buffer. A protein peak, which proved to contain mast cell-degranulating factors, was eluted at this molarity. Proteins comprizing the ascending part of the peak were pooled, concentrated by ultrafiltration and rechromatographed as before. The protein fraction, obtained after rechromatography, appeared to fulfill criteria of purity when tested on acrylamide gel electrophoresis.

Histamine assay. Mast cell suspensions were incubated with the specified concentrations of colchicine or vinblastine in a final volume of 1 ml for 60 min before exposure to compound 48/80 at 0.2 µg per ml or Ascaris degranulator at 1 mg in proteins per ml. After a further 10 min, the reaction was stopped by centrifugation (500 *g* for 10 min) and aliquots of the supernatants and boiled cell debris were analyzed for histamine content by a fluorometric method[17].

Drugs. Colchicine (Sigma), vinblastine sulfate (Eli Lilly) and compound 48/80 (Burroughs Wellcome) were dissolved in Tyrode solution immediately before use.

Results and discussion. The results in Table I and II show that preincubation of mast cells with colchicine or vinblastine for a suitable time interval inhibited the selective histamine release induced by compound 48/80 and by Ascaris degranulator. By contrast, both drugs did not affect the non-selective histamine release caused by decylamine. Compound 48/80 and Ascaris degranulator both release histamine from mast cells within seconds by an energy-dependent process, whereas decylamine is a surface active agent which disrupts mast cells by a non-enzymatic mechanism not involving the biochemical steps required for granule exocytosis[18].

The mechanism of histamine release induced by compound 48/80 has been investigated in detail[19,20]. Morphological evidence suggests that the first event which follows 48/80 interaction with cell membrane is a pore formation by fusion of the cell membrane with the perigranular membrane of adjacent granules. Exposure of histamine-containing granules to the extracellular fluid then results and histamine is released by a simple cation exchange between granule amine and extracellular sodium. On the basis of this mechanism, it seems justified to equate histamine release with degranulation and to conclude that colchicine and vinblastine affect the degranulation process, probably by interfering with microtubule function. This suggestion is supported by the electron microscopic demonstration of microtubules in mast cells[21] and by the findings[13,22] that colchicine and vinblastine bind to microtubule subunits which are in equilibrium with formed microtubules thereby preventing their formation.

Microtubules, in connection with microfilaments, may provide a framework which helps to support peripheral cytoplasmic structures and guide the movements of amine-containing granules towards the plasma membrane or, if PADAWER's suggestion[23] that granules are stored in folds external to the cell prove to be correct, microtubules may favour the opening of the fold and extrusion of the granule.

Riassunto. Il rilascio selettivo di istamina da mastociti peritoneali di ratto è significativamente ridotto in seguito a preincubazione delle cellule con colchicina o vinblastina. Poichè entrambi i farmaci agiscono alterando il grado di organizzazione degli elementi strutturali del citoplasma, primariamente i microtubuli, viene suggerito che tali elementi esplicano un ruolo preminente nel processo di exocitosi delle amine farmacologicamente attive.

G. TOLONE, L. BONASERA and N. PARRINELLO

Istituto di Patologia Generale ed Istituto di Zoologia dell'Università, Corso Tukory 211, I-90134 Palermo (Italia), 24 September 1973.

[17] P. A. SHORE, Meth. Biochem. Analysis suppl. (1971).
[18] B. HÖGBERG and B. UVNÄS, Acta physiol. scand. *48*, 133 (1960).
[19] I. L. THON and B. UVNÄS, Acta physiol. scand. *71*, 303 (1967).
[20] B. UVNÄS, in *Biochemistry of the Acute Allergic Reactions* (Blackwell, Oxford 1971).
[21] J. PADAWER, J. Cell Biol. *35*, 180 (1967).
[22] M. L. SHELANSKI and E. W. TAYLOR, J. Cell Biol. *38*, 304 (1968).
[23] J. PADAWER, J. Cell Biol. *40*, 747 (1969).

