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# Thymectomy and Immunological Ageing in Mice: Precocious Emergence of Scrapie-Like Antigen

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*Abstract.* Thymectomy in neonatal mice leads to the rapid emergence of a new antigen in the tissues which, when injected into guinea pigs, results in their lymphocytes showing marked sensitization to scrapie mouse brain as compared with normal brain. The same antigenic changes occur though much more slowly in normal ageing animals so that thymectomy appears to have accelerated an ageing process. Lymphocyte sensitization was determined by the macrophage electrophoresis migration (MEM) test. The relation of scrapie to normal ageing is discussed.

*Key Words*
Thymectomy
Mouse
Scrapie
MEM test
Antigen changes

It has recently been found that when tissue from either mouse or human of increasing age was injected into guinea pigs, the lymphocytes of these animals developed greater reactivity to a preparation of scrapie mouse brain or spleen than they did to corresponding tissue from an equally aged normal mouse. This scrapie normal difference (SND) in lymphocyte sensitization was measured by the newly introduced macrophage electrophoretic mobility (MEM) test [FIELD and CASPARY, 1970, 1971; CASPARY and FIELD, 1971] which enables accurate quantitative assessment of cellular sensitization. In view of this increasing SND with age of the animal (either mouse or man) from which the inoculum was derived, the effect of neonatal thymectomy upon the rise in SND with time was studied. It has been found that the operation markedly accelerates the change in all tissues though individual differences exist between the tissues.

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## *Materials and Methods*

Webster Swiss mice have been used throughout. Some have been subjected to total thymectomy within 24 h of birth, others have suffered a 'sham' operation.

At intervals after operation animals have been killed and brain, liver, spleen, kidney removed, made up as a $10^{-1}$ suspension in sterile saline and cleared by 10 min centrifugation at 1,000 *g*. 0.1 ml of suspension was injected intradermally into the dorsum of the right foot of a Hartley guinea pig (ca. 500 g body weight) of either sex. After 8 days cardiac puncture was performed and 8–10 ml of blood removed from which lymphocytes were separated by the method of COULSON and CHALMERS [1967] as modified by HUGHES and CASPARY [1970] using methyl cellulose and carbonyl iron.

A $10^{-1}$ sterile suspension of scrapie mouse brain in saline was prepared from an animal with clinical disease some 5 months after intracerebral inoculation of 0.03 ml mouse adapted material obtained originally from Dr. R.L. CHANDLER and since passaged many times through Webster-Swiss mice in this unit. A similar suspension was prepared from brain of a mouse of the same age which had been inoculated at the same time intracerebrally with normal mouse brain suspension. In each case, too, the spleen was removed and subjected to 5,000 r irradiation before being made up as a $10^{-1}$ suspension as test antigen.

Guinea pig lymphocyte sensitization was measured by the MEM test which is very sensitive and highly discriminatory. In principle it depends upon the observation that lymphocytes in the presence of specific antigen liberate some 'lymphokine' with property of causing normal guinea pig macrophages to travel more slowly in an electric field (macrophage slowly factor, MSF, which may be identical with MIF). If $t_e$ = migration time of macrophages when Ag-lymphocyte reaction has taken place; $t_c$ = migration time when no Ag is present, then $t_e > t_c$ and

$$\frac{t_e - t_c}{tc} \times 100$$

is a measure of lymphocyte sensitization. It is these percentage slowings which are presented in the Results. A full description of the method together with an experimental protocol *in extenso* has been given by CASPARY and FIELD [1971]. All measurements were, of course, made 'blind'.

## *Results*

It will be seen from table I that the percentage figure obtained in the MEM test when normal mouse brain is used as test antigen remains constant and is independent of the age of the animal. Only at 39 days was there a slight drop but this was not statistically significant. The brain material from sham thymectomized animals also gave the same constant figure throughout the period spanned by the experiments. The same consistency was found for the liver and kidney injected guinea pigs. In each case the response of the guinea pigs lymphocytes to normal tissue (either brain or spleen) remained the same. The response to brain from thymectomized mice on the other hand

