THE CARBOHYDRATE OF γ-GLOBULIN AND MYELOMA PROTEINS

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(Received for publication, April 26, 1956)

Fractions of serum consisting primarily of γ-globulin as determined by electrophoresis have been shown to contain protein-bound carbohydrate (1–3). However, recent evidence concerning the electrophoretic inhomogeneity of γ-globulin (4–6) as well as the finding of secondary components in the ultracentrifuge (7) which might be high in carbohydrate has caused uncertainty regarding the existence of carbohydrate as a distinct constituent of the various species of proteins making up this fraction of serum.

The purpose of the present study was to investigate quantitatively the carbohydrate content of various components of serum γ-globulin as well as the pathological myeloma proteins which are immunologically related to these normal proteins (8–10). Five preparations of γ-globulin and twelve myeloma proteins were isolated electrophoretically and analyzed for hexose, fucose, hexosamine, and sialic acid. A method of zone electrophoresis was developed employing a polyvinyl chloride supporting medium which was particularly suited for carbohydrate analyses of separated fractions. It was found that various γ-globulin preparations from normal and pathological sera which were homogeneous in the ultracentrifuge contain a similar amount of carbohydrate. This was also true of certain of the myeloma proteins of the γ-globulin type. The β-myeloma proteins showed a higher carbohydrate content.

Gamma globulin separated from normal serum using filter paper electrophoresis has been variously reported to be periodic acid–Schiff (PAS) negative (11, 12) and positive (13, 14). Myeloma proteins have been stained with PAS after paper electrophoresis and were found to exhibit an intense PAS reaction (11, 15, 16). This finding has been interpreted to indicate that the myeloma proteins represent abnormal glycoproteins. These studies, however, were not quantitative and the protein-carbohydrate ratio could not be determined for comparison with normal γ-globulin. Various myeloma proteins have been found to differ markedly in electrophoretic mobility (17) and in their immunological specificity (8, 18, 19). The possible role of the carbohydrate constituents of these proteins in accounting for their differences has not been ascertained, although some evidence on this point has recently been reported by Smith and associates (10) and from this laboratory (20). Determination

of the amino acid composition has not completely accounted for the differences in mobility between the γ- and β-myeloma proteins.

Materials and Methods

Sera and Protein Preparations.—The normal sera employed in this study were obtained primarily from laboratory workers. They all contained between 0.7 and 1 gm. γ-globulin per 100 cc. The myeloma sera were obtained from multiple sources. Approximately half were examined within a few days of collection (Nos. XXI, XIII, XVI, X, XX, XV, XVIII). The remainder were stored for variable periods at 4°C. or in the frozen state. Three sera were shipped from North Carolina by air but not in the frozen state. All the myeloma sera contained more than 5 gm. of the myeloma protein per 100 cc.

Three preparations of γ-globulin were isolated in considerable amounts by preparative zone electrophoresis in the polyvinyl medium. One was obtained from the serum of a normal laboratory worker and another from a 12 year old patient with liver cirrhosis. The latter serum contained approximately 4 gm. γ-globulin per 100 cc. The third preparation was isolated from pooled normal serum. Preparations of Cohn fraction II γ-globulin from two different pooled lots were obtained from Lederle Laboratories. These consisted of approximately 98 per cent γ-globulin with only a trace of albumin and other proteins.

Electrophoretic Methods.—The electrophoretic separation of normal and myeloma sera was performed in a polyvinyl chloride supporting medium. This material did not interfere with any of the colorimetric methods used for carbohydrate and protein analysis. Preliminary experiments employing starch and powdered cellulose were considerably less satisfactory because of contamination from carbohydrate derived from the media. The polyvinyl particles were employed in a block form in a manner similar to that published previously for starch (21). The details of this procedure will be published separately (22). Starch zone electrophoresis was used in certain experiments particularly those demonstrating the heterogeneity of the γ-globulin fractions. Similar results in regard to protein separation were obtained by the two methods. The protein solutions were separated in barbital buffer at pH 8.6, ionic strength 0.1 or 0.05.

Separation of the carbohydrates obtained by hydrolysis of the various proteins was also carried out in the polyvinyl chloride medium. In this case sodium borate buffer 0.05 molar, pH 9.2 was used. A potential gradient of 12.5 volts/cm. and a time of 6 to 8 hours were employed. The comparative mobility of the different γ-globulins and myeloma proteins was determined by paper electrophoresis employing broad sheets as well as from the individual separations in the polyvinyl medium.

Ultracentrifugal Methods.—For analytical ultracentrifugation a Spinco model E machine was used. The proteins were dissolved in barbital buffer pH 8.6, ionic strength 0.1. The concentration of the samples was 4 to 5 mg. per ml. The temperature throughout the experiment was 20.0°C., speed 52,640 R.P.M. Analyses of the schlieren patterns were carried out using a micrometer comparator (23).

