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Stabilization of Serum Albumin to Heat, and Inactivation of the Hepatitis Virus

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In the blood plasma fractionation program, during the Second World War, which was centered at Harvard Medical School, under the direction of *Edwin J. Cohn*, the most urgently needed product was serum albumin for transfusion, especially for cases of shock [*Cohn*, 1948]. In the course of developing a standard procedure for producing purified albumin on a industrial scale many experimental variations in the fractionation techniques were tried out in the Harvard Pilot Plant. Naturally there was constant need for testing the purity and stability of the product. One criterion of quality was stability to heat. We knew that the albumin would be sent all over the world, including regions of intense heat such as the north African desert or the southwest Pacific. It had to remain undenatured by exposure to heat for months, indeed for years, if it was to serve its purpose. Hence one test imposed on each lot of albumin, before its release for clinical use, was a thermal stability test. Samples were held for at least 12 days at a temperature of 50 °C, and the light scattering before and after was measured in a nephelometer. Any significant rise in the nephelometric reading was sufficient ground for rejecting that preparation of albumin. It was found that raising the concentration of sodium chloride in the solution from 0.15 to 0.3 *M* significantly enhanced heat stability. The most favorable pH for stability was found to be 6.8 [*Scatchard* et al., 1944].

In cooperation with the Harvard Laboratories, Prof. *J. Murray Luck* and his associates at Stanford University undertook a systematic study of the factors affecting the thermal stability of albumin. From work of some investigators studying shock in animals experimentally, there were suggestions that the presence of some small organic anions (lactate and succinate) might be benefi-

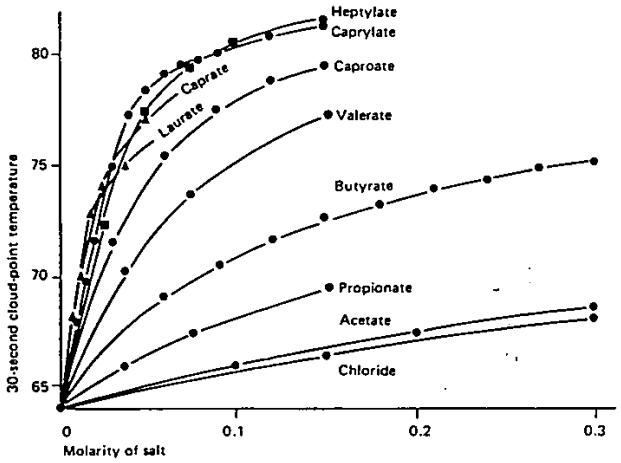

Fig. 1. The stabilization of serum albumin to heat by fatty acid salts [from *Boyer* et al., 1946].

cial. In fact, as part of the fractionation process, acetate buffers had been used for adjustment and stabilization of pH in the various steps. Generally the acetate had been removed during the final reworking of the albumin in the Harvard Pilot Plant, before clinical testing. However, the interest in organic anions led us to send Dr. *Luck* some albumin preparations from which the acetate had not been removed, and he soon found that thermal stability in the presence of acetate was higher than with an equivalent amount of chloride. In one of our weekly seminar discussions in Dr. *Cohn's* office I made the rather obvious suggestion that the next step should be to try propionate, to see whether the added CH_2 group would enhance stability. I believe that this was my only recorded contribution to the whole development [*Ballou* et al., 1944, p. 605; *Cohn*, 1948, p. 409, footnote 1].

The procedure developed in *Luck's* laboratory [*Ballou* et al., 1944] involved heating the albumin solutions in thin capillary tubes at a steady rate, and noting the temperature at which the solution became cloudy within 30 s (the '30-second cloud-point temperature'). With a standardized procedure these measurements were highly reproducible on any given preparation in a well-defined medium. The results, in the presence of a series of fatty acid anions, were dramatic (fig. 1). The effects of acetate were only slightly greater than those of chloride, but propionate had fully twice the stabilizing action of acetate, and the limiting slope of the curve for caprylate in figure 1 was some 20 times as great as for acetate. Similarly impressive results were obtained for alkyl sulfonates and for salts of various aromatic acids, such as phenylacetate and mandelate [*Ballou* et al., 1944; *Boyer* et al., 1946].

In discussing these impressive results with us, Prof. *Hans T. Clarke* of Columbia University suggested that the sodium salt of acetyl phenylalanine would be a physiologically appropriate stabilizing agent; and *Laurence E. Strong*, who was then directing the work of the Harvard Pilot Plant, suggested using the salts of the acetyl derivatives

of the amino acids in which albumin is deficient for nutrition (tryptophan and isoleucine). It proved that sodium acetyltryptophanate was a highly effective stabilizer, and its combination of stabilizing action, safety, and possible nutritional value made it the first such agent to be accepted for albumin solutions destined for clinical use. *George Scatchard* (Massachusetts Institute of Technology), who had worked closely for years with *Cohn*, in peace and war [*Edsall and Stockmayer*, 1980] pointed out that the stabilizing action of 0.04 *M* acetyltryptophanate was so great that it would permit a heat treatment of the albumin in the final container, which would eliminate the need for adding a bacteriostatic agent and could quite likely destroy the virus of serum hepatitis. In this medium albumin could be heated for 10 h at 60°C, and by nephelometric and other tests remained more stable than earlier albumin preparations that had not been subjected to heat. This also made possible the clinical use of albumin preparations of low sodium content if this were desired [*Scatchard* et al., 1945]. Such heat treatment of albumin that was destined for clinical uses soon became standard practice, and the evidence that the hepatitis virus was destroyed by the treatment soon became clear. Indeed, in a review of the history of albumin transfusion, a few years ago, it was concluded that no case of serum hepatitis had ever been traced to a transfusion with heat-treated albumin over a period of more than 35 years. [personal commun. from Dr. *James L. Tullis*, who took part in this meeting].

In basic research, these discoveries led to a flood of research on the binding of anionic substances to albumin, in the years after the war. It is a striking fact that serum albumin remains unique in its capacity for binding anions with attached nonpolar groups and for the resulting resistance to heat denaturation. No other protein, as far as I know, has been found to resemble it in this respect.

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