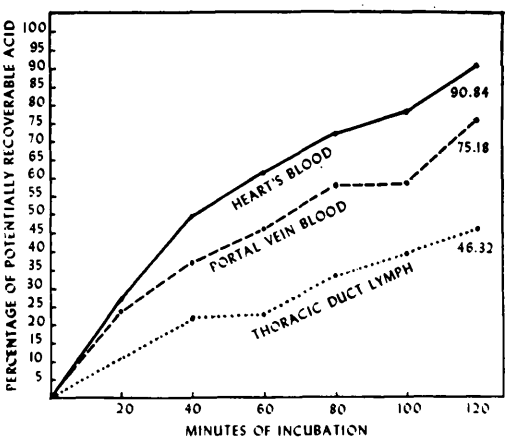

FIG. 1. Percentage recovered of potentially recoverable salicylic acid after *in vitro* incubation of acetylsalicylic acid in the respective fluids (rat).

of the potentially recoverable acid, using the means of 4 separate pools of each fluid at each of the time intervals. The highest recoveries at all intervals were from heart's blood, next highest from portal vein blood, and lowest from lymph. Throughout the entire 2-hour period the hydrolysis of aspirin proceeded at only about one-half the rate in lymph as in heart's blood. It is doubtful if the findings in the portal vein blood are truly representative of the enzymatic-hydrolysis-potential of that fluid directly as it leaves the site of principal aspirin absorption in the small intestine, because the specimens were taken without tying off the confluent veins from the stomach, spleen and pancreas, and therefore they contain some blood that had recently traversed the organs named. The presently designated portal vein blood is therefore actually such blood as it is delivered to the liver but it is not truly the first sanguine fluid that is encountered as aspirin is absorbed.

Discussion. The data establish that aspirin is hydrolyzed much less rapidly in lymph than in blood. It will be the objective of subsequent studies to determine whether the respective rates can be influenced by any adjuvant measures in the hope that the findings may help in the development of a satisfactory technic for determining salicylate antiphlogistic action in the laboratory.

Summary. The *in vitro* enzymatic hydrolysis of acetylsalicylic acid (aspirin) has been found to proceed only about one-half as fast in the abdominal thoracic duct lymph of the rat as in the heart's blood of the same animal.

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Two Types of γ-Globulin Differing in Carbohydrate Content. (22690)

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Considerable evidence has accumulated suggesting that certain antibodies in human (1,2,3) as well as horse sera(4,5) are of higher molecular weight than other antibodies and the bulk of γ-globulin. A sedimentation constant of approximately 19 S has usually been observed in the ultracentrifuge indicating a molecular weight close to 1 million instead of the ordinary 150,000. The possibility has been raised that these large proteins are aggregates of the low molecular weight components(6). Recent experiments employing zone electrophoresis(7) have furnished evidence that normal human γ-globulin contains from 5-10% of 19 S material as a constant constituent. This is localized primarily in the faster migrating portion of the γ-globulin. Gamma globulin prepared by

Deutsch employing alcohol fractionation has been found to contain significant amounts of this heavy component(1).

Preliminary observations(7) indicated that this high molecular weight γ-globulin was very rich in carbohydrate as compared to the main 7 S fraction. The latter was found to contain approximately 2.5% of carbohydrate consisting of hexose sugars, hexosamine and sialic acid which remained quite constant in various preparations. The present report describes further observations on the considerably higher carbohydrate content of the 19 S component of γ-globulin which has been purified by repeated preparative ultracentrifugation.

