

retain its enigmatic character until similar conditions may possibly be encountered at the foot of a hill where a lakeshore environment favored better preservation of a prehistoric hunting skill which, to my knowledge, has not been recognized so far at a prehistoric site in North America.

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## Iodinated Protein in Milk<sup>1</sup>

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Iodide has been demonstrated in the milk of women (1, 2) and of animals (3). We have reported that rats on a synthetic low iodine diet containing purified casein<sup>3</sup> do not develop goiter (4). This suggested the possibility of a substance in casein which prevented goiter. We found purified casein to contain 0.11 µg of iodide/g. This iodide was not removed by 6-hr soxhlet extraction with butanol or ethanol. The following experiment was performed to test for the production of protein bound iodine in milk.

Two lactating dogs were injected with 2.5 and 8 millicuries, respectively, of I<sup>131</sup>. After 4 and 24 hr milk was collected. The milk was dialyzed against running water for 2-4 days and the dialyzate subjected to paper electrophoresis in a barbital buffer at pH 8.6 for 3 hr. Human serum and the dog serum were used as controls. An autoradiograph was prepared from the electrophoresis paper. The area containing radioactivity was then cut out and counted in an internal gas counter. Figure 1 shows the nondialyzable radio-

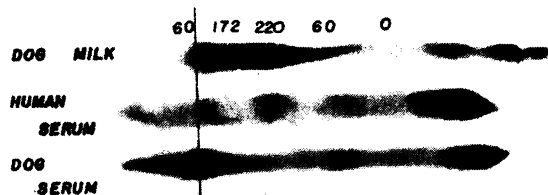


FIG. 1. Paper electrophoresis patterns. The nondialyzable I<sup>131</sup> was found with the slow moving protein of the dog milk. Counts/minute of the various areas of the dog milk patterns are indicated above.

activity of milk to move with a slow moving protein under these conditions.

The dialyzed milk was hydrolyzed in 2 N NaOH for 12 hr at 100° C, then acidified and extracted with butyl

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alcohol. This extract was subjected to paper chromatography by the method of Gross *et al.* (5). Two radioactive substances were demonstrated using butyl alcohol, dioxane, and ammonia. Their R<sub>f</sub> values were 0.25 and 0.35. Using butyl alcohol and formic acid, a radioactive compound with R<sub>f</sub> = 0.58 was shown. The acid-butanol extract from less severe hydrolysis (0.5 N NaOH for 4 hr at 100° C) was chromatographed using *n*-butanol and acetic acid. One dominant radioactive compound was separated with R<sub>f</sub> = 0.87.

These data are interpreted to indicate that milk contains an iodinated protein and purified casein possesses a thyroid-like property of goiter prevention.

*Added in proof.* Further experiments have demonstrated detectable quantities of I<sup>131</sup> in all protein fractions of the electrophoretic separation of milk.

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## Changes in Concentration of Hydrogen Ion During Precipitation Reactions Between Neutral Salt Solutions

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In discussing changes of hydrogen-ion concentration in chemical and biological systems all writers appear to assume without question that when two dilute neutral solutions of normal salts of strong or moderately strong acids and bases are mixed the resulting solution is also neutral, and that when two such solutions differ in hydrogen-ion concentration and from exact neutrality the hydrogen-ion concentration of the resulting solution will be between that of the two solutions. Neither appears to be generally true, however, when a precipitate is formed as a result of a chemical reaction between the two solutions.

As a good illustrative example, changes observed on mixing solutions of barium chloride and potassium sulfate may be cited. In the experiment in which 0.1 M barium chloride of pH 6.6 was slowly added to 0.1 M potassium sulfate of pH 6.4, the pH of the mixture or supernatant liquid as measured with a glass electrode rose steadily to a maximum of 9.5 just before the equivalence point and then dropped more rapidly through the equivalence point to about 7.2. When the solutions were mixed in the reverse order, the pH fell to a level of 5.7 before the equivalence point and rose to a maximum of 8.9 just after. The graphs of the changes in pH during the two titrations were

<sup>1</sup> National Science Foundation Predoctoral Fellow.

rough mirror images of each other. The extent and the pattern of the changes in hydrogen-ion concentration were found to vary considerably with the rate of mixing. Also, when the solutions contained a miscible organic solvent both the range of change in hydrogen-ion concentration and the sharpness of the change at the equivalence point were increased. For example, in an experiment in which 0.1 *M* barium chloride was added to a 50% ethanol solution of 0.1 *M* potassium sulfate the pH was 10.2 just before the equivalence point, and 6.5 just after.

