magnetization decreases as we decrease the ratio $T_{cA}/T_{cB}$ and near the triple point in the P-T phase diagram the B transition becomes of second order, again being consistent with the experimental findings[6]. The NMR frequency shift $\nu^2_{liq}(T, H) - \nu^2_{sol}(T, H)$ was found[1] to be proportional to $(1 - T/T_{cA})$ and also independent of H in the phase-A. For zero field case this would imply $\nu^2_{liq}(T, H = 0) \propto (1 - T/T_{cA})$ in the phase-A. The shift vanishes abruptly at B transition. It is found that the solution of eq. (5), for the above-mentioned values of the parameters. gives a linear behaviour of $M_s^2(T)$ in the phase-A (H=0) and an abrupt vanishing at B, confirming the experimental facts. The other experimental observations like the specific heat jumps at A transition and the constancy of the transverse magnetic susceptibility $[\chi_\perp(T)]$ in phase-A and then an abrupt fall in $\chi_\perp(T)$ at B[1] also follow from our theory. The specific heat jump is found to be proportional to D. Adding a transverse field term to the Hamiltonian and ignoring commutators involving the total spins, we get $\chi_\perp(T) \simeq 2/D$, a constant in the phase-A, and at the B transition a discontinuous drop given by,

$$\frac{\chi_\perp(T \to T^-_{cB})}{\chi_\perp(T \to T^+_{cB})} \simeq \frac{D}{k_B T_{cB}\left[\frac{1}{g}\exp(\Delta/k_B T_{cB}) + 3\right]} \simeq \cdot 25. \quad (6)$$

In conclusion, we have been able to explain the anomalous nature of the two phases in liquid $^3$He below 3 mdeg K on the basis of an effective spin Hamiltonian with semiphenomenological parameters. Detailed calculations will be published elsewhere.

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## CONCENTRATION OF JAPANESE ENCEPHALITIS (JBE) VIRAL ANTIGENS PREPARED FROM VERO CELL CULTURE BY SUCROSE DIALYSIS

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ABSTRACT

Concentration by sucrose dialysis of HA and CF antigens of JBE virus prepared from Vero cell culture is described. This method is rapid, reliable, economical and does not require any special equipment or material. Antigens thus concentrated can be employed in serological tests.

### INTRODUCTION

THE production of high titred arboviral antigens from the infected suckling mouse brains is a procedure routinely employed in most of the laboratories working on arboviruses. Clarke and Casal's[1] sucrose acetone (SA) extraction method is most suitable for the preparation of antigens for haemagglutination-inhibition (HI) as well as complement fixation (CF) tests. However, the procedure is rather laborious, potentially hazardous and requires voluminous acetone. The tissue culture system for the production of arboviral antigens is more convenient and less hazardous, but usually the titres of antigens are comparatively poor[2-6]. Several procedures are employed for concentrating viral antigens[4,6-20]. Concentration by sucrose dialysis of HI and CF antigens prepared from Vero cell culture is described in this communication. The Japanese encephalitis virus, a Togavirus, (Andrewes)[21] is employed in the study.

### MATERIAL AND METHODS

Sucrose is commonly used for preparing density gradient. It is hygroscopic and highly soluble in water and has little effect on viruses. Infected tissue culture fluids (ITCF) harvested on different days were dialysed against solid sucrose or saturated sucrose solution and the concentrated antigens were tested for haemagglutination (HA), HI and CF tests to find out the potentialities of antigens for use in the routine diagnostic serological tests.

Growth and maintenance of Vero culture, methods for inoculation of virus JBE P3 (P 20778) and harvesting ITCF are described separately (Rai, J. *et al.*, in preparation).

*Concentration of Antigens by Dialysis against Sucrose.*—The antigen to be concentrated was placed in a dialysis bag. Both ends of the bag were tied and the bag was kept in a beaker or a measuring cylinder. Solid sucrose covered the dialysis bag all around and the container was kept at room

temperature or at 4° C. After 8 to 24 hours the antigen was collected from the dialysis bag and processed further to evaluate its potentialities for routine serological tests. The proportion of solid sucrose to fluid containing antigen is not critical, but should cover completely the dialysis bag in the container. Whenever saturated sucrose solution was used, it was kept in a bottle or beaker containing a magnet and the dialysis bag was immersed in the solution which was stirred with magnet. The proportion of saturated sucrose solution was ten times more by volume to fluid containing antigen in the dialysis bag.

An alternative procedure to the above method was also tried and found to be satisfactory. LKB filter frame was inserted into an inflated dialysis bag and solid sucrose was placed in it. The dialysis bag was then immersed in a beaker containing antigen and kept for concentration. After 24 hours antigen concentrate was collected from the beaker.

The concentrated tissue culture antigen was compared with SA extracted mouse brain antigen in HI and CF tests employing hyperimmune mouse sera/peritoneal fluid against JBE, West Nile, dengue-1, dengue-2, dengue-3 and dengue-4 viruses. A similar comparative study was carried out with limited number of convalescent human sera/survey sera collected from different epidemics and surveys.