Preparative ultracentrifugation of γ-globulin was performed in a Spinco model L at 114,400 *G* for 6 hours. The concentration of the protein solution was 5 mg./ml. After ultracentrifugation the top portions were decanted and the remaining material resuspended in buffer. Samples of whole γ-globulin, an electrophoretically fast fraction and a slow fraction of γ-globulin were subjected to this procedure.

Carbohydrate Analysis.—Hexoses were determined by the anthrone reagent using essentially the method described by Mokrasch (24). In the case of quantitative assay of protein-bound hexose the measured optical density was corrected for color due to protein sulfuric acid interaction by subtracting the optical density of a duplicate which was devoid of anthrone. The standard deviation (S.D.) for 50 gamma hexose in water in the anthrone reaction was

3.2 per cent of the mean. The S.D. of the hexose concentration was 6.9 per cent of the mean when a protein solution was examined containing approximately 14 gamma of protein-bound hexose.

For the determination of hexosamine Elson and Morgan's method (25) was used. The proteins were hydrolyzed 14 to 15 hours to allow complete liberation of hexosamine (26). Hexosamine was separated from interfering chromogens by passing the hydrolysate over a dowex 50 column as suggested by Boas (26). The treatment of the test solution with alkali was carried out for 45 minutes at 90°C. to prevent the formation of non-specific chromogens. These were described by Schloss (27) to occur under alkali treatment in the boiling water bath. The S.D. for the hexosamine concentration of a solution containing about 42 gamma of protein bound hexosamine was 3.8 per cent of the mean.

The cysteine method described by Dische and Shettles was employed for the assay of fucose (6-deoxygalactose) (28). The color developed by the protein when heated in sulfuric acid in the absence of cysteine was subtracted from the color obtained in the cysteine reaction. The S.D. of the optical density in per cent of the mean was 5.4 for 20 gamma of fucose.

Sialic acid was determined using Bial's reaction as described by Werner and Odin (29). This method is regarded as the most specific one. Sialic acid is an acid carbohydrate which was first isolated from submaxillary mucin by Blix (30). Although the structure is not yet known it appears certain that it is not identical with hexuronic acid. It is closely related to neuraminic acid which was described by Klenk (31). Both these substances recently have been crystallized from serum proteins, neuraminic acid by Böhm and Baumeister (32), and sialic acid by Odin (33). Substances other than these which might give color in Bial's orcinol reaction are not known to occur bound to serum proteins. In this study the color produced by γ-globulin and myeloma proteins in Bial's reaction was interpreted as being caused by the acid carbohydrate which has been isolated from normal serum proteins and it has been called sialic acid. Two experiments designed to verify the presence of sialic acid demonstrated that the material in Fr II γ-globulin which gave the Bial reaction showed on separation from the protein the same electrophoretic mobility and the same absorption spectrum for the Bial reaction as crystalline sialic acid. Crystalline sialic acid from human ovarian cyst fluid for use as a standard was obtained through the kindness of Dr. Odin in Uppsala, Sweden.

Protein Determination.—Protein was assayed by both a modified Folin procedure (5) and by the ninhydrin method (8). The color was compared with the color produced by known amounts of albumin and regardless of the type of protein measured the results were expressed in weight units of human albumin. This protein was used as the standard because it was found to be free of carbohydrate (22). The intensity of ninhydrin color depends very much on the type of serum protein analyzed. This is not as true for the color obtained in the Folin reaction. For the latter standard curves for albumin and of γ-globulin are more similar. Therefore only the values obtained by the Folin method have been used in the calculation of molar ratios. In this method the S.D. of the concentration of a protein solution was found to be 5.3 per cent of the mean. In calculating molar ratios of carbohydrate and protein a molecular weight of 160,000 has been assumed for γ-globulin and the myeloma proteins. In specific instances ratios were checked by direct analyses based on the dry weight of the individual proteins.

RESULTS

1. Subfractionation of γ-Globulin by Zone Electrophoresis.—An attempt was made to determine the extent of separation of γ-globulin fractions in a typical experiment employing zone electrophoresis. In the bottom of Fig. 1, the protein curve obtained by such a fractionation of Fr. II γ-globulin is illustrated. Comparison with a normal serum separated at the same time

indicated that this curve corresponded very closely to the distribution of γ-globulin in whole serum. Fractions 3 to 10 from this experiment were each concentrated by ultrafiltration and then separated individually on one broad

