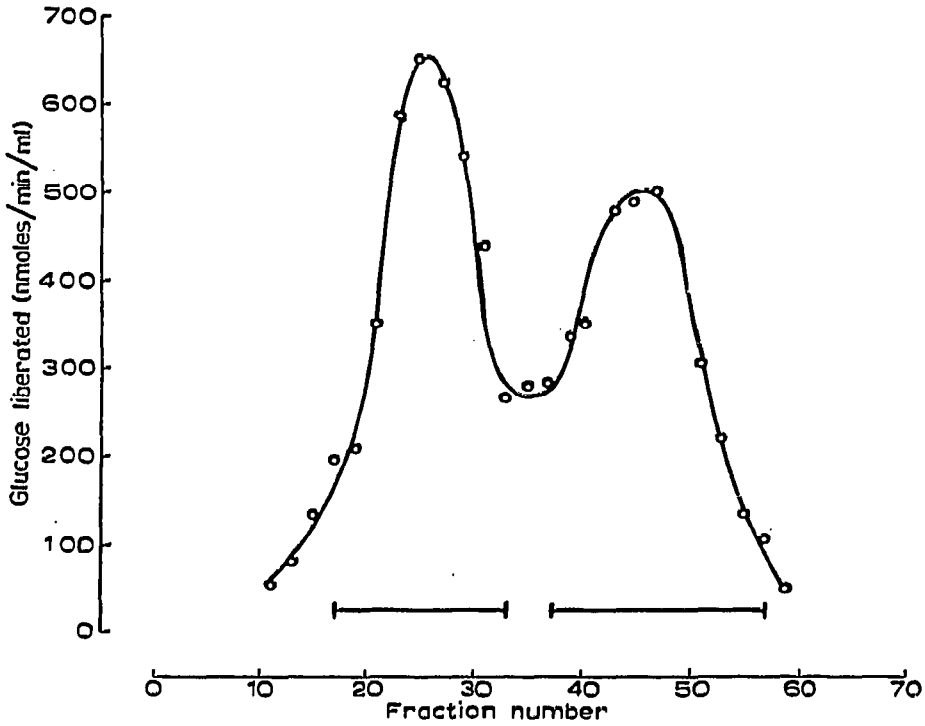

Fig. 2. Elution pattern of maltase activity from a Sephadex G-200 column: pooled, concentrated fractions, corresponding to peak I of Fig. 1, were applied to the column, and enzyme activity was determined in the eluate with maltose as a substrate.

