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The Type of Naturally Occurring Sialic Acids*

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The results obtained in this laboratory and others about the type of sialic acids contained in materials from the animal kingdom (mammals, birds, fishes and echinoderms), alga and bacteria, are summarized and distributed according to their source.

It may be deduced that sialic acids are widely distributed in Nature. The same sialic acid can be found in very different species, although not at the same concentration.

In contrast, in very close species (from a taxonomic point of view) the type and concentration of their sialic acids may be different for the same material (generally, serum).

Our results with some vegetal materials (kidney beans, pea seeds, lentils, sweet almonds, orange seeds and peels, and green peppers) giving a positive thiobarbituric acid reaction and identical color in some cases to that obtained with the acylneuraminic acids, do not permit to admit the occurrence of sialic acids in these materials.

It is not possible to find a correlation between the occurrence and concentration of N-glycolylneuraminic acid and acetylneuraminic acids in different taxonomic groups of the most evolved beings.

The neuraminic acid (a molecule not found in Nature) may be regarded as a condensation product of 2-amino-2-deoxy-D-mannose (mannosamine) with pyruvic acid. It is known that the acylneuraminic (or sialic) acids are essentially the mono, di, tri or tetra acetyl or the glycolyl derivatives of the neuraminic acid.

The trivial name of the main naturally occurring sialic acids are (54): N-Acetylneuraminic acid (NANA); N-acetyl-4-O-acetylneuraminic acid; N-acetyl-7-O-acetyl-neuraminic acid; N-acetyl-8-O-acetylneuraminic acid; N-acetyl-7,8-di-O-acetylneuraminic acid; N-glycolylneuraminic acid (NGNA). The structure of other sialic acids (N-acetyl-4,x-di-O-acetylneuraminic acid and N-acetyl-x,y,z-tri-O-acetylneuraminic acid) has not been well established

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still. Other forms of sialic acids, such as N-glycolyl-4(?)-O-acetylneuraminic acid, N-glycolyl-8-O-methylneuraminic acid and others (see next Tables), have been found in a few materials. Finally, the colominic acid — a polymer of NANA (82, 99) — has been detected in some bacteria.

In a survey on the acylneuraminic acids, the following steps can be considered: A) Discovery. B) Knowledge of their chemical structure. C) Adaptation of methods for their identification and easy quantitative determination. D) Study of their distribution in Nature. E) Study of their metabolism. F) Progress in the knowledge on their biological significance. (The present work concerns only with point D.)

The occurrence of sialic acid in various materials has been the object of several publications. The results up to 1960 (concerning only with mammals) have been summarized by GOTTSCHALK (53). Later, WARREN (128) has detected and determined the sialic acid of many species not previously studied. At the present time there are numerous data on the concentrations of sialic acids. Yet, their type in several materials remains often unestablished. It seems that a summary of the results up to date is lacking. The present paper summarizes our own results on the type of the acylneuraminic acids and those of other authors (when they indicate such type), but it does not pretend to be exhaustive.

Materials and Methods

Samples, from the sources indicated in the following Tables, have been studied for detection and identification of sialic acid by the quoted authors.

The following materials have been investigated in our laboratory: Tears, urine, colostrum, milk, bile, serum and saliva from the human specie; colostrum, milk and dairy products from cows; colostrum, milk, serum, erythrocyte and liver from goats; platelets from the equine (horse, donkey and mule), ovine, porcine and bovine species; leucocites from horse, mules, donkey and pig; eggs and liver from three fish species. Some vegetal materials were also studied (kidney beans, pea seeds, lentils, sweet almonds, orange seeds and peels, and green peppers), but with a negative result.

For the separation and purification of the acylneuraminic acids, we have employed the method of KLENK - UHLENBRUCK (86), as described in a previous paper (48). The assays of sialic acid have been carried out with paper chromatography and colorimetric procedures as reported in other publication (16, 48). The modified Ehrlich spray (9) was generally used for staining chromatograms; quantitative determinations were always performed by the resorcinol modified procedure (103, 116) and the thiobarbituric acid method (3, 124); in some cases, the Eeegriwe test was used to determine NGNA (86).

