

cations present in normal feces, except as noted above.

Summary. Rats receiving sulfonic acid resin respond by increasing their urinary output of acid phosphate and ammonia, but not in amounts sufficient to compensate for their fecal alkali loss. Those receiving moderate amounts of potassium resin show little change in acid-base balance, while those on high potassium resin intakes show a partially compensated alkali retention. The net fecal alkali loss amounts to 2.4 meq per g resin fed, but a large part of this represents increased phosphate absorption, presumably due to the binding of calcium and/or magnesium by the resin. There is suggestive evidence that some of the extra alkali appearing in the feces as a result of resin ingestion is not in direct combination with the resin, and this may have

some bearing on the problem of neutrality regulation.

1. McChesney, E. W., *Am. J. Physiol.*, 1952, v168, 44.
2. ———, *J. Lab. Clin. Med.*, 1951, v38, 199.
3. Irwin, L., Berger, E. Y., Rosenberg, B., and Jackenthal, R., *J. Clin. Invest.*, 1949, v28, 1403.
4. Greenman, L., Peters, J. H., Mateer, F. M., and Danowski, T. S., *J. Clin. Invest.*, 1951, v30, 1027.
5. Flanagan, T. L., Sax, M. F., and Heming, A. E., *J. Pharm. and Exp. Therap.*, 1951, v103, 215.
6. McChesney, E. W., *Fed. Proc.*, 1952, v11, 103.
7. McChesney, E. W., and McAuliff, J. P., *Am. J. Physiol.*, 1950, v160, 264.
8. McChesney, E. W., *J. Lab. Clin. Med.*, 1952, v39, (May).
9. Chen, J. S., and Freeman, S., *J. Lab. Clin. Med.*, 1950, v35, 99.
10. Gamble, J. L., *J. Biol. Chem.*, 1922, v51, 295.

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Protective Effect of Moisture on Denaturation of Human Serum Albumin By Heat (19436)

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It is well known that various proteins and living organisms are more resistant to heat when dry than when in the liquid state. However, certain materials when dried to a critical level (5-10% moisture) are more unstable than the same material in solution(1). While studying this interesting fact it was noted that dialyzed albumin, though denatured by heat when thoroughly dry, was protected when containing a certain amount of moisture.

This observation is published since it may have some bearing on the discordant results obtained in studies on viability of dried bacterial cultures.

Procedure. Twenty-five per cent human serum albumin containing 1/15,000 merthiolate was dialyzed for 24 hours at 4°-6°C against several changes of distilled water. This was made up to 5% solution with distilled water and 4.5 ml quantities distributed into a number of 20 ml ampoules. To one-third of these was added 0.5 ml of water, to another third 0.5 ml of M NaCl and to the last third

0.5 ml of 5 M NaCl. This material was then dried from the frozen state in the ampoules. They were then divided in 6 groups and placed in desiccators over sulfuric acid of the following specific gravities: 1.83, 1.73, 1.61, 1.53, 1.42, and 1.31. They remained for over a week in the desiccators at room temperature under a vacuum of 1-10 mm of Hg. During this time the moisture content of the albumin in each desiccator reached a predetermined level depending upon the concentration of the sulfuric acid(2). One ampoule of each preparation was then removed from the desiccators and the moisture content determined by the U.S.P. method. The remainder of the ampoules were then sealed under air. The sealed ampoules were then autoclaved at 250°C for 2 hours, opened and reconstituted with 5 ml of distilled water. A photoelectric colorimeter (Lumetron) was used to measure the turbidities using a green (530) filter. The results were plotted as percent transmission of a water control.

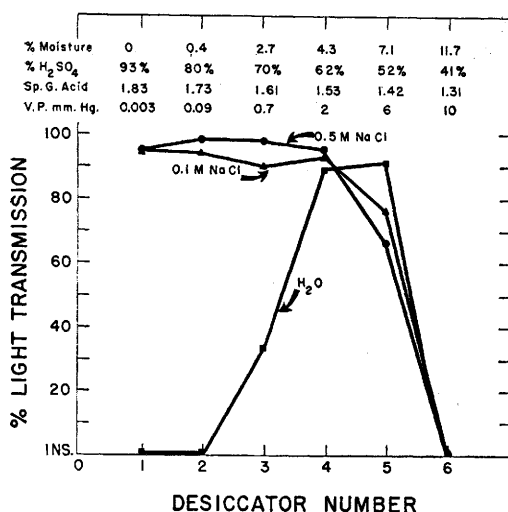


FIG. 1.

Results. Fig. 1 expresses the findings we obtained. The percent transmission roughly indicates denaturation, 100% indicating no denaturation and ins. indicating insoluble or nearly complete denaturation. The ampoules from the 6 desiccators are represented on the horizontal axis. The following additional information is recorded: Percent sulfuric acid by weight, specific gravity of the acid, water vapor pressure over the acid at room temperature, and moisture content of the albumin. The denaturation curves of the 0.1 M and 0.5 M NaCl albumin are similar to the curves which have previously been obtained for plasma and serum. These together with the albumin in water tend to become unstable at about 7% moisture and are quite unstable at 12% moisture. The albumin in water curve is most unusual in that it indicates that albumin is more stable when it contains 4% moisture than when it contains 0.4% moisture. We have found that essentially the same thing occurs when *S. marcescens* is dried in distilled water. Stability is greater when dried over 70% sulfuric acid than when dried over 93% acid.

Discussion. An explanation for the above findings is not apparent. However it is interesting to conjecture as to why this apparent anomaly should occur. It is possible that there are a number of points in the albumin molecule which have affinities for other mole-

cules or for themselves. It is of course well known that albumin does react by binding fatty acids, certain dyes, salts, etc. Normally electrolytes or other substances would be attached to the molecule at these points. This would tend to prevent the union of these points to each other or to similar points on other albumin molecules. Such a union might result in polymerization resulting in insolubility. It seems reasonable, in the absence of such electrolytes, that water molecules might take their place, thus preventing polymerization. Calculation of the number of molecules involved makes this assumption possible. Each

ampoule contains: $\frac{.214 \text{ g albumin}^*}{69000 (\text{Mol wt albumin})} =$

3×10^{-6} mols albumin. Each ampoule with 0.1 M NaCl contains 5×10^{-4} mols NaCl. In the absence of salt the number of mols of water to prevent denaturation is 6.5×10^{-4} . This compares well with the figure 5×10^{-4} indicating that possibly approximately the same number of molecules of either water or salt are needed to protect against denaturation. If we divided the number of mols of water needed by the number of mols of albumin we have the figure 210 which is the number of molecules of water per molecule of albumin. Brand(3) has shown that there are 550 molecules of amino acids derived from one molecule of albumin. It is not clear what relationship these figures have to each other. However it is possible that experiments along this line may be useful in the study of the structure of albumin and other substances.

Summary. Dried dialyzed albumin is denatured by heat. One tenth M NaCl or 4 to 7% moisture prevented coagulation under the conditions of the experiment.

* Actual wt. of albumin in the ampoule.

1. Florsdorf, Earl W., Hull, Lewis W., and Mudd, Stuart, *J. Immunol.*, 1945, v50, 21.
2. Hornibrook, J. W., *J. Lab. and Clin. Med.*, 1949, v34, 1315.
3. Brand, Erwin. Conference on Amino Acid Analysis of Proteins. New York Academy of Sciences, Section of Physics and Chemistry October 12-13, 1945.

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