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Sialic Acids. XII. On the Nature of the Acylneuraminic Acids from Human Normal Urine *

A. Carrión, R. Bourrillon and J. A. Cabezas **

Department of Biochemistry. Faculty of Pharmacy. Santiago de Compostela (Spain), and Laboratoire de Biochimie. Faculté de Médecine. Paris (France)

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After a survey of the bibliography on the nature of the acylneuraminic (or sialic) acids from several human products, it may be deduced that N-acetylneuraminic acid is the main sialic acid; but the possibility of the existence in the human specie of other acids as N-glycolylneuraminic acid and pluriacetylneuraminic acids, even at little amounts, may not be excluded.

Using mild methods for fractionation of the adialyzate of human urine with Sephadex, BioGel, etc., and paper chromatography for analysis, it seems that a N,O,diacetylneuraminic acid (probably the N-acetyl-4-O-acetylneuraminic acid) is present, bound and free, in some fractions of the material; yet, further work is required to confirm this result.

It is generally said that tissues and secretions of the human specie contain only NANA *** and none of the other neuraminic acids derivatives. NANA has been found at a relatively high concentration in human serum, milk, tears, liver, kidney, etc. (3, 4, 8, 16).

But there is an increasing evidence (or, at least, a reasonable doubt) about the occurrence of other sialic acids, as NGNA, in this specie; for example, the percent of NGNA in crystallized sialic acids from human serum is estimated as less than 1% (17) and that from erythrocyte stroma, 0-5 % (13); furthermore, NGNA has been identified in human chorionic gonadotropin (15), and in a non-dialyzable urinary fraction soluble in 65 % ethanol (5); the occurrence of NGNA in the mingin (a urinary trypsin inhibitor) has been also suggested (2), and lately its presence in HeLa S3 cells has been reported (11, 12). Probably, the difficulty in assuring the general occurrence of NGNA in the human specie comes from the almost impossibility in determining it exactly when it exists at a little concentration (20) as it is the case for the quoted materials.

On the other hand, pluriacetylneurami-

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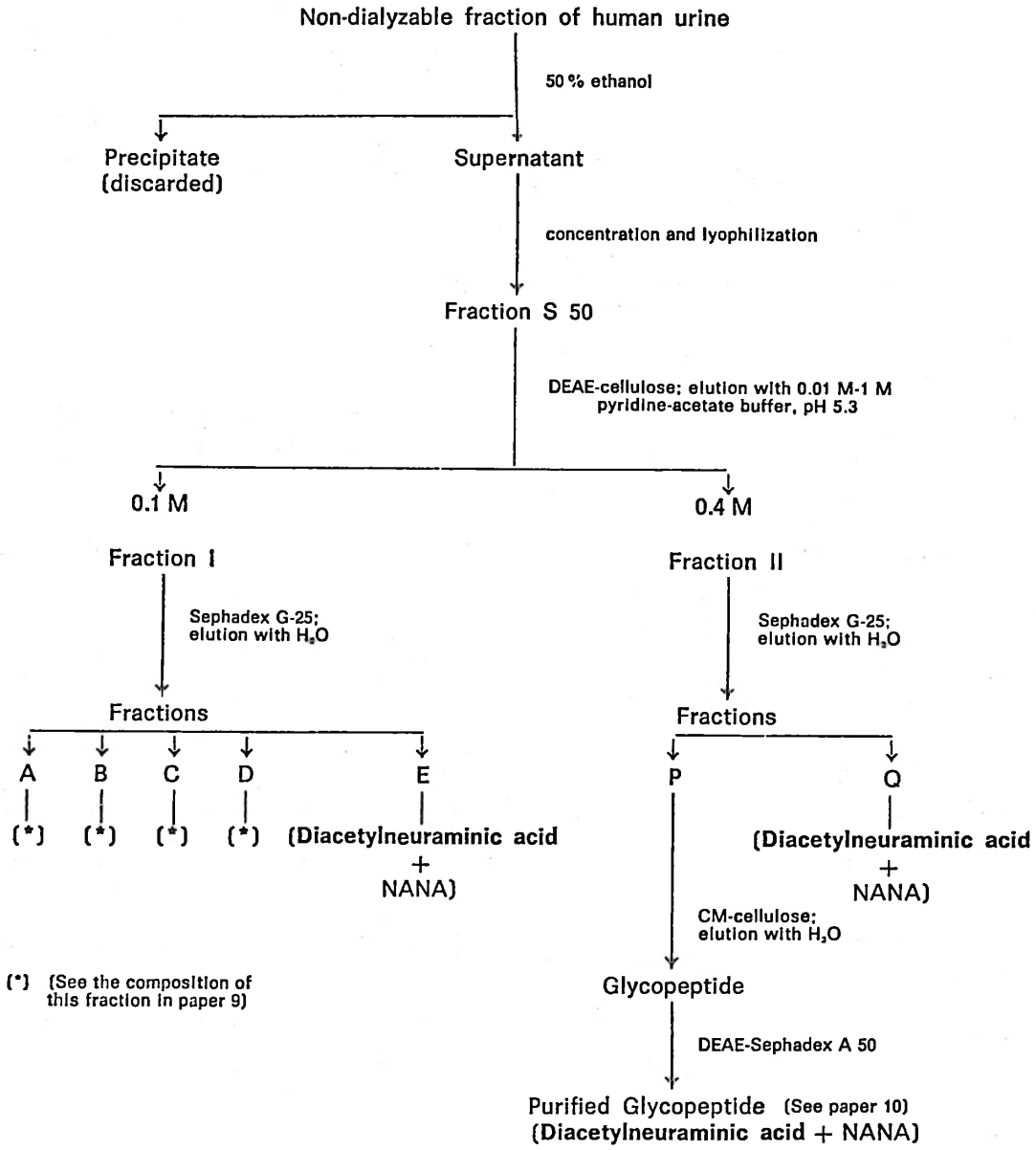
** Present address (to whom reprints may be asked) Department of Biochemistry Faculty of Sciences, University. Salamanca (Spain).

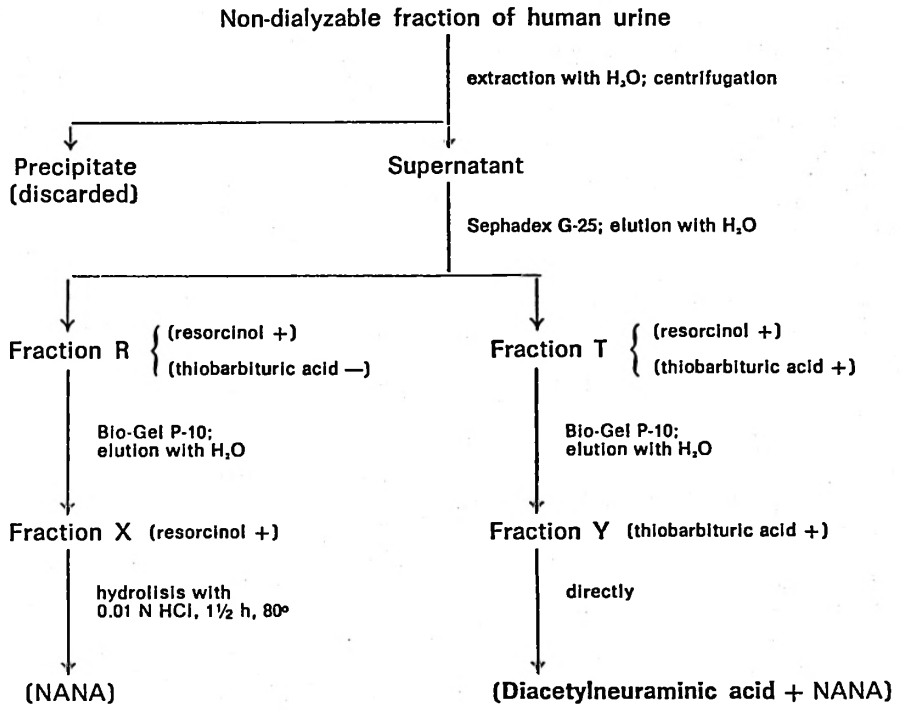
*** Abbreviations: NANA, N-acetylneuraminic acid; NGNA, N-glycolylneuraminic acid.