Methods. Zone electrophoresis was carried out primarily in a polyvinyl chloride supporting medium(7) because this was particularly suitable for carbohydrate analyses of isolated fractions. Starch was also used as supporting medium but not in those instances where carbohydrate analyses were done. Both media were employed in a similar manner and the amount of 19 S γ-globulin isolated by the two methods was quite comparable. Several preparations highly enriched in heavy material were prepared from pooled normal serum by preparative ultracentrifugation in a Spinco Model E ultracentrifuge. In one instance 864 cc of normal human serum were recycled 8 times at 114,000 x G. The times chosen were sufficient to sediment the heavy material into the bottom of the tube, and depended on the total protein concentrations of the solutions. Beginning with the fifth centrifugation albumin was added in an attempt to prevent denaturation of the heavy material. The final preparation, consisting of primarily 19 S material and albumin, was separated by zone electrophoresis employing polyvinyl chloride as a supporting medium. Analytical ultracentrifugation of the fractions was carried out in a Spinco Model E ultracentrifuge and the composition was determined by methods described by Trautman (8). Hexose sugars were determined colorimetrically using the anthrone method according to Mokrasch(9). The coefficient of variation (V) was 3.2%. The color obtained in the anthrone-glycoprotein reaction was corrected for the color caused by protein-sulfuric acid interaction. The O.D. of an equally treated protein-sulfuric acid blank was subtracted from the O.D. measured after the anthrone reaction. The hexose sugars present in serum glycoproteins which are responsible for the anthrone color are mannose, galactose and 6-deoxygalactose. The latter sugar was separately assayed by employing the cystine method described by Dische and Shettles(10). $V = 5.4\%$. For the determination of hexosamine the procedure described by Boas(11) was used chiefly. The proteins were hydrolyzed in 1.5 N HCl for 14 hours. Dowex 50 was used to eliminate non-specific chromogens and alkali treatment which is part of Ehrlich's reaction was carried out for 45 minutes at 90°C. $V = 3.8\%$.

Sialic acid was assayed according to the method of Werner and Odin(12) which is based on Bial's orcinol reaction. Although pentoses and hexuronic acid produce color in this reaction the absorption maxima of their color products differ greatly from the maximal absorption of the color caused by sialic acid. The absorption curves of 19 S γ-globulin and 19 S α_2-globulin after the orcinol reaction are almost parallel to that of sialic acid, and their absorption maxima coincide with that of the latter at 570 mμ. No other maximum was observed which could indicate the presence of either pentose or hexuronic acid. Crystalline sialic acid from ovarian cyst fluid was obtained through the kindness of Dr. Odin, Uppsala.

Results. Ultracentrifugal analysis of the various fractions of serum separated by zone electrophoresis in a starch or polyvinyl supporting medium has demonstrated that the 19 S component observed in whole serum is distributed in two electrophoretic fractions with peak concentrations in the γ_1 and α_2 regions(13). A similar distribution is obtained when the serum is first subjected to repeated cycling in the preparative ultracentrifuge and then separated by electrophoresis. Fig. 1 shows the ultracentrifuge pattern obtained after concentration of the heavy components in the preparative ultracentrifuge. The sample contained less than 15% material with a sedimentation constant below 19. All the

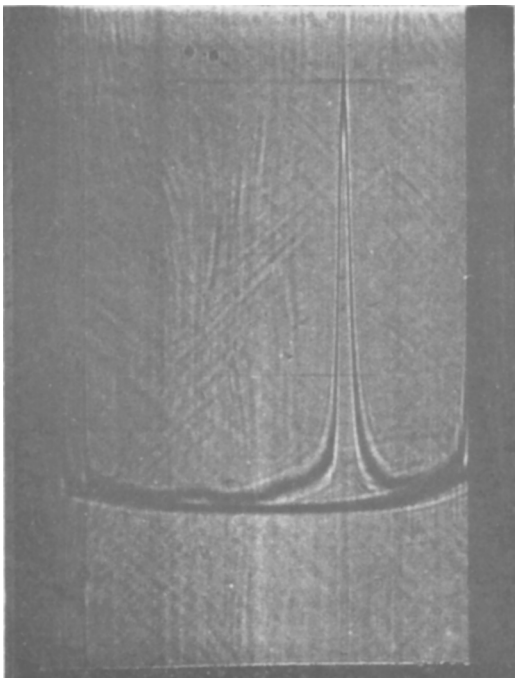

FIG. 1. Schlieren pattern obtained by ultracentrifuge analysis of a preparation of heavy component ($S_{20,w} = 19$ S).

albumin had been removed and only a small amount of 12 S and 7 S fractions remained. In this case no further albumin was added. The main peak after analysis at various concentrations was calculated to be $S_{20,w} = 19$ S. This sample was then subjected to zone electrophoresis in the starch medium and the protein pattern obtained is shown in Fig. 2. Two peaks are evident, one in the γ_1 and the other in the α_2 regions as indicated by comparison with the pattern of the original whole serum separated on the same starch block.

