References and Notes

1. J. Bellin and D. Laszlo, *Science* **117**, 331 (1953); J. Bellin, A. Hausinger, and H. Spencer, *Proc. Am. Assoc. Cancer Research* **1**, 4 (1953).

2. This study was supported by a research grant C-1540 (C3) from the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service. The assistance of Eva Berger, Joseph Greenberg, Estelle D. Gottesman, and Mary Perrone is gratefully acknowledged.

3. This technique was first used for the calculation of indogenous fecal phosphorus in man by G. Hevesy [*Radioactive Indicators* (Interscience, New York, 1948)] and for the calculation of endogenous fecal calcium in cattle by C. L. Comar *et al.* [*J. Nutrition* **50**, 23 (1953)].

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Partition of Neuraminic Acid among Human Serum Proteins

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Ever since the original description by Klenk, neuraminic acid has been recognized as a constituent of gangliosides (*1, 2*), and the specific color reaction that it gives with Bial's reagent (*3*) has been used to distinguish gangliosides from other water-soluble glycolipids (*4*). Neuraminic acid has been shown to be a constituent of gangliosides not only in lipid storage diseases (*1*), but also in normal mammalian nerve tissue, liver, spleen, and erythrocytes (*2, 5*). Attempts to demonstrate neuraminic acid in serum had, however, failed until recently, when Bohm *et al.* (*6*) showed that neuraminic acid was bound to serum proteins. Precipitation of serum proteins with trichloroacetic acid and subsequent treatment of the precipitate with Bial's reagent gave the characteristic Klenk-Langerbeins reaction for neuraminic acid. Since these workers were also able to isolate neuraminic acid from serum, the presence of neuraminic acid in serum appears established.

Our own attempts were directed at determining to which of the serum protein fractions neuraminic acid is normally bound (*7*). Fresh human serums obtained from (i) individual subjects, (ii) random hospital patients (10 pooled), and (iii) American Red Cross blood donors (40 pooled) showed neuraminic acid values ranging between 440 and 496 μg/ml serum according to the procedure of Bohm *et al.* (*6*). That the binding of neuraminic acid to certain of the serum proteins exists independent of a coprecipitation phenomenon which might arise in the course of trichloroacetic acid precipitation of proteins was confirmed by pressure ultrafiltration of serum. The ultrafiltrate contained no neuraminic acid. Treatment of the trichloroacetic acid precipitates with boiling ethanol and chloroform-methanol (2:1) did not extract neuraminic acid from the protein moiety.

Electrophoretic separation of serum proteins was undertaken with a Durrum-type paper electrophoresis apparatus, using 0.05*M* diethylbarbiturate buffer at *p*H 8.64 and a potential difference gradient of 15 v/cm. The paper was Whatman No. 1 with a strip width of 3.7 cm. In order to effect clearing of the starting point by the gamma-globulin zone, the serum was applied 4 cm away from the center (apex) toward the cathode. Four strips each carrying 20 μlit of serum were used in each run. After allowing electrophoretic separation of the zones to proceed for 6 to 8 hr, a guide strip was oven-dried and stained with bromphenol-blue in the usual manner. The untreated strips that were to be analyzed for neuraminic acid were not allowed to dry. Segments corresponding to the albumin, alpha-1 globulins, alpha-2 globulins, beta and gamma globulins, as well as the line of origin, were carefully cut out. The respective segments from three or four strips were combined and extracted overnight with distilled water. The aqueous extracts were individually lyophilized and analyzed for neuraminic acid by the modified procedure of Bohm *et al.* (*6*).

The results of the analyses indicate (Fig. 1, Table 1) that the maximum amount of neuraminic acid in human serum is located in the alpha-2 globulin fraction. The lesser amounts found in the alpha-1 and beta-globulin segments can be ascribed to the fact that portions of these fractions are overlapped by the edges of the alpha-2 globulin zone. The same preponderance of neuraminic acid in the alpha 2 globulin fraction was found in individual serums as well as in pooled serums from 10 hospital patients and also from 40 normal blood donors.

When the serum proteins were precipitated with 10 vol of ethanol, redissolved in 0.9 percent saline, and

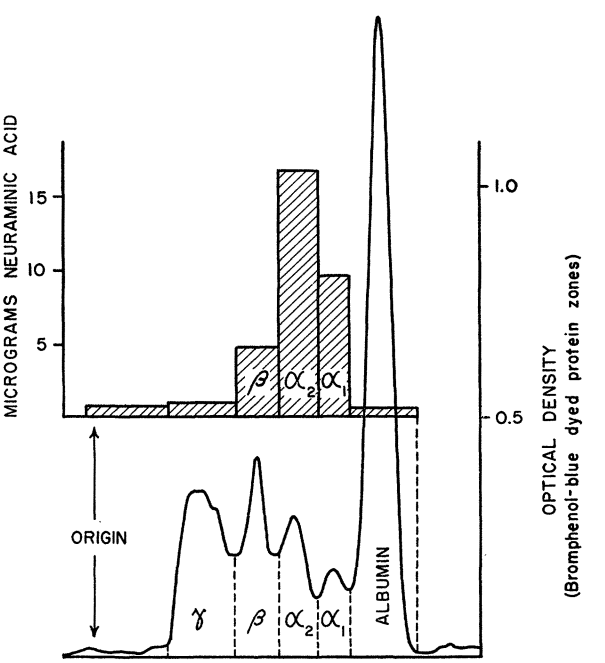

Fig. 1. Distribution of neuraminic acid with respect to serum proteins on electropherograms. The scanning of the bromphenol-blue-dyed protein zones represents a strip with 20-μlit serum. The neuraminic acid values correspond to four such strips (80-μlit serum).

