Neuraminic Acid as a Constituent of Human Cerebrospinal Fluid.* (22796)

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Recent investigations on the distribution of neuraminic acid (NA) have revealed that its occurrence in the body is not restricted to its role as a constituent of water-soluble glycolipids of brain(1,2), nor to states of disturbed glycolipid metabolism(1,6), but that NA occurs in a wide variety of body organs and fluids without necessarily constituting an integral unit of a glycolipid molecule(2,3,4,8). Thus, Böhm *et al.*(7,8) have isolated NA from serum, and have shown that normally it is tightly bound to serum proteins, being wholly precipitable with trichloracetic acid. These findings were confirmed by Uzman and Rosen(9) who also established that the bulk of serum NA is bound to alpha-2 globulins, and present in non-dialyzable form though not as a constituent of gangliosides. The NA values for normal serum recorded by Böhm *et al.*(7) was 40-65 mg/100 ml serum. Those recorded by other workers(9) ranged between 44 and 49.6 mg per 100 ml serum. Thus, assuming a normal serum protein of 6g%, the NA would be expected to correspond to approximately 0.67-1.1% of the total serum protein according to Böhm *et al.*(7) and 0.78-0.83% according to Uzman and Rosen(9). If the NA in the cerebrospinal fluid (CSF) were solely derived from serum, the amount of CSF neuraminic acid would be expected to be approximately proportional to the protein content of the CSF, provided that specific protein fractions having a high NA content were not preferentially concentrated in the CSF. It is a well known fact, however, that the bulk of CSF protein consists of albumin, with the globulin fractions constituting a much smaller proportion of the total than in serum(10-12). Evidence for enrichment of specific glycoproteins with high NA content, like the fetuin (6% NA) of Klenk(13) and the mucoprotein (8% NA) of Winzler(13, 14), in human CSF is totally lacking. The extremely low values (2.4 $\pm$ 0.9 μg NA per ml CSF) reported(15) on the basis of analysis of CSF total glycoproteins is further evidence against any selective increase of NA-rich glycoproteins in CSF with respect to serum.

The present investigation was undertaken to establish whether a relationship between the NA and protein content of human CSF did indeed exist, and to ascertain if, in addition, the CSF contained NA in a different state of binding than that found in serum. The latter consideration assumes importance in view of the possibility of release of NA from tissue glycolipids in the course of cerebral metabolism. Our findings demonstrate that human CSF contains 3 to 20 times more NA than would be expected from the CSF protein content, and that only the non-dialyzable NA moiety of the total NA reflects that portion of CSF neuraminic acid derived from serum proteins.

Material. The samples studied represented cerebrospinal fluid obtained by spinal puncture in the course of pneumoencephalography(10-30 ml), myelography (ca. 10 ml) or routine spinal fluid examinations, as well as samples obtained during diagnostic ventriculography (10-15 ml) from unselected cases studied on the Harvard Neurological Unit at the Boston City Hospital. All samples grossly contaminated with blood, or containing more than 5 white cells/cu mm were rejected.

Methods. The determination of neuraminic acid was effected by the method outlined by Böhm *et al.*(7). Total neuraminic acid was usually determined on 3 ml samples of CSF. 3 ml of Bial's reagent was used. This modification did not affect the linearity, if a 3 ml water blank was used, and blank recordings subtracted from the test samples. Duplicates

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checked within ± 1.0%. Non-dialyzable NA was determined by subjecting 3 ml of CSF to dialysis in 27/32 inch viscose tubing overnight. The dialyzed CSF was quantitatively recovered from the dialysis bags (with distilled water washings), made up to a known volume and aliquots taken for NA determination. These were compared with the total NA, and expressed as per cent non-dialyzable NA. Duplicates agreed within ± 2.0%. TCA-precipitable NA was determined by adding 3 ml of 10% TCA to 3 ml of CSF. centrifuging off the ensuing precipitate, and determining the NA content in the precipitate in the manner originally described by Böhm *et al.*(7).