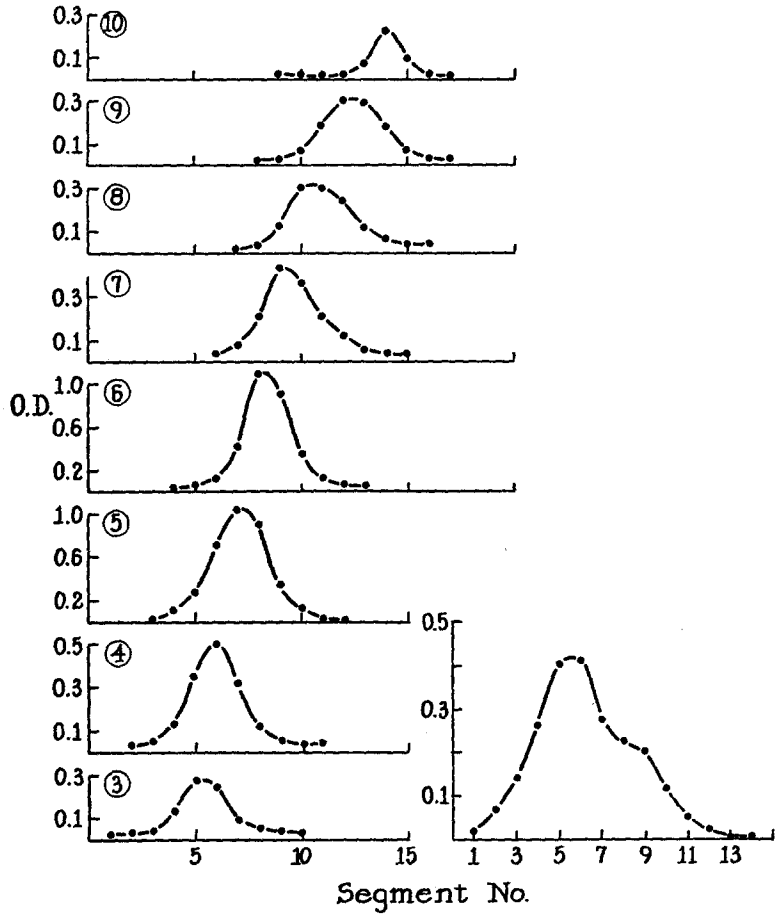


FIG. 1. Subfractionation of Fr. II γ-globulin by zone electrophoresis. The right lower curve represents the pattern obtained for the whole γ-globulin. The series of curves on the left show fractions 3–10 of the whole γ-globulin following separation in a second experiment employing one broad block. The relative mobilities are indicated by the distribution according to segment number. The site of application was segment 10 in each case.

starch block so that they could be compared directly under the same conditions. The upper curves of Fig. 1 show the distribution of the proteins in each of these fractions. It can be seen that each fraction has a slightly different mean mobility although there is considerable overlap of the peaks.

Two experiments with whole serum gave essentially similar results for the

subfractionation of the γ-globulin by these techniques. The degree of separation was primarily a function of the width of the initial band relative to the final distribution. By special selection of fractions, it was possible to obtain as many as four non-overlapping components. It was apparent that the zone electrophoretic distribution of γ-globulin reflects the presence of a broad spectrum of proteins of different mobilities.

TABLE I

Ultracentrifugal Composition and Carbohydrate Content of Various γ-Globulin Preparations

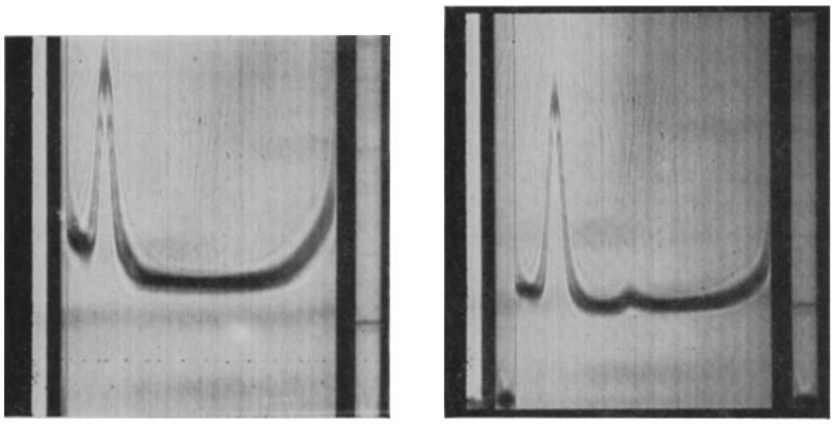
No.	Gamma globulin preparation	Ultracent. Comp.		Carbohydrate	
		7 S	19 S	$\frac{\text{Mg. hexose}}{\text{Mg. protein}} \times 10^{-3}$	$\frac{\text{Mg. hexosamine}}{\text{Mg. protein}} \times 10^{-3}$
		per cent	*per cent*		
1.	Cohn Fr. II (*a*)	100		11.9 ± 0.5	11.5 ± 0.3*
2.	Cohn Fr. II (*b*)	100		11.3	11.2
3.	γ-globulin (normal)‡			14.1	13.3
4.	γ-globulin (cirrhosis)‡	99	1	12.1	13.0
5.	γ-globulin (pooled, normal)‡				
	Whole	93	7	15.3	14.3
	Ultracent. top	97.5	2.5	12.5	12.6
	Slow fraction				
	Whole			11.3	10.4
	Ultracent. top			11.3	11.2
	Fast fraction				
	Whole	91.2	8.8	17.6	17.2
	Ultracent. top	96.5	3.5	14.9	14.2
	Ultracent. bottom	77.2	22.8	24.7	

* Standard deviation.

‡ Electrophoretically isolated.