A Simple Procedure for the Preparation of Ehrlich Ascites Cells for the Induction of Immunity

Although Ehrlich ascites tumour (EAT) cells have been used extensively for biochemical and immunological studies, little is known regarding the nature of the antigens associated with the cell which are responsible for immunizing mice against a lethal challenge with living cells. As a result of immunizing mice with EAT cell heterokaryons, CHEN and WATKINS[1] concluded that the antigens responsible for protecting immunized mice from a subsequent lethal challenge with living EAT cells were not of the class of strong H-2 histocompatibility antigens. These authors attributed the protection to weak antigens of an unknown nature.

A variety of methods have been used to produce devitalized ascites cells suitable for immunizing mice[2-7]. These methods suffer from one or more of the following disadvantages. 1. The antigenic components might be damaged by the procedure e.g. X-irradiation or chemical treatment resulting in the denaturation of proteins; 2. the preparative procedures are involved and 3. the recovery of antigenic material is low. This report describes a simple, mild method for the production of devitalized EAT cells which will induce a high level of immunity in the host mice, with an excellent recovery of antigenic material.

EAT cells were grown routinely by serial passage in female CFLP mice (Carworth Europe) by the inoculation of 0.1 ml. of freshly drawn ascitic fluid (concentration 2×10^7 cells) i.p. The cells were harvested after 10 days. Hypotonic lysis of the cells was effected after washing the suspension twice in PBS (0.85% NaCl, 0.01 *M* phosphate buffer pH 7.4) and subsequently resuspending in 10 to 20 times the packed cell volume of 0.01 *M* phosphate buffer pH 7.4. After standing at 4 °C for 5 min the cells were sedimented by centrifugation at 1,000 *g* for 5 min at

[1] L. CHEN and J. F. WATKINS, Nature, Lond. *225*, 734 (1970).
[2] A. C. APFFEL, B. G. ARNASON and J. H. PETERS, Nature, Lond. *209*, 694 (1961).
[3] M. FURUSAWA, Expl. Cell Res. *47*, 63 (1968).
[4] M. GIAMBOI and A. MINUTOLI, Annali Sclavo *9*, 844 (1968).
[5] G. D'ALESSANDRO, G. GIAMMANCO and P. BRANCATO, Riv. Ist. sieroter. ital. *42*, 332 (1967).
[6] G. HAUGHTON, Ann. N. Y. Acad. Sci. *101*, 131 (1962).
[7] D. L. MCCOLLESTER, Cancer Res. *30*, 2832 (1970).

4 °C and the hypotonic treatment was repeated twice. The cells were finally washed once then resuspended in sterile PBS to a cell population density of 1×10^8 cells per ml. This preparation of cells will subseqeuntly be referred to as 'ghosts', since the method used was similar to that used by DODGE et al.[8] for the preparation of erythrocyte ghosts.

Female CFLP mice were immunized by i.p. injection of a suspension of 'ghosts'. A challenge with viable tumour cells was made by i.p. injection 10 days after the last immunizing dose of 'ghosts'. The challenged mice were examined daily and those animals with palpable ascites tumours were recorded and sacrificed. The experiments were terminated after 30 days. Protein was measured by the method of LOWRY et al.[9].

Over 80% of the original cell numbers were recovered as 'ghosts', of which up to 20% were still viable by dye exclusion[10]. A loss of 25% of the TCA precipitable protein was found to occur during the preparation of 'ghosts' but a 100% recovery of the plasma membrane marker enzyme – $(Na^+ + K^+)$ ATPase and 90% recovery of the antigenic material – measured with ^{125}I-labelled IgG from 'ghost'-immunized mice, was found on a per cell basis (preliminary results).

Despite the high viability of the 'ghosts', determined by dye exclusion, the incidence of tumour development after 2 inoculations with 1×10^6 'ghosts', at an interval of ten days, was less than 5%.

The incidence of tumours in female CFLP mice immunized with a single dose of lysed cells and challenged with 10^5 EAT cells

Immunizing dose No. lysed cells	Incidence of tumours at 30 days
0	14/15[a]
10^1	13/15
10^2	14/15
10^3	15/15
10^4	14/15
10^5	10/15
10^6	5/15
10^7	2/10

[a] Ratio of the number of mice with tumours at 30 days to the total number of mice in the group.

