nance of a high level of JH, which is a consequence of virus multiplication. The high JH titre in the diseased larvae could be ascribed to a) the CA remaining active during the course of polyhedrosis as indicated by the ultrastructure (unpublished observation) and b) the fact that as the fat body is one of the major sites of multiplication of virus leading to its degradation, it is likely that the lipid soluble JH would not have been stored in large quantities but have remained in an active state in the haemolymph. Apart from this, a low activity of the haemolymph enzymes that degrade JH and the inefficient excretion of JH through Malpighian tubules might be the other possibilities.

Prothetely due to nuclear polyhedrosis virus (NPV) infection in *Lambdina fiscellaria somniaria* and *Orgyia pseudotsugata*[10], and metathetely due to entomopox virus infection in *Choristoneura biennis*[11], were observed earlier, In the case of prothetely it was suggested that virus multiplication might have inhibited synthesis/release of JH, whereas in the case of metathetely, the effects were similar to those caused by excess JH. The reduction in JH titre to a very low level, which is essential for pupation, fails to occur due to NPV infection; this eventually prevents pupation in *S. litura*. This report presents direct evidence of a hormonal imbalance which occurs due to NPV infection.

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PRO EXPERIMENTIS

A colloidal gold prepared with ultrasonics

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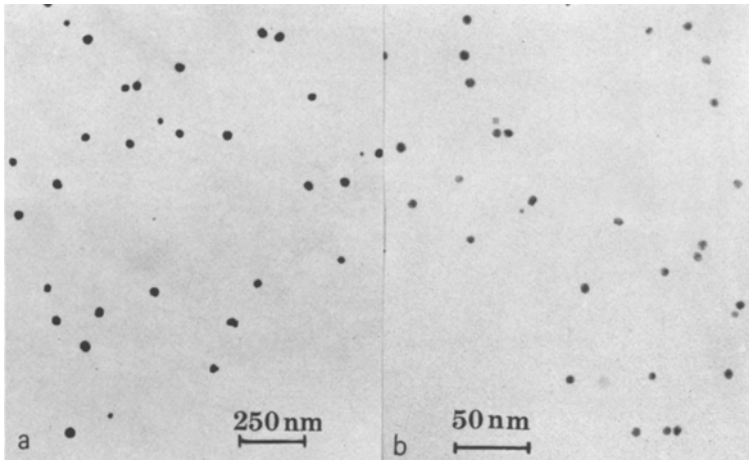
Summary. With the application of ultrasonic energy a colloidal gold may be easily prepared with a particle diameter of less than 10 nm and suitable for use as an immunological marker for TEM-studies. This new approach replaces the use of phosphorus, traditionally used for producing gold sols of the smallest diameter.

Traditionally, the most commonly used methods for the preparation of colloidal gold are based on the reduction of gold chloride with a variety of substances such as phosphorus[1,2], formaldehyde[3], ethyl alcohol[4], tannic acid[4] and, more recently, sodium citrate[5], usually in association with heat. Such reducing agents produce colloids varying considerably in particle size but only the use of a solution of phosphorus in ether[6] results in diameters averaging less than 10 nm, and suitable as an immunological marker for our studies at the ultrastructural level. It was therefore decided to investigate the possibility of making a similar gold sol using a less hazardous substance by substituting ultrasonics for the conventional energy source.

The basic technique chosen for investigation with ultrasonics was that of Oswald[4] in which ethyl alcohol is used as the reducing agent. A neutral solution of gold chloride was prepared as described in the legend. Half of the solution was heated prior to the addition of ethyl alcohol and the



Random electron micrographs of heat (a) and ultrasonically (b) induced gold colloids showing degree of dispersion. Preparation: 0.2 ml of a 1% $H(AuCl_4)$ solution was diluted to 100 ml and made neutral with 0.2 M K_2CO_3. 50 ml was heated to 75 °C and 0.5 ml ethyl alcohol was added. After approximately 5 min a reddish pink colour reached a maximum in intensity with an absorption at 540 nm (a). To the remaining 50 ml, 0.5 ml ethyl alcohol was added. Sonication was carried out at 20 kc and 125 W by immersing a flat ended probe approximately 1 cm under the surface. After 2 min a pinkish colour reached a maximum in intensity with an absorption at 520 nm (b). For electron microscopy the grids were dipped into a 0.1% aqueous solution of poly-L-lysin, washed twice in aqua dest. and after being submerged into the respective sols for approximately 2 min were again washed and finally dried on filter paper.

remainder subjected to ultrasonics after its addition, until the appearance of the respective colloid-associated colours. In order to assess the particle size and exact dispersion of the colloids poly-L-lysine treated[7] pioloform-F coated grids were submerged in the respective suspensions (see legend) and examined electronmicroscopically.

