The Thymus Gland: recent studies relating to 'thymosin', ageing and 'slow' infection of the nervous system

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The thymus, not so long ago a seemingly uninteresting appurtenance of the lymphatic system, has recently yielded a crop of surprises that in their turn raise fundamental problems. This brief survey is limited to evidence of continuing thymic function throughout adult life enabling antigen recognition (by T cells) and regulating the appearance of new antigens in the tissue associated with normal ageing. These antigens are in some way related to the scrapie-Kuru-Jakob-Creutzfeldt complex which still remains a most enigmatic problem (Lancet, 1967).

THE EFFECTS OF THYMECTOMY

Miller (1961) introduced neonatal thymectomy as a most valuable tool in experimental immunology. While a good deal depends upon the strain of mouse or rat used, it is now recognised that animals so treated show a high mortality in the first 3 to 4 months of life; an increased susceptibility to various disease conditions including infections (bacterial and fungal), tumours and so on; prolonged acceptance of allogeneic skin grafts, and inability to give a normal cellular immune response to commonly used antigens. In some strains of mouse, neonatal thymectomy leads to lesions in the central nervous system (Field and Joyce, 1970) of a remarkable primary demyelinating character (Field and Peat, 1970). The severity of effect of neonatal thymectomy appears to be related to the bacterial load in the animals' environment; germ-free animals, for example, do not develop lethal runt disease (McIntyre et al., 1964; Wilson et al., 1964). However, outbred Swiss mice thymectomised at birth show only mild illness and rapidly recover their immunological competence (Dukor et al., 1966). In contrast, thymectomy in adult mice is without dramatic change in the immunological status of the animal. Miller (1965) reported that decline in immunological capacity becomes significant only after a period of 6 to 9 months and that 'thymectomy in the adult unlike thymectomy in the newborn, has no immediate effect on immunological capacity, presumably because an adequate pool of competent cells has already been constructed'. Dukor et al. (1966) also found that adult
thymectomy did not alter responsiveness within a period of eight weeks. The present review will show that, on the contrary, thymectomy in the adult does have an immediate effect upon the immunological capacity of T cells to react to antigens, and that this deficit persists for months.

**ESTIMATION OF THYMOSIN**

With a more refined method of studying T cell activity (the rosette method) Bach *et al.* (1971) found that adult thymectomy in the mouse suppressed sensitivity of spleen cells to anti-theta serum and azathioprine within five days, i.e. changes are produced much more rapidly than had previously been supposed. In further experiments, Bach and Dardenne (1972) re-affirmed that adult thymectomy reduces theta positive rosette forming cells (RFC) in the spleen within six days but does not do so in lymph nodes, and showed that normal azathioprine and anti-theta sensitivity of spleen RFC is restored *in vivo* and *in vitro* by treatment with low doses of thymus extract (thymosin). This thymosin-like activity disappears from serum after thymectomy, with a half-life of three hours. With the macrophage electrophoretic mobility test, it has been possible to show that thymectomy in the adult human and guinea-pig leads to an immediate effect (i.e. within one hour) on the ability of lymphocytes in the blood to recognise antigen, and this is dependent upon circulating thymosin. It has also been possible to devise a simple method of estimating thymosin in serum.

These developments started from the observation that circulating lymphocytes in the blood of patients suffering from myasthenia gravis were sensitised to muscle, to basic protein of human sciatic nerve and, unexpectedly, to encephalitogenic factor (EF) of human brain as well as (in a small degree) to thymus but not to lymph node extract. When such patients were thymectomised therapeutically, in two days there was a dramatic reduction in the measured reactivity of their lymphocytes to these as well as PPD and thyroid antigens (Field *et al.*, 1973).

All measurements of lymphocyte reactivity to antigens were made by the macrophage electrophoretic mobility (MEM) test (Field and Caspary, 1970) fully described by Caspary and Field (1971) with technical details set out by Shenton *et al.* (1973), Shenton and Field (1975) and Field and Shenton (1975). The MEM test has been shown to run parallel with lymphocyte transformation (Bloom and Bennett, 1966) and the well-known macrophage migration inhibition (MMI) method (Mertin *et al.*, 1974). In principle, the method depends upon the interaction of sensitised lymphocytes with antigen to liberate some lymphokine (which may be the same as the well-recognised MIF (Caspary, 1973)), possessing the property of causing normal guinea-pig macrophages to travel more slowly in an electric field, so that normal macrophages are used as an indicator system for lymphocyte-antigen interaction. The slowing produced is expressed as a percentage of the original macrophage speed. If $t_c =$ migration time of macrophages in the presence of lymphocyte-antigen mixture, $t_c =$ migration time of
macrophages with no antigen present, then \( t_e t_c \) and \( (t_e - t_c / t_c) \times 100 \) is a measure of the lymphocyte-antigen interaction. (It would have been more accurate to use the statistic \( t_e - t_c \times 100 \), but with the magnitude of the figures involved no serious error is introduced.)

