overall immune response to tumour membrane immunization therefore was nonprotective and moreover treated animals were refractory to subsequent immunization with irradiated hepatoma cells which in normal rats produces tumour resistance (Baldwin & Moore 1971).

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Classification of Immunological Unresponsiveness and Tolerance

Traditionally tolerance or specific unresponsiveness was conceived as the abolition or diminution in parallel of all the classes of immune response to an antigen. However, unresponsiveness may selectively depress antibody production (Parish & Liew 1972) or delayed hypersensitivity (*see* Asherson 1967). This underlines the possibility that unresponsiveness may sometimes be due to one type of immune response depressing another type.

The classical concept of tolerance is that antigen, e.g. in the body fluids, directly affects antigen-sensitive cells and deletes or inactivates them (Billingham *et al.* 1956). This concept may be called 'direct antigen-mediated unresponsiveness'. A special version of this concept is that antigen on the surface of a cell, such as a macrophage, may selectively trap, inactivate or kill antigen-sensitive cells (Ada & Parish 1968). This classical view has been modified for three reasons. Firstly, the concept that tolerance is due to the deletion or long-term inactivation of cells does not apply to systems in which unresponsiveness can be reversed by simple manipulations. Secondly, there is evidence that tolerance is sometimes a positive phenomenon, i.e. one set of cells or their products (e.g. antibody) may block the response of a second set of cells. Finally, the role of the thymus in depressing immune responses and in the induction of positive unresponsiveness in certain systems is against the general applicability of the classical view.

Short and long lasting inactivation of cells in unresponsive animals: The classical view of tolerance suggested that cells were deleted or inactivated for a long period and that recovery depended on the genesis of new antigen-sensitive cells. This view was supported by the finding that thymectomy delayed recovery from unresponsiveness to bovine serum albumin in the mouse (Taylor 1964). However, there are several systems in which antigen-binding cells can be found in apparently unresponsive animals and in which cells from tolerant animals can be reactivated by simple procedures. For instance, unresponsiveness to *E. coli* lipopolysaccharide and other antigens can be ended by injecting the unresponsive cells into irradiated recipients or by incubating unresponsive cells into irradiated recipients or by incubating unresponsive cells overnight *in vitro* (*see* Sjöberg 1972). This reversal of unresponsiveness may be due to the genesis of new antigen-sensitive cells but the removal of surface antibody, antigen or immune complexes seems a more likely mechanism. These unresponsive animals possess cells able to bind antigen and this indicates that not all cells able to interact with the antigen have been eliminated.

Positive unresponsiveness: An alternative concept of tolerance is that one set of cells or their products, e.g. antibody, blocks the response of a second set of cells. This has been called 'positive unresponsiveness' (Asherson *et al.* 1971*a*) or 'infectious tolerance' Gershon & Kondo 1971) and where the detailed mechanism is known it may be further qualified as 'thymus dependent', 'antibody mediated' or 'immune complex mediated unresponsiveness'.

There are four criteria for positive unresponsiveness:

(1) Failure of normal lymphoid cells to restore immune competence to unresponsive recipients.

This will also occur in direct antigen-mediated unresponsiveness (classical tolerance) where there is residual antigen in an effective tolerogenic form.
(2) Blocking of the ability of normal lymphoid cells to restore tolerance to irradiated (normal or unresponsive) recipients by unresponsive cells.
(3) Blocking of the immune response of normal animals by unresponsive cells.
(4) Blocking of the immune response of normal animals by serum from unresponsive animals, i.e antibody-mediated unresponsiveness. This is a special case of positive unresponsiveness which is called 'immune enhancement' when cellular immunity is depressed and 'antibody feedback inhibition' when antibody production is depressed.

