

bines with water, which it does very readily at 37° or above, it gives not asparagine only but *both* asparagine and *iso*asparagine. While we are grateful to Drs. Sondheimer and Holley for furnishing evidence of the kind which we ourselves would have been glad to obtain, there still remain some features of the asparagine-glutamine problem that require explanation.

Asparagine and glutamine behave differently toward ninhydrin and they also behave differently toward nitrous acid in acetic acid solution. Asparagine reacts toward ninhydrin in two distinct ways. In solution in the cold or on paper at 60° C., it reacts with ninhydrin without being decarboxylated to form a brown compound. This reaction is typical neither of the  $\alpha$ -amino-acids nor of glutamine. (In respect to the brown ninhydrin colour, asparagine resembles amino-succinimide more than glutamine.) In hot solutions, however, asparagine, like glutamine, may be made to react with ninhydrin with decarboxylation to give a blue colour and in a manner which is typical of most  $\alpha$ -amino-acids. In the reaction with nitrous acid, the amide group of asparagine does not react, whereas that of glutamine does.

These anomalies and interesting physiological differences between glutamine and asparagine still require to be fully explained—even though, from the work of Sondheimer and Holley, one cannot now regard L-asparagine as a simple hydrate of L-amino-succinimide.

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<sup>1</sup> Steward, F. C., and Thompson, J. F., *Nature*, **169**, 739 (1952).

<sup>2</sup> Steward, F. C., and Thompson, J. F., *Nature*, **171**, 1063 (1953).

<sup>3</sup> Sondheimer, E., and Holley, R. W., *J. Amer. Chem. Soc.* (in the press).

### Hydrogen Bonding and the Volume of Water

WATER behaves approximately as a normal liquid with respect to its volume at a high temperature and pressure. The expected volume of the liquid at room temperature is obtained by extrapolation, for example, using the formula of Jacobson and Heedman<sup>1</sup>. The volume thus deduced corresponds to a state without hydrogen bonding (at 0° C. about 8 per cent denser than ordinary water). Each hydrogen bond formed at a given temperature ( $t < 174^\circ \text{C.}$ ) is now assumed to affect the ideal liquid in the same way, raising the molecular volume to that of ice when two moles of hydrogen bonds are established. (Ice is supposed to exhibit the same thermal expansion above 0° C. as immediately below.)

Introducing the observed volume of water, these assumptions render possible the determination of the molecular fraction of hydrogen bonds,  $X$ , as a function of temperature. At 0° C.,  $X$  is calculated to be 0.44, decreasing to 0.23 at 100° C. When no hydrogen bond is broken, the number being two per molecule,  $X$  is one.

Following Finbak<sup>2</sup>, the law of mass action is applied to the formation of hydrogen bonds. The breaking of a bond gives two sites for bond formation. Thus a graph of  $2 \ln(1 - X) - \ln X$  against  $1/T$  was investigated. An almost straight line was obtained, corresponding approximately to an enthalpy change of 2.6 k.cal./mole water and an entropy change of 8.9 cal./mole.

A hydrogen-bond energy of 1.3 k.cal./mole seems quite low, compared with the current estimates for water. There has been no general agreement, however, on the energy of the hydrogen bond. This is probably due to difficulties in defining and sharing out the different energies. The present investigation indicates that 2.6 k.cal./mole is responsible for the tendency towards tetrahedral configuration in water. This is ascribed to the hydrogen bond. The van der Waals's energy superimposed gives only a very loose orientation effect, though it is probably partly included in most hydrogen-bond energy determinations.

A detailed discussion will appear later elsewhere.

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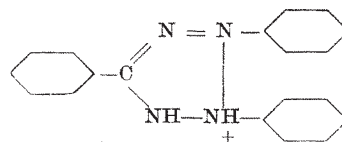
<sup>1</sup> Jacobson, B., and Heedman, P. A., *Acta Chem. Scand.*, **7**, 705 (1953).

<sup>2</sup> Finbak, Chr., *Norske Vidensk. Akad.*, Avh. I. Matem.-Naturv. Kl. No. 10 (1945).

### Reduction of Tetrazolium Salt

SOME two hundred papers dealing with the chemistry of triphenyltetrazolium chloride and its use as a redox-indicator have been reviewed by Ried<sup>1</sup> and Smith<sup>2</sup>. In spite of its wide use in biology, up to the present little is known about its reducing mechanism, the influence of experimental conditions on it, on its redox-potential, etc., and therefore the results obtained with it are empirical in character. Investigations of the salt by means of polarography and spectrophotometry carried out in this laboratory revealed some aspects which should be considered when using it as a chemical indicator of reducing power in biological reactions.

Polarographic study has shown that, in media of pH lower than 6, the reduction of triphenyltetrazolium chloride yields chiefly a colourless product:



In more alkaline solutions the reduction product is mainly the red formazan. This behaviour is due to the change of redox-potentials in the reduction of nitrogen atoms 1 and 2, and atoms 2 and 3 respectively (Fig. 1), and explains why reddening can be achieved only in solutions which are not too acid. The redox-

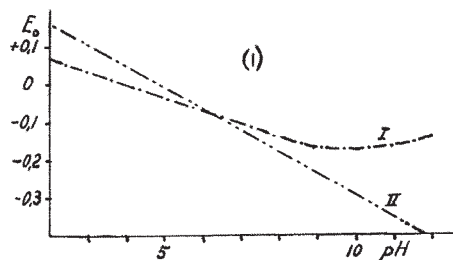


Fig. 1. Redox-potentials of triphenyltetrazolium chloride. Reduction of nitrogen atoms 2 and 3 (curve I) and of atoms 1 and 2 (curve II)