Before thymectomy, for example, a myasthenic patient showed a 17.8 per cent slowing with PPD and 7.4 per cent two days after operation. His EF result before operation was 8.4 per cent and 0.1 per cent afterwards. This surprising loss of ability of blood lymphocytes to react with antigen was studied more precisely by withdrawing blood at intervals after complete severance of the blood supply to the thymus at operation, and collecting samples at intervals from half an hour onwards (Fig. 1). It can be seen that reactivity of the blood lymphocytes to three antigens (Thyroid F1 fraction, PPD of tubercle, and EF) begins to drop within

![Graph showing % Slowing over Hours post Thymectomy](image)

Fig. 1. Sensitisation of blood lymphocytes to F1 (thyroid), PPD (of tubercle) and encephalitogenic factor (EF) at intervals after exclusion of the thymus from the circulation during thymectomy for myasthenia gravis. Note rapid fall significant within half an hour, well marked by three hours, and almost total by 24 hours. Sensitisation measured by MEM method and expressed as percentage slowing of macrophages (ordinate).
half an hour of elimination of the thymus from the circulation, is very marked by 3½ hours, and sinks practically to zero after 24 hours. The same is not found after other serious operations. In another patient, aged 60, a similar drop in lymphocyte reactivity was observed even though the ‘thymus’ removed contained no more than 1 to 2 g of lymphoid tissue. Following these unexpected results in the human, experiments were carried out on adult Hartley guinea-pigs. These were sensitised to PPD by intracutaneous injection of 10 µg PPD in Freund’s complete adjuvant, and about 10 days later lymphocyte sensitisation to PPD was measured. It was about 25 to 30 per cent in the MEM test. The animals were then subjected to thymectomy and small samples of blood were withdrawn at intervals to estimate lymphocyte reactivity to PPD (Fig. 2). Sham thymectomy (exposing and mobilising the two gland lobes) produced no effect, but removal was followed by a profound fall in the cellular reactivity, just as in the human. Clearly, the presence of the thymus gland within the circulation is essential if lymphocytes are to be capable of ‘recognising’ antigen. Two explanations appear possible: the thymus usually produces a humoral factor that disappears rapidly from the blood when the gland is removed, as suggested by Bach and Dardenne (1972), or cells
must pass through the thymus for a short period immediately before being able to recognise antigen.

Attempts were therefore made to restore recognitional ability by addition of normal serum (both human and guinea-pig) to the in vitro system (Fig. 3). Normal serum contains a lymphocyte depressing factor (LDF), active up to a titre of 1:60 (Field and Caspary, 1971a). This interferes with restoration of recognitional capacity of normal serum. In practice, therefore, dilutions of 1:120 and more, outside the range of LDF activity, are more active in restoring recognitional capacity than is a dilution of 1:60 with its active LDF (Field and Caspary, 1971a) and, in systematic studies of thymosin, LDF, which is associated with a macroglobulin, will have to be removed by chromatography. The restorative power of the animal’s own serum is greater than that of another normal animal, and normal guinea-pig serum is active to a higher dilution than normal human serum. Activity is thus not species restricted. The pre-thymectomy serum does not reach maximal capacity until a dilution of at least 1:240 is reached and this may well be due to the higher LDF that accompanies sensitisation (Field and Caspary, 1971a; Field and Caspary, 1972; Field and Caspary, 1971b). Serum from the thymectomised guinea-pig is without any restorative capacity.

