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THE CLINICAL SIGNIFICANCE OF SERUM SIALIC ACID

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Submitted in Partia Fulfillment for the Degree of
Doctor of Medicine

College of Medicine, University of Nebraska

April 1, 1957

Omaha, Nebraska

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I. Introduction

In the last few years many investigators have demonstrated that the concentration of human serum glycoprotein is abnormally high in a number of physiological and pathological states (1). Evidence has accumulated showing that there are a number of distinct serum glycoproteins, and that the concentrations of these may vary in an independent manner.

The main purpose of this thesis is an attempt to review the literature on available information on the rise of the serum sialic acid in pathologic states and also to present results of the determination of serum sialic acid in certain patients of this hospital.

II. Terminology

At the present time there is no generally accepted terminology used regarding proteins conjugated with carbohydrate and particularly with respect to the carbohydrate-containing proteins of serum. Investigators have generally accepted the recommendation of the Committee on Protein Nomenclature of the American Physiological Society and the American Society of Biochemists (2) "that compounds of the protein molecule with a substance or substances containing a carbohydrate group, other than nucleic acid, be classified as glycoproteins." Serum carbohydrate (other than free glucose) is

a constituent of certain serum proteins, i.e., glycoproteins. Thus the amount of protein bound carbohydrate in serum is a direct measurement of the serum glycoprotein levels.

Werner and Odin (4) have shown "sialic acid" to be a component of serum glycoprotein. Sialic acid was isolated in 1936 from beef submaxillary mucin by Blix (4,5,6) and Swedish workers gave this substance that name in view of its origin (6,7).

III. Nature of Sialic Acid

Sialic acid has not yet been characterized chemically except that it was shown to be a nitrogen containing polyhydroxy, acidic reducing substance with an empirical formula approximating $C_{14}H_{24}O_{11}N$ (8). It gives strong humin formation and liberation of carbon dioxide in heating with mineral acid. It gives a purple color even without previous treatment with alkali on heating with Ehrlich's p-dimethylamino-benzaldehyde reagent and a violet color when treated with Bial's orcinol reagent (7). Sialic acid, neuraminic acid isolated by Klenk in 1941 from the ganglioside fraction of brain lipid (9,19) and the prehemataminic acid obtained by Yamakawa and Suzuki (10) are identical products (11).

Several methods have been used in the determination of serum sialic acid namely, the diphenylamine reaction of Dische (12) for desoxypentoses, the tryptophane-perchloric acid reaction described by Cohen (13) for desoxypentose, the "direct Ehrlich reaction"

described by Werner and Odin (8) and also by the Bial's reagent method(8).

Sialic acid has also been isolated from urine proteins (14), from red cell stroma lipids (15) and from milk (16) and it has been shown that it occurs in combination with lactose in the mammary gland of lactating rats (11). Kuhn et al have isolated what appears to be another closely related compound from glycoproteins of bovine colostrum and have designated this compound "lactaminic acid" (20). Gottschalk (17) and Hiyama (18) have isolated 2-carboxy pyrrole from mucoids hydrolized with barium hydroxide, and it is thought that quite possibly this compound is a component part of "sialic acid" or that it is produced from "sialic acid" during alkaline hydrolysis (8).

IV. Sialic Acid versus E S R

Two groups of investigators (20,21,22) in order to determine the relation to erythrocyte sedimentation rate of serum diphenylamine (DPA) reaction in rheumatic fever did parallel blood determinations weekly on 32 children with rheumatic fever and it is their opinion that there is a close correlation between the DPA reaction value and the Erythrocyte Sedimentation Rate and that of the two determinations, they found the DPA reaction to be more sensitive, to rise and fall with less lag time, and to be free of errors associated with heart failure or anemia, both of which

affect the ESR while the DPA reaction parallels the rheumatic activity.

Simkin, Bergman and Prinzmetal (41) in their studies on sialic acid following the occurrence of myocardial infarction have noted that the rise in serum sialic acid was more accurate than the ESR or the leukocyte count in reflecting the presence or absence of myocardial necrosis.