2. Ultracentrifugal Studies.—Table I indicates the results obtained by ultracentrifugal analysis of various types of γ-globulin used in this study. It is evident that electrophoretically isolated γ-globulin contains a heavy ($s_{20,w}$ = 19 S) component which is not present in the preparations of Fr. II γ-globulin (Fig. 2). The myeloma proteins which migrated in the γ-globulin region all showed only one component in the ultracentrifuge ($s_{20,w}$ = 7 S). The myeloma proteins of faster mobility were more complex when examined in the ultracentrifuge and showed a 9.5 S component in addition to the usual 7 S. The

concentration of the former varied considerably ranging from approximately 8 per cent (No. XVIII) to 50 per cent (No. XV and XII). In addition, an 11.6 S component was observed in some instances. Evidence was obtained that the amount of the 9.5 S and 11.6 S fractions varied considerably even in the same myeloma protein examined at different times after concentration and dialysis. It was also possible to produce this material irreversibly at the expense of the $s_{20,w}$ = 7 S component by lowering the pH to 5.5 This suggested that the $s_{20,w}$ = 9.5 S and 11.6 S material represents at least in part an aggregate of the lower molecular weight component.



2 *a* 2 *b*

FIG. 2. Schlieren patterns obtained by ultracentrifuge analysis of γ-globulin. Fig. 2 *a* represents Fr. II; Fig. 2 *b*, electrophoretically isolated γ-globulin. A small 19S component is visible in the latter pattern in addition to the major 7S fraction.

3. The Carbohydrate of γ-Globulin.—Assay of ten normal sera for neutral hexose following separation by zone electrophoresis always showed carbohydrate to be present throughout the γ-globulin as well as in the other globulin fractions. One of these experiments is illustrated in Fig. 3. A definite carbohydrate peak is evident in segments 4 to 9 which represent the γ-globulin. Quantitative determination of carbohydrate was carried out on five different preparations of whole γ-globulin and the results are shown in Table I. Hexose and hexosamine determinations on two different preparations of Fr. II and on the three electrophoretically isolated γ-globulin fractions described previously are illustrated.

The values obtained for the two preparations of Fr. II are in close agreement, but smaller than those obtained for whole γ-globulin isolated by electrophoresis. The two γ-globulin preparations from normal serum show similar carbohydrate content, whereas the γ-globulin from a patient with cirrhosis

is lower in carbohydrate and only slightly higher than Fr. II. The γ-globulin from 2 other patients with an abnormal elevation also showed an intermediate carbohydrate content. The difference between the various γ-globulins might be due to proteins of higher carbohydrate content included in the electro-

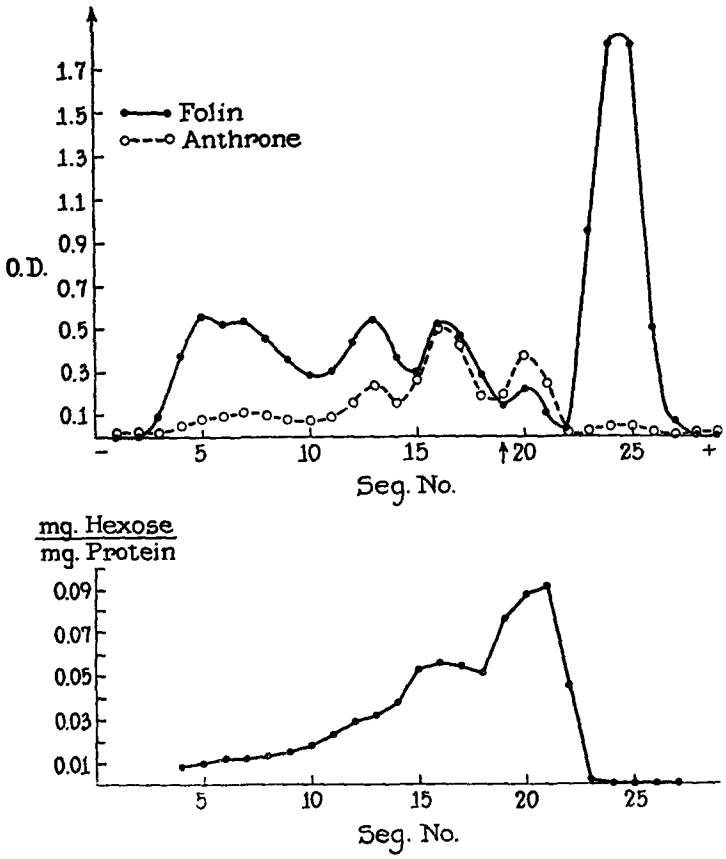


FIG. 3. Upper curves: protein and hexose levels for fractions of a normal serum after electrophoresis in polyvinyl chloride medium. Lower curve: The hexose-protein ratio of each of the fractions of the separated serum.

