

THE CONCENTRATION OF GLUCOSAMINE IN NORMAL AND PATHOLOGICAL SERA

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The development of a convenient colorimetric method for the determination of glucosamine and chondrosamine by Elson and Morgan (1) has made possible the quantitative determination of these substances in sera. In a preliminary communication (2) glucosamine values of 40 to 60 mgm. per cent were reported for normal human sera together with elevated values in the serum of individuals with various infections, advanced malignant disease, and sterile infarcts (coronary occlusion). Palmer and Meyer (3), however, found that the color developed does not obey Beer's law over the range originally suggested, and Palmer, Smyth and Meyer (4) have developed a method which, with slight variations, we have employed. Their values for normal human sera varied from 90 to 130 mgm. per cent. Nils-son (5, 6) has described a modification of the technique of Elson and Morgan and has determined the glucosamine content of the sera of several animals, and of man. His average value for adult human serum is 77 mgm. per cent, for umbilical cord serum 32 mgm. per cent, and for pneumonia serum 145 mgm. per cent with a maximum of 183 mgm. per cent.

Rimington (7) isolated a carbohydrate containing glucosamine and mannose from the proteins of horse serum, but Bierry (8) and Sørensen and Haugaard (9) have presented evidence that galactose is also present. Hewitt (10) detected only traces of carbohydrate and no glucosamine in a crystalline fraction of horse serum albumin and describes a carbohydrate rich serum glyco-
id. Lustig and Langer (11) studied the "sugar" content of various human serum proteins finding increased "sugar" values in patients having carcinoma or pneumonia.

METHODS

One cc. of serum is diluted to 10 cc. with physiological salt solution and 1 cc. of the dilute serum is hydrolyzed with 1 cc. 2 N HCl for 5 hours in a boiling water bath.

The hydrolysis is carried out in a test tube (125 × 16 mm.) calibrated at 5 cc., using a rubber stopper into which has been inserted a small-bore glass tube as an air condenser to prevent evaporation.

At the end of the hydrolysis the tubes are cooled in tap water for a few minutes, distilled water is added to the 5 cc. mark, the tube is thoroughly shaken to mix the contents, and the liquid is filtered to remove the coagulum. Then 0.5 cc. of the above filtrate is carefully neutralized with 0.5 N NaOH. A trial titration is used to determine the amount of alkali required.

A stock standard solution of glucosamine hydrochloride containing 10 mgm. of glucosamine per cc. is diluted daily to make two standards containing 10 γ and 20 γ of glucosamine per cc. One cc. samples of the dilute standards are treated in the same manner as the neutralized serum hydrolysate.

The determination is carried out in 10 cc. volumetric flasks which have ground glass stoppers. After neutralization of the hydrolysate, 1 cc. amounts of acetyl acetone reagent (1 cc. acetyl acetone dissolved in 50 cc. 0.5 N Na₂CO₃) are added to the hydrolysate and standards. The stoppers are placed loosely in the necks of the flasks, and the flasks are immersed in a boiling water bath for 25 minutes. A few minutes later, the stoppers are pushed firmly into place to avoid loss of acetyl acetone by evaporation.

At the end of the 25 minute condensation period the flasks are cooled for 2 minutes in water, and 3 cc. of redistilled 95 per cent ethyl alcohol and 1 cc. Ehrlich's reagent (0.8 *p*-dimethylaminobenzaldehyde in 30 cc. 95 per cent ethyl alcohol plus 30 cc. concentrated HCl) are added to each flask. They are shaken gently to mix the contents and placed uncovered for 3 minutes in hot but *not boiling* water since the alcohol boils violently when heated too suddenly. The flasks are then cooled for 3 minutes, made up to 10 cc. with redistilled 95 per cent ethyl alcohol, the tops replaced, and the fluid mixed.

After standing for 30 minutes, the rose color is read in a Klett photoelectric colorimeter using a green glass filter with maximal absorption at 5250 Å reading against the 10 γ or 20 γ standards according to the depth of color. Standard curves must be worked out owing to the lack of a linear relationship between color and amount of glucosamine present.