Results

Next Tables show the data on the type of the acylneuraminic acids from animals, alga and bacteria, ranged by species (see Tables).

Another problem which has called the attention of several authors is the following: Do the acylneuraminic acids exist in the vegetal kingdom?

In 1964, MAYER *et al.* (98) reported the occurrence of a «sialic acid» (not structurally characterized to the present time) in soybeans and alfalfa. Later, other authors (106, 112) have also reported numerous other plant material which, they say, contain sialic acids.

From 1964, our assays (20, 23) with several fractions of kidney beans seeds gave a very intense thiobarbituric reaction by the procedures of WARREN (124) and AMINOFF (3) and the absorption spectra was identical to that of NANA. Another sensible and specific reactions such as the resorcinol-butyl acetate, Ehrlich (direct), orcinol (Bial) and that of Hestrin for O-acetyl-groups gave no result. By paper chromatography one spot was obtained, of R_f near to that of NANA, and the typical color was produced with the thiobarbituric acid, but not with the other sprays. Almost the same happened with the products isolated from lentils and sweet almonds, although the thiobarbituric acid reaction gave less intensive color. The purified product isolated from the testas of pea seeds was also assayed (23); but similarly to what occurred with other vegetal materials recently studied by us (such as orange seeds and peels) the Ehrlich, Bial and resorcinol reactions gave a negative result, although assays with the thiobarbituric acid resulted to be generally identical to those obtained with NANA. We also deduced from the test with neuraminaldolase that the problem product was not an acylneuraminic acid, but probably a very similar or related substance (23).

Discussion

Very often the comparison between the results obtained by several authors is difficult, because the methods employed for the identification of sialic acids have been different. Or, several reactions have been adapted to the determination of sialic acids (orcinol, resorcinol, thiobarbituric and p-dimethylaminobenzaldehide), but none of these reactions is specific. Two or three of these reactions, after purification or isolation of the sialic acids, should be employed before certifying that sialic acid has been found in a new material.

Besides, a more rigourous comparison should be made not on the whole sample (as serum, for example) but on the macromolecule in which sialic acids are generally bound; yet, the isolation of such macromolecules has been achieved in a few cases only.

With regard to the general occurrence of the acylneuraminic acids in Nature, WARREN'S (128) study permits to deduce that these acids are scarcely and irregularly present in beings of a not very high degree of specialization and, exceptionally, in bacteria; when the evolution process is more advanced, they can be found in a constant manner from the echinoderma to the vertebrates.

In connection with the problem of the possible occurrence of sialic acid in higher plants, it must be outlined that the results published are generally based on the determination by the thiobarbituric acid method, but no isolation or crystallization has been reported to the present time. Even their characterization has not been confirmed. Taking into consideration the lack of specificity for sialic acids of the thiobarbituric reaction, it seems that much attention must be paid before certifying the general existence of acylneuraminic acids in higher plant. Our negative results agree with those obtained by WARREN (128). Later, GIELEN (51) reported also that bananas and soybeans gave a positive thiobarbituric acid reaction, but no reaction was obtained with the Bial's reagent (in its adaptation for sialic acids), and he suggests that these materials contain one 2-ceto-3-deoxialdonic acid.

Finally, data of Tables show that it is not possible to find a correlation between the occurrence and concentration of NGNA and acetylneuraminic acids in different groups of the most evolved beings. Thus, in materials from very far taxonomous groups, such as human beings, submaxillary ovine mucins, sea lamprey and other fish eggs and in some bacteria, only the acetyl derivative is practically found; in contrast, in other materials such as pig submaxillary mucin, it is mainly NGNA; and finally, in the other products of animal origin there are NGNA and acetylneuraminic acids in relative concentrations of wide variability. If one takes into consideration the fact that the biosynthesis of the same acylneuraminic acid, Table *I*. *Sialic acids identified in materials from the animal kingdom (mammals, birds, fish and echinoderms), alga and bacteria.* (Abbreviations: NANA = N-acetylneuraminic acid. NGNA = N-glycolylneuraminic acid. NODANA = N,O,diacetylneuraminic acid)