A number of different preparations of γ-globulin with varying amounts of heavy component were examined in the analytical ultracentrifuge and subjected to carbohydrate analysis. The amount of carbohydrate found was compared with the relative concentration of 19 S material. These results are plotted in Fig. 3. One of the major problems encountered in this work was progressive insolubility of the 19 S material after concentration and storage in the cold in the purified state. Associated with these alterations was the appearance of components with increased S

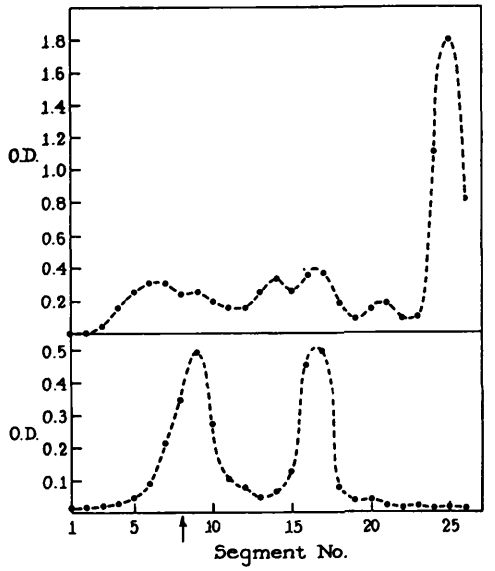

FIG. 2. Upper curve represents protein pattern obtained after electrophoretic separation of normal human serum. Lower curve represents a preparation of heavy component separated under identical conditions on the same starch block.

rates in the ultracentrifuge pattern. Since these materials were formed at the expense of the 19 S peak, they were included in Fig. 3 as belonging in the 19 S class. The question whether all of these heavier components arose in this manner was not entirely answered. Trace amounts were also found in some freshly isolated γ-globulin preparations. No evidence was obtained that the major 7 S fraction of γ-globulin aggregated in this manner to material with an S rate higher than 12.

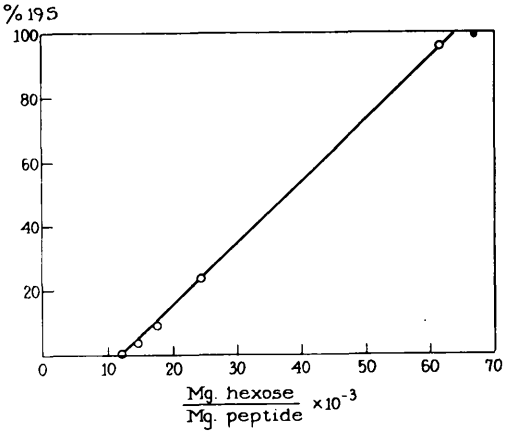

FIG. 3. Correlation between amount of 19 S material present in several γ-globulin preparations and the hexose-peptide ratio.

TABLE I. Carbohydrate Content of γ-Globulin and Various 19 S Proteins.

Protein	% N	% hexose	% fucose	% hexosamine	% sialic acid	% total carbohydrate	Hexosamine / Hexose
γ-glob. (7 S)	15.64	1.22	.29	1.14	.22	2.58	.94
γ_1 (19 S)	14.47	5.20	.62	2.90	1.70	9.80	.55
α_2 (19 S)	14.22	4.55		2.68	2.30	10.53	.59
Macroglob. I*	14.50	4.85	.62	2.66	1.71	9.22	.55

* Kindly furnished by Dr. H. F. Deutsch.

As shown in Fig. 3, there was a direct relationship between the percentage heavy components found in γ-globulin preparations and the carbohydrate-protein (peptide) ratio. The lowest point on the curve represents the ratio obtained for γ-globulin which is completely free of heavy components. The latter was obtained as previously described(7) from certain preparations of Fr. II-γ-globulin and from electrophoretic isolation of the slowly migrating material. The results obtained with 2 preparations which were close to 100% heavy component are indicated in the upper portion of the curve. The point, indicated as a solid dot, was not utilized in the plot because only a small amount of this preparation was available and the analyses were not considered to be as accurate as for the other points. Duplicate analyses were obtained for all the other preparations.