*Table I.* Thymectomized mice

| | | Normal brain | Scrapie brain | Normal spleen | Scrapie spleen | Brain SND | Spleen SND |
|---|---|---|---|---|---|---|---|
| *Sensitivity of GP lymphocytes injected with mouse tissues of different ages after thymectomy* | | | | | | | |
| Brain injected | 1 day | 9.7 | 10.2 | 4.5 | 5.0 | 0.5 | 0.5 |
| | 2 days | 9.5 | 10.2 | 4.6 | 5.3 | 0.7 | 0.7 |
| | 3 days | 9.6 | 10.4 | 4.5 | 5.2 | 0.8 | 0.7 |
| | 5 days | 9.6 | 11.0 | 4.4 | 5.8 | 1.4 | 1.4 |
| | 6 days | 9.7 | 11.3 | 4.5 | 6.3 | 1.5 | 1.8 |
| | 14 days | 9.7 | 12.8 | 4.6 | 7.9 | 3.1 | 3.3 |
| | 17 days | 9.9 | 13.5 | 4.7 | 8.3 | 3.6 | 3.6 |
| | 20 days | 9.6 | 13.5 | 4.5 | 8.4 | 3.9 | 3.9 |
| | 30 days | 9.6 | 13.8 | 4.5 | 8.7 | 4.2 | 4.2 |
| | 35 days | 9.8 | 14.0 | 4.6 | 9.1 | 4.2 | 4.5 |
| | 39 days | 8.3 | 12.6 | 4.2 | 8.2 | 4.3 | 4.0 |
| | 48 days | 9.4 | 14.4 | 4.3 | 9.4 | 5.0 | 5.1 |
| | 76 days | 9.7 | 15.6 | 4.6 | 10.5 | 5.9 | 5.9 |
| | 87 days | 9.8 | 16.1 | 4.6 | 11.3 | 6.3 | 6.7 |
| Liver injected | 1 day | 4.8 | 5.2 | 4.5 | 5.0 | 0.4 | 0.5 |
| | 2 days | 4.5 | 5.5 | 4.6 | 5.6 | 1.0 | 1.0 |
| | 3 days | 4.5 | 6.0 | 4.0 | 5.7 | 1.5 | 1.7 |
| | 6 days | 4.7 | 7.8 | 4.7 | 7.9 | 3.1 | 3.2 |
| | 14 days | 4.5 | 8.6 | 4.5 | 8.7 | 4.1 | 4.2 |
| | 20 days | 4.1 | 8.2 | 4.5 | 8.8 | 4.1 | 4.3 |
| | 30 days | 4.6 | 9.2 | 4.6 | 9.4 | 4.6 | 4.8 |
| | 48 days | 4.5 | 9.8 | 4.6 | 9.8 | 5.3 | 5.2 |
| | 76 days | 4.6 | 11.6 | 4.5 | 11.3 | 7.0 | 6.8 |
| | 87 days | 4.5 | 12.1 | 4.5 | 12.3 | 7.6 | 7.8 |
| Kidney injected | 6 days | 4.5 | 5.3 | – | – | 0.8 | – |
| | 14 days | 5.9 | 8.5 | 4.5 | 7.1 | 2.6 | 2.6 |
| | 20 days | 4.5 | 7.5 | 4.5 | 7.4 | 3.0 | 2.9 |
| | 35 days | 4.6 | 7.7 | 4.6 | 7.8 | 3.1 | 3.2 |
| | 48 days | 4.5 | 7.7 | 4.4 | 7.9 | 3.2 | 3.5 |
| | 76 days | 4.5 | 8.8 | 4.5 | 8.8 | 4.3 | 4.3 |
| *Sensitivity of GP lymphocytes injected with mouse tissues of different ages after sham thymectomies* | | | | | | | |
| Brain injected | 1 day | 9.7 | 10.1 | 4.5 | 5.0 | 0.4 | 0.5 |
| | 2 days | 9.6 | 10.1 | 4.4 | 4.9 | 0.5 | 0.5 |
| | 3 days | 9.4 | 10.2 | 4.5 | 5.3 | 0.8 | 0.8 |
| | 5 days | 9.6 | 10.4 | 4.5 | 5.2 | 0.8 | 0.7 |
| | 6 days | 9.6 | 10.2 | 4.6 | 5.1 | 0.6 | 0.5 |
| | 14 days | 9.2 | 9.9 | 4.3 | 4.9 | 0.7 | 0.6 |
| | 17 days | 9.7 | 10.2 | 4.4 | 4.9 | 0.6 | 0.5 |
| | 20 days | 9.3 | 10.0 | 4.1 | 4.9 | 0.7 | 0.8 |