The CSF total protein was determined by the sulfosalicylic acid method of Ayer *et al.* (16) as modified by Merritt and Fremont-Smith(17), inasmuch as this procedure gave the most reliable and reproducible results in the protein concentration ranges involved (duplicates agreed within 1%). The possibility of incomplete precipitation of some glycoproteins is a handicap shared by all other methods of CSF protein determination. In those cases where the partition of neuraminic acid was studied in the serum as well as in the CSF of a subject, the alpha-2 globulins were also determined by paper electrophoresis, using the Spinco-Durrum electrophoresis apparatus, quantitation being effected by use of the Spinco "Analytrol" photometric recording scanner and integrator.

Results. In 49 samples of human CSF examined, the neuraminic acid concentration was found to vary between 10.7 and 33.5 μg per ml. In those samples containing normal amounts of protein (15-30 mg%) the neuraminic acid corresponded in amount to about 10% of the protein (on the average). The concentration of NA did increase with increasing amounts of total protein in the CSF, but there was no proportional relationship. On the other hand, the NA/Total Protein ratio decreased with increasing protein concentration, so that in samples with a total protein above 500 mg%, the value of NA as per cent of total protein approached 1.0%, which is close to the average value in serum (0.8%).

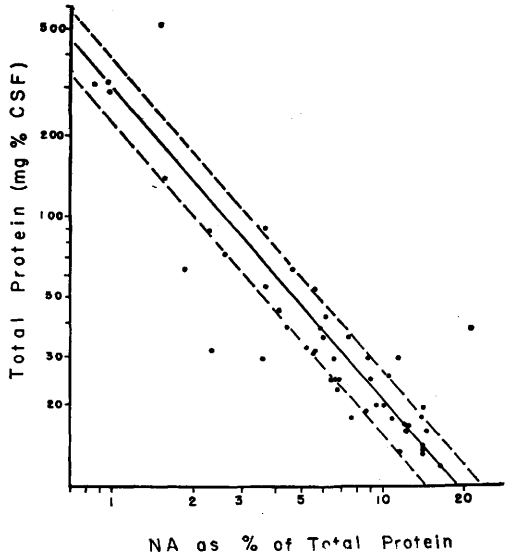

FIG. 1. Showing variation of NA-protein relationship in CSF with increasing conc. of CSF protein.

Thus, although some of the CSF neuraminic acid content is dependent on the NA accounted for by serum proteins, this constitutes a minor portion. Fig. 1 represents the relationship of the CSF neuraminic acid expressed as per cent of total protein to the CSF total protein concentration. It is evident that NA as % total protein decreases with increased protein concentration and rises with decreased protein concentration. Furthermore, this relationship is an exponential one both with the ordinate as well as the abscissa of the graph, so that the majority of values fall along a straight line when plotted against logarithmic ordinate and abscissa. The 5 exceptions represent gross deviations, presumably depending on the pathology presented by the patients involved. In CSF samples with very low protein content (6-10 mg%) obtained by ventricular drainage the NA was proportionately much greater, so that it represented 20-30% of the total protein (top 3 points on Fig. 2).

In those samples where the non-dialyzable NA was estimated, the results indicated that it is this fraction which is derived from serum. In Fig. 2, the log of the non-dialyzable NA, as per cent of the total NA, is plotted against the log of total neuraminic acid as per cent of the total protein. It is evident that as the

TABLE I.

Serum alpha-2 globulins as % total protein	CSF total protein, mg %	NA as % of total protein		Non-dialyzable NA as % total NA		TCA-precipitated NA as % total NA	
		Serum	CSF	Serum	CSF	Serum	CSF
12.8	62	.82	4.6		40.0	95.0	
11.4	13	.81	14.4		21.0	92.4	
12.6	43	.63	6.12	96.5	31.0	88.0	17.85
11.2	64	.49	1.84	100.0	43.0	88.5	22.9
12.1	56	.63	3.66	100.0	43.0	88.0	
9.8	20	.84	9.35	99.5	23.8	92.0	11.7

non-dialyzable NA fraction increases and approaches 100%, the NA/protein ratio drops to levels approaching those prevalent in serum since the NA value expressed as per cent of total protein approaches the serum value of 1.0%. Similarly, low values for per cent non-dialyzable NA correspond to those samples that had low total protein and therefore a high NA/protein ratio.