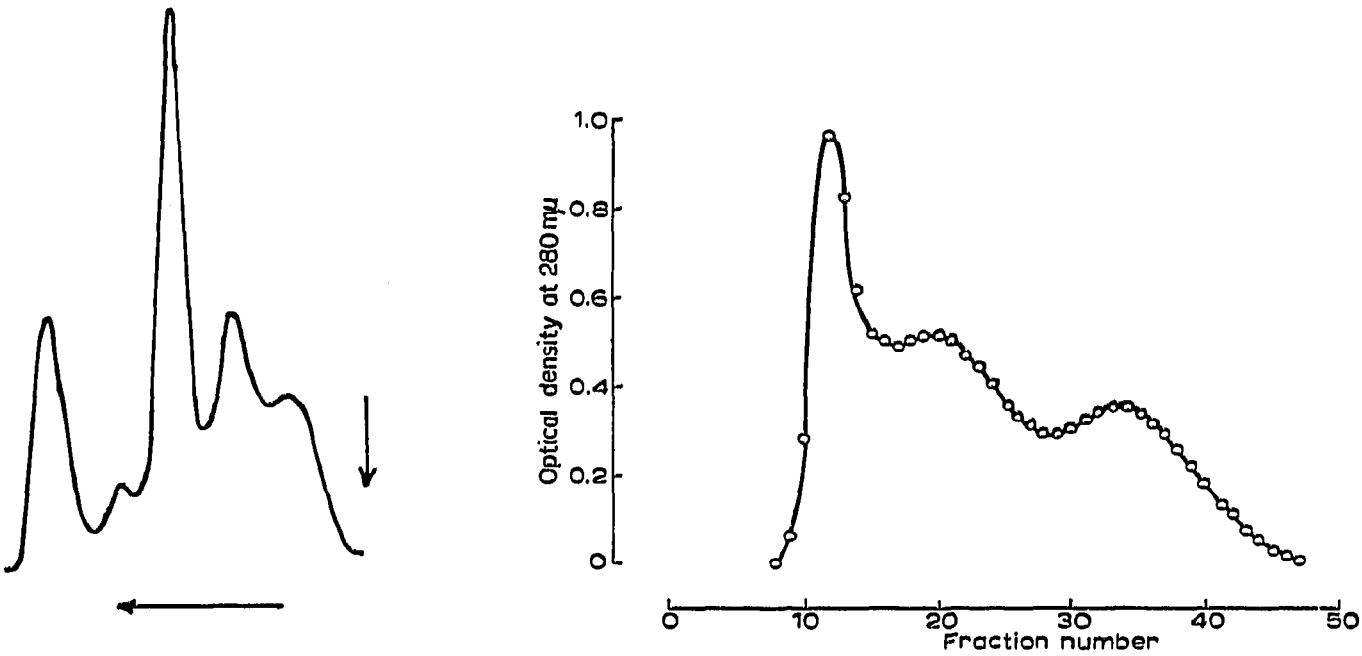

Fig. 3. Electrophoretic pattern (paper electrophoresis) of serum proteins from a patient suffering from malignant granuloma. Note the high increase of α_2-globulin.

Fig. 4. Gel filtration pattern of the same serum as in Fig. 3. Note the increase in the 19 S fraction.

to the "maltase" previously described in human urine[6], to the enzymes referred to by Dr. AURICCHIO in this Symposium[7], and to some enzymes described by several workers in some animal organs (*cf.* refs. 8 and 9).

SERUM PROTEINS

A method has been studied for the quantitative determination of γ_1- and α_2-macroglobulins, by combined Sephadex G-200 gel filtration and cellulose acetate electrophoresis. The method has been applied to the study of the sera from patients suffering from malignant granuloma, where an increase of α_2-globulin takes place[10]. In some cases we found this to be due, at least in part, to an increase of the α_2-macroglobulin[11]. A clear example is shown in the following figures: Fig. 3 shows the electrophoretic pattern of the serum from a malignant granuloma patient, with a very high increase in α_2-globulin. Fig. 4 shows the gel filtration pattern of the same serum (on Sephadex G-200), with the increase in the 19 S fraction. Fig. 5 shows the electrophoretic pattern of the 19 S fraction, compared with whole serum. Clearly most of the 19 S fraction is made up of α_2-macroglobulin, which accounts for about 20% of total serum proteins.

The possibility of assessing the molecular size of antibodies is a further application of gel filtration in the field of serum proteins[12,13]. The opportunity of studying a serum with a very high level of cold agglutinin[14] was presented. By means of gel filtration on Sephadex G-200 an increase of the 19 S fraction was demonstrated, which is in accordance with data obtained by means of ultracentrifugal analysis[15], and the molecular class of the agglutinin itself, which, as known, belongs to the 19 S group (Fig. 6) was characterized.