Schema I. *Method for fractionation of human normal urine.*

nic acids are also frequently found together with NANA in many materials from the animal kingdom (3, 6, 16), but the occurrence of a diacetylneuraminic acid in man, precisely in urine, has not been reported until recently (10). The present study has confirmed this result and deals with the identification of a free diacetylneuraminic acid detected in another fraction obtained also from human normal urine.

The fractionation procedures employed



Schema II. *Mild method for fractionation of human normal urine.*

for the preparation of some rich sialic acid glycopeptides from urine and the identication of the sialic acids from them, have been previously described (9, 10). Schema I indicates some of the techniques employed and summarizes some of the results; peak E from fraction I and peak Q from fraction II have been studied independently.

Besides, urine was also fractionated by other very mild procedures (see schema II) to avoid the eventual possibility of artifacts formation; in this view, acetate, pyridine exchange ion resins, etc., were not used.

One-dimensional descending paper chromatography was carried out with the following solvent systems: *a*) *n*-Butanol-acetic acid-water (120:30:50, v/v), during 45-60 hours; *b*) *n*-Butanol-acetic acid-water (40 : 10 : 50, v/v), during 60 hours; and c) *n*-Butanol-*n*-propanol-0.1 N HCl (10:20:10, v/v), during 15-40 hours; standard sialic acids [from Sigma Chem. Co., and from young goat serum (7)] were always used as reference. Staining with Ehrlich (direct) (8), Bial and thiobarbituric acid reagents (14), and color reactions in tubes were performed as previously quoted (9, 10). Furthermore, preparative paper chromatography was employed to isolate by elution with water the problem acylneuraminic acid from not stained zones.

As it can be observed, fraction E (schema I) contains only sialic acids: NANA and other sialic acid the Rf of which is that of a N,O,diacetylneuraminic acid; fraction Q contains also the same acids. Fractions R and T (schema II) differ between them by their behaviour against resorcinol and thiobarbituric acid reactions in test tubes; both fractions contain acylneuraminic acids, only bound in the for-

mer and also free in the latter. Fractions X and Y, respectively, were obtained, after filtration through BioGel P-10 and elution; in fraction X, using paper chromatography (staining with Ehrlich and thiobarbituric reagents) NANA and two other components were detected; the Rf of the latter were different from those of NGNA and N-acetyl-4-O-acetylneuraminic acid. Fraction Y seems to contain NANA and N,O,diacetiylneuraminic acids.

The positive test of the thiobarbituric acid should indicate that the N,O,diacetylneuraminic acid is probably the N-acetyl-4-O-acetylneuraminic acid, according to the peculiarities of this reaction (1, 19, 21). This acid is bound in the case of the purified glycopeptide (10), but in fractions E and Q (schema I) and in fraction Y (schema II) the direct reaction with the thiobarbituric acid (without previous hydrolysis) shows that it is free. [In connection with this fact, the excretion of free NANA from human urine, in high concentration, has been reported in a pathological case (18)].

Further work is required to confirm these results; yet it seems that a N,O,diacetylneuraminic acid (probably the N-acetyl-4-O-acetylneuraminic acid) may be considered as a component of human normal urine, besides NANA. Taking into consideration what has been said about NGNA, the qualitative composition in sialic acids of the human specie should not be different from that of other mammals.

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