The PPD sensitised thymectomised guinea-pig can be used as a test model for
Table 1. Adult guinea-pigs sensitised by intracutaneous injection of 10 μg PPD in Freund’s complete adjuvant. Sera added to the in vitro test system 1:240.

| Day 8: before thymectomy | 1 Day: post-thymectomy | Addition of pre-thymectomy serum | Addition of post-thymectomy serum |
|--------------------------|-------------------------|---------------------------------|----------------------------------|
| Time                     | % slowing               | Time                            | % slowing                        |
| CON.                     | 5.96 ± 0.05             | 5.93 ± 0.08                     | 5.93 ± 0.06                      |
| PPD.                     | 7.66 ± 0.06             | 5.98 ± 0.06                     | 7.68 ± 0.05                      |

Marked reduction in sensitisation one day after thymectomy: full restoration by pre-thymectomy serum but not by post-thymectomy.

| Time                     | % slowing               | Time                            | % slowing                        |
|--------------------------|-------------------------|---------------------------------|----------------------------------|
| CON.                     | 5.96 ± 0.05             | 5.95 ± 0.06                     | 5.95 ± 0.06                      |
| PPD.                     | 6.00 ± 0.05             | 5.98 ± 0.05                     | 7.22 ± 0.06                      |

Sensitisation after thymectomy, can only be expressed in the presence of normal serum.

| Time                     | % slowing               | Time                            | % slowing                        |
|--------------------------|-------------------------|---------------------------------|----------------------------------|
| CON.                     | 5.91 ± 0.07             | 5.95 ± 0.06                     | -                                |
| PPD.                     | 5.96 ± 0.04             | 7.11 ± 0.07                     | 19.4                             |

Persistence of defect caused by adult thymectomy.
estimating the power of a serum to restore recognitional capacity, i.e. for thymosin estimation (Field and Shenton, 1975b), and this might well be of value in estimating completeness of thymic removal (Meyer-Rienecker et al., 1975; Kark, 1975). Bach et al. (1973) used a ‘rosette test’ in which sheep red blood cells form a corona around T lymphocytes, and have applied it to the estimation of thymosin necessary for the phenomenon. They failed to find thymosin in normal subjects over the age of 30 years, though the MEM assay test shows it to be present until well over the age of 60 years. They also reported that serum thymosin activity in the human declined within a few hours of thymectomy. They failed to demonstrate thymosin activity in 11 of 12 young patients with systemic lupus erythematosus.

Thus, two quite different techniques — the ‘rosette’ method and the MEM assay — have demonstrated functional activity of the adult thymus gland. The rapid and easily reproducible MEM method detects thymosin in older subjects and appears to be more sensitive than Bach’s rosette cell method.

PERSISTENCE OF EFFECT OF ADULT THYMECTOMY

Two further questions arise —

1. If the presence of a functional thymus is needed for maintenance of an adequate level of blood thymosin to enable sensitised T cells to interact with antigen, is the presence of the thymus also necessary to allow primary sensitisation against an antigen? To this a clear negative answer may be given. When a thymectomised adult guinea-pig is immunised by intracutaneous inoculation of 10 μg PPD in Freund’s complete adjuvant, lymphocytes fail to respond to PPD in the MEM test. However, when diluted normal serum is added, there is a normal response (see Table 1). The integrity of the thymus is thus immaterial to the development of full sensitisation, but essential for the expression of this sensitisation.

2. How long does the effect of adult thymectomy persist? Experiments carried out so far in adult guinea-pigs sensitised to PPD show that the failure of lymphocytes to respond to PPD persists for at least four months, but that reactivity to PPD can be restored at any stage by addition of normal guinea-pig serum (but not that of the thymectomised animal). There would appear to be no physiological mechanism at hand by which the effect of removal of the thymus in the adult guinea-pig can be quickly overcome and lymphocyte reactivity to antigen restored.

The thymus in the adult guinea-pig apparently functions throughout life. From the profound effect produced in humans by removal of even a very small remnant of functional thymic tissue in myasthenia gravis, it is clear that even a small fraction of the original gland suffices to maintain normal T cell reactivity. How long the effect of total thymectomy persists in the human remains to be studied in patients who have been thymectomised many years previously.
THYMUS; AGEING; AND 'SCRAPIE-LIKE' ANTIGENS

