THERE is a difficulty which can occur regarding the interpretation of heats derived from Clapeyron's equation applied to phase equilibria involving clathrates. This difficulty is not thermodynamic in origin but arises from the non-stoicheiometry of the phases. Before this non-stoicheiometry was realized, heats evaluated using Clapeyron's equation were considered to refer to fully stoicheiometric reactions, for example:

$$CHCl_3 + 17H_2O \rightarrow CHCl_3.17H_2O \quad (1)$$

the values 1 and 17 here being regarded as fixed integers. However, equation 1 should be formulated as:

$$xCHCl_3 + 17H_2O \rightarrow xCHCl_3.17H_2O \quad (2)$$

where x is less than 1, has not a fixed value but may vary with P and T, was unknown to earlier experimenters and indeed in this and many analogous systems is still unknown. x could vary from one experimenter to another according to the physical conditions employed. The heats derived have therefore referred to uncompleted reaction equations and so have an uncertain meaning[1], until x is determined at the same time as the heat of reaction. Fortunately, x in many cases does not differ greatly from an integer, so that uncertainties resulting from non-stoicheiometry are not large.

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BIOCHEMISTRY, PHYSIOLOGY and MEDICAL SCIENCES

Glucuronide and Sulphate Conjugation *in vivo* and *in vitro*

EARLY studies[1] of the metabolism of the polycyclic hydrocarbon 3 : 4 benzypyrene showed that this compound was converted within mouse and rat tissues to one or other of two water-soluble derivatives. These were characterized physically, but not chemically identified, and for convenience in later work were labelled as BpX_1 and BpX_2.

Later, Calcutt and Payne[2] showed that within mouse or rat liver—the major site of benzpyrene metabolism—BpX_1 was formed only in association with the heavy components of the soluble proteins representing the supernatant fraction of cell homogenates. On the other hand, BpX_2 was formed in the nuclei, mitochondria, microsomes and lighter components of the supernatant. The major site of formation of this derivative was the microsomes[3].

Harper[4] has now obtained evidence that BpX_1 is a mixture of the sulphuric acid conjugates of fully aromatic benzpyrenols, while BpX_2 is a mixture of the glucuronic acid conjugates of the same phenols.

It now becomes possible to correlate the above results concerning the primary metabolism of benzpyrene with other information in the literature dealing with *in vitro* systems of conjugation. It has been shown by Bernstein and McGilvery[5] and De Meio *et al.*[6] that sulphate conjugation *in vitro* is based upon an enzyme system present in the high-speed fraction of the supernatant from liver homogenate. Activity is dependent upon the presence of adenosine triphosphate and magnesium ions. The identification of the benzpyrene X_1 derivative as a mixture of sulphuric acid esters now gives an example of correspondence between *in vivo* and *in vitro* conjugation systems. In both cases sulphate conjugation has been found to be associated with the same cellular sub-fraction.

In the case of glucuronide synthesis *in vitro* activity has been found in the microsome fraction of liver homogenate[7,8] and to a lesser extent in nuclei and mitochondria[9]. With the recent proof that benzpyrene X_2 is a mixture of glucuronic acid conjugates we again have an exact parallel between *in vivo* and *in vitro* behaviour. In this case the parallel is not only with regard to the cellular sub-fractions concerned but also with the relative amounts of conjugate formed.

So far as we are aware this evidence, based on work with 3 : 4 benzpyrene, is the first example of known *in vitro* systems for glucuronic and sulphuric acid conjugation proving to be an accurate reflexion of behaviour in whole tissues under *in vivo* conditions. Furthermore, it is also apparent that the processes of homogenization and ultracentrifugation involved in the extraction of these enzyme systems for *in vitro* use do not result in the loss or redistribution of these systems.

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Conjugated Forms of Urobilinoid Pigments

TAKASHI NORO[1] reported a fraction of urinary urobilinoids not extractable with chloroform but extractable by saponification with potash; this fraction he assumed to be in the ester form of the urobilinoids. We have found that some of the urinary urobilinoids not extractable with chloroform are characterized by their adherence to the gel-like precipitate formed during the extraction. Shaking normal urines, or preferably those from cases of hæmolytic jaundice, with Sevag's chloroform–amyl alcohol (9 : 1) mixture[2] results in partition of their urobilinoid contents. After centrifugation, part of the urobilinoids are found bound to the precipitate which collects at the interface and are extractable from it with ethyl acetate but not with chloroform, while the other part passes into the chloroform – amyl alcohol phase.

The paper chromatographic behaviour of these fractions was different. (Solvent: chloroform saturated with water–butanol–pyridine saturated with water (2·2 : 1·8 : 1); the urobilinoids were visualized by their green fluorescence in ultra-violet light after spraying with Schlesinger reagent.) The fraction

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