## Results and Discussion

About 10 fold increase of HA and CF titres of the antigen was achieved by dialysing the antigens against sucrose (Table I). The results of comparative study with the two antigens (SA extracted mouse brain antigen and Vero tissue culture sucrose concentrated antigen) showed no significant difference in the HI and CF titres of the convalescent and immune sera (VRC unpublished data, 1974). Therefore, it is suggested that the sucrose concentrated JBE antigens can be used in serological tests.

Table I

*HA and CF titres of JBE antigens before and after concentration**

| Antigen lot number | HA titres at pH 6·4–6·6 | | CF titres† | |
|---|---|---|---|---|
| | Before concentration | After concentration | Before concentration | After concentration |
| 1 | 40 | 480 | 16 | 128 |
| 2 | 80 | 960 | ND | ND |
| 3 | ND | ND | 4 | 64 |

* About ten-fold concentration in volume obtained.
† The highest dilution of antigen which fixed 2·5 units of compliment in the presence of hyperimmune ascitic fluid. ND=Not done.

One of the applications of dialysis method is the concentration of solutions of macromolecules. Virus concentration is carried out by utilising hygroscopic macromolecules surrounding the dialysis bag. Polyethylene Glycol (PEG) has been used successfully for the concentration of virus and viral antigens including Togaviruses (Klemperer and Pereira ; Della-Porta and Westaway).[13,6] Viral antigens have also been concentrated by dialysis method using Carboxymethyl Cellulose (Versteeg)[19].

It is relatively easy to produce large volumes of infected tissue culture fluid for many arboviruses, and if HA and CF antigens are present in low titre the method described by us will provide a valuable supplement to the more commonly used antigens prepared from suckling mice. The method is rapid, reliable, economical and no special equipment or expensive material is required. Under the experimental condition described above, about ten-fold concentration was achieved in 8–24 hours. No denaturation effects were observed in spite of sucrose being present in the antigen concentrate. After dehydrating the sucrose, the same material could be reused repeatedly and was found to be satisfactory. Saturated sucrose solution could also be reused. Comparative studies on different methods of concentration of virus antigens are in progress to evaluate the merits of the technique described in this communication.

## Conclusion

Sucrose dialysis can be employed to obtain high titred JBE, HI and CF antigens prepared from Vero culture.

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## A MOLECULAR ORBITAL TREATMENT OF SOME HALOGEN SUBSTITUTED AMIDES

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ABSTRACT

A Huckel molecular orbital treatment of some halogen substituted formamide, acetamide and benzamide series has been carried out to study the effect of substitutions (Cl, Br, I) on the charge delocalization in these molecules. The net charges on carbon, oxygen, nitrogen and halogen atoms and the bond orders of the C = O and C — N bonds have been calculated and the results are discussed. The barrier heights for the internal rotation about the C — N bond and resonance energies are also calculated.

### INTRODUCTION

IT is well known that in amide molecules, no single valence bond structure is consistent with all their properties. This is due to the delocalization of the carbonyl π-electrons and lone pair electrons of nitrogen, resulting in the double bond character of the C — N bond[1-5]. Infrared, Raman and NMR Spectroscopic studies of halogen substituted amides have been carried out by Petterson[6], Laches[7] and recently by Deklein[8,9] and Devia[10]. In the present work, the authors have attempted to make a systematic study of electronic charges on O, C, N and halogen atoms and bond orders of C = O and C — N groups to study the charge delocalization due to different substitutions.

### METHOD OF CALCULATION, RESULTS AND DISCUSSION

The Huckel molecular secular determinant of a representative secondary amide is as shown in Fig. 1. Where the $\alpha$ and $\beta$ are the coulomb and resonance integrals respectively of the atoms O, C, N and the substituent to $R_1$ and X.

The secular determinant was solved using a CDC 3600 computer. The solution of the determinants provides the eigen values and eigen functions of the energy levels. Treating this as a six electron problem, the three lowest levels are taken as filled.

The mobile bond orders and the π-electronic charge defined in the usual way were determined. The net charge was determined as the difference between the charge the atoms would have in the absence of delocalization and its actual calculated electronic charge. A positive sign denotes a deficit and a negative sign an excess of electronic charge. T. Yanezawa *et al.*[11] parameters are used in the calculations.

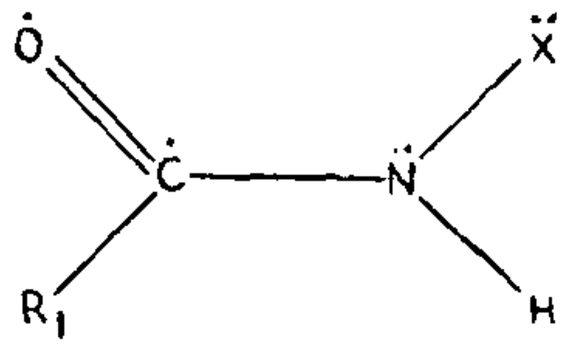


FIG. 1

The results are tabulated in Tables I and II. As the aim of the present investigation is to study the relative delocalization of the charge due to the different halogen substitutions, the HMO method is used. This method is well suited for comparative study and the results depend upon the choice of the parameters of the coulomb and the resonance integrals,