phoretic preparations but absent from Fr. II γ-globulin. Since the heavy component ($s_{20,w}$ = 19 S) was absent in preparations 1 and 2 and of low concentration in 4 (Table I), its contribution to the carbohydrate of γ-globulin was investigated. An attempt was made to see whether the carbohydrate content of the γ-globulin could be changed by preparative ultracentrifugation which increases the relative percentage of this heavy component in the bottom fraction and decreases it in the supernatant. For these experiments γ-globulin

prepared by electrophoresis from normal serum was divided into two equal parts, one of slow mobility and the other fast. This could be done readily by dividing the γ-globulin peak through the center. In the case of the fast fraction of γ-globulin, for which experimental data are most complete, it is apparent from Table I that a close correlation exists between the percentage of 19 S component and the elevation in carbohydrate-protein ratio. The same correlation also is seen when whole γ-globulin is compared with the upper fraction obtained by preparative ultracentrifugation. The carbohyrdate of the γ-globulin of slow mobility did not change significantly after ultracentrifugation. It is comparable to that of Fr. II. The high carbohydrate content of the 19 S component was confirmed in a separate experiment where a γ-globulin preparation was obtained by repeated ultracentrifugation which contained 95 per cent of this heavy component. A hexose-protein ratio of approximately 60×10^{-3} was obtained. This figure is in close agreement with that calculated for the heavy component from the data of Table I.

Since the major 7 S portion of γ-globulin is a mixture of electrochemically specific components, experiments were carried out to determine whether these subfractions differ in carbohydrate content. It has been shown above that zone electrophoresis at pH 8.6 leads to a resolution of γ-globulin into multiple overlapping components. Differences in the carbohydrate composition of these subfractions may be detected by analysis of a group of adjacent segments of electrophoretically separated γ-globulin. Such an analysis was carried out on one preparation of Fr. II which contained no heavy component (Fig. 4). The greater part of the separated Fr. II γ-globulin contains per molecule of protein about 10 molecules of hexosamine and 10 to 12 molecules of hexose of which 2 are fucose. The other hexose molecules are mannose and galactose as was found by electrophoresis of hydrolysates of γ-globulin in borate buffer. In addition sialic acid was determined on the eluates of segments 11 to 16. Approximately 1 molecule was found per molecule of protein (molar ratio 0.86 to 1.10). Only in the very fast portion of Fr. II γ-globulin were proteins found which appeared to be slightly richer in carbohydrate. Gamma globulin prepared by electrophoresis was difficult to study in this manner because of the additional presence of the 19 S component. The latter showed maximum concentration just ahead of the main γ-globulin peak and the carbohydrate protein ratio increased in the faster migrating γ-globulin fractions where the relative concentration of heavy component was greatest. This can be seen in the lower curve of Fig. 3 where there is a rise from approximately 0.01 to 0.018 in hexose-protein ratio.

An attempt was also made to subfractionate the homogeneous preparation of Fr. II by dialysis against distilled water. A euglobulin precipitate was obtained consisting of approximately 12 per cent of the total material. This showed a slighly higher carbohydrate content than the pseudoglobulin fraction

and the original material. However, it was not possible to determine whether or not this was due to trace amounts of the 19S fraction not detectable by ultracentrifuge analysis that might have contaminated the starting material and become concentrated in the euglobulin fraction. The 19S fraction is certainly a euglobulin and water dialysis was used as one of the procedures for its concentration.

4. Myeloma Proteins.—The sera of 18 patients with multiple myeloma were separated by electrophoresis in the polyvinyl-supporting medium and the

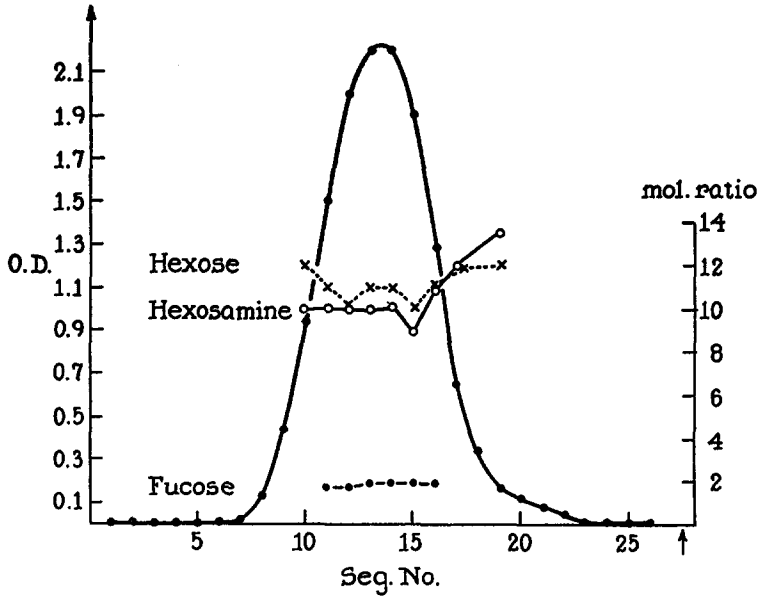


FIG. 4. Carbohydrate-protein ratios obtained at different points over the curve of Fr. II γ-globulin separated by zone electrophoresis.

