Attempts to identify echinacein were greatly hampered by the fact that the crystals are highly unstable, polymerizing in the air after 1 hr at room temperature and after 2 days in a nitrogen atmosphere at −10°C (a natural antioxidant is apparently present in the crude extract of the roots). The compound showed a maximum in the ultraviolet at 259 mμ, characteristic of a conjugated triethenoid structure, and analyzed fairly well for $C_{16}H_{25}NO$. Hydrogenation in ethanol solution with a platinum catalyst gave colorless needles, mp 51° to 52°C, identical with N-isobutyllauramide. Permanganate oxidation of echinacein resulted in a mixture of acids, none of which were present in sufficient amount for identification. One of these acids, volatile with steam, consisted of a few drops of a colorless, highly corrosive oil with a penetrating unpleasant odor. Accidental contact of a trace of this acid with the skin of the hand caused an immediate burning sensation and rapid blistering of the skin at the site of contact, followed by peeling of the skin after 2 days.

Echinacein appears to be the isobutylamide of a highly unsaturated 12-carbon straight-chain acid and may be identical with neoherculin, mp 63° to 65°C, isolated by Crombie (*9*) from the bark of *Zanthoxylum clava-herculis*. Further identification must await the isolation of larger quantities of pure material.

References and Notes

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Utilization and Intestinal Excretion of Calcium in Man

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Data on Ca^{45} metabolism in man have been reported and measurements of endogenous fecal calcium and the utilization of ingested calcium have been discussed (*1*). In this study the rate of absorption and utilization of ingested calcium, the excretion of digestive juice calcium into the gastrointestinal tract and its reabsorption were measured with the Ca^{45} technique and metabolic balances (*2*). Two patients without bone disease, B and L, maintained on low calcium diet, received a single dose of 50 μc Ca^{45} with 30 mg of calcium carrier orally. Serum, urine, and stool were analyzed for calcium and Ca^{45} (Fig. 1). Balances of calcium, phosphorus, and nitrogen were measured for four 6-day periods (Table 1).

The top graph of Fig. 1 shows the specific activities of serum and urine and the serum Ca^{45} levels of patient B in the first 24 hr. Radioactivity was first detected in blood at 15 min; a 5-min sample had no activity. The specific activities of blood and urine from the second day to the termination of the experiment are plotted on a semilogarithmic scale in Fig. 1. There is a simple exponential decline with a half-time of 8 days. The first stool collected at 2 hr contained no activity; the next specimen at 25 hr, 5.0 percent; after a 5-day period a total of 58 percent of the dose was excreted. The specific activity of these samples is not plotted, since it was considerably higher than 1 percent of the dose per 100 mg of Ca. Figure 1 shows