Israel, Webster, and Maher following the determination of serum polysaccharide by the tryptophane-perchloric acid reaction on ninety patients from the Woman's Medical College Hospital in Philadelphia have noted that while rapid ESR is almost invariably present during pregnancy and the postpartum period, the serum polysaccharide are apparently rarely elevated in uncomplicated pregnancy and hence this determination may prove useful in the detection of intercurrent diseases.

V. Serum Sialic Acid in Pathologic States

A. Arthritis:

Coburn and Haninger in their experimental arthritis using the diphenylamine reaction (DPA) observed that the values obtained in arthritis were higher than those of the control group (19).

B. Rheumatic Fever:

It has been shown by several workers (43,21,22,23,24) that

the serum sialic acid by the Diphenylamine Reaction appears to be significantly elevated during active rheumatic fever, to decrease with lessening of the rheumatic activity and to return to normal upon recovery.

C. Burns:

Serial determinations with the tryptophane-perchloric acid reaction made in six burn cases by Keyser (25) clearly indicated pronounced rise in serum sialic acid which reached a maximum usually several days after the burn and generally returned only slowly to a normal level. The most severe burns gave the highest values.

D. Cancer:

Niazi and state (26) by the addition of the diphenylamine reagent to "a certain fraction of human serum" result in a purple color, the intensity of which is greater in patients with malignancy than in apparently normal individuals. Other workers (23,27,28,29, 30,31,32) too have observed malignancies, however, they added that its determinations as such is of no value either to indicate the presence of malignancy or to indicate whether or not a malignant growth has been successfully removed, and that perhaps its measurement may be of value as a supplementary procedure to other laboratory and diagnostic tests in determining the extent and character of certain pathologic states.

E. Sarcoidosis:

Fisher and Davis (33) and others (23) have demonstrated that the sera of proven cases of sarcoid show that those without clinical signs of activity the sera were almost normal there being only a slight elevation. Those with active lesions were found to have marked elevations.

F. Disseminated Lupus Erythematosus:

No specific determination for sialic acid had been done except that there was observed a rise in serum polysaccharide (34) however, since sialic acid is a component of polysaccharide, one can postulate that perhaps sialic acid too is elevated.

G. Diabetes Mellitus:

/Berkman, Rifkin and Ross (35) after determining the serum polysaccharide (sialic acid) in a total of sixty six diabetic patients with and without degenerative vascular diseases came to the conclusion that the increased levels of polysaccharide substances in the sera of diabetic patients are associated with the existence of degenerative vascular complications rather than with the primary metabolic defect in diabetes mellitus.

H. Tuberculosis:

Several workers (23,29,36,37,38,39) have done determinations of serum sialic acid in tuberculosis and they are in agreement that

there is no rise in serum sialic acid in those patients who have minimal tuberculosis of questionable clinical significance but in cases of active tuberculosis there was significant elevation in the serum sialic acid and as the disease progressed to moderately and far advanced stages, sialic acid progressively increased and one group (40) went further to say that extremely high values in sialic acid are usually associated with a fatal outcome.

I. Myocardial Infarction:

Simkin, Bergman and Prinzmetal (41) reported that serum sialic acid was definitely elevated by the third day after clinical indications of coronary occlusion with myocardial infarction were evident. On the sixth day the peak of the rise occurred and this elevation was usually maintained for another week after which the serum sialic acid gradually declined and normal values at the end of a month were approached. Serum sialic acid values in patients with chest pain due to causes other than myocardial infarction were within the normal range in cases including angina pectoris, neurocirculatory asthenia, paroxysmal tachycardia fibrositis and hypertension. As a contrast the erythrocyte sedimentation rate was found elevated in paroxysmal tachycardia and angina pectoris. Hence it is their opinion that the increase in serum sialic acid appeared to reflect more accurately than the erythrocyte sedimentation rate or leukocyte count the presence or absence of myocardial necrosis

and they suggested that although serum sialic acid changes following myocardial infarction are not specific for this condition alone determination of serum sialic acid may be useful as a diagnostic aid in evaluating clinical syndromes which are caused by coronary artery diseases.