The glucosamine stock standard, the acetyl acetone reagent and the Ehrlich's reagent should be kept in the ice box when not in use. The acetyl acetone reagent should be made up fresh every 3 or 4 days and the Ehrlich's reagent every week or 10 days.

As previously stated, the failure of the color developed to obey Beer's law made it necessary to prepare special charts. On these, the scale readings of the colorimeter were plotted against 5, 10, 15, and 20 γ glucosamine standards read against each other. The color developed in the serum was read against the nearest 10 γ or 20 γ standard, and the value determined from the chart. All determinations were done in duplicate and discarded if they did not agree within ± 3 per cent.

Samples of serum were hydrolyzed for 1.5, 3, 5, and 7 hours to determine the time of hydrolysis suitable for maximal subsequent color development. This was found to be 5 hours and included sera with both normal and maximally elevated glucosamine contents. Glucosamine could not be detected in unhydrolyzed serum, nor in the filtrate hydrolyzed after precipitation of the serum with sulphosalicylic acid. This precipitant does not interfere with quantitative development of the color in pure glucosamine solutions nor in hydrolyzed sera. Glucosamine added to serum which was then hydrolyzed for 5 hours was quantitatively accounted for. This was also true for a 6 per cent casein solution, and a 6 per cent casein 1 per cent glucose solution.

Whether all of the color developed by this method in the hydrolyzed serum is due to glucosamine, it is of course impossible to say. However, the peak of the absorption spectrum for the color derived from hydrolyzed serum and pure glucosamine coincide, and heating with alkali destroys the ability of hydrolyzed serum and of glucosamine to develop any appreciable color. All blood samples were collected in the postabsorptive state, excepting those of the pregnant and postpartum women. This was probably an unnecessary precaution as in two individuals the only change in glucosamine concentration before and one hour after a generous breakfast was accounted for by a change in the water content of the serum as judged by serum protein concentration.

RESULTS

One hundred and eighty satisfactory determinations of glucosamine have been carried out on the sera of normal and pregnant individuals and of patients with various diseases. In the majority of instances the serum protein, albumin, globulin, euglobulin, and fibrinogen (12) were also determined together with the erythrocyte sedimentation rate (13).

The resulting values were analyzed with two objects in view, first to determine whether any relationship existed between the level of glucosamine and of that of any of the other serum constituents studied, and secondly, to determine the glucosamine level in normal individuals and the variations from it in disease.

Lack of correlation of the glucosamine level with serum proteins and sedimentation rate

Glucosamine concentrations were plotted against total serum protein, albumin, globulin, euglobulin, and fibrinogen, and no correlation could be established. There was a tendency for elevation of the sedimentation rate to occur with high glucosamine values, but exceptions were numerous, even in the absence of anemia and of markedly altered fibrinogen levels. The number of determinations and their ranges was as follows: total protein 152 varying from 3.1 to 8.6 per cent, albumin 140 from 1.2 to 5.5 per cent, globulin 140 from 1.3 to 5.2 per cent, euglobulin 140 from 0.1 to 1.9 per cent, fibrinogen 109 from 0.2 to 1.0 per cent, and sedimentation rate 120 from 4 to 130 mm. per hour. The glucosamine values varied from 71 to 210 mgm. per cent. It is interesting that glucosamine, which is part of the carbohydrate complex supposed to be attached to some serum proteins, did not vary proportionately with any of the protein fractions studied. This may indicate that fractionation of the serum proteins by Howe's method does not separate chemical individuals, or that the carbohydrate complex may exist as a serum mucoid independently of protein. Hewitt's isolation of a crystalline serum albumin free from carbohydrate, and Kendall's