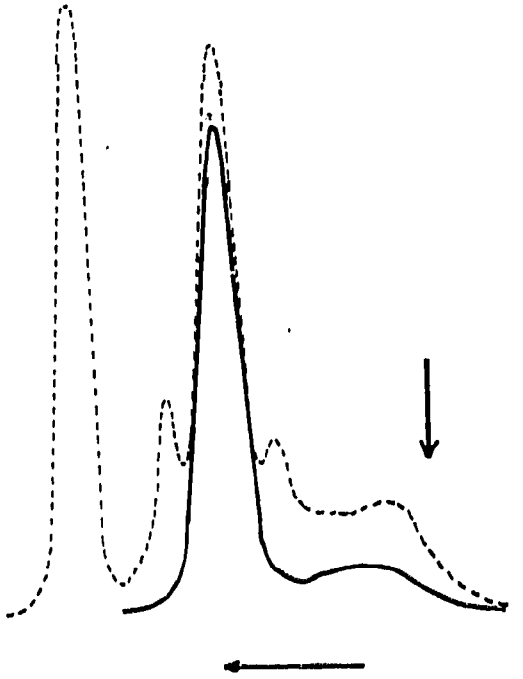

Fig. 5. Electrophoretic pattern (cellulose acetate) of the 19 S fraction obtained by the gel filtration experiment of Fig. 4 (continuous line), in comparison with the whole serum from the same subject (dotted line). The 19 S fraction is made up almost entirely of α_2-globulin.

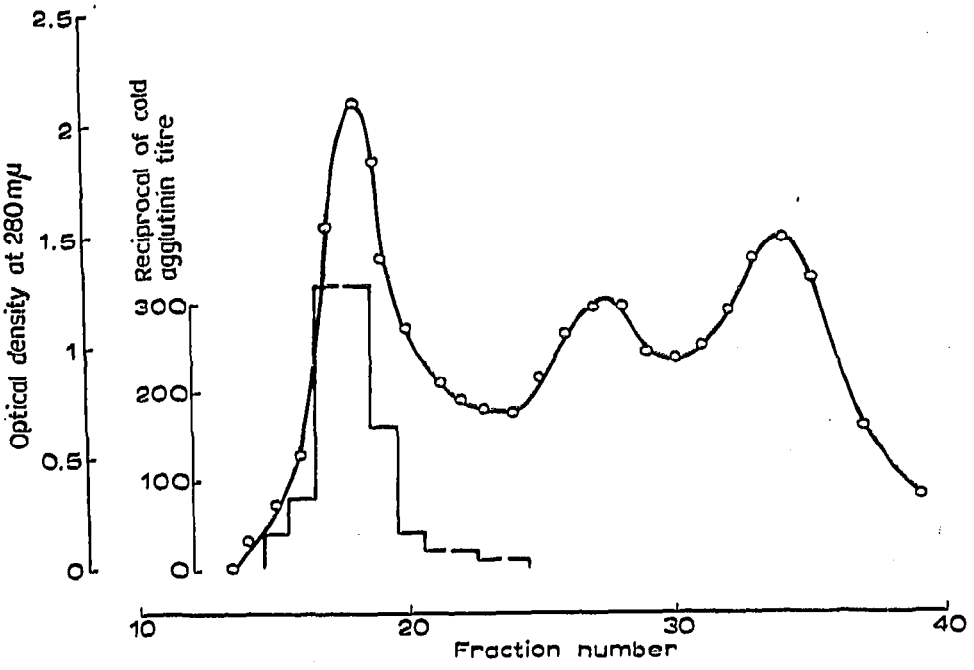

Fig. 6. Elution pattern of protein and cold agglutinin from a Sephadex G-200 column. 2 ml of a serum with a very high cold agglutinin titre (1:51, 200) were fractionated. The cold agglutinin is eluted with the 19 S peak.