Positive unresponsiveness may interfere with the induction or manifestation of an immune response. For instance immune complexes may block the ability of immune cells to kill cytotoxically and by inference to mediate effective tumour rejection *in vivo* (*see* Hellström *et al.* 1971). It may also interfere with the induction of immune responses. It is useful to distinguish two possibilities:

(1) On the initial exposure to antigen one set of cells may produce factors, e.g. antibody, which, acting locally or systemically, perhaps in combination with antigen, render other cells unresponsive. If this set of cells then renders itself tolerant it may be difficult to distinguish this form of tolerance from direct antigen-mediated tolerance and the four criteria of positive unresponsiveness would not apply.
(2) Following the initial exposure to antigen one set of cells produces factors, e.g. antibody, which, acting locally or systemically, interfere with the response to a second exposure to antigen. These factors may only be produced on re-exposure to antigen. This situation would be detected by the criteria of positive unresponsiveness.

Diener & Feldman (1970) produced an *in vitro* model for the role of immune complexes. They showed that immune complexes blocked the response to antigen *in vitro* and that the concentration required was far lower than in antibody- or antigen-mediated unresponsiveness.

There are now several examples of positive unresponsiveness: tolerance to sheep red cells in mice (Gershon & Kondo 1971); tolerance to human serum albumin in mice (Terman *et al.* 1971); tolerance to picryl chloride in the mouse (Asherson *et al.* 1971*a*); tolerance to tumour antigens, maternal tolerance to the fœtus and tolerance in allophenic mice (Wegmann *et al.* 1971); and tolerance induced by neonatal injection of allogeneic cells in the mouse (Voisin *et al.* 1972). It is possible that the difficulty in passive transfer of experimental autoimmune disease is due to positive unresponsiveness.

Role of the thymus and thymus-derived cells in immunological unresponsiveness: For certain antigens collaboration between T and B cells is required for an immune response and this raises the question whether unresponsiveness affects T or B cells. Chiller *et al.* (1971) found that both populations may be affected but did not look for positive unresponsiveness.

There are now several lines of evidence for the controlling influence of thymus-derived cells on immune responses. Allison *et al.* (1971) review the literature. Firstly, normal thymus cells reduce the IgE type response to ascaris in irradiated rats (Okumura & Tada 1971) and the response to red cells in chickens. Secondly, thymus cells reduce the cell division seen in irradiated mice restored with normal thymus cells and injected with sheep red cells (Gershon *et al.* 1972). Cortisone-treated thymus cells have the same effect, which in this case appears to be non-specific. Finally, positive unresponsiveness to sheep red cells does not occur in thymectomized mice (Gershon & Kondo 1971). These results suggest that there is a distinctive inhibitory thymus cell. There are two main hypotheses about the mode of action of inhibitory thymus cells:

(1) The inhibitory thymus cell on exposure to antigen specifically liberates a nonspecific factor which depresses the immune responses. Provided the animal is not simultaneously tested with the tolerated antigen and an unrelated antigen this will simulate specific unresponsiveness.
(2) The inhibitory thymus cell on exposure to antigen generates a cell-bound or free inhibitory factor specific for the antigen. One possibility is the production of an antibody which together with antigen blocks immune responses.

Unresponsiveness to picryl chloride in the mouse: Against this background it is interesting to summarize the data on unresponsiveness to picryl chloride in the mouse, much of which are already published (Asherson *et al.* 1971*a*, *b*). Repeated injection of picryl sulphonic acid alters the immune response to picryl chloride in a number of ways:

Antigen-induced movement of lymphocytes to lymph nodes: Part of the enlargement of lymph nodes following immunization with picryl chloride is due to the arrival of circulating lymphoid cells. In fact the arrival of ^{51}Cr-labelled normal lymph node cells at the draining lymph nodes is 13.0% one day after immunization with picryl chloride

