I refer to the situation where irradiation of a group of lymphomatous glands, with a single small dose, results in rapid clinical regression of other lymphomatous masses at distant sites in the body.

The possibility of an immune mechanism has frequently been suggested, but immunological proof of this has not been forthcoming for man. While accepting the dangers of converting *in vitro* results to living biological systems, the coincidence is too close to ignore.

Hardy and Ling also indicate that activation may require two populations of living cells and ponder on the differences between live and dead cells in this regard. Enquiry into the membrane potentials of cells in various states may be of assistance.

Spangler and Cassen[2] reported that the electrophoretic mobility of rabbit lymphocytes increased after treatment with doses of cobalt-60 irradiation of 200–400 r. They found that the magnitude of the effect decreased steadily with time, reaching normal levels after 30 min.

Could it be that a membrane potential on both populations of cells is an essential requirement for the activation phenomenon ? Extrapolating further, could it be that an increase in the membrane potential of a cell population confers ability to enhance this activation ?

R. D. H. RYALL

Department of Radiotherapy,
Groote Schuur Hospital,
Observatory, Cape,
South Africa.

Received April 14, 1969.

[1] Hardy, D. A., and Ling, N. R., *Nature*, **221**, 545 (1969).
[2] Spangler, G., and Cassen, B., *Radiat. Res.*, **30**, 22 (1967).

Dr Hardy and Dr Ling write: It is most encouraging that Dr Ryall should consider our results in the light of whole body immunity to lymphoma cells. We feel that something very similar to the *in vitro* reaction of normal lymphocytes to irradiated cultured lymphoma cells could occur in the body. It would also be reasonable to suppose that the lymphoproliferation so induced is the first stage in an immune response resulting in the elimination of the lymphoma cells. Cell destruction, however, is not a feature of a normal mixed lymphocyte reaction, suggesting that either there are further events in the body not detected *in vitro* or X-irradiated cells induce a different response.

The work of Moore's group[1] and others in establishing lymphoid cell lines from "normal" individuals could be interpreted to indicate that there are "abnormal" (? potentially malignant) lymphoid cells even in healthy people. The establishment of malignant clones would usually be prevented by an immunological process analogous to that suggested above. Where, however, a tumour did arise, perhaps because of unrecognized antigens or fast proliferation, it might perhaps be only after irradiation that the immune system would be effective. At present there is no direct evidence for this hypothesis and we are investigating it in a mouse system.

The suggestion that the activation observed is somehow dependent on the membrane potentials of the cells involved, we agree, deserves the most serious attention. As far as we are aware it has never been studied. Several observations may be relevant. (1) Agents acting on the cell surface, and thus presumably altering the membrane potential, block the reaction. Examples are anti-lymphocyte antisera and anti-light chain antisera (described by M. F. Greaves, G. Torrigiani and I. M. Roitt in a paper presented to the British Society of Immunology in May this year). (2) We know of no work reporting changes in membrane potential after irradiation of lymphoma cells at 6,000 r. Any changes which do occur, if they are important in the stimulation, must be relatively stable because irradiated cells incubated for 72 h before mixing are virtually as effective as stimulators of normal lymphocytes as those irradiated a few hours before. (3) The stimulation obtained using X-irradiated small lymphocytes in a "one way" reaction was studied by Kasakura and Lowenstein[2]. They reported that several "non-specific" factors affect the strength of a unidirectional reaction. It is possible that the factors mentioned were a result of changed membrane potential. (4) Antigenic differences do seem to be necessary in the mixed lymphocyte reaction[3] and in the reaction against lymphoma cells. We can obtain a stimulation with lymphoma cells heated to 50° C for 10 min. X-irradiated small lymphocytes when added to the lymphocytes of the same donor give no stimulation, showing that X-irradiation in itself is not responsible for the activation.

We think the problem of the relative contributions of antigenic differences and metabolic factors (perhaps as differences in membrane potential) is very interesting both with regard to tumour immunity and immunological concepts in general.

D. A. HARDY
N. R. LING

Department of Experimental Pathology,
University of Birmingham.

Received May 9, 1969.

[1] Moore, G. E., and McLimans, W. F., *J. Theoret. Biol.*, **20**, 217 (1968).
[2] Kasakura, S., and Lowenstein, L., *J. Immunol.*, **101**, 12 (1968).
[3] Ling, N. R., *Lymphocyte Stimulation* (North Holland, Amsterdam, 1968).

Disodium Cromoglycate, an Inhibitor of Mast Cell Degranulation and Histamine Release induced by Phospholipase A

DISODIUM cromoglycate (INTAL) has been shown to be an effective inhibitor of experimental allergen-induced bronchoconstriction in asthmatics[1,2]. The compound is novel in that it specifically inhibits the allergic release of spasmogens in immediate hypersensitivity reactions in several animal systems[3]. A recent suggestion was that disodium cromoglycate does not inhibit the reagin antibody/antigen union *per se* but that it inhibits discharge of the mediators from mast cells[4].

It is generally accepted that in the immediate hypersensitivity type reaction antibody is attached to the mast cell membrane and union with antigen occurs at this site[5]. Fine structure studies using "reaginic" antiferritin sera have revealed antigen on the mast cell membrane[6] and some evidence of membrane rupture during passive cutaneous anaphylaxis[7]. This suggests that the release of spasmogen is initiated on the mast cell membrane. Thin-layer and column chromatography have shown that various phospholipids are important constituents of cell membranes[8]. Phospholipase A, known to degranulate and release histamine from mast cells[9], has recently been demonstrated on the surface of mast cells[10]. It therefore seemed worthwhile to investigate the effect of disodium cromoglycate on mast cell disruption and histamine release mediated by phospholipase A, and to compare the results with those obtained in the antigen-induced immediate hypersensitivity reaction using the *Nippostrongylus brasiliensis* system[11].

Preliminary work confirmed Uvnäs's findings that phospholipase A induced histamine release from subcutaneous connective tissue mast cells, but that phospholipase C and D in comparable concentrations had no effect.

For our *in vitro* experiments we used Tyrode solution and pieces of rat subcutaneous connective tissue, approximately 10 mg fresh weight, removed from the flanks of female Sprague–Dawley rats weighing 100 g. Tissues for the antibody/antigen reaction were sensitized 24 h before removal by intradermal injection of 0·1 ml. of anti-*Nippostrongylus brasiliensis* serum (diluted 1 : 256) into normal rats[11]. Unsensitized tissues for testing with phospho-

©1969 Nature Publishing Group