VI. Determination of Serum Sialic Acid

The method used in the determination is that of Odin, Werner and Bjornesjo, Institute of Medical Chemistry, University of Uppsala, Sweden:

To 0.50 ml of blood serum is added 1.00 ml physiological saline. Of this mixture 0.40 ml is poured drop by drop with stirring into 10 ml cold absolute ethanol. After centrifuging (about 2000 rev./min. for 10 min.) the supernatant is decanted. The precipitate is washed in the centrifuge tube with 10 ml cold absolute ethanol. It is then dissolved in 5 ml physiological saline. Orcinol reagent 3.5 ml and $FeCl_3$ - solution 0.5 ml are added. The determination is then conducted according to Odin and Werner (Acta Soc. Med. Upsal., 57, 230 (1952). The mixture is warmed to + 108degrees Centigrade (paraffine bath) for 10 min. and then cooled to room temperature by tap water. Redistilled isoamylalcohol 5.0 ml is added, the mixture thoroughly shaken and then centrifuged. The light absorption of the solution is measured

within an hour at 570 mu.

The precipitation and the colour reaction are best carried out in the same centrifuge tube, equipped with a ground glass stopper.

Orcinol reagent. - 0.50 g orcinol is dissolved in 200 ml concentrated hydrochloric acid and 50 ml distilled water.

$FeCl_3$-solution. - M/150 in hydrochloric acid of the same strength as in the orcinol reagent.

As standard is used pure sialic acid of the ovine type, which is the form of the substance also occurring in the human materials.

A. Serum Sialic Acid in "Normal" Subjects:

As a control serum sialic acid determinations were done on 12 subjects ranging in age from 5-48 years of age. There were 6 males and 6 females.

	Name	Age	Sex	Serum Sialic Acid
1)	W.J.	5	M	.226
2)	S.A.	5	M	.280
3)	R.H.	35	M	.304
4)	J.P.	42	M	.399
5)	J.S.	44	M	.318
6)	B.M.	48	M	.404
7)	D.R.	9	F	.247

* Expressed as optical density

	Name	Age	Sex	Serum Sialic Acid *
8)	D.D.	11	F --------	.273
9)	L.D.	21	F --------	.225
10)	I.M.	27	F --------	.235
11)	R.S.	28	F --------	.297
12)	M.P.	40	F ---------	.253

* Expressed as optical density

The Average Density ---------- .288

B. Serum Sialic Acid in Pathologic States:

1. Rheumatoid Arthritis:

Case #1: V. P., age 15, Female, Hospital #15960:

V. P. is a 16 year old Mexican female with complaints of painful and swollen joints since May, 1956. Joints involved were temporomandibular, shoulder, elbows, wrists, proximal interphalangeal, knee and metatarsal.

Sedimentation rate: 55 corrected 34 mm

C-reactive protein - 2 positive

Serum Sialic Acid --- .409

Case #2: B.W., age 61, Female:

B.W. is a 61 year old white female who has had rheumatoid arthritis for a number of years with

typical fusiform deformity of her prominal interph joints.

Sedimentation Rate: 12 mm

C-Reactive protein -- negative

Serum Sialic Acid -- .310

Observation: In case #1 the SSA is .409 which is .121 higher than the average normal of .288 while in case #2 it is only .022 above the normal average and actually lower than the high normal value, however, while case #1 has a sedimentation rate of 34 corrected and positive c-reactive protein, case #2 has a sedimentation rate of only 12 mm and negative C-reactive protein while the SSA in case #1 is not very significantly elevated, yet it is encouraging to note that it was higher in the case of faster ESR and lower in the case of slower ESR.

2. Rheumatic Fever:

Case #1: K.D., age 5, Female:

This 5 year old female was admitted to the hospital because of fever of 5 days duration, history of pain in both wrists which migrated to the knees, and also had malaise and headache.

C-reactive protein: positive

Date	Sed. Rate	Serum Sialic Acid
1-24	59 mm	.553

Date	Sed. Rate	Serum Sialic Acid
1-28	57 mm	.514
2-7	30 mm	.495

Observation: The SSA is quite significantly elevated in the acute phase of the disease and decreases with improvement of the condition.