Specie	Material	Molecule	NANA %	NGNA %	NODANA %	Other sialic acids %	Remarks	Reference
			MAMMALS					
Human	Serum		~ 99	< 1				Martinsson et al. (94)
»	Plasma	α_1 Acid Glycop.	+++					Eylar et al. (44)
»	»	Ganglios.	100					Yu et al. (130, 131)
»	Immuno-globulins		++++					Miller (102)
»	Erytroc.		100					Klenk et al. (87) Faillard (46)
»	Liver		100					Klenk et al. (85) Martinsson et al. (93)
»	Submax. mucin		100					Klenk et al. (84)
»	Milk		100					Zilliken et al. (133)
»	»	Oligosach.	100					Grimmonprez et al. (56, 57)
»	»	Casein	100					Alais et al. (2)
»	Colostrum		100					Cabezas et al. (22)
»	Urine		100					Klenk et al. (84)
»	»		+++		Traces			Cabezas et al. (29, 30, 31)
»	»					Different from NANA & NGNA		Goussault et al. (55)
»	Bile		+++		+			Cabezas et al. (24)
»	Tears		100					Cabezas et al. (16)
»	Gastric mucoids	(«Intrins. fact.»)	100					Faillard et al. (47)
»	Hela cells			+ (?)				Carubelli et al. (32, 33)
»	»		100					Hof et al. (64)
»	Sem. Plasma		100					Herrman et al. (63)
»	Skel. Muscle	Sphingolip.	100					Svennerholm et al. (117)

Monkey (several species)	Serum		+++	+				Uhlenbruck et al. (121)
» (Velvet)	»		61	39				Dzulynska et al. (39)
»	Erythroc.		+	+++				Uhlenbruck et al. (121)
Calf	Serum (fetal)	Fetuin	100					Spiro (114)
»	Serum		77	23				Faillard et al. (48)
»	»		90-58	10-42			5 days-18 months, resp.	Dzulynska et al. (40)
»	Erythroc.		40	60				Eylar et al. (45)
»	»		+	+				Cabezas et al. (26)
»	Skin (fetal)			100				Bourrillon et al. (14)
»	Platelets			+				Cabezas et al. (25)
»	Bile		++	37	(Pluriacetylneur.) +			Cabezas (18)
»	Brain	Ganglios.		+				Yu et al. (130)
Cow/Ox	Serum		36	64				Martinsson et al. (94)
»	Plasma	Acid glycopr.	+	++		+		Bezkorovainy (9)
»	»	Ganglios.	++	++				Yu et al. (131)
»	Serum	γG Immunoglobulins	++		++	(N-Acetyl-NGNA) +		Kickhöfen et al. (80)
»	Colostrum	»	++		++			Kickhöfen et al. (80)
»	»		+++					Clark et al. (34)
»	»			+				Kuhn et al. (90)
»	»		87	13				Cabezas et al. (17)
»	Milk	Glyco-α-lactalbumin	+++	+				Barman (5)
»	»	Casein	+++					Alais et al. (1)
»	Erythroc. stroma			95				Klenk et al. (88)
»	»		+	++				Cabezas et al. (26)
»	Submax. mucin		+	13-17	++			Blix et al. (13)
»	»			21-25				Klenk et al. (86)