It is also evident that the relationship of non-dialyzable neuraminic acid to the NA/protein ratio is an exponential one, inasmuch as the values (excepting those marked with arrows), plotted in Fig. 2 fall along a straight line if logarithmic ordinate and abscissa are used. The 4 values, indicated by arrows, that deviate grossly from the straight-line trend represent an extreme type of pathological deviation, the significance of which remains to be elucidated.

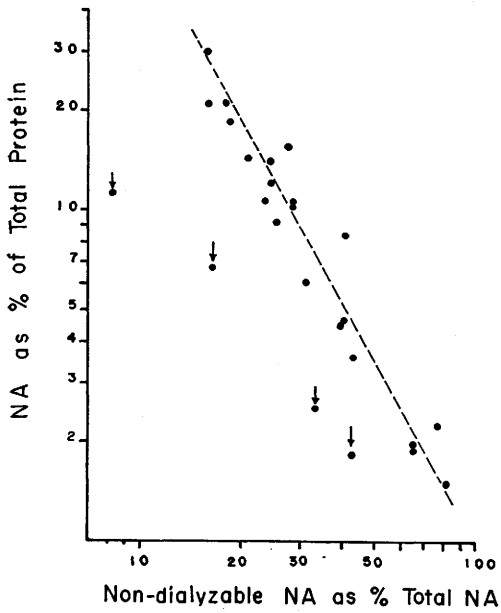

FIG. 2. Variation of non-dialyzable NA moiety of CSF with changes in total NA/protein ratio. Dotted line represents theoretical curve that would be obtained if the whole non-dialyzable NA fraction of the CSF were derived from serum.

That the variations in the NA/protein ratio and the per cent of non-dialyzable NA fraction in the CSF are not due to variations of these values also in the serum of any individual subject is demonstrated by the comparison of values for serum and CSF in 6 randomly selected subjects (Table I). It is apparent that the different state of binding and different NA/protein ratio in the CSF cannot be explained by the NA/protein ratio of the serum. Nor was there any significant difference in the serum alpha-2 globulin concentrations to account for the elevated NA/protein ratios in the CSF. Similarly, the different proportions of the nondialyzable NA fraction in the CSF in contrast to the serum could not be explained on the basis of differences in the alpha-2 globulin content of the sera of the same subjects.

Discussion. From the foregoing information it appears that human cerebrospinal fluid is notable for the high proportion of dialyzable neuraminic acid it contains with respect to its total NA content. The disparity between the dialyzable and bound neuraminic acid in CSF on one hand, and serum on the other, most probably is explained by considerations involving a 2-fold origin for the CSF neuraminic acid. One of these, accounting for the non-dialyzable neuraminic acid fraction, is probably derived from the serum neuraminic acid carried into the CSF by serum proteins. The relationship of the non-dialyzable fraction to the CSF total neuraminic acid with increasing protein levels (NA as per cent of total pro-

tein) as indicated in Fig. 2, clearly supports this view. The second source of neuraminic acid could be the metabolic processes of the central nervous system itself which, presumably in the course of ganglioside turnover, may contribute to the dialyzable, and probably free, neuraminic acid moiety. The fact that it is the dialyzable fraction that is responsible for the disproportionate elevation of NA with respect to protein in CSF clearly argues against the high NA/protein ratio of CSF as being due to selective entry of specific glycoproteins with high NA content. Admittedly, none of the samples studied can be assumed to represent entirely normal values for human CSF, inasmuch as there is always suspicion, if not evidence, of neurological disease when a spinal or ventricular puncture is performed. This fact had, however, the advantage of providing a survey of variations of neuraminic acid content with different levels of CSF protein, and establishing the relationship of the non-dialyzable fraction to the serum-derived neuraminic acid, as shown by our data in Figs. 1 and 2. A further advantage would be evinced in the correlation of neurological pathophysiology and gross deviations from the straight-line trend depicted in Fig. 1. Any increase in NA concentration due solely to increase in CSF total protein would be predicatable within the margin of variation indicated by the dotted lines† in Fig. 1 by the relationship

$$\log (TP) = -1.16 \log (NA) + 2.4771$$

which is the equation for the solid line, where (TP) represents total protein in mg% CSF, and (NA) stands for NA as per cent of total protein. Gross deviations from this numerical relationship, plotted in the same manner as in Fig. 1, would indicate a decreased amount of dialyzable NA if the points fall to the left of the line, and an increased amount of dialyzable NA, if the points fall markedly to the right of the line. It is hoped that extensive correlative studies between the gross deviations of the neuraminic acid concentration from those values expected from the total protein content and the neurological conditions giving rise to these states will prove fruitful in defining the source for the free (or dialyzable) moiety of neuraminic acid in the CSF.

