PN 1275

Lipophilic sialic acid derivatives in human erythrocytes

Material from human erythrocytes which behaves chromatographically like gangliosides and gives the colour reaction for sialic acids with Bial's reagent has recently been detected[1-3], although previously reported by others to be absent.

Three times saline-washed erythrocytes were haemolysed with 9 vol. distilled water, and the stroma, centrifugally sedimented at pH 5–6, were lyophilised and extracted with 10 ml of chloroform–methanol (2:1, v/v) per ml of cells. Water to 2% (v/v) was added with vigorous shaking before filtering through sintered glass. The more water was added to the lipid solvent, the more vigorously it was necessary to shake to combat the increased rate of agglutination of the membrane dispersion. Whole cells were extremely difficult to extract because of a similar clumping phenomenon.

The Bial's positive material remained in the lower phase on KCl partition[4] and with water partition[5].

The R_F of the material in chloroform–methanol–water–conc. ammonia (60:35:6:2, v/v)[6] was indistinguishable from that of the very fast-moving monosialoganglioside found in normal human brain, preponderating in Tay–Sachs disease[7]. In this system, mucopolysaccharides, proteins, and peptides stay on or near the origin.

The material, after dialysis and hydrolysis in 0.1 N H_2SO_4 at 80° in the presence of silica gel, was assayed by a periodate–thiobarbiturate method[8] and by an HCl–diaminobenzoic acid fluorimetric method[9], extinctions being corrected against unhydrolysed samples. The spectral maxima of these chromophores and that with Bial's reagent were identical in wavelength to those from synthetic *N*-acetylneuraminic acid, and the three methods gave similar estimates of the amount of lipophilic sialic acid, in the region of 0.5 μg/ml of cells.

Variations in the rate of release of sialic acid during such hydrolysis of the material further suggested a similarity to brain gangliosides[10]. The pattern of such variations, particularly a phase of very slow release of sialic acid around 21–27 min, was common to five chromatographically separated types of ox-brain gangliosides, and to the erythrocyte material. The effects were observable below the critical micelle concentration of gangliosides[11] and so are not likely to be reflecting progressive

TABLE I

ANALYSIS OF VARIANCE AMONG DIFFERENT TIME PERIODS IN THE RATE OF SIALIC ACID RELEASE BY ACID HYDROLYSIS OF SIALIC ACID-CONTAINING LIPIDS

	Degrees of freedom	*Variance*	*Probability of F-ratio to residual variance*
Between types of material (5 brain ganglioside bands and 1 erythrocyte band)	5	60	> 0.1
Between periods of fast and of slow sialic acid release (Low rate: 24–27, 40–50, 60–90 min; high rate: 27–40, 50–60, 90–120 min)	1	824	$\ll 0.01$
Interaction between types and periods	5	88	$\triangleq 0.1$
Residual variance	28	31	—
Total No. of readings	39		

changes in micellar organisation. An analysis of variance (Table I) of the rates of sialic acid release at periods of low and of high rate showed significant differences between the graphically selected groups of rate measurements, but no significant differences between types of sialic acid derivative, including the erythrocyte material.

Further characterisation of this material is being carried out concurrently with a survey of its occurrence in erythrocytes from human subjects in normal and in a variety of pathological conditions.

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Isolation and analysis of mono-, di-, and trisialogangliosides

The heterogeneity of ganglioside preparations in chromatographic systems has been well documented; however, the number of resolvable components and their composition reported by various investigators has varied (see refs. 1–5). This investigation sought to confirm or disprove some of the reports which were in conflict regarding the number of components and their composition. The resolution of 8 gangliosides by thin-layer chromatography has been noted, 5 of which are readily demonstrable. Homogeneous preparations of 4 of these 5 have been isolated, and their compositions closely correspond to theoretical values.

Gangliosides prepared from normal human gray matter as previously described[6,7] were subjected to thin-layer chromatography on Silica Gel G (E. Merck, A. G. Darmstadt, Germany) utilizing chloroform–methanol–water (60:35:8, v/v) (ref. 8). The dried plates were sprayed with resorcinol reagent[9] and the color developed in an HCl-saturated closed chamber at 110–120°. When minimal amounts of material were spotted, areas representing 4 major gangliosides were observed which were designated 1-G, 2-G, 3-G, and 4-G in order of decreasing chromatographic mobility. A fifth, faster-moving ganglioside designated FM was seen when larger amounts of gangliosides were chromatographed. 3 additional gangliosides with the fastest migration on