Table I *(Follow-up)*

Specie	Material	Molecule	NANA %	NGNA %	NODANA %	Triacetyl neur. acid %	Other sialic acids %	Pluriacetylneur. acid or Acetylneur. acids (together), %	Reference
					MAMMALS				
Cow/Ox	Submax. mucin		20-25	15	20-25 (N-acetyl-7-0-acet.); 20-25 (N-acetyl-8-0-acet.)	5-10			Schauer et al. (111)
»	Bile		10	23	(Pluriacetyl neur.) 67				Cabezas et al. (24)
»	Bone		+++						Herring (62)
»	Brain	Glycoproteins	+						Brungraber et al. (15)
»	»	Ganglios.		+					Tettamanti et al. (118, 119)
»	»	»		+					Yu et al. (130)
»	Kidney	»	+	+					Puro (109)
»	Aorta		+						Meier et al. (100)
»	Myocardium	Glycoprot.	++	+					Golovatskii et al. (52)
Horse	Serum		35	5	(4-0-Acetyl-NANA) 50		(4-0-Acetyl NGNA) 10		Pepper (107)
»	»		86	14					Martinsson et al. (94)
»	»		85	15					Dzulynska et al. (39)
»	Erythroc.		5-10	95-90					Klenk et al. (88)
									Eylar et al. (45)
»	»						(0-Acetyl-NANA) +		Hakomori et al. (58)
									Klenk et al. (88)
»	Brain	Ganglios.	100						Svennerholm (115)
									Blix et al. (10)
»	Kidney		89	11					Martinsson et al. (92)
»	Submax. mucin			1-2					Blix (12)
»	»				+++				Blix (13)
»	»		15-20	1-2		5			Schauer et al. (111)

Horse	Platelets			+++					Cabezas et al. (25)
»	Leucocytes		+	+++				Traces	Cabezas et al. (27)
Donkey	Serum		86	14					Dzulynska et al. (39)
»	Erythroc.			++					Cabezas et al. (26)
»	Platelets		Traces	+++					Cabezas et al. (25)
»	Leucocytes		+	+++				Traces	Cabezas et al. (27)
Mule	Erythroc.		+++						Cabezas et al. (26)
»	Platelets		Traces	+++					Cabezas et al. (25)
»	Leucocytes		+	+++				Traces	Cabezas et al. (27)
Pig	Serum		85	15					Martinsson et al. (94)
									Klenk et al. (88)
»	»		90	10					Dzulynska et al. (39)
»	»	Ceruloplasmin	+++						Matsunaga et al. (94)
»	Erythroc.		34	66					Klenk et al. (88)
									Eylar et al. (45)
»	»		+	++	+				Cabezas et al. (26)
»	Leucocytes		+	+++					Cabezas et al. (27)
»	Submax. mucin		10	90					Blix et al. (11)
»	»		-	++++					Salegui, De, et al. (110)
»	»	Glycoprot.	+	+++					Katzman et al. (75)
»	»	Oligosacch.		++++					Carlson (28)
»	Mucoidis	(«Intr. fact.»)	40	60					Faillard et al. (47)
»	Gastric mucin		80	20					Atterfelt et al. (4)
»	Pancreas		38	62					Petterson et al. (108)
»	Aorta				+++	+ (?)			Klemer et al. (83)
»	Kidney		86	14					Martinsson et al. (92)
»	Platelets			++++					Cabezas et al. (25)
»	»	Sphingolip.		++++					Heckers et al. (60)
»	Bile		48	13				Pluriacetylneur. acid 39	Cabezas et al. (24)
»	Brain	Gangliosid.		+					Yu et al. (130)
Goat (young goat)	Serum		+++	12				Acetylneur. acids (together) 88	Cabezas et al. (21)
»	Erythroc.		+	69				» 31	Cabezas et al. (21)
»	Liver		41	10	21			Pluriacetylneur. acid 28	Cabezas et al. (21)