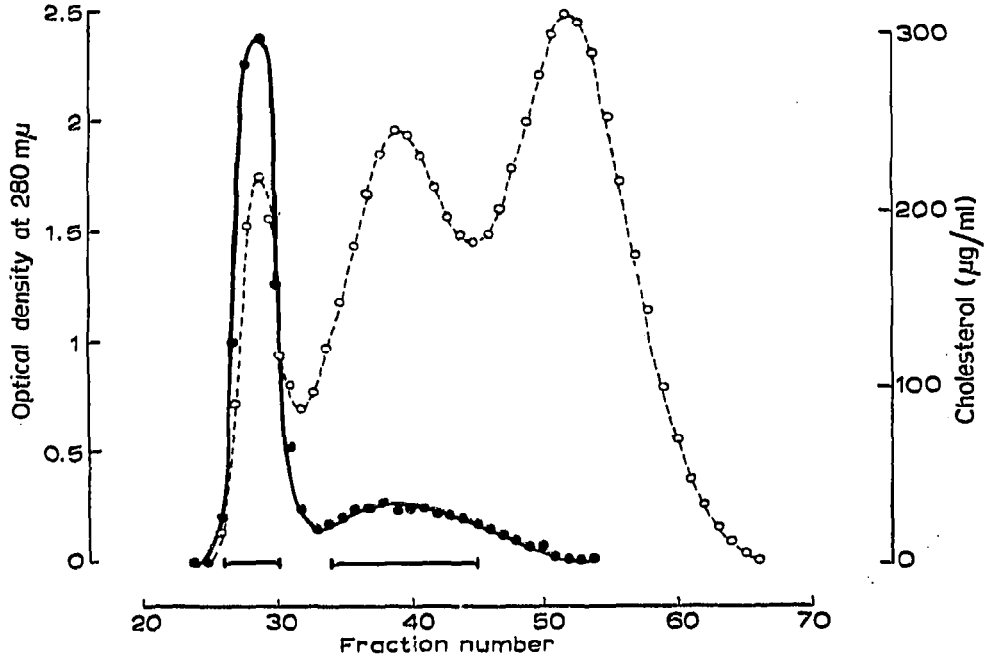

Fig. 7. Elution pattern of cholesterol (continuous line) and protein (as optical density at 280 mμ, dotted line), obtained by fractionating whole serum on a Sephadex G-200 column. Cholesterol is eluted into two peaks.

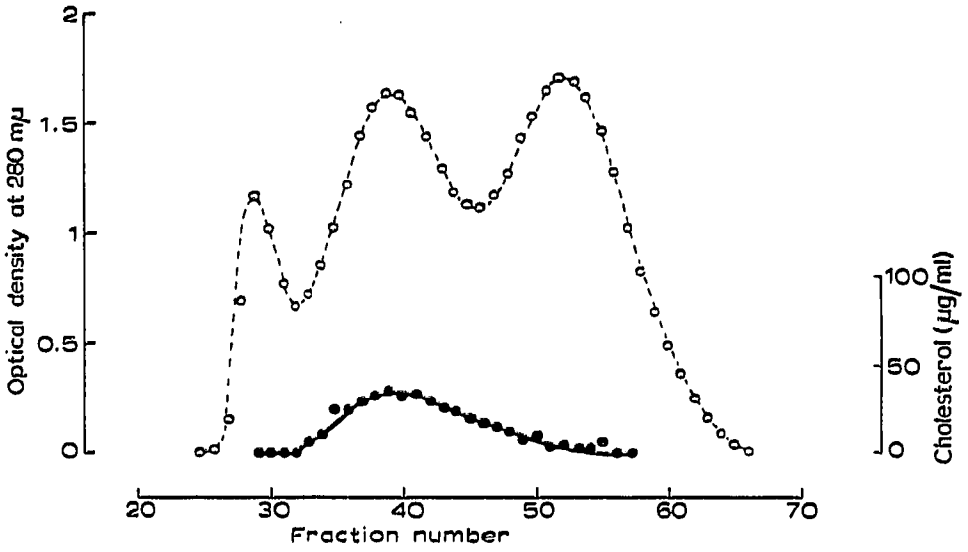

Fig. 8. Same as Fig. 7, obtained by fractionating β-lipoprotein-depleted serum.