Rheumatic Fever with Chorea:

Case #2: W. P., Male, age 5:

A 5 year old white male was admitted to the hospital because of nervousness and unsteady movements. For 2 weeks before admission patient had cough, neck stiffness, later he developed "nervousness" with arms shaking when he talked as well as at rest. He also had difficulty maintaining his balance and he complained of aching and stiffness in his joints with easy fatiguability.

Sedimentation Rate: 56 mm on admission

Serum Sialic Acid: .279 taken about 1 week later when ESR was only 20 mm.

Observation: The SSA appears to be less than the normal average value and this seems to indicate that in rheumatic fever the SSA is below normal, but one must realize that the ESR was only 20 mm and one wonders if the SSA would not be significantly elevated had the SSA been done when the ESR was accelerated as in case #1.

3. Diabetes Mellitus

Case #1: R. M., age 13, Male, Hospital #8105:

This 13 year old white male who is a known diabetic of 8 years duration was hospitalized because of upper respiratory infection and diabetes out of control. Patient was afebile but had been taking 52 UNPH.

Blood sugar -- 400 mgm

Serum Sialic Acid -- .304

Case #2: C. A., age 14, Female, Hospital #16232:

C. A., white female, is a 14 year old who has a 9 year history of diabetes and is in the hospital because of neglected control of her diabetes.

Blood sugar -- 620

Serum Sialic Acid -- .344

Observation: In both cases of diabetes mellitus, the SSA does not seem to be significant being .304 and .344 respectively which are less than the high normal of .404.

4. Acute Glomerulonephritis:

Case #1: C. S., age 2, Female:

This 2 year old white female enters hospital with complaints of "kidney trouble". A history of having had cold with sore throat and earache 3 weeks previously followed by puffiness around the eyes, dark urine just before admission.

Urinalysis: Albumin -- 4+
25-30 RBC/HPF
1-2 WBC/HPF

Sedimentation Rate: not done

Serum Sialic Acid: .356

Observation: SSA of .356 cannot be considered significant in view of the high normal of .404, unfortunately no sedimentation rate has been done.

5. Galactosemia:

Case #1: H. S., age 2, Male:

H. S. 's mother gave the history of his having protuberant abdomen and masses in the neck. Patient was found to have umbilical hernia, palpable cervical, axillary and inguinal nodes. Both liver and spleen are palpable.

Ceph. Flocc.: negative

Thymol Turbidity: 0.75 units

Blood Galactose: 25 mgm %

Serum Sialic Acid: .282

Observation: There doesn't appear to be any significant elevation of SSA in this disease entity.

6. Celiac Disease:

Case #1: C. H., age 4, Female:

C. H. was admitted into the hospital because of failure to gain any weight for 2-3 years. History of soft

brownish to tan color stools with oil like substance and foul smelling.

Sweat and duodenal drainage: normal

Sedimentation rate: 33 mm

Serum Sialic Acid: .297

Observation: In this case the ESR is accelerated while the SSA does not show any evidence of significant elevation.

VII. Summary

Terminology, origin and nature of sialic acid have been given. Sialic acid is reported to parallel the ESR and in several conditions to excel the ESR in accuracy. The literature shows elevation of SSA in such pathologic states as arthritis, rheumatic fever, burns, cancer, sarcoidosis, disseminated lupus erythematosus, tuberculosis, myocardial infarction.

VIII. Conclusion

After reviewing the literature on serum sialic acid in the various pathologic states and its comparison with the erythrocyte sedimentation rate one gets the impression that the determination of serum sialic acid may be of value as a supplementary diagnostic tool and that it may even replace the ESR.

In the determination of serum sialic acid on the few patients

in the University Hospital and the Children's Memorial Hospital, we can say that only in the case of acute rheumatic fever is the serum sialic acid significantly elevated and that it paralleled the ESR. However, in view of our limited number of cases and only one good case, we are in no position to either confirm or contradict the reports in the literature.

Acknowledgement

I wish to express my appreciation and gratitude to Dr. Gordon E. Gibbs for his valuable suggestions, direction and review of this paper and also to extend my thanks to Mr. Roger H. Hutchings who did the actual determination of Serum Sialic Acid.

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