Table I *(Follow-up)*

Specie	Material	Molecule	NANA %	NGNA %	NODANA %	Triacetylneur acid %	Other sialic acids %	Pluriacetylneur. acid or Acetylneur. acids (together), %	Reference
				MAMMALS					
Goat (young goat)	Brain	Glycoprot.		28				Pluriacetylneur. acids 19	Cabezas et al. (21)
»	»	Ganglios.	++	+					Cabezas et al. (21)
Goat (adult goat)	Serum		+++	18				Acetylneur. acids (together) 82	Cabezas et al. (21)
			53						
»	»		85	15					Dzulynska et al. (39)
»	Liver		45	18	14			» 23	Cabezas et al. (21)
»	Colostrum		89	11					Cabezas et al. (21)
»	Milk		46	37	17				Cabezas et al. (21)
»	»	Casein	++	+					Alais et al. (1)
Lamb	Erythroc.		58	42					Eylar et al. (45)
»	Platelets		++	++					Cabezas et al. (25)
»	Bile		64	15		+			Cabezas et al. (25)
Sheep	Serum		87	13					Martinsson et al. (94)
»	Erythroc.		26	74					Klenk et al. (88)
									Eylar et al. (45)
»	»		+	++	+				Cabezas et al. (26)
»	Submax. mucin		97-99	3-1					Blix (12)
»	Milk	Casein	+	++					Alais et al. (2)
»	Brain	Ganglios.		+					Yu et al. (130)
Bactrian camel	Serum		++	+	+				Walkowiak et al. (123)
Llama	»		++	+	+				Walkowiak et al. (123)
Elk	Serum		68	32					Blix (12)
»	Submax. mucin		97	3					Blix (12)
Fallow deer	Serum		76	24					Dzulynska et al. (39)
Reindeer	»		++	+	Traces				Walkowiak et al. (123)

Red deer	»		++	+	Traces			Walkowiak et al. (123)
Dog	»		91	9				Dzulynska et al. (39)
»	Erythroc.		+					Klenk et al. (89)
»	Gastric mucin		+					Kim et al. (81)
Silver fox	Serum		100					Dzulynska et al. (39)
Dingo	»		92	8				Dzulynska et al. (39)
Jackal	»		95	5				Dzulynska et al. (39)
Wolf	»		100					Dzulynska et al. (39)
Cat	»		93	7				Dzulynska et al. (39)
»	Erythroc.	Ganglios.		(di) ++				Handa et al. (59)
Tiger	Serum		85	15				Dzulynska et al. (39)
Lion	»		86	14				Dzulynska et al. (39)
Brown bear	»		95	5				Dzulynska et al. (39)
Mink	»		100					Dzulynska et al. (39)
Rabbit	»		90	10				Dzulynska et al. (39)
»	»		+++	+				Hudson et al. (68)
»	Plasma	Ganglios.	+++	Traces				Yu et al. (131)
»	Urine		+		+			Neuberger et al. (105)
Eur. brown hare	Serum		83	17				Dzulynska et al. (39)
Guinea pig	»		90	10				Dzulynska et al. (39)
Golden hamster	»		+	++	Traces			Walkowiack et al. (123)
»	Seminal secr.		++				++	Fouquet (50)
Rat	Serum		++	+	+			Walkowiack et al. (123)
»	Submax. mucin		++	++				Warren et al. (126)
»	Brain		+++					Benedetta et al. (8)
»	»		+++					Irwin (70)
»	Skin			+++				Herp et al. (61)
»	Urine		++	+	+			Maury (96, 97)
Mouse	Submax. mucin		+					Warren et al. (126)
»	Immunoglob.			+				Melchers (101)
Whale			100					Nakagawa et al. (104)
»	Cartilage		100					Toda et al. (120)

Table I *(Follow-up)*

Specie	Material	Molecule	NANA %	NGNA %	NODANA %	Plurlacetyl neur. acid %	Remarks	Other sialic acids %	Reference
					BIRDS				
Hen	Serum		93	7					Dzulynska et al. (39)
»	»		90	9					Faillard et al. (48)
»	Erythroc.		95-100	5-0					Klenk et al. (88)
									Eylar et al. (45)
»	Egg White		+++	+ (?)	+ (?)				Feeney et al. (49)
Turkey	Serum		95	5					Dzulynska et al. (39)
Goose	»		93	7					Dzulynska et al. (39)
Duck	»		98	2					Dzulynska et al. (39)
Collocalia	mucin		++++						Howe et al. (67)
»	»				+++ N-acetyl-4-0-acetylneur.				Kathan et al. (75)
					FISH				
Loach	Ext. mucous subs.		+++						Enomoto et al. (41)
Sing ray	Ext. mucous cartil.		+++						Enomoto et al. (42)
Eel	Ext. mucous		+++				Unidentif. Ehrlich +		Enomoto et al. (42)
» (electric)	Electric organ		++++						Yu (132)
Shark	Cartilage		++++						Enomoto et al. (42)
Hammerhead	»		+++				»		Enomoto et al. (42)
Lamprey	White cells		80-90	20-10					Eylar et al. (43)
»	Eggs, liver		+++			+			Cabezas et al. (19)
Sucker	Eggs		+++			+			Cabezas et al. (19)