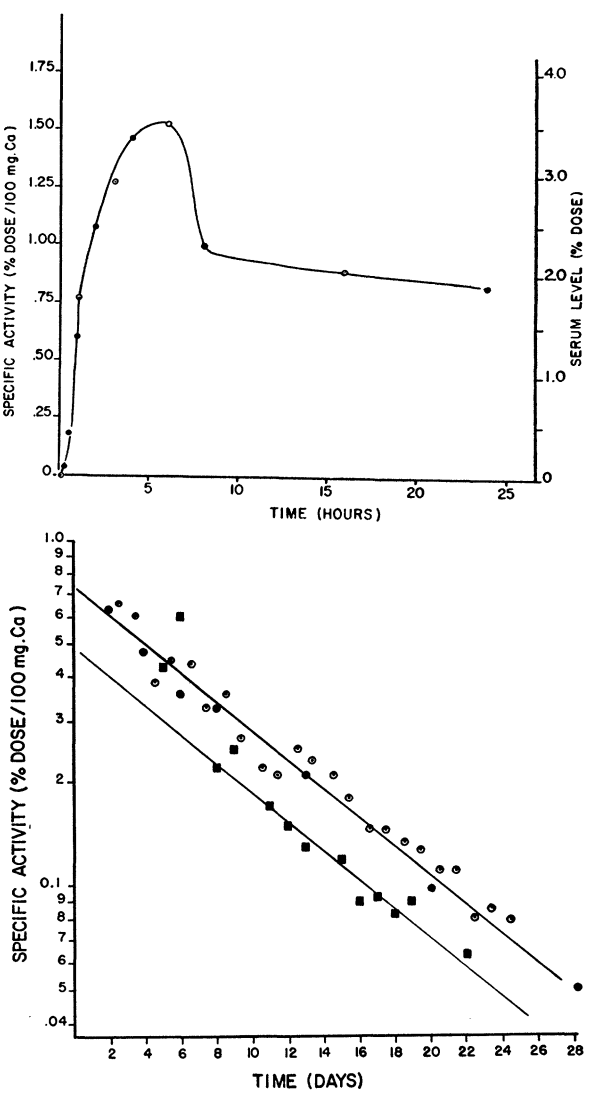

Fig. 1. Calcium and Ca^{45} content in serum, urine, and stool of two human patients. Symbols: ● plasma, ⊙ urine, ■ stool.

the specific activity of stool collected after the fifth day. After 1 wk the specific activity of the stool calcium falls exponentially on a line parallel to, and below, the blood and urine line.

Data obtained in the simultaneous study of patient L were similar to those of B. Blood taken at 15 min contained no Ca^{45}, whereas it was radioactive at 30 min, with the peak occurring at 2 hr (3.4 percent of dose). Urine collected in the first hour was radioactive; the 2- to 4-hr sample had the highest specific activity. The specific activities of plasma and urine were equal and fell exponentially with a half-time of 9 days. The specific activity of the stool was high for several days and then fell on a line parallel to, and below, the urine and serum level.

The radiocalcium content and the specific activities of serum and urine in the early phases (top graph, Fig. 1) illustrate the rapidity with which ingested calcium is absorbed, probably from the uppermost segments of the intestinal tract. The specific activities of serum and urine become rapidly equal, urines of the first and second hour, respectively, fall already on the serum curve. After this early phase, the specific activity of serum and urine decline exponentially with an 8- or 9-day half-time. Similar results were obtained in patients given Ca^{45} intravenously, which implies that the metabolic pattern is independent of the route of administration following the initial period of absorption.

In the early phase of the experiment the stool radiocalcium derives from two sources: unabsorbed ingested Ca^{45} and Ca^{45} carried into the tract with digestive juices. These juices probably become radioactive almost immediately after the dose is given and have the same specific activity as blood and urine. During the passage of the unabsorbed dose (about 1 wk) the stool has a high specific activity, whereas at the end of this time values fall on a line parallel to, and below, the urine-blood line (Fig. 1). At this time the only source of stool Ca^{45} is digestive juice calcium, which enters the tract with the specific activity of plasma calcium but is diluted by nonradioactive unabsorbed food calcium. Ratio of the specific activities of stool: plasma or urine represents the fraction of stool calcium that is endogenous, that is, intestinal juice calcium (*3*). The endogenous calcium calculated in this manner averaged 91 mg/day for B, 117 mg/day for L. The remainder of 60 mg and 45 mg fecal calcium per day, respectively, is unabsorbed food calcium. Subtracting these values from the intake (Table 1), the utilization of ingested calcium was calculated to be 44 percent for B and 67 percent for L.

The utilization can also be determined from the unabsorbed Ca^{45} in the stool. The total excretion of Ca^{45} in stool for B during the period the unabsorbed dose was passed (first week) was 60 percent. During this time, however, endogenous fecal Ca^{45} had also been excreted, for which a correction must be made. By extrapolating the straight line portion of the stool specific activity plot toward zero time, the endogenous Ca^{45} of this period was estimated to be 3 percent of the dose; therefore, 57 percent passed unabsorbed and 43 percent was utilized. For patient L utilization was calculated to be 67 percent. The results obtained by this method and independently by the ratio of specific activities are in close agreement.