Conclusions. 1. The neuraminic acid (NA) content of 49 samples of human cerebrospinal fluid (CSF) was studied. In all instances the quantitative relationship of neuraminic acid to the cerebrospinal fluid protein was far in excess of that prevalent in serum. In 26 samples in which the total protein was 30 mg % or less, the neuraminic acid was 4-18 times in excess of that expected if it were derived from serum and bound to protein, as is the case in blood. Unlike its state in serum, most of the CSF neuraminic acid is in freely dialyzable form (60-80%). That moiety of CSF neuraminic acid which is derived from serum is in protein-bound, non-dialyzable form. 2. The origin of the dialyzable NA is unknown, but is not related to ingress of serum proteins into the CSF. The implications of these findings are discussed.

† Arbitrarily chosen to include 80% of values. The normal spread is obviously a function of two variables, *i.e.* CSF protein concentration and dialyzable NA, and can only be determined by a study of a very much larger case population.

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Effect of Dietary Fat on Requirement of Vitamin B_{12} by the Chick.* (22797)

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The young chick's dietary requirement for vit. B_{12} may be influenced by several factors. That the hen's diet affects the vit. B_{12} requirement of her progeny was recognized even before isolation of the vitamin. The requirement of chicks from hens depleted of their vit. B_{12} stores has been shown by most workers to fall between 15 and 30 γ/kg diet(1-4). The vit. B_{12} requirement of chicks from hens fed typical breeder diets was determined by Davis and Briggs(5) to be 1.5 to 2 γ/kg of diet. Similarly, Miller, Norris, and Heuser (6) found that chicks with moderate stores of vit. B_{12} upon hatching required 1.25 γ/kg diet. These values are somewhat below 8 γ, the level that has been tentatively recommended by the Committee on Animal Nutrition of the National Research Council(7). The relationship between vit. B_{12} intake of the hen and requirement of the chick has been quantitated by Milligan, Arscott, and Combs (8). They found that chicks fed a basal corn-soybean oil meal diet (25% protein) require 27, 12, 3, and 0 γ vit. B_{12}/kg diet when the dam's diet contained 0, 4, 8, and 16 γ per kg, respectively.

The influence that certain nutrients of the chick's diet may have upon the requirement of vit. B_{12} has been reviewed by Smith(9). Spivey, Briggs, and Ortiz(10) reported that a severe vit. B_{12} deficiency could be produced in undepleted chicks by the addition of 20% fat to a corn-soybean oil meal diet. The present paper deals with the increase in the vit. B_{12} requirement of chicks receiving this high fat diet.

Methods. The chicks were from a commercial hatchery. They were distributed into groups of 6 chicks each when 1 day old and were kept on the experimental diets for a 4-week period. The chicks were maintained in standard electrically heated batteries with screen wire floors. Feed and water were supplied *ad libitum* and total amount of diet consumed by each group during the experiment was recorded. The chicks were weighed at weekly intervals, and the final weight was the chief criterion of response used in evaluating the results of the experiments. The diets were similar to those used previously(10,11). Diet C29 had the following composition/kg: soybean oil meal 350 g, ground yellow corn 575 g, glucose 10 g, corn oil 5 g, chick salts A 60 g(12), riboflavin 8 mg, vit. D_3 0.02 mg, and 2-methyl-1, 4-naphthoquinone 1 mg. The small amounts of glucose and corn oil served as vehicles for the vitamins. Diet C30 was identical with diet C29 except that 200 g of lard were substituted for an equivalent amount of corn in a kg of diet. The total fat content of diet C29 was approximately 3%, by calculation, and that of C30, 22%. The vit. B_{12} content of each diet was varied in these experiments at levels between 5 and 2,000 γ/kg of diet. For parenteral administration, an aqueous solution of the vitamin was injected subcutaneously in the abdomen

* Part of these data was presented at 39th Meeting of Fed. of Am. Soc. for Exp. Biol., San Francisco, Apr. 11, 1955.