Table 1. Neuraminic acid values (in micrograms) for different zones of paper electropherograms.
(80 μlit serum)

Serums	Albumin	Globulins				Starting point
		alpha-1	alpha-2	beta	gamma	
Single case	0.6	7.8	12.0	2.4	2.0	1.0
10 patients (pooled)	.7	9.6	16.8	4.8	0.8	0.8
40 normal adult males (pooled)	.9	7.5	14.5	7.0	3.0	1.3

heated for 30 min in a boiling water bath and then reprecipitated with 8 vol of ethanol, neuraminic acid was released and could be detected in the alcoholic supernatant about 80 percent of total originally present). Since this procedure is unlikely to result in hydrolysis of a polysaccharide or glycolipid, it appears unlikely that neuraminic acid is a constituent of an independent polymer that happens to have the same mobility as alpha-2 globulins; rather it indicates that neuraminic acid is either bound to alpha-2 globulins or is present as a constituent of the polysaccharide or glycolipid moieties of alpha-2 globulins. The relationship of neuraminic acid levels to alpha-2 globulin levels in various disease states is under investigation.

References and Notes

* Captain, MC, AUS.
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7. We are indebted to Stanley M. Levenson for his interest and advice and to E. Durrum, MC, for helpful suggestions concerning paper electrophoresis.

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Fractional Total-Body Irradiation and Thyroid Function in the Burro

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Thyroids of burros exposed to acute or fractional total-body irradiation have shown histological evidence of extreme stimulation by hyperplasia of the epithelial cells and marked colloid depletion.

Increased uptake of iodine-131 in thyroids of irradiated animals has been reported by Monroe *et al.* (*1*), Evans *et al.* (*2*), and Hursh *et al.* (*3*). Botkin *et al.* (*4*), however, noted that total-body irradiated rats had a decrease that followed a small initial rise. In the present study (*5*) burros were exposed to total-body cobalt-60 gamma irradiation, 25 r/day, until death and the thyroid uptake of intraperitoneally administered iodine-131 (*6*) followed.

Of 13 healthy burros (*Equus asinus asinus*), ten were simultaneously exposed, and three served as controls for stable iodine determinations (*7*). Exposures were made on a large exposure field previously described (*8*). Burros were allowed orchard grass hay, water, and noniodized salt *ad libitum* throughout the study. Each animal was its own control, by comparison with preirradiation rate of uptake. To increase the number of observations, the group was divided and, at staggered intervals, each group of five received intraperitoneal injections of 10 μc of carrier free iodine-131. Thyroid activity was measured by external counting with a scintillation counter (NaI crystal). The iodine-131 that accumulated in the thyroid 8 hr after injection was chosen for purposes of comparison. The fractionation and determination of total stable iodine and stable thyroxin iodine were done by the method of Taurog and Chaikoff (*9*).

After 600 to 800 r some animals began to concentrate iodine-131 (Fig. 1*b*) to a marked degree; others

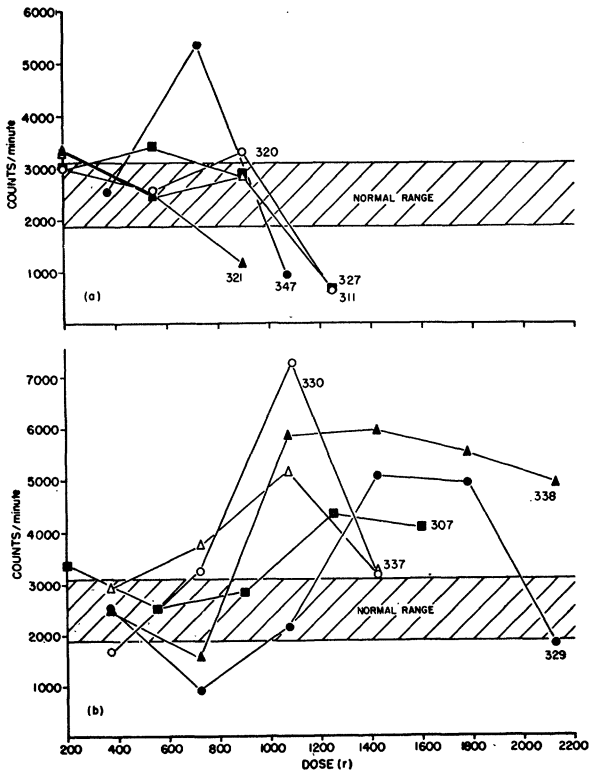

Fig. 1. Counts per minute over the thyroid at various accumulated dose levels. The plots are separated for ease in reading.