Initial observations indicated that the average particle diameter of the ultrasonically induced colloid was less than 10 nm whereas that of the heat preparation was considerably larger at around 25 nm. Both preparations were highly disperse. To quantify this observation a number of ultrasonic and heat induced sols were randomly photographed and the particle diameters measured on a projection screen. Arithmetical means and standard deviations were calculated for each preparation. An analysis of the data showed that the mean diameters of the ultrasonically induced colloids differed significantly (t-test, $p < 0.05$) within a range of 6–10 nm with an occasional average of less than 5 nm. Heat produced colloids also varied significantly in diameter within a range of 20–30 nm. Furthermore the standard deviations within each group differed significantly (F-test applied to the variances, $p < 0.05$) and coefficients of variation of 8–50% were found in both groups.

The sols were still disperse after several weeks without stabilization[8]. The ease of preparation, however, allowed fresh batches to be made whenever required. The colloidal gold adsorbed γ-globulins and protein A[9] readily, as demonstrated with sodium chloride flocculation[10], and therefore it is suitable as an immunological marker. A similar colloidal gold was successfully prepared using a laboratory ultrasonic cleaner.

Although the mechanism with which the ultrasonic energy induces the colloid is unclear, further limited observations showed that variations in pH of 5.0–8.5 as well as in alcohol concentration of 0.5–10% appeared not to influence the results obtained, contrary to experience obtained with conventional methods.

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Chemotaxis of human neutrophils against gravity: A new method[1]

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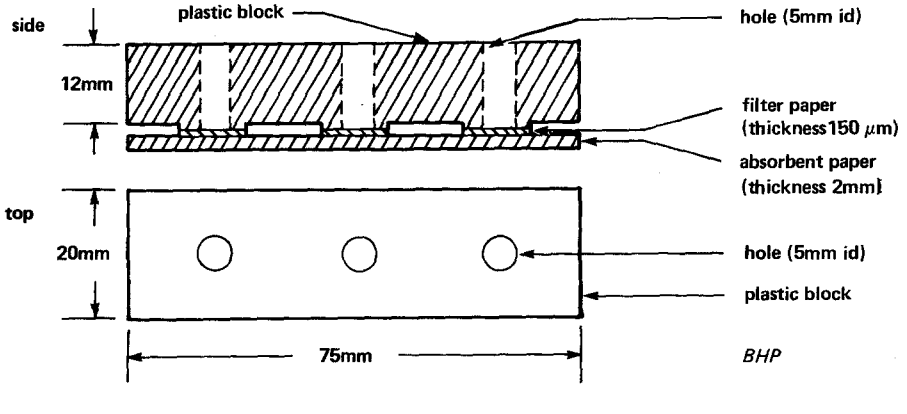
Summary. A new method is described in which human neutrophils were made to migrate upward and against gravity. Thus, the possible effect of gravity on cell migration and consequent detachment of cell after migration have been eliminated.

The Boyden Chamber method[2] is widely used for studies in chemotaxis, and is based on the assumption that the migrated cells would remain attached to the lower surface of the filter where they are counted. However, a variable proportion of migrated cells may detach from the lower surface[3]. Thus, the cell count at the lower surface of the filter may not always represent the actual number of cell migration. To overcome this inherent problem, I have devised a simple method in which the cells are made to migrate upward through the filter membrane, and thus the possibility of spontaneous detachment after migration is eliminated. 3 ml of fresh venous blood (heparin 20–40 units/ml) was centrifuged in a 5 ml plastic tube at 300 g for 10 min. The plasma, buffy coat and the upper layer of red blood cells were transferred into a new 5 ml plastic tube and mixed gently. The tube was placed upright in a test tube rack at room temperature and the red blood cells settled for 30–45 min. The leukocyte rich plasma layer was then transferred into a new plastic tube, the total cell as well as polymorphonuclear leukocytes (PMN) were counted by the



A diagram showing the apparatus used for leukocyte deposition.