The association of the thymus in some way with ageing has long been recognised. If only because of its well-known involution. In recent years, however, a more direct influence upon the ageing process has been established (Fabris et al., 1972). Certain similarities are found between the morphological changes that take place in the brain of the mouse in normal old age and those in the young mouse with scrapie — as if the scrapie process had telescoped the time co-ordinate (Field, 1967). With the development of the MEM test and the demonstration that specific sensitisation to saline extracts of scrapie (as opposed to normal) brain occurred in mice and sheep with the disease (Field and Shenton, 1973e; Field and Shenton, 1973f; Field and Shenton, 1974), tests were made with lymphocytes from both humans and chimpanzees with natural and experimental Kuru and Jakob-Creutzfeldt disease because of their resemblance to scrapie. Lymphocytes from both diseases were found to react to a much greater degree with scrapie material than with normal (Field and Shenton, 1973a). The reaction occurs before the onset of signs and does not take place in chimpanzees injected with multiple sclerosis brain material (Field and Shenton, 1975a). These experiments suggested that new antigenic determinants emerged as the 'slow' infection established itself. Because the agent of scrapie is widespread in tissues other than the nervous system, it was looked for by injecting suspensions of tissue into guinea-pigs and 9 days later measuring the sensitisation of the guinea-pig lymphocytes to scrapie brain extract as compared with normal brain extract. It was found that reaction to scrapie brain was always greater than to normal brain so that the 'scrapie normal difference' (SND) was positive. The next step was to look at normal ageing tissue from both mice and humans. It was found that as the ageing progressed the SND found in the lymphocytes of an injected guinea-pig increased, so that it was possible to construct an 'immunological' ageing curve depending upon the appearance of scrapie-like antigens in the tissues of the aged (Field and Shenton, 1973c). It is of great interest that the lymphocytes of old people (in whose tissues new scrapie-like antigens are developing) do not show high SND when tested directly, i.e. their lymphocytes regard the new antigens as 'self'. It is only when the old tissues are exposed to the immunological recognition system of the guinea-pig that they are recognised as different from young tissues. Because of the general association of the thymus gland with the ageing process, litter-mate mice were subjected to thymectomy or sham operation at birth and their tissues tested at intervals by injecting suspensions into guinea-pigs whose lymphocytes were then studied for increased SND reaction after 9 days. The results were striking. Neonatal thymectomy resulted in a very rapid rise in the response to scrapie test antigen (that to normal remaining unchanged) so that the SND was markedly increased. At 14 days after operation a mouse showed a greater SND value than a normal animal at 203 days; a 48-day-old animal showed greater SND from both brain and spleen injection than a normal of 840 days (Field and Shenton, 1973b).
Later it was found that implants of 10-day-old thymic tissue would hold up the precocious development of the high SND after thymectomy (Field and Shenton, 1973d). Removal of the thymus gland from the neonatal rodent thus leads to rapid changes in its tissue antigenicity, determinant(s) appearing that cross-react strongly with those found in young scrapie animals. How the integrity of the thymus gland prevents the development of the antigenicity is not known. Some modern theories of scrapie lay emphasis on the molecular rearrangement of all membranes (Gibbons and Hunter, 1967; Field, 1976). Perhaps thymosin exerts a generalised stabilising effect on membrane structure. There would thus appear to be two apparently unrelated effects of thymectomy, an immediate one (occurring within 2 to 3 hours) involving inability of circulating lymphocytes (presumably T cells) to recognise antigen so that thymosin may be looked upon as having an 'opsonising' effect upon recognitive lymphocytes; and a precocious development of scrapie-like antigens in the tissues similar to that which takes place much more gradually during normal ageing. Such ageing antigens also occur in the 'slow' infections of man, Kuru and Jakob-Creutzfeld disease.

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J. Roy. Coll. Phycns Vol. 11 No. 2 January 1977

Book Review

Introductory Medical Statistics by R. F. Mould. Pitman Medical 1976. Price £4.00.

The great virtue of this book is its readability. For this reason alone it can be confidently recommended to those medical students, undergraduate and postgraduate, who feel insecure when they first dip into statistical waters. Quite apart from this, examples are drawn from an area of medicine with which they are likely to be on nodding terms. This results in a closer relationship with the author than could exist, for instance, with Moroney’s Facts from Figures, which, at £1.00 in paperback, sells for a quarter of the price but aims at a wider, less specific, readership.

Dr Mould, whose chief interest appears to lie in the field of oncology (judging from a detailed analysis of the calculation of survival rates in Chapter 10), has clearly worked closely with medical doctors and it may be that the effectiveness of his book, which appears to be based on the London M.B. syllabus, owes something to collaboration with the professorial medical staff at the Westminster Hospital. The outcome is certainly a happy one and any prospective M.R.C.P candidate can be assured that as much statistical information as he will be expected to have acquired is included between the hardback covers of this book.

The series of articles recently published in the British Medical Journal under the umbrella title of ‘Statistics at Square One’ bears witness to the growing recognition of the importance of this branch of medical expertise. Dr Mould’s book, liberally peppered with 40 diagrams in 90 pages of text, including a useful glossary of symbols, provides a commendable introduction to medical statistics.

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