*Table I* (continued)

| | | Normal brain | Scrapie brain | Normal spleen | Scrapie spleen | Brain SND | Spleen SND |
|---|---|---|---|---|---|---|---|
| | 25 days | 9.6 | 10.2 | – | – | 0.6 | – |
| | 30 days | 9.4 | 9.9 | 4.5 | 5.0 | 0.5 | 0.5 |
| | 35 days | 9.6 | 10.2 | 4.6 | 5.2 | 0.6 | 0.6 |
| | 48 days | 9.3 | 10.0 | 4.0 | 5.0 | 0.7 | 1.0 |
| Controls | | | | | | | |
| Spleen injected | 6 days | 4.5 | 5.0 | 9.5 | 10.1 | 0.5 | 0.6 |
| | 14 days | 4.4 | 4.9 | 9.5 | 10.0 | 0.5 | 0.5 |
| | 20 cays | 4.6 | 5.3 | 9.5 | 10.2 | 0.7 | 0.7 |
| | 30 days | 4.5 | 5.0 | 9.6 | 10.1 | 0.5 | 0.5 |
| | 35 days | 4.5 | 5.1 | 9.6 | 10.1 | 0.6 | 0.5 |
| | 48 days | 4.5 | 5.2 | 9.5 | 10.2 | 0.7 | 0.7 |
| Liver injected | 1 day | 5.0 | 5.3 | 4.6 | 4.9 | 0.3 | 0.3 |
| | 2 days | 4.5 | 5.0 | 4.5 | 4.9 | 0.5 | 0.4 |
| | 3 days | 4.5 | 5.2 | 4.5 | 5.2 | 0.7 | 0.7 |
| | 6 days | 4.6 | 5.1 | 4.5 | 5.2 | 0.5 | 0.7 |
| | 14 days | 4.5 | 5.1 | 4.7 | 5.0 | 0.6 | 0.3 |
| | 20 days | 4.5 | 5.1 | 4.5 | 5.0 | 0.6 | 0.5 |
| | 30 days | 4.5 | 5.0 | 4.5 | 4.9 | 0.5 | 0.4 |
| Kidney injected | 6 days | 4.5 | 5.3 | – | – | 0.8 | – |
| | 14 days | 4.5 | 5.1 | 4.5 | 5.2 | 0.6 | 0.7 |
| | 20 days | 4.1 | 4.5 | 4.2 | 4.8 | 0.4 | 0.6 |
| | 35 days | 4.5 | 5.0 | 4.4 | 5.0 | 0.5 | 0.6 |

soon began to rise above this base line, significantly so by 14 days. (In the MEM test with present level of experience, a difference of more than 2.5% signifies $p<0.01$.) Even before this time, the trend for the reaction to scrapie to exceed that to normal brain asserted itself and as time went by the SND became more marked (fig. 1). Exactly the same phenomenon was observed when scrapie and normal spleen were substituted for brain as test materials for the lymphocyte sensitization.

Whilst the SND of the guinea pigs lymphocytes increases consistently for all tissues from thymectomized animals there were clear differences in the rate at which it developed. These are brought out in figure 1, where it will be seen that the change sets in earliest in the case of liver tissue and reaches a higher level than it does in the case of kidney, with brain and spleen being similar and intermediate.

In each case the 'ageing' reaction occurs most quickly during the first 20 days after thymectomy.

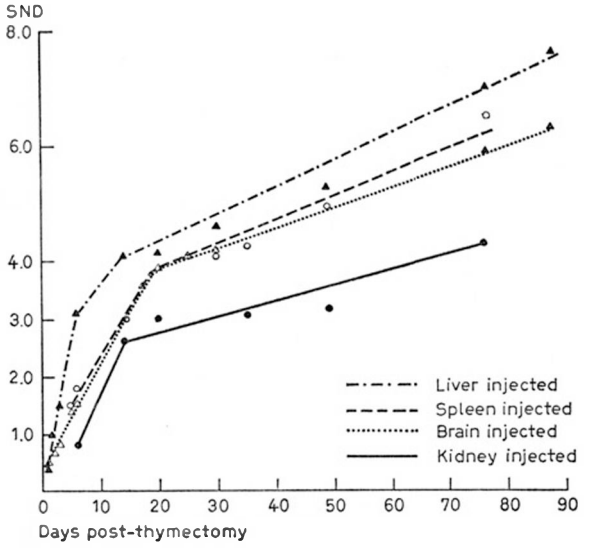


*Fig. 1.* Relation of SND of lymphocytes from guinea pigs inoculated 8 days previously with tissues from thymectomized mice.

Table I and figure 1 indicate that the same pattern of reactivity occurs for all the tissues tested and may be summarized as follows: (a) The sensitization of guinea pig lymphocytes to normal mouse brain (or spleen) