

## CARTAS AL EDITOR

---

### Neuraminic Acids. — VIII. Identification of the N-acetyl- and N-glycolyl- neuraminic Acids in Rat Serum, and Study of their Absorption by the Rat Intestine

Since the discovery of the acylneuraminic (or sialic) acids about thirty years ago by KLENK and BLIX independently, numerous publications have appeared concerning these substances, and we know actually that they exist in a wide distribution in the animal kingdom (8). Their main metabolic pathways have been intensely studied, but some of them have not been completely established, nor the nature of the several acylneuraminic acids in different biological materials. In this paper the identification of these acids in rat blood serum and their absorption by the intestinal wall is reported.

For the separation and purification of the acylneuraminic acids, we have mainly followed a procedure similar to the KLENK-UHLENBRUCK's method (6), as described in a previous work (4). 16 ml of serum from five white Wistar decapitated rats were employed; after precipitation with ethanol, boiling, decantation, reboiling with the same amount of ethanol, two hydrolysis with  $H_2SO_4$  were made. The acylneuraminic acids were characterized by ion exchange methods, specific color reactions and paper chromatography, in which the following solvents were used:

n-butanol —acetic acid— water (4:1:5, v/v), n-butanol-pyridine-0.1N HCl (5:3:2, v/v), and n-butanol —n-propanol— 0.1N HCl (1:2:1, v/v), on Schleicher & Schüll 2043b paper, by ascending and/or descending modalities, during 24 to 60 hours; a mixture of N-acetylneuraminic acid (NANA) and N-glycolylneuraminic acid (NGNA) (87:13), isolated in this laboratory from cow colostrum, was employed as reference. Other details concerning these experimental procedures are indicated in our previous publications (1, 2).

For the absorption assays, the technique of successive absorptions *in vivo* (7) was used, employing twelve white rats, weighing from 100 to 200 g; and NANA (synthetic, from Sigma Chemical Co.) and NGNA —synthesised by FAILLARD and BLOHM (3)— at the concentrations and experimental conditions indicated below. In many cases four successive absorptions were made.

The results of all the assays of identification of the acylneuraminic acids in the rat serum seem to show the existence of NANA as the main component, followed by NGNA; another third unidentified neuraminic acid derivative was also found.

Absorption of NANA and NGNA through the  
TABLE I

Intestine of rats *in vivo*.

N.º Absorp.	Substance ( $\mu$ M)	Intestinal absorption ( $\mu$ M/cm)	
		Resorcinol method	Thiobarbituric method
9	NANA, 0.27	0.038 $\pm$ 0.007	0.045 $\pm$ 0.009
2	" 0.57	0.069	
5	" 1.29	0.284 $\pm$ 0.003	
4	NGNA, 0.23	0.070 $\pm$ 0.01	0.061 $\pm$ 0.009

NANA = N-acetylneuraminic acid.

NGNA = N-glycolylneuraminic acid.

Time of absorption, 30 min; in some cases also 15 and 60 min.

The results obtained in the case of the first two acids are similar to others studied in different mammal serums.

Table I summarizes the chief values referring to the absorption of the two acylneuraminic acids by the intestine wall of the rat. It may be observed that the two acids, as normal components of the rat serum, as we have seen, can be absorbed by the intestine. The NANA values show a correlation between intestinal absorptions and concentrations, at least in the conditions of this study. From other undetailed results, it seems that the absorption does not increase with the time, ranging between 15 and 60 min. Although the assays with NGNA are few (because of the difficulty in getting this product), it is obvious that its absorption is bigger than that of NANA; the existence of one hydroxyl group more in its molecule is perhaps involved in this process. Finally, in view of the fact that the acylneuraminic acids are composed by a rest of an acylhexosamine, we have tried to compare the absorption of the N-acetyl-D-glucosamine, studied in our previous work (5), with those of the two acylneura-

minic acids; it seems that the NANA and NGNA absorptions are bigger than that of the N-acetylglucosamine.

In brief, the N-acetyl- and N-glycolylneuraminic acids have been identified in the blood serum of the rat, and it has been showed that these acids may be absorbed by the intestine of the rats.

This work has been made with the help of the Spanish «Ministerio de Educación y Ciencia».

We are grateful to Dra. P. FERNÁNDEZ-OTERO for her advice in reference with the absorption technique; and to Prof. H. FAILLARD and Dr. M. BLOHM for their generous gift of the sample of NGNA.

## References

1. CABEZAS, J. A., *et al.*: *Biochim. Biophys. Acta*, **83**, 318, 1964.
2. CABEZAS, J. A.: *Bull. Soc. Chim. Biol.*, **48**, 381, 1966.
3. FAILLARD, H., and BLOHM, M.: *Hoppe-Sey. Z. Physiol. Chem.*, **341**, 167, 1966.
4. FAILLARD, H., and CABEZAS, J. A.: *Hoppe-Sey. Z. Physiol. Chem.*, **333**, 226, 1963.
5. FERNÁNDEZ-OTERO, P., PORTO, E., and CABEZAS, J. A.: *R. esp. Fisiol.*, **23**, 141, 1967.
6. KLENK, E., and UHLENBRUCK, G.: *Hoppe-Sey. Z. Physiol. Chem.*, **307**, 266, 1957.
7. SOLS, A., and PONZ, F.: *R. esp. Fisiol.*, **2**, 283, 1946.
8. WARREN, L.: *Comp. Biochem. Physiol.*, **10**, 153, 1963.

J. A. CABEZAS  
A. CARRIÓN  
M. C. GÓMEZ-GONZÁLEZ  
M. RAMOS

Department of Biochemistry  
University of Santiago de Compostela  
(Spain)

(Received for publication, October 13, 1967)