Extrusion of Water in Antigen-Antibody Reactions

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By using thermodynamic data the conclusion was reached that the association of various antigens with their respective antibodies results in water extrusion (e.g. Karush, 1962). The question naturally arose as to what mechanisms may be involved in water extrusion, and it was suggested that hydrophobic associations could be involved (e.g. Kleczkowski, 1965). However, antigen-antibody reactions can be reversed by high salt content (Heidelberger, Pedersen & Tiselius, 1936) and by lowering of the pH to about 1-2 (Haurowitz, Tekman, Bilen & Schwerin, 1947), which activities are compatible with ionic reaction involvement rather than hydrophobic rearrangements; also, the rigidity of the antigen-antibody complexes is higher than would be expected from hydrophobic associations only. I have therefore re-examined the problem using correctly proportioned molecular models (Corey-Pauling-Koltun) and energetic principles, compatible with the entropy decrease involved in the formation of the antigen-antibody complex itself and the considerable entropy increase involved in the associated water extrusion. The conclusions are that the following systems are likely to be co-operatively involved.

(1) Formation of the guanidinium (arginine)-carboxylate (glutamate or aspartate) linkage. The formation of this linkage involves co-operative resonances of the positive charge on the guanidinium group and the negative charge on the carboxylate group. (Lewin, 1969a,b). Because the maximum neutralization of the charge involves in this case two-dimensional electric fields, water has to be extruded, and a very rigid linkage is formed (Lewin, 1970).

(2) Formation of ϵ -ammonium (lysine)-carboxylate (glutamate or aspartate) linkage. The formation of this linkage involves some water extrusion, but probably to a smaller extent than that of the arginine-carboxylate linkage; it also possesses a smaller degree of rigidity.

(3) Random coil (hydrated) $\rightarrow$ helix and water. A particular decrease of entropy in the formation of the antigen-antibody complex, compensated for by the entropy increase in water extrusion, can arise from the transformation of the hydrated random-coil form (which is bound to be water hydrogen-bonded by its peptide carbonyl and imino groups) into the α -helical structure, which is anhydrous. Such a position can be enhanced when the antigen and the antibody complementary-association sites

require the existence of rigid structures, such as α -helices. Indeed, optical-rotatory changes accompanying the formation of antigen-antibody complexes are compatible with the involvement of conformational changes noted (e.g. Henney, Stanworth & Gell, 1965; Henney & Stanworth, 1966).

The above linkage formation can be accompanied by hydrophobic-group associations between the antigen and the antibody; but this association involves smaller degrees of rigidity.

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Use of Electrofocusing for the Detection of Carrier Proteins and their Differentiation from Antibodies

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It is generally accepted that a number of biological activities utilize carrier proteins. For example, some immunological reactions require not only the presence of the antigen and the antibody but also that of a third entity, e.g. bovine serum albumin in the reaction of insulin with its antibody. Clinically this may be crucial since lack of the carrier proteins may exert an inhibitory action on the immune response. I have therefore examined a number of antigen-antibody reactions with a view to elucidation of the need for carrier proteins. The investigation included electrofocusing with Ampholines (LKB-Produkter AB, Stockholm, Sweden), pH range 3-10, which cover both serum albumins, which are generally considered to contain carrier proteins, and the antibody, γ -globulin, region. Established procedures (for references see Lewin, 1969 & 1970) and in particular pre-electrofocusing, which led to improved repeatable patterns and interpretations (S. Lewin, unpublished work), were used. The principle employed in the detection of carrier proteins was based on the comparison of electrofocusing patterns of human sera before and after the addition of a particular antigen (or hapten). Disappearance of original bands in the overall serum

albumin region after antigen addition was taken as evidence for the existence of carrier proteins; a smaller extent, or disappearance, of bands in the γ -globulin region accompanied by the formation of new bands elsewhere was considered evidence for antibody reactivity.

These principles were applied to insulin reactivity with human sera. Insulin addition to the sera of diabetic and normal patients resulted in a differential. In the former both considerable weakening of the bands in the overall albumin region and decrease in their number in the presence of γ -globulins, as well as the development of new bands, were displayed; in normal sera only considerable weakening of the bands in the overall albumin region was noted, but no weakening of the γ -globulin bands was observed. These findings are compatible with the existence of insulin carrier proteins in both normal and diabetic patients, and with the interpretation that some diabetic patients possess insulin antibodies.

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Countercurrent Studies of Porcine Liver Porphyrins

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During investigations of the ether-soluble porphyrin of various tissues (Smith, Belcher & Mahler, 1969) a group of porphyrins was found in extracts from fresh pig liver that differed from those present in bile, blood, meconium and bone marrow. Examination of countercurrent fractions derived from these extracts showed the presence of coproporphyrin and protoporphyrin together with three porphyrin fractions with Soret maxima at 420, 415 and 423nm in 5% HCl (described below as the 'S420', 'S415' and 'S423' porphyrins).

Six livers from pigs killed at the abattoir and three livers from pigs that had been starved for 2 days and then anaesthetized were examined. The 'S415' and 'S420' fractions were found in seven of the livers. In two of the abattoir livers, which were not fresh, only small amounts of the 'S420' fraction was found together with 2-carboxyl and 3-carboxyl porphyrins of the type found in bile (Smith *et al.*

1969). The three livers from starved anaesthetized pigs and one of the abattoir pig livers showed all three atypical fractions. The countercurrent tube positions of the fractions with the system described by French & Thonger (1966) were as follows: 'S420', tubes 19–32; 'S415', tubes 34–48; 'S423', tubes 69–81.

These porphyrins, which were characterized by a 'smoky' red-brown fluorescence, were stable for at least 56h in 5% HCl, and exhibited sharp Soret maxima typical of porphyrins. Sufficient 'S415' and 'S420' material was collected to demonstrate that these porphyrin fractions had four-banded aetioporphyria-type spectra in visible light.

The 'S415' and 'S420' fractions both separated into two bands, occupying 2-carboxyl and 3-carboxyl positions on lutidine chromatograms, the separated bands having the same Soret wavelength as the parent fraction.

The 'S423' fraction ran as a single band in the 2-carboxyl position.

Preliminary experiments indicate that the 'S415', 'S420' and 'S423' fractions are not protein-bound conjugates and that it is not possible to incorporate zinc into the nucleus.

It was also noted that the coproporphyrin/protoporphyrin ratio in the extracts from the abattoir pig livers was always less than 1, whereas the extracts from starved anaesthetized pigs gave ratios greater than 1.

The 'S420' fraction was also found in a human liver removed at autopsy from a road-accident casualty.

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Two Techniques for the Study of Hepatic Membrane-Containing Fractions

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For membrane fragments as found in liver homogenates, it would be useful to have techniques for separating plasma-membrane vesicles from endoplasmic-reticulum vesicles and for ascertaining the location of marker enzymes within a population of vesicles. Exploratory work is now reported.

Earlier work on the zonal centrifugation of postlysosomal supernatant (El-Aaser *et al.* 1966; Hinton *et al.* 1967) achieved a partial separation of 5'-nucleotidase (plasma-membrane marker) from