TABLE I
*Serum glucosamine concentration of maternal and umbilical cord serum **

Hospital number	Day postpartum or months pregnant	Maternal serum		Umbilical cord serum	
		Glucosamine	Protein	Glucosamine	Protein
		mgm. per cent	grams per cent	mgm. per cent	grams per cent
518181	Day 1	94	5.2	57	5.2
513792	Day 1	99	5.3	59	5.4
491321	Day 1	95	5.3	60	5.7
389363	Day 1	104	5.8	63	5.9
522890	Day 1	107	5.4	64	6.2
517966	Day 2	144	6.0	53	5.5
511418	Day 2	127	6.6	67	5.7
387816	Day 2	121	6.2	68	5.5
511990	Day 5	127	6.7	59	6.5
517836	Day 5	130	7.1	64	6.7
517726	Month 8	88	6.6		
510011	Month 8	99	7.0		

* Range of serum glucosamine of normal adults is 75 to 110 mgm. per cent.

studies on α -globulin and globulin-X (14) are consistent with this interpretation.

Glucosamine level in normal adult individuals, pregnant women, and their newborn

Twenty-three determinations of glucosamine and serum protein were made on twenty-one normal individuals aged 25 to 50 years, ten of whom were women. The values varied from 76 to 110 mgm. per cent with an average of 93. No difference was found between the sexes. The thirteen determinations made in June averaged 90, the ten from December through March averaged 98, and the level in one individual fell from 110 in December to 76 the following June. Although colds were not noted, subclinical upper respiratory

infections may have been present in the winter months. Two individuals showed no variation not accounted for by a change in serum water as judged by protein concentration before and one hour after a large breakfast.

Ten determinations made on umbilical cord blood at birth and on maternal blood postpartum are shown in Table I. The fetal cord serum varied from 53 to 64 mgm. per cent, the maternal venous blood serum on the first day postpartum from 94 to 107. However, on the second and fifth days, values varying from 121 to 144 mgm. per cent were obtained. Two women in the eighth month of pregnancy had values of 88 and 99 mgm. per cent. No infected cases were included. The values are higher than those reported by

TABLE II
Serum glucosamine concentration in malignant disease †

Hospital number	Diagnosis	Glucosamine	Erythrocyte sedimentation rate	Protein	Albumin	Globulin	Euglobulin	Fibrinogen
		mgm. per cent	mm. per hour	grams per cent	grams per cent	grams per cent	grams per cent	grams per cent
536408	Lymphatic leukemia. W.b.c. 16,000	89		7.2				
502636	Multiple myeloma	99	94	7.8	3.3	4.5	0.8	0.3
436416	Liver metastases from carcinoma of breast removed 2 years before and carcinoma of cecum removed 4 months before	100	12	5.7	2.5	3.2	0.8	0.2
523334	Carcinoma of stomach with liver metastases	110	30	6.1	2.3	3.8	0.9	0.3
523391	Carcinoma of stomach with moderate bone metastases	110*		6.1	3.6	2.4	0.5	
518101	Lymphatic leukemia. W.b.c. 12,520	113	27	6.2	4.2	2.1	0.5	0.5
508528	Carcinoma of cervical lymph gland, secondary, following carcinoma of lung?	120	35*	6.4	3.8	2.6	0.7	
515311	Carcinoma of head of pancreas with liver metastases	127	122	6.2	3.1	3.1	0.2	0.6
509288	Lymphatic leukemia. W.b.c. 6,520	127	153	7.3	3.7	3.6	0.9	0.5
523589	Carcinoma of cecum	127		5.8	3.4	2.5	0.4	0.4
504628	Hodgkin's disease	146	53	6.4	4.6	1.8	0.2	
498548	Hodgkin's disease?	152	80*	6.2	4.4	1.8	0.2	0.6
513075	Carcinoma of common hepatic duct	154	45	7.0	4.0	2.9	0.2	0.6
274592	Hypernephroma of left kidney with metastases to cervical lymph nodes, humerus, and liver	155		7.1	3.1	4.0	1.4	
515229	Carcinomatosis with metastases to omentum and lymph nodes	157	44	7.2	3.2	4.0	1.0	0.4
522385	Acute basophilic leukemia. W.b.c. 35,000	163		7.5				
511965	Reticulum cell sarcoma of all lymph nodes	163	131*	7.5	4.1	3.5	0.6	0.3
510543	Carcinoma of bronchus with extensive metastases to liver, bones, mediastinum and lymph nodes	170	40*	5.5	2.9	2.6	0.5	0.2
506271	Carcinoma of stomach with metastases to lymph nodes, heart, liver, lungs, spleen, pancreas, kidneys, vertebrae and ribs	172	60	5.6	3.8	1.8	0.2	
510615	Carcinoma of colon with metastases to liver and mesenteric lymph nodes	173	94*	7.7	4.6	3.2	0.6	0.6
515082	Carcinoma of ovary with metastases to peritoneum, bones, and myocardium	175	86	5.7	3.4	2.3	0.4	0.5
512625	Carcinoma of lung	180		6.5	4.1	2.4	0.6	
509775	Carcinoma of bronchus with very extensive bone metastases	183	106*	7.3	4.3	3.0	0.4	0.7
Private patient	Hodgkin's disease with extensive bone involvement	195		8.1	3.9	4.3	1.5	

