The effects of temperature on the response of human plasma kinin-forming system to promoting and inhibiting agents

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In native human plasma or serum there is a kinin-forming system which may be reversibly activated by cooling (37°–0° C). Its activities have been attributed to a disaggregation or unfolding on cooling, thus revealing the active sites on an enzyme molecule which is normally held together (inactive) by the operation of hydrophobic bonds. Following cooling, kinin appears and is destroyed and kininogen concentration decreases; nevertheless, the kinin-forming enzyme system does not show deterioration (Armstrong & Mills, 1965; Armstrong, Mills & Stewart, 1967). In high concentration, hexadimethrine bromide delays onset of this kinin formation and kininogen depletion (Armstrong & Mills, 1965; see also Fig. 1). Kinin formation which is induced by means of dilution, ε-amino caproic acid, or "ammonium sulphate precipitation" is sensitive to hexadimethrine bromide (10^{-8}–10^{-6} g/ml.), inhibiting it at 20° C (Armstrong & Stewart, 1962). Moreover, con-

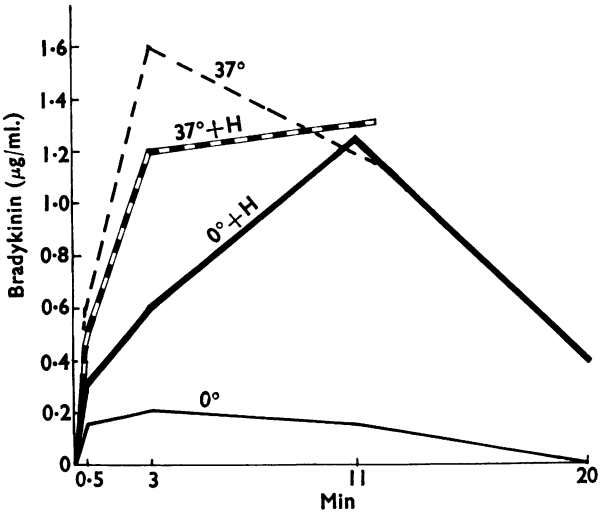

FIG. 1. Kinin, estimated in terms of synthetic bradykinin (Sandoz; rat uterus test), generated at 20° C by human saliva, 10%, added 50/50 to human plasma. 37° and 0° indicate temperature (° C) at which the plasma was stored for 23 hr before experiment. H=hexadimethrine bromide (1 mg/ml.) present throughout.

centrations of Hageman factor (5 μg/ml.) which produce kinin generation at 20° C are inhibited by hexadimethrine bromide (10^{-7}–10^{-6} g/ml.) at that temperature (see Ratnoff & Miles, 1962; Eisen, 1964). The efficacy of this inhibitor increases with rise in temperature and decreases with fall in temperature. Furthermore, the ability of Hageman factor to utilize kininogen decreases with rise in temperature and increases at lower temperatures. Thermal sensitivities to heparin, to ε-amino caproic acid, and the responses to acidification, and to heat (37°–50° C) also suggest that the plasma kinin-forming system may normally be held inactive by the operation of an inhibitor with the properties of the C'1 esterase inhibitor of Lepow and his associates.

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Changes of the intracellular water space produced by antibacterial drugs in relation to membrane permeability and membrane lesions

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Some conflicting results on membrane response to antibacterial action have led to a systematic investigation of methods commonly used to assess membrane damage. Measurement of protein leakage is of no value, at least in the case of *E. coli* K 12 and B (Rausa, Cannizzaro, Arena & Gebbia (1966); Rausa, Arena, Gebbia & Guardo (1967); Rausa, Gebbia, Guardo & Persico (1967); Rausa, Arena, Gebbia & Guardo (1967)). The CTR–strain of *E. coli* B is classified as cryptic to citrate: that is, it is classified as a strain impermeable to citrate, although possessing the enzymes required to oxidize it. We have, however, found that CTR + and CTR – strains take up equal quantities of ^{14}C-citrate, although the CTR – mutant has been said to utilize citrate only after membrane damage (Krampitz, 1961). Part of the label which is retained by the cryptic cells is recovered as $^{14}CO_2$ or in the various fractions of the extract, especially in protein suggesting that accumulation within the micro-organisms is partly due to incorporation.

Measurement of water space has also given conflicting results. Thus measurement of water space in *E. coli* K 12 gives different results according to the technique and type of tracer used: for instance, after centrifugation at 12,000 *g* the water space is 25–30% by $Na_2\,^{35}SO_4$, 60–70% by ^{14}C-urea, and 80–90% by ^3H_2O. The water space of a *Shigella sonnei* strain, of which the cells are normally unable to oxidize lactose, increases substantially after centrifugation at 200,000 *g* and the cells then oxidize lactose. In several mutants, labelled lactose crosses the membrane of "cryptic" cells to the same extent as that of ordinary ones, apparently by diffusion. This