

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 Milk¹

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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 casein³ 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 I¹³¹. 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-

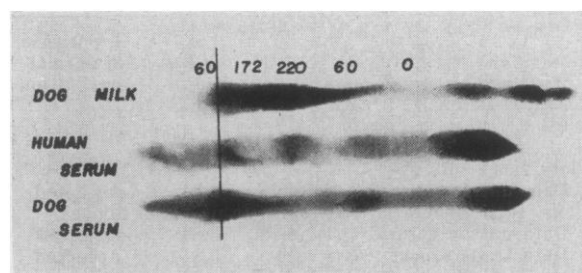


FIG. 1. Paper electrophoresis patterns. The nondialyzable I¹³¹ was found with the slow moving protein of the dog milk. Counts/minute of the various areas of the dog milk patterns are indicated above.

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

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_f values were 0.25 and 0.35. Using butyl alcohol and formic acid, a radioactive compound with R_f = 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_f = 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 I¹³¹ in all protein fractions of the electrophoretic separation of milk.

References

1. HONOUR, A. J., MYANT, N. B., and ROWLANDS, E. N. *Clin. Sci.*, **11**, 447 (1952).
2. NURNBERGER, C. E., and LIPSCOMB, A. J. *Am. Med. Assoc.*, **150**, 1398 (1952).
3. COURRIER, R., *et al. Compt. rend. soc. biol.*, **143**, 599 (1949).
4. VAN MIDDLESWORTH, L., and BERRY, M. M. *Am. J. Physiol.*, **167**, 576 (1951).
5. GROSS, J., *et al. Science*, **111**, 605 (1950).

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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

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