† Range of serum glucosamine of normal adults 75 to 110 mgm. per cent.

* Anemia.

TABLE III
Serum glucosamine concentration in infections other than pneumonia †

Hospital number	Diagnosis	Glucosamine	Erythrocyte sedimentation rate	Protein	Albumin	Globulin	Euglobulin	Fibrinogen
		mgm. per cent	mm. per hour	grams per cent	grams per cent	grams per cent	grams per cent	grams per cent
478973	Meningococcus bacteremia, subsiding	97	27*	7.2	5.1	2.1	0.2	0.4
395722	Grippe	100	11	6.7	4.7	2.0	0.5	0.3
514573	Tuberculosis, lungs (moderate), lymph nodes and peritoneum	121	38	8.1	3.8	4.3	1.0	0.4
478973	Meningococcus bacteremia, febrile	122	63	7.4	5.2	2.2	0.4	0.7
513535	Rheumatic heart disease	125	78	7.7	4.8	2.9	0.6	0.5
395722	Grippe	127	19	6.7	4.7	2.0	0.2	0.4
522462	Rheumatic fever	128		7.4				
506378	Onset of grippe	130	43	6.1	3.7	2.4	0.5	0.4
506374	Grippe	130	20	6.8	4.3	2.5	0.5	0.3
500599	Infectious mononucleosis	142	45	7.7	4.1	3.6	1.0	0.4
507862	Colitis, polyposis	142	90*	6.4	4.1	2.4	0.2	0.5
503845	Bacterial endocarditis	143		5.8	3.5	2.3	0.5	0.4
510580	Hemolytic streptococcal pharyngitis	145	47	7.6	4.8	2.8	0.3	0.5
376786	Rheumatic fever	146		8.6	4.8	3.8	1.0	
509435	Rheumatoid arthritis	154	106	6.9	3.9	3.1	0.6	0.7
427376	Lymphogranuloma inguinale	154		7.8	3.9	3.8	1.3	
511151	Chronic ulcerative colitis	158	76	7.2	4.1	3.1	0.6	0.5
510580	Hemolytic streptococcal pharyngitis	159	42	8.0	5.1	2.9	0.5	0.6
243838	Tuberculosis, ileocecal	161	38	7.9	4.0	3.9	1.2	0.4
390321	Lymphogranuloma inguinale	161	113	8.0	4.4	3.6	0.7	0.9
511953	Lymphogranuloma inguinale	161	80	8.4	4.0	4.4	1.0	0.5
513259	Lymphogranuloma inguinale and lues	164	38	7.8	3.9	3.9	0.9	0.5
507336	Bacterial endocarditis	165	40	6.7	3.8	2.9	0.9	0.4
407163	Rheumatic fever	167	112	7.3	3.9	3.4	0.6	0.8
500519	Periarteritis nodosum	167	94	6.4	3.8	2.6	0.4	0.6
508826	Acute psoas abscess	174	62	6.7	4.2	2.5	0.5	0.8
514219	Tuberculosis, right upper lobe; pleurisy with effusion	175	25	7.4	4.2	3.2	0.6	0.7
510876	Gonococcal arthritis	178	83	8.5	4.8	3.7	1.0	0.7
485198	Bacterial endocarditis	178	121*	6.1	3.5	2.6	0.7	0.6
521274	Lymphogranuloma inguinale	179		7.4	4.3	3.2	1.0	
512583	Rheumatic fever	180	98	8.6	4.2	4.5	1.1	0.3
523752	Rheumatic fever	182	114	7.1	3.8	3.2	0.6	0.9
500519	Periarteritis nodosum	182	125*	6.0	3.6	2.4	0.5	0.4
341046	Rheumatic fever	184	135*	8.3	3.7	4.7	1.7	1.1
478850	Rheumatoid arthritis	187		6.9	4.0	2.9	0.8	
498482	Lymphogranuloma inguinale	187	65*	8.4	3.2	5.2	1.3	0.6
316874	Bacterial endocarditis	187	100	7.5	3.9	3.5	0.9	
513617	Acute rheumatoid arthritis	189	106	8.1	4.2	3.9	1.3	