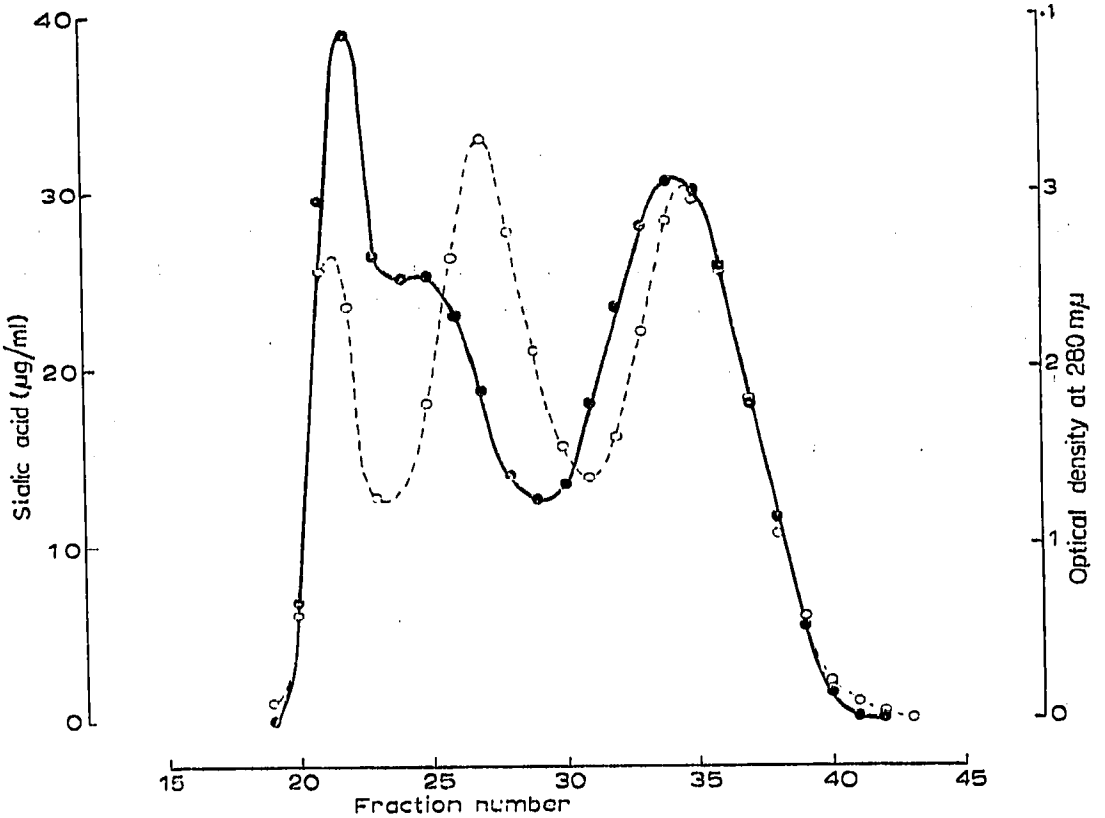

Fig. 9. Elution pattern of sialic acid (continuous line) and protein (as optical density at 280 mμ dotted line) obtained by fractionating adult serum on Sephadex G-200.

Conjugated proteins of human serum were also studied, in our laboratory, by means of gel filtration.

Lipoprotein distribution in the effluent fractions from a Sephadex G-200 column was monitored by means of cholesterol determination. Two peaks of cholesterol were obtained with whole serum (Fig. 7), and one only with β-lipoprotein-depleted serum (Fig. 8). The correspondence between the two peaks and β- and α-lipoproteins, respectively, was established by several criteria, including electrophoretic mobility and cholesterol/phospholipid ratios[16].