Table I shows the results of more detailed quantitation of the different carbohydrate components of one preparation which contained less than 5% of material with S rates lower than 19 S. For comparison the results obtained with 7 S γ-globulin and the α_2 heavy component are also indicated. It is apparent that the high molecular weight γ-globulin contains approximately 5 times as much hexose sugars as does the 7 S fraction. It has a low hexosamine-hexose ratio and is relatively rich in sialic acid. It is of interest that the α_2 fraction which is very similar to the γ_1 heavy component in size and shape(13) is also similar in carbohydrate content. Table I also gives the results obtained with a preparation of γ_1 material obtained from a case of Waldenström's macroglobulinemia. Immunological evidence obtained in this laboratory(14) and also by Korngold(15) has indicated a close relationship between the normal heavy component and the pathological macroglobulins. The carbohydrate content of this macroglobulin is similar to the normal preparation.

The sera of 4 patients (3 children and 1 adult) with agammaglobulinemia* were investigated to determine the concentration of the 19 S component migrating in the γ_1 region. When compared with normal sera a reduction to one-tenth or less of the normal level was found in 3 of the 4 cases. The methods used were not sensitive enough to indicate whether or not there was an even further reduction. Carbohydrate analyses on the fractions from one of these sera showed a complete absence of anthrone positive material throughout the γ_2 and γ_1 regions. The α_2 heavy component was not reduced in concentration in two of these sera in which it was examined.

Discussion. The high carbohydrate content of the 19 S group of proteins which migrate in the γ-globulin region demonstrates that these are not simple aggregates of the main 7 S type. They may represent polymerized molecules but if this is the case the basic units differ in hexose, hexosamine and sialic acid from other γ-globulins. Immunological studies which will be published separately (14) indicate that there are distinct antigenic differences between these 2 types of γ-globulin but that there is also considerable cross reaction. A similar immunological relationship was observed between 19 S and 7 S antibodies in horse sera by Treffers and associates(5). These observations suggest that the basic unit of the 19 S group of γ-globulins is similar to that of the 7 S class. The question of whether all the antigenic differences can be accounted for on the basis of the increased carbohydrate along with the large size of the heavy com-

* The authors are indebted to Dr. Robert Good for 3 of these sera.

ponents is not as yet answered.

It should be emphasized that the 19S γ globulin preparations analyzed in this study were never obtained 100% pure. Low molecular weight proteins could be almost completely removed and they presented a relatively minor problem. However the components with a sedimentation rate greater than 19S could never be eliminated entirely. The possibility remains that these materials may not be solely aggregates of the 19S fraction and cause slight alterations in the measured carbohydrate-protein ratio.

In the human, the blood group antibodies, certain abnormal red cell antibodies, and part of the Wasserman antibodies have been thought to be localized in the 19 S fraction of γ globulin. The possibility is raised that these and perhaps other antibodies are rich in carbohydrate. The increased carbohydrate, particularly sialic acid, would influence such properties as the isoelectric point and solubility and might confer unique characteristics to such antibodies.

The absence of detectable 19 S components of the γ_1-globulin type in the sera of 2 patients with agammaglobulinemia was reported previously(13). Further observations have confirmed these findings although small amounts were found in one case where some 7 S material was also present. These observations offer further evidence for a possible relationship between the heavy components under discussion and antibodies.

Summary. 1. The heavy components (19 S) have been highly concentrated from normal human serum by repeated cycling in the preparative ultracentrifuge. Electrophoresis of such preparations has permitted isolation of a high molecular weight γ-globulin fraction. 2. Analyses of this material indicate a high carbohydrate content with hexose sugars and sialic acid present at approximately 5 times the concentration found for the main 7 S γ-globulin. The hexosamine-hexose ratio is approximately 0.5. 3. Markedly reduced levels were found in the sera of 4 patients with agammaglobulinemia. The possibility that certain antibodies fall into the 19 S fraction and are similarly rich in carbohydrate is discussed.

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Oxidation of Acetate and Malonate in Extrahepatic Tissues.* (22691)

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Previous studies from this laboratory have shown that a rapid "explosive-like" oxidation of acetate occurs in the extrahepatic tissues (1). To investigate the pathway of terminal oxidation of acetate, the ability of C^{14} acetate to be oxidized in the presence of malonate, a competitive inhibitor of succinic dehydrogenase, was tested. This procedure, which has been successfully applied to *in vitro* studies (2,3) and which has been reported effective in whole animal experiments(4,5), proved to be non-inhibitory to acetate oxidation at concentrations of malonate that were not lethal to the eviscerated animals. Radioactive mal-

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