Two i.p. injections of 1×10^6 'ghosts' at an interval of 10 days completely protected mice against a challenge with 5×10^5 viable EAT cells. The combined washings, obtained during the preparation of 'ghosts', when administered i.p., failed to protect mice against a challenge with 5×10^5 viable EAT cells. These inoculations were administered in an amount equivalent, on a protein basis, to 10^6 'ghosts'; approximately 250 µg protein. It is apparent therefore that the immunizing antigens are associated with the membrane systems of the EAT cell.

Mice immunized with a single dose of 'ghosts' were not protected against a challenge with 1×10^5 EAT cells until the immunizing dose of 'ghosts' was equivalent to or greater than the challenge dose of cells (Table).

It has been suggested that the immunogenicity of weak antigens of membrane origin is imapired if the integrity of the membrane is destroyed[11–13]. The simple expedient of hypotonic lysis offers a rapid method for the recovery of the membrane components of the EAT cells in an apparently intact form and high yield. Such a preparation is most suitable for the production of sera against membrane bound antigens without recourse to the use of adjuvants. The protection of mice which such a preparation provided was better than[4,5] or equivalent to[14] that reported by others.

Résumé. On décrit quelques caractéristiques biochimiques et immunologiques des «ghosts» de cellules de la tumeur ascitique d'Ehrlich, produits par une méthode facile de lyse hypotonique. Ces «ghosts» ne forment pas de tumeurs quand on les injecte aux souris par voie i.p. et ils protègent celles-ci contre les cellules EAT viables.

P. ATKINSON and I. ab I. DAVIES

School of Pharmacy, City of Leicester Polytechnic, P.O. Box 143, Leicester LE1 9HB (England),
24 September 1973.

[8] J. T. DODGE, C. MITCHELL and D. J. HANAHAN, Archs Biochim. Biophys. *100*, 119 (1963).
[9] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[10] J. P. KALTENBACH, M. H. KALTENBACH and W. B. LYONS, Expl. Cell Res. *15*, 112 (1958).
[11] R. W. BALDWIN and D. GLAVES, Clin. exp. Immun. *11*, 51 (1972).
[12] R. W. BALDWIN and M. MOORE, Nature, Lond. *220*, 287 (1968).
[13] A. WOLF, Transplantation 7, 49 (1969).
[14] J. F. WATKINS and L. CHEN, Nature, Lond. *223*, 1018 (1969).

Immunofluorescent Studies in Isoproterenol-Induced Myocardial Necrosis in Rats

The presence of circulating antibodies against human heart has been reported in the post-myocardial infarction and post-pericardiotomy syndromes, in acute ischaemic heart disease and in a variety of myocardial diseases. Circulating antibodies have been demonstrated by haemagglutination, complement fixation, antiglobulin consumption and immunofluorescent techniques[1–4]. It was possible to produce circulating anti-heart antibodies in experimental animals immunized by xenogenic and allogenic heart extracts. Studies on rats kept at intermittently reduced barometric pressure demonstrated the appearence of circulating anti-heart antibodies[5].

RÓNA et al.[6] described that isoproterenol (IPR) given s.c. once daily for 2 days caused infarct-like myocardial necrosis in rats. WEXLER and KITTINGER[7] considered the pathogenesis and repair of IPR-induced myocardial necrosis as similar to infarct in man. We supposed that the IPR-induced necrosis might liberate antigenic substances and provoke immune reaction. The purpose of the present study was to demonstrate anti-heart antibodies after IPR injections by immunofluorescence.

Material and methods. Male Wistar rats with initial weights in the range of 180–200 g were used in 3 groups: 1. Animals were injected s.c. once daily for 2 days with 400 mg/kg IPR. 2. Animals were treated once daily for 7 days with i.p. injections of 5 mg/kg IPR. Sera from animals of group 1. and 2. were drawn weekly for 6 weeks for indirect immunofluorescence (sandwich technique). 3. Animals were treated as the first group, they were killed weekly, their hearts were examined by direct immunofluorescence. Control animals were treated with isotonic saline solution. Circulating antibodies were demonstrated