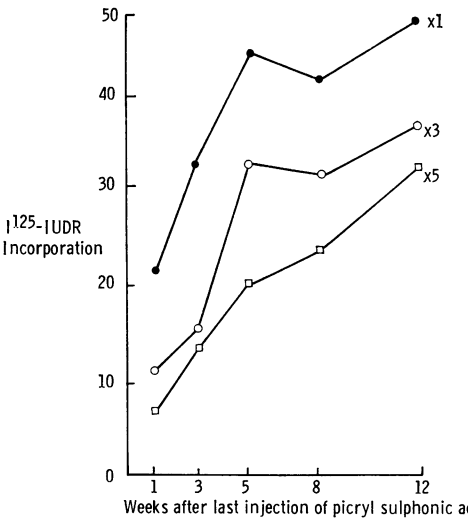

Fig 1 *I^{125}-IUDR incorporation* in vivo: *the effect of pretreatment with picryl sulphonic acid on the response to picryl chloride. Mice were given 1, 3 or 5 injections of picryl sulphonic acid. At the stated time afterwards they were sensitized with picryl chloride, injected with I^{125}-IUDR 3 days later and the radioactivity in the draining lymph nodes measured the next day. The results are expressed as a percentage of the incorporation in controls which were not pretreated with picryl sulphonic acid*

as compared with 5.7% in unimmunized lymph nodes. However, in unresponsive animals this arrival is reduced to 8.4% one day after immunization. (The arrival in unimmunized unresponsive mice was 3.7%.) This effect is specific and pretreatment with picryl sulphonic acid does not affect the arrival of cells after immunization with oxazolone. This experiment shows that part of the inflow of cells caused by picryl chloride in animals painted with picryl chloride for the first time is due to an immunological phenomenon.

Antigen-induced cell division: Painting of the skin with picryl chloride causes cell division in the draining lymph node. This can be assessed by incorporation of the radioactive nucleic acid precursor iododeoxyuridine-125 (IUDR). This technique measures both local DNA synthesis and the inflow of cells synthesized elsewhere, e.g. in the bone marrow. Fig 1 shows that a single injection of picryl sulphonic acid reduces ^{125}IUDR incorporation which normally follows painting with picryl chloride and that this effect lasts for at least twelve weeks. Five injections of picryl sulphonic acid cause a more profound reduction.

Antigen-induced production of inflammatory lymphocytes: Four days after immunization with picryl chloride the draining lymph node population shows an increased movement to sites of inflammation as judged by dissociation of the lymph node, labelling with radioactive chromium and injecting into recipients. This appearance of inflammatory lymphocytes is reduced in mice pretreated with picryl sulphonic acid (Asherson & Allwood 1972).

Contact sensitivity and antibody production: Asherson *et al.* (1971*a*) showed that 5 injections of picryl sulphonic acid completely abolished contact sensitivity to picryl chloride. This depression was specific as contact sensitivity to oxazolone was unaffected. Dr B Carr (*see* Asherson *et al.* 1971*b*) showed that unresponsiveness to picryl chloride reduced the antibody response to picryl chloride by about two tubes.

Blast transformation: Mouse lymph node cells taken from mice immunized with picryl chloride divide *in vitro* when exposed to picrylated antigens (*see* Milner 1971). Table 1 shows that several different picrylated proteins are effective. Pretreatment of mice twelve days beforehand with picryl sulphonic acid greatly reduces this response (Fig 2).

In a preliminary experiment mice given the standard course of 5 injections of picryl sulphonic acid and then left for six weeks gave an impaired *in vitro* response to picrylated mouse serum. It is particularly interesting that the dose response curve was U-shaped and that high doses of antigen led to a reduced response (Fig 3).

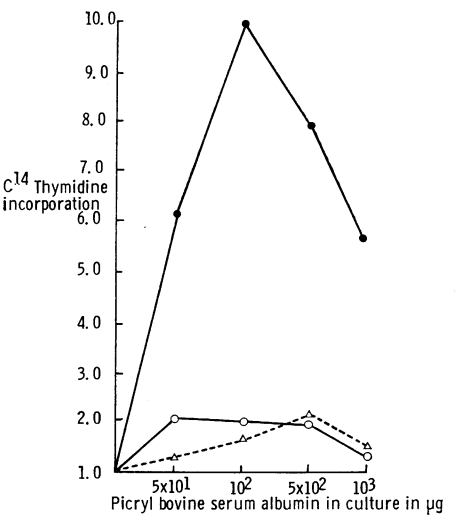

Fig 2 *DNA synthesis* in vitro *in normal and partially unresponsive mice. Normal mice were immunized with picryl chloride at −7 days. Other mice were pretreated with picryl sulphonic acid at −12 days. The incorporation is expressed as the ratio in culture with and without antigen. See legend to Table 1*