pathological fraction was tested with anthrone. With one exception all the myeloma proteins were found to give a distinct anthrone color throughout the fractions making up the protein peak. Fig. 5 illustrates the curves of three sera containing myeloma proteins of different mobilities. It can be seen that the two faster proteins yielded more anthrone color relative to protein than the slow one. In the case of protein XV a brownish green color developed instead of the blue-green color which most sugars produce with anthrone. This color can be caused by interfering amino acids or by certain desoxyhexoses (35). To exclude the presence of anthrone-reactive compounds other than mannose and galactose, which are known to occur in serum proteins, the hydrolysates of myeloma proteins X, XV, XVIII were subjected to electrophoresis in borate buffer on polyvinyl. A solution of mannose and galactose

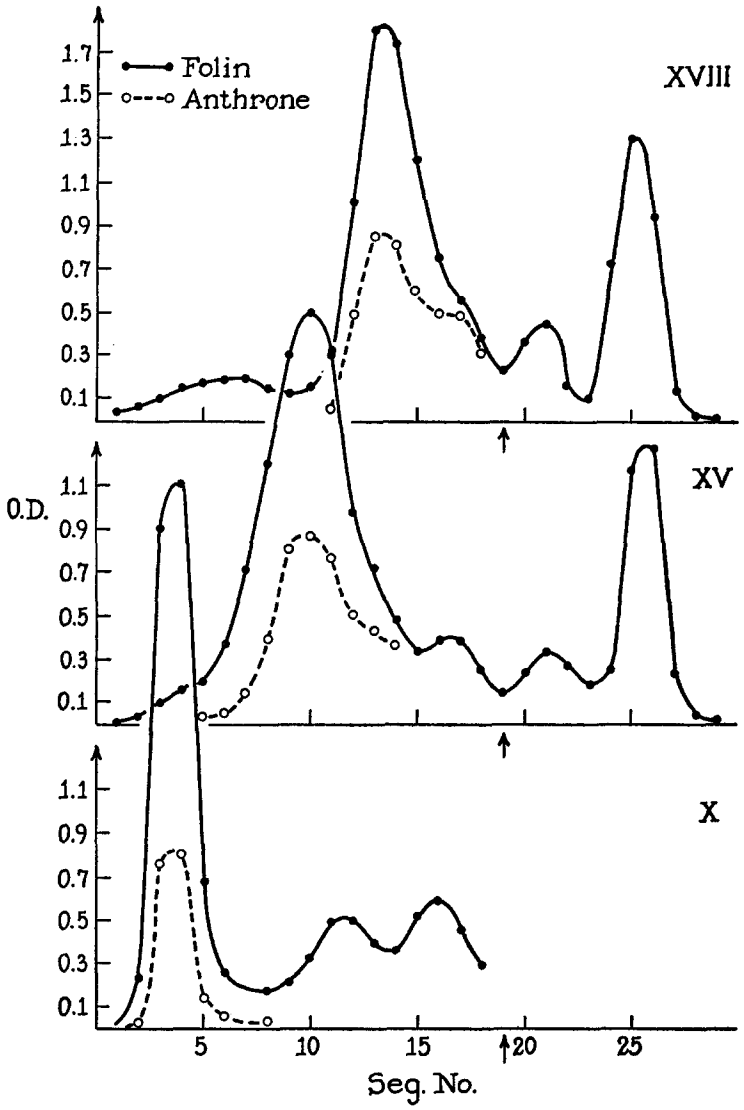


FIG. 5. Protein and hexose curves for three sera from patients with multiple myeloma after electrophoresis in the polyvinyl medium. The abnormal protein component has a different mobility in each serum and contains definite carbohydrate.

was separated simultaneously on the same block. After separation the sugar borate complexes were detected and quantitated by anthrone. As demonstrated in Fig. 6 carbohydrate was present in each of the hydrolysates and could be identified as mannose and galactose. The observed color was a pure blue-green.

The one serum which showed a myeloma protein that failed to react with anthrone had been stored for several months at 5°C. prior to examination. On electrophoretic analysis of the protein-free ultrafiltrate of this serum, glucose and mannose were identified. In addition, a third component was obtained exhibiting a mobility less than that of mannose. On acid hydrolysis

