

ately to 451 m $\mu$  (curve 4—solution non-fluorescent), and on subsequent addition of a drop of hydrochloric acid it reverted to 430 m $\mu$ . In this case the spectral shifts were barely detectable visually.

Since only acid-reversible reaction of the zinc ions with the rubins could be responsible for the foregoing spectral shifts (control experiments having shown that the acetate ions were not), this evidence indicates that bilirubin and mesobilirubin form non-fluorescent zinc complexes which, in common with the zinc complexes of other bile pigments, are readily decomposed by dilute acid, the original pigments being released.

The zinc complexes of many bile pigments are oxidized rapidly on addition of a few drops of a solution of iodine in chloroform, whereas the free pigments are oxidized very much more slowly. Iodine did not oxidize free bilirubin or mesobilirubin rapidly in chloroform-methanol solution, but on addition of a drop of zinc acetate in methanol, oxidation by iodine took place rapidly (giving mixtures of purpurins and choletelins). However, when zinc complex formation was prevented by addition of a drop of acid with the zinc acetate, rapid oxidation by iodine did not take place.

This additional evidence seems to support the conclusion that the rubins form non-fluorescent complexes with zinc ions. The structures of these complexes are being investigated.

I thank Dr. C. Ó hEocha for his advice and for providing facilities for this work, which was supported by the U.S. Air Force, through its European Office (contract No. AF 61(052)-409). I also thank Prof. C. H. Gray and Dr. D. E. Nicholson for a sample of mesobilirubin.

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### Acetone Isotherms

Casado, Massie and Whytlaw-Gray<sup>1</sup> measured the density of five organic vapours at 22° C with a quartz-fibre microbalance and obtained results which permit the calculation of second virial coefficients. However, with acetone, the data imply a contribution over the experimental pressure range (26–136 mm) of a positive third virial coefficient sufficiently large to cause a two-fold change in the slope of the  $PV/P$  line, a finding quite at variance with Lambert's<sup>2</sup> linear isotherms. Work on acetone by Pennington and Kobe<sup>3</sup> and Zaalishvilli and Kolysko<sup>4</sup> was conducted by methods which do not provide direct evidence on the  $PV/P$  curvature. Recent  $PVT$  determinations at sub-atmospheric pressure for other organic compounds boiling near 50° C indicate that any supposed curvature is scarcely distinguishable from observational errors.

To clarify the position we have recently examined acetone using the differential compressibility apparatus described previously<sup>5</sup> and offer a comparison with previous determinations (Table 1).

Table 1. SECOND VIRIAL COEFFICIENTS, CM<sup>3</sup>/MOLE

	Present work	Lambert	Zaalishvilli	Pennington
50° C	-1,439	-1,560	-1,535	-1,480
22° C	-2,111	-1,960	—	-2,166

We have used  $PV = nRT(1 + nB/V)$ ; Lambert and Zaalishvilli used  $PV = nRT + nBP$ ; the final column is the value interpolated from the Stockmayer parameters given by Pennington and Kobe.

Our technique, which permits measurement of the several  $PV$  products in one expansion to 1 in 10<sup>5</sup> shows that, as with other organic vapours similarly examined if there is any significant departure from linearity after the slight corrections applied for adsorption on mercury and glass surfaces, it is less than 2 or 3 parts in 10<sup>5</sup> for the pressure ranges examined (100 mm at 22° C, 200 mm at 50° C), at least ten-fold less than claimed previously, and favours a negative third virial. Whytlaw-Gray's acetone results by the microbalance method, which is particularly sensitive to the purity of materials, decomposition of the acetone, and accidental contamination of the balance parts by minute particles of grease, we believe to be in error.

To obtain further information about the general curvature of isotherms at reduced temperatures below 0.80, where the Stockmayer intermolecular potential indicates that the third virial coefficient becomes negative, it will be necessary to increase the available precision at least one order of magnitude. In a very limited number of high-precision experiments,  $T_r = 0.5-0.7$ , recorded in the literature there is just an indication of such a curvature.

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### Dissociation of Proteins into Sub-units by Succinylation: Hæmerythrin

In the course of investigation of the molecular weight of the marine oxygen-carrying pigment hæmerythrin (obtained from *Golfingia gouldii*), indications arose that the macromolecule of about 105,000 molecular weight might be constituted of several smaller units. A few sedimentation experiments in solutions of sodium dodecyl sulphate, following a suggestion of Schachman<sup>1</sup>, did reveal sub-units, but complete dissociation could not be obtained and the presence of detergent created a number of difficulties in experimental procedure. Since the effectiveness of the detergent in splitting the macromolecule probably depends in part on the electrostatic repulsion produced by the large negative charge of the bound ions, it seemed worth while to examine the effect of the introduction of additional negative charges covalently linked to the macromolecule. Reaction of protein with succinic anhydride<sup>2,3</sup> should replace each cationic  $\epsilon\text{-NH}_3^+$  charge by an anionic carboxylate group. Since hæmerythrin