† Range of serum glucosamine of normal adults 75 to 110 mgm. per cent.

* Anemia.

Nilsson. The elevation of the maternal values on the days following delivery may well represent the reaction of the body to tissue destruction.

Glucosamine levels in various diseases

Malignant disease. Hodgkin's disease has been arbitrarily placed in this group. Table II presents a summary of the results and shows that cases of early malignancy may have normal values but that with advanced malignancy, the glucosamine level is elevated. Fibrinogen determinations were essentially normal, and the lack of correlation of the glucosamine level with the sedimenta-

tion rate, serum protein, albumin, and globulin is clearly shown. Whether the elevation of glucosamine has to do with the malignant process or extensive tissue destruction, it is impossible at present to say, but the latter possibility seems more probable.

Infections. Table III shows the results obtained in infections other than pneumonia. The extent and activity of the infectious process in general parallels rising glucosamine values. It is uncertain whether tissue necrosis or the immune mechanism or both are responsible for the high glucosamine level. Several of the cases of pneu-

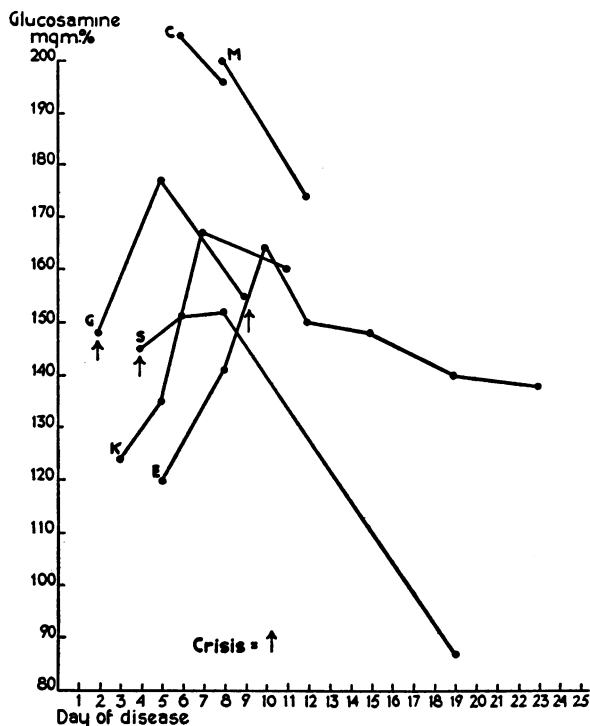


FIG. 1. SERUM GLUCOSAMINE LEVEL IN PNEUMONIA

C—Hospital Number 506687, Type III, positive blood culture; died. M—Hospital Number 510503, Type I, empyema, drained Day 20. G—Hospital Number 506309, Type I. S—Hospital Number 509385, Type XVI. K—Hospital Number 508228, bronchopneumonia. E—Hospital Number 514989, Type I, positive blood culture.