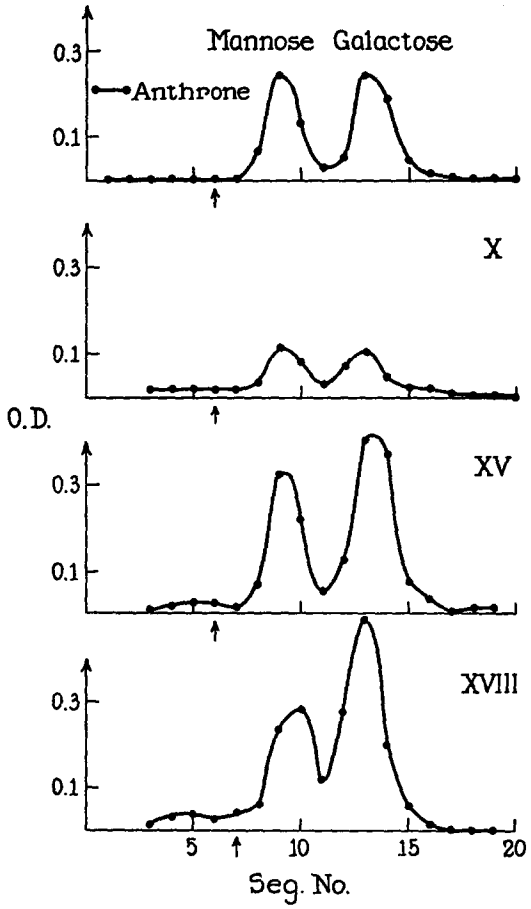


FIG. 6. Hexose curves of hydrolysates of myeloma proteins after electrophoresis in borate buffer. The upper pattern was obtained with a test solution of mannose and galactose (1:1)

this component split into galactose and mannose. During storage this myeloma protein apparently had been partially degraded with liberation of carbohydrate. It is of interest that this protein without the carbohydrate showed the lowest mobility of all the myeloma proteins in this series. The serum was sterile.

Twelve purified myeloma proteins isolated electrophoretically on polyvinyl were analyzed for total hexose, hexosamine, fucose, and sialic acid. In Table II

the results of these analyses are compared with those obtained for Fr. II γ-globulin. The results are given as "approximate" molar ratios. The reasons for this are twofold. First the protein has not been measured by weight but colorimetrically. Secondly, the greater part of the carbohydrate determinations on the myeloma proteins could not be performed repeatedly because of lack of material. The proteins have been arranged according to their electro-

TABLE II

Carbohydrate Content of Different Myeloma Proteins Compared to Normal γ-Globulin

No.	Electrophoresis	mg. Ninh. / mg. Fol.	Carb. Moles per Mole Protein				Hexosamine / Hexose ‡
			Hexose	Hexos-amine	Fucose	Sialic acid	
♂ Fr.II			10.5±0.4*	10.3±0.3*	2.0±0.1*	1.0	0.98
XXI		0.58	16.0±0.8*	11.6±0.3*	2.8	1.9	0.74
XIII		0.55	7.8	8.6	2.0	0.8	1.10
XVI		0.50	6.8	8.7	2.0	0.5	1.28
VI		0.50	10.0	8.6		1.2	0.86
X		0.50	9.0	8.9		0.9	0.99
XVII		0.50	9.0	9.0	2.0	0.6	1.00
XX		0.50	8.3±0.1*	7.5±0.2*	2.0	1.2	0.91
III		0.50	24.2	15.1		1.0	0.62
XII		0.45	25.0	21.2	2.3	3.0	0.85
XV		0.42	26.0	21.1		3.0	0.81
XI		0.42	23.4	18.8		5.7	0.80
XVIII		0.44	22.9	22.4	3.0	4.0	0.98

* Standard Deviation
‡ Molar Ratio

phoretic mobilities as indicated by the location of each spot relative to the broad γ-globulin distribution. It is apparent that the myeloma proteins fall into 2 main groups. One of these includes myeloma proteins with a carbohydrate moiety close to but slightly less than that of Fr. II γ-globulin and a mobility falling into the range of the mobility of γ-globulin (XIII, XVI, XVI, XVII, XX). The other group consists of protein containing more than twice as much carbohydrate as normal γ-globulin and migrating with the mobility of the β-globulins (XII, XV, XI, XVIII). Myeloma protein III appears to represent an intermediate type with regard to both electrophoretic mobility

and carbohydrate content. Myeloma protein XXI also appears to be an intermediate type in respect to carbohydrate but not in mobility. The mean values for the two major groups are as follows:

	Hexose	Hexosamine
Group I	8.50 ± 1.01	8.50 ± 0.49
Group II	24.30 ± 1.24	20.80 ± 1.30

For both properties the confidence limits of the means of Group I and Group II do not overlap at $p < 0.001$. Hexose and hexosamine values for Fr. II γ-globulin shown in Table II are slightly higher than those for Group I ($p < 0.001$). This difference was also apparent in the case of myeloma XX from which replicate analyses were obtained ($p < 0.001$). Small differences in the hexosamine-hexose ratio also appeared to be present for some of the myeloma proteins. In addition the β-myeloma proteins show 3 to 6 times more sialic acid than the γ-myeloma proteins and normal γ-globulins.

The pathological γ-globulins from 2 cases of Waldenström macroglobulinemia were also examined for carbohydrate. Hexose:protein ratios of 50×10^{-3} and 52×10^{-3}[1] were obtained.