We ascribe all such changes in hydrogen-ion concentration to the adsorption or desorption of hydrogen ions or hydroxide ions on the surface of the precipitates. The extent of the changes depends not only on the specific nature of the precipitate, but also on its state of subdivision, in other words its surface area. The different results observed with differences in rate of mixing and the greater changes caused by the presence of a miscible organic solvent may all be ascribed to differences in particle size and corresponding surface area.

This general effect is not only of theoretical interest but appears to be capable of practical application to analytical measurements. For example, by the use of a glass electrode as an indicator, we have been able to attain an accuracy of about 0.5% in the titration of sulfate with a standard barium solution.

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## Biased Forest Stand Estimates Due to Sample Size<sup>1</sup>

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There has been a tendency toward the use of small samples in American forest sampling for ecological purposes. Relatively few investigations have used sample plots larger than the 100 sq. m. (approximately 1/40 acre) recommended by Cain (1), and an entire state survey (Wisconsin) has been based on sets of two-tree samples, the "random pairs" of Cottam and Curtis (2). Recent comparisons of the precision and efficiency of various forest sampling procedures in the Great Smoky Mountains (3) illustrate a generally unrecognized small-sample bias, which may lead to important errors in stand estimates.

Cumulative distributions of sample basal area values for tenth-acre, fortieth-acre, and random-pair samples taken at the same random points in a spruce-fir stand are plotted on probability paper, on which a normal distribution would lie along a straight line (Fig. 1). The mean of the random-pair samples is considerably higher than the median and is an unreasonably high stand estimate, but the estimate based on fortieth-acre plots, which has the same sort of bias in lesser degree,

<sup>1</sup> Contribution from the Botanical Laboratory, The University of Tennessee, N. Ser. 154.

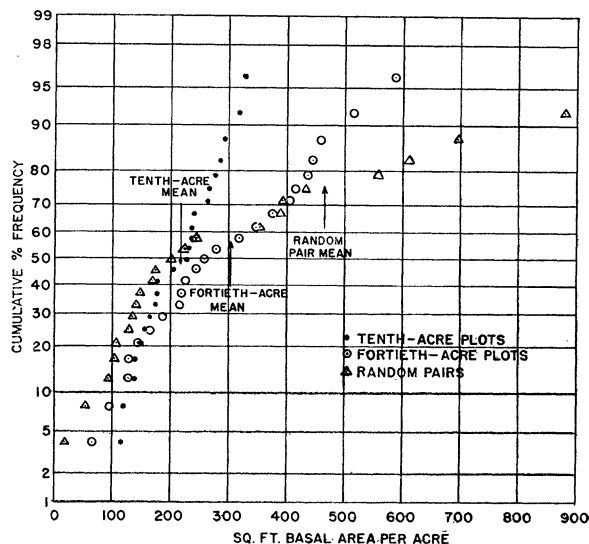


FIG. 1. Cumulative distributions of sample basal areas obtained from samples of different sizes, spruce-fir stand, Great Smoky Mountains. (The 24th point in each series is at infinity, and the 23rd point in the random-pair series lies off the graph, at 1680 sq. ft.)

is near enough to a reasonable range to be accepted in the absence of comparative data or detailed analysis.

The form of several small-sample curves, including those in Fig. 1, suggested a logarithmic transformation, which brought the data into satisfactory agreement with the normal distribution, as is evident from the chi-square test (Table 1). This transformation made possible the application of conventional statistical procedures to the small-sample data, and resulted

TABLE 1  
CHI-SQUARE TESTS OF GOODNESS OF FIT TO A  
NORMAL DISTRIBUTION

	$\chi^2$	$\chi^2_{.95}$	Hypothesis of normal distribution
<i>Spruce-fir forest type, all-aged</i>			
Plots, tenth-acre (24)			
Data untransformed	1.00	3.84	Accepted
Plots, fortieth-acre (24, at the same points as above)			
Data untransformed	7.67	3.84	Rejected
Data transformed	1.33	3.84	Accepted
Plots, fortieth-acre (40, including the 24 above)			
Data untransformed	3.46	7.81	Accepted
Data transformed	2.79	7.81	Accepted
Random pairs (40, at the same points as above)			
Data untransformed	Very large	7.81	Rejected
Data transformed	3.57	7.81	Accepted
<i>Deciduous forest type, buckeye-basswood-sugar maple, all-aged</i>			
Plots, tenth-acre (25)			
Data untransformed	2.68	3.84	Accepted
Random pairs (39)			
Data untransformed	Very large	7.81	Rejected
Data transformed	.83	7.81	Accepted