Table 1

Incorporation of ^{14}C-thymidine *in vitro*

	Immunizing agent	*Antigen in culture*	*^{14}C-thymidine incorporation* ●
Exp. 1	Picryl chloride	PIC-mouse serum 100 μg	5.0
	Picryl chloride	PIC-bovine serum albumin 100 μg	5.4
	Oxazolone	PIC-mouse serum 100 μg	2.1
	Nil	PIC-mouse serum 100 μg	2.3
Exp. 2	Picryl chloride	PIC-human gamma globulin	4.6
	Picryl chloride	PIC-bovine serum albumin	4.7

● The thymidine incorporation is expressed as the ratio of incorporation in the presence of antigen to incorporation in the absence of antigen. 2×10^6 viable lymph node cells in 1.5 ml Eagle's MEM supplemented with 10% RPMI 1640 and 10% fœtal bovine serum (glutamine 3 mmol/l., Hepes 3.3 mmol/l., sodium bicarbonate 0.176% with penicillin and streptomycin) were incubated in 8% oxygen, 6% carbon dioxide and 86% nitrogen. ^{14}C-thymidine 0.1 μc (62 Ci/M) was added at 66 hours and the cells harvested 6 hours later. The mice were immunized with picryl chloride 7 days beforehand

Evidence for positive unresponsiveness: These findings show that a wide range of immune responses are reduced in animals pretreated with picryl sulphonic acid. The evidence for positive unresponsiveness in mice treated with picryl sulphonic acid is that normal lymph node cells fail to restore responsiveness to unresponsive animals unless the recipients are irradiated; unresponsive lymph node cells reduce the immune response to picryl chloride in normal mice; unresponsive lymph node cells interfere with the ability of normal lymph node cells to restore immune response to irradiated recipients and unresponsive lymph node cells interfere with the ability of immunized lymph node cells passively to transfer contact sensitivity.

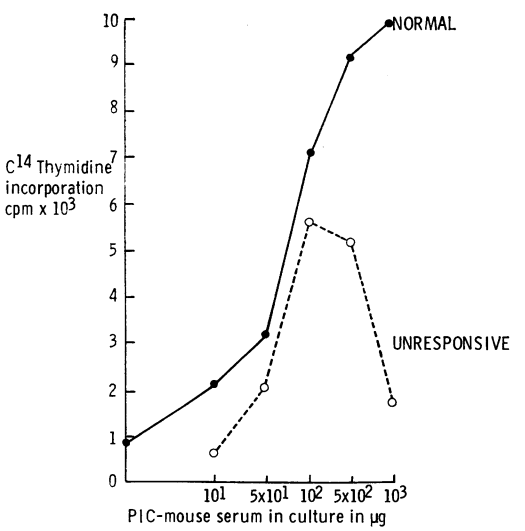

Fig 3 *DNA synthesis* in vitro *in normal and unresponsive mice. Mice were given 5 injections of picryl sulphonic acid and left for 5 weeks. These unresponsive mice and normal mice were then immunized with picryl chloride and lymph nodes taken 7 days later.*
See *legend to Table 1*

This evidence for positive unresponsiveness is based on measurement of contact sensitivity as assessed by ear swelling. The question now arises whether the other alterations of immune responses and in particular those apparent early after immunization, such as the inflow of lymphocytes into immunized lymph nodes, are also due to a positive phenomenon or to a classical direct antigen-mediated tolerance.

Acknowledgments: We are grateful to the Medical Research Council for a junior fellowship to R M R Barnes and the Cancer Research Campaign for supporting M Zembala with whom these studies were begun.

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