DISCUSSION

A considerable subfractionation of γ-globulin is possible by simple techniques of zone electrophoresis in a starch- or polyvinyl-supporting medium. These fractions differing slightly in mean mobility are very similar in the ultracentrifuge and consist primarily of an $s_{20,w} = 7$ S component. Each fraction contains carbohydrate and the amount appears quite constant. Gamma globulin isolated directly from serum by zone electrophoresis contains an additional ultracentrifugal component ($s_{20,w} = 19$ S) which is not always present in γ-globulin of similar mobility isolated by the Cohn procedures or by electrophoresis convection (6). The reason for this difference is not entirely clear. Apparently the heavy component is sometimes precipitated in a different fraction than the main γ-globulin when isolated by the Cohn procedures. Fractionation of γ-globulin by electrophoresis convection is usually carried out at a pH lower than that used for isolation by zone electrophoresis and it is possible that the heavy component has a different mobility relative to the main γ-globulin at a pH nearer the isoelectric point of these proteins than at pH 8.6. In the present study it was possible to concentrate the heavy fraction by differential centrifugation and it was found to be considerably higher in carbohydrate than the main 7 S component indicating that it did not represent a simple aggregate of the latter.

[1] Crystalline preparation kindly furnished by Dr. Harold Deutsch.

A certain amount of evidence has accumulated (34, 10) in support of the idea that the myeloma proteins represent huge elevations of individual components of the normal spectrum of γ-globulins. There remain a few observations, however, that are difficult to explain in terms of this concept. This is particularly true of some immunological data showing extreme differences in myeloma proteins of very similar mobility both in regard to their relationship to each other and to normal γ-globulin (8, 19). The present observations on the carbohydrate content of the myeloma proteins indicates that those migrating in the γ-globulin region resemble the 7 S component of γ-globulin, with slight yet significant differences in certain instances. Although these results are difficult to interpret, they are not easily reconciled with the concept that these are completely normal proteins.

The problem with the β-myelomas is more complex because of the lack of information regarding the carbohydrate content of the normal β-globulin constituents to which these myeloma proteins might be related. It is known that there is some 7 S material with this mobility in normal serum (8) but the quantity is relatively small and it has not been separated from the iron-binding protein which makes up a large part of this fraction. All of the β-myeloma proteins encountered in this study were found to contain considerably more hexose, hexosamine, and sialic acid than the γ-myeloma type or normal γ-globulin. Smith and associates (10) in a detailed study of the amino acid composition of four myeloma proteins also noted that one, with more rapid mobility, contained more carbohydrate than the others.

One of the advantages of working with the myeloma proteins and other pathologically elevated γ-globulins is that their extremely high concentration in serum makes isolation in a relatively pure form comparatively easy and eliminates much of the possibility of contamination with other materials that might be high in carbohydrate. The variable mobility of the different myeloma proteins also helps in identifying the carbohydrate as a distinct constituent of these proteins. The results with the myeloma proteins and particularly with other pathologically elevated γ-globulins complement the observations with normal γ-globulin.

The high carbohydrate content, particularly of the portion that appears to be sialic acid, may contribute to the more acid characteristics of the more rapidly migrating β-type proteins discussed above. The difference in carbohydrate may also account for some of the immunological differences between the γ- and β-type proteins (8, 10).

All of the various normal γ-globulins examined in this study contained at least 20 moles of various carbohydrates as definite constituents of the total protein. This increases the number of possibilities for variation in the spatial configuration of these proteins which may have bearing on the problem of antibody specificity. The very high carbohydrate content of the 19 S com-

ponent of normal γ-globulin is of particular interest because of the evidence indicating that certain antibodies fall into this high molecular weight fraction (36).

SUMMARY

Various preparations of γ-globulin homogeneous in the ultracentrifuge showed a similar content of hexose, hexosamine, fucose, and sialic acid. Subfractionation of Fr. II γ-globulin by zone electrophoresis revealed multiple components of different mean mobilities but containing similar amounts of carbohydrate.

Gamma globulin isolated directly from normal serum by zone electrophoresis showed a heavy component in addition to the usual 7 S material. The heavy component ($s_{20,w}$ = 19 S) concentrated by preparative ultracentrifugation was found to be considerably richer in carbohydrate than the rest of the γ-globulin and accounted for small differences in carbohydrate content between different preparations of γ-globulin.

Pathological sera with marked elevation in γ-globulin showed a carbohydrate-protein ratio for the γ-globulin similar to that found for the corresponding 7 S fraction in normal serum. This was only partially true of the myeloma proteins with a mobility in the γ-globulin region. Certain of these proteins showed slight but significant differences. The myeloma proteins of faster mobility (β-myelomas) contained considerably more carbohydrate. The possible role of these carbohydrates in accounting for some of the mobility and immunological differences in the myeloma proteins is discussed. The pathological proteins found in two cases of macroglobulinemia showed a high carbohydrate content similar to but slightly lower than the normal 19 S component of γ-globulin.

The authors are indebted to Drs. Wayne Rundles, Leonard Korngold, Bruno Volk, and William Eisenmenger for certain of the myeloma sera used in this study. Drs. Arno Motulsky and Wade Volwiller kindly furnished serum from a patient with Waldenström macroglobulinemia.

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