The endogenous fecal calcium is total digestive juice (T.D.J.) calcium minus reabsorbed digestive juice calcium. The T.D.J. calcium may be estimated by

$$\frac{\text{T.D.J. Ca}}{\text{Food Ca} + \text{T.D.J. Ca}} = \frac{\text{specific activity of stool}}{\text{specific activity of plasma}}.$$

This estimation assumes that the calcium of food mixes homogenously and exchanges with the radiocalcium of digestive juices and thereby dilutes its specific activity, which was initially the same as that of the plasma. The absorption then proceeds from this homogenous mixture of food and digestive juice calcium, the nonabsorbed portion, that is, endogenous fecal calcium plus unabsorbed food calcium, being excreted in stool. Since the amount of food calcium and the specific activities of plasma and stool can be measured, the total digestive juice calcium can be calculated: 160 mg/day for B, and 370 mg/day for L. Both values are somewhat lower than the estimates, based on the estimated total volume of intestinal juices and their calcium concentration: 400 to 560 mg/day. However, if dietary calcium were absorbed before mixing with digestive juices or if exchange would take place across the intestinal wall, the T.D.J. calcium would be even lower.

Thus, by simultaneous measurements of Ca^{45} and metabolic balances, it is possible to measure the utilization of ingested calcium, its speed of absorption from the intestinal tract, and excretion and reabsorption of calcium of the digestive juices. This technique has also been used to measure the utilization of dietary calcium at various intake levels and to study the calcium compartments of the body in normal and pathologic states. These data will be reported separately.

Table 1. Metabolic balances.

	Patient B				Patient L			
	Nitrogen (mg/day)	Phosphorus (mg/day)	Calcium (mg/day)	Ca^{45}* (% dose)	Nitrogen (mg/day)	Phosphorus (mg/day)	Calcium (mg/day)	Ca^{45}* (% dose)
Intake	8990	534	107	100	11250	676	135	100
Urine	5120	269	90 ± 16	7.0	8150	366	51 ± 8	4.1
Stool	910	153	151	62.6	1100	225	162	42.3
Balance	+2960	+112	−134	30.4†	+ 2000	+ 85	− 78	53.6‡

* At end of experiment (26 days). † < 0.12 percent in the total plasma volume. ‡ < 0.20 percent in the total plasma volume.

References and Notes

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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

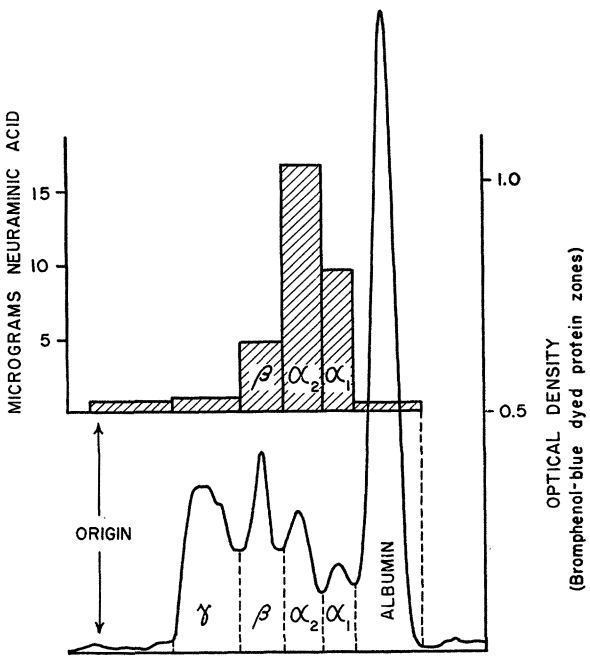

Fig. 1. Distribution of neuraminic acid with respect to serum proteins on electropherograms. The scanning of the bromphenol-blue-dyed protein zones represents a strip with 20-μlit serum. The neuraminic acid values correspond to four such strips (80-μlit serum).