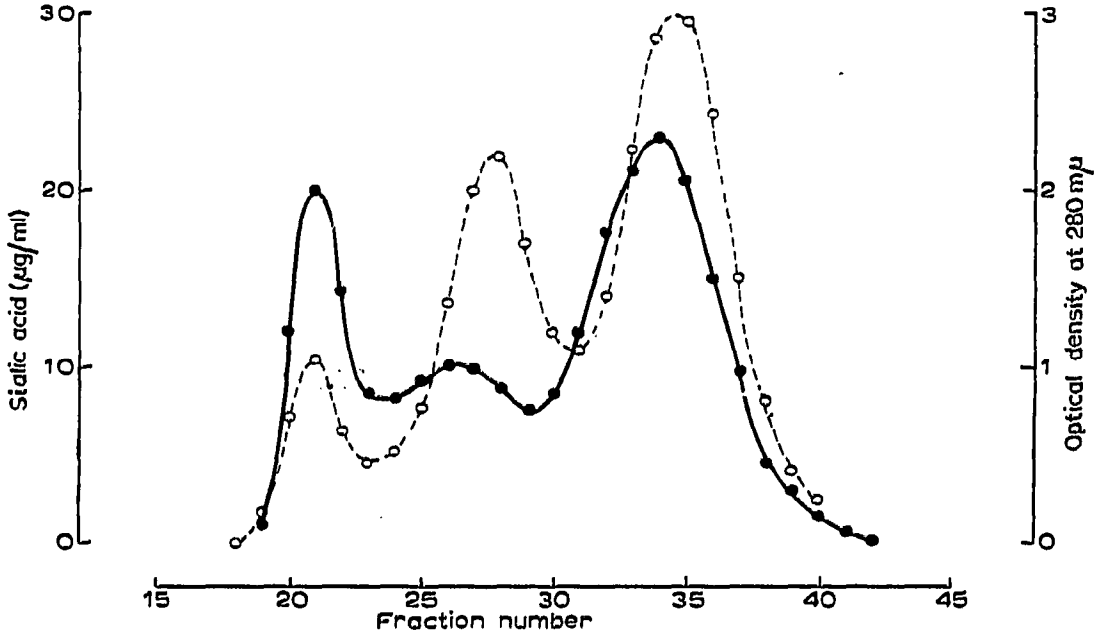

Fig. 10. Same as Fig. 9, obtained by fractionating cord serum on the same column.

Glycoprotein distribution was studied by determining the sialic acid content in the effluent fractions from a Sephadex G-200 column[17]. In search for an explanation for the low sialic acid levels in cord sera[18], both adult and cord sera were fractionated. Results are shown in Fig. 9 for an adult serum, and in Fig. 10 for a cord serum. In both cases sialic acid was recovered in three main peaks, roughly corresponding to the three protein peaks, the second being somewhat displaced. Quantitative distribution of sialic acid among the three peaks is shown in Table I. All the three fractions from

TABLE I

DISTRIBUTION OF SIALIC ACID AMONG THREE FRACTIONS OBTAINED BY SEPHADEX G-200 GEL FILTRATION

The data are the mean values from five experiments and are expressed both as mg/100 ml serum and as percentage of total recovered.

	19 S fraction		*7 S fraction*		*4 S fraction*	
	mg/100 ml	%	*mg/100 ml*	%	*mg/100 ml*	%
Adult sera (5)	16	21	20	24	43	55
Cord sera (5)	12	25	9	18	29	57

cord sera contained a lower amount of sialic acid, the more pronounced lowering being found in the 7 S fraction. The reasons for these findings, as well as for the displacement of the second peak of sialic acid, are now being investigated in our laboratory.

All the gel filtration experiments referred to, herein, were performed with columns of cross-linked dextran gels, marketed by Pharmacia (Uppsala) under the trade name Sephadex.

SUMMARY

When gel filtration was applied to the study of human amylolytic enzymes, it was possible to show in human urine the occurrence of two α-glucosidases, which could be separated from α-amylase.

In the field of serum proteins the following results were achieved: the hyper-α_2-globulinemia of malignant granuloma was found to be due, at least in part, to a high level of α_2-macroglobulin; an elevation of the 19 S peak was found to occur in cold agglutinin disease, and the antibody itself eluted in the same peak; α- and β-lipoprotein eluted separately; the quantitative distribution of sialic acid among the gel filtration fractions was studied in adult and cord sera.

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