monia studied are presented in Figure 1. In addition to glucosamine, total serum proteins and their various fractions were determined. There was a slight decrease in total protein on the third and fourth days of the disease, possibly because of the formation of the exudate, and, as has been previously reported (15, 16), a subsequent rise in the globulin fraction and total protein was observed. The glucosamine level was elevated on the second day of the disease and reached its highest value three or four days after the crisis. The rate of return to normal levels varied greatly as Case E and Case S show. Unfortunately, it was not possible to study the appearance of antibodies in the serum, and the relative importance of the rôles played by the immune mechanism and the resolution of consolidation remains to be determined.

Coronary occlusion. The three cases with coronary occlusion present the best examples of a

sterile infarct. Cases S and W clinically represent single infarctions of arteriosclerotic etiology. Case M probably had a single infarct with a marked pericardial reaction, a rub, some pericardial fluid, and an unusually prolonged elevation of the sedimentation rate. These cases were free from obvious infection and suggest that autolyzing sterile infarcts give rise to high glucosamine values. It is recognized that the changes are not great, but the infarcts are comparatively small, and the larger infarcts encountered were complicated by infection. It is possible that the elevation of maternal serum glucosamine, postpartum, also represents the reaction of the body to the absorption of necrotic tissue.

Miscellaneous conditions. Six cases of Laennec's cirrhosis of the liver, all with inverted albumin globulin ratios, two with marked ascites, had glucosamine values varying from 100 to 137 mgm. per cent. No correlation could be detected with the various protein fractions studied, nor with ascites. Cases with the following diseases had "normal" glucosamine values, that is, 71 to 110 mgm. per cent: pernicious anemia, Ménière's disease, congestive cardiac failure, hyper- and hypothyroidism, pulmonary emphysema, infestation with *Clonorchis sinensis*, peptic ulcer with hemorrhage, chronic nephritis, Paget's disease (bone), and diabetes mellitus. On the other hand, sev-

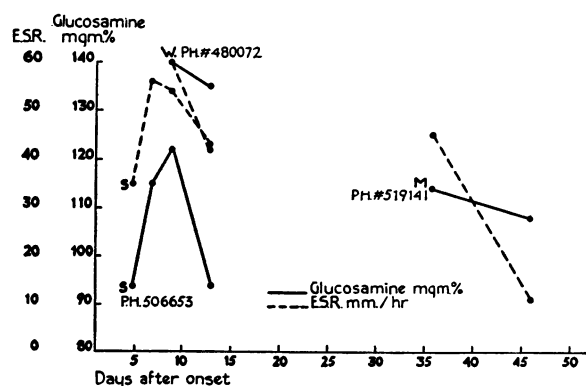


FIG. 2. SERUM GLUCOSAMINE LEVEL AND ERYTHROCYTE SEDIMENTATION RATE IN CORONARY OCCLUSION

eral cases of advanced nephritis, nephrosis, and cholecystitis had definitely elevated values. It was extremely difficult to determine whether infection, infarction or thrombosis might not have been present in these last individuals, in addition to their underlying disease.

SUMMARY

One hundred and eighty determinations of serum glucosamine have been carried out. In the majority of instances, total protein, albumin, globulin, euglobulin, fibrinogen, and the erythrocyte sedimentation rate were also estimated.

No correlation could be detected between the concentrations of total protein or its various components and that of glucosamine.

There was a tendency toward correlation between the erythrocyte sedimentation rate and the glucosamine concentration of the serum, but exceptions were numerous.

The serum glucosamine level in twenty-one normal adults varied between 76 and 110 mgm. per cent.

Elevated glucosamine values were observed in the sera of patients suffering from infections, disseminated malignant disease, and sterile infarcts.

The level of serum glucosamine in newborn infants is much lower than in adults.

The maternal serum glucosamine level is normal on the first day postpartum, but elevated by the fifth day.

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