5 Scomber	»		+++			+			Cabezas et al. (19)
Trout	»		++	++					Warren (125)
Carp	Brain	Ganglios.	++		N-Acetyl-8-acetyl-neur. ++				Ishuzuka et al. (73)
Goldfish	»	»			++				Seiter (113)
»	»	»		+					Yu et al. (130)
Codfish	»	»	++		++				Ishuzuka et al. (73)
»	»	»	+++						Ishuzuka et al. (74)
Haddock	»	»	++		N-Acetyl-8-acetyl-neur. ++				Ishuzuka et al. (73)
Halibut	»	»	++		» ++				Ishuzuka et al. (73)
ECHINODERMS									
Sea urchin	Eggs			(NGNA or NG-deriv.)					Hotta et al. (65)
				+++				+	Hotta et al. (66)
»	»			++					Isaka et al. (71)
»	»			+++					Immers (69)
»	»		++						Warren (125)
»	»			+					Uhlenbruck et al. (122)
» Starfish	»			+				(8-Methoxy-NGNA) ++	Warren (128)
Sea cucumber				+					Warren (128)
» (Holoturia forskali)								Hf-neuraminic acid (new)	Isemura et al. (72)
Bittle star				+					Warren (128)
Sea lily				+					Warren (128)

Table I *(Follow-up)*

Specie	NANA %	NODANA %	Colominic acid %	Reference
		ALGA		
Chlorella pyrenoidosa	+			Correl (36)
		BACTERIA		
E. coli K_{235} (and other)			+	Barry et al. (6, 7)
E. coli 07 & 016			+	Kimura (82)
E. coli	+	N-Acetyl-7-0-acetyl-neur. +		Dewit (37, 38)
Salmonella dahlem (and other)	+			Kedzierska (77, 78, 79)
Neiss. meningitidis	+			Watson et al. (129)
»	+			Liu et al. (91)

NANA, is different for bacteria and for mammals (127), and the irregular distribution of NGNA, it is possible to think of a polyphiletic origin of the acylneuraminic acids. COHEN (35) has suggested that the presence of sialic acid in procaryotic (bacteria) and eucaryotic cells seems to reflect a convergent evolutionary mechanism to form a single substance by different paths.

Resumen

Se reúnen los datos, obtenidos por este Departamento y por otros autores, acerca de la naturaleza de los ácidos siálicos o acilneuramínicos contenidos en materiales procedentes del reino animal (mamíferos, aves, peces y equinodermos), una alga y algunas bacterias, intentando hacer una revisión (no exhaustiva) que facilite el conocimiento de la distribución de dichos ácidos en los distintos materiales agrupados por especies.

Puede deducirse que los ácidos siálicos se hallan ampliamente distribuidos en la Naturaleza. El mismo ácido siálico (generalmente el ácido N-acetilneuramínico) se encuentra en especies muy alejadas desde el punto de vista taxonómico, pudiendo ser su concentración bastante diferente de unas a otras especies animales. En cambio, distintos ácidos siálicos (y a concentraciones diferentes) pueden hallarse en un mismo material, como el suero sanguíneo, de especies muy próximas entre sí, en el caso de los mamíferos por lo menos.

Desde 1964, nuestros resultados relativos a la eventual existencia de ácidos siálicos en materiales procedentes de plantas superiores (distintas partes de judías, lentejas, pimientos verdes, almendras y naranjas) indican que el producto que da positiva la reacción del ácido tiobarbitúrico no corresponde a los ácidos siálicos.

No es posible encontrar una correlación entre la existencia y/o concentración del ácido N-glicolilneuramínico y la concentración del ácido N-acetilneuramínico en los distintos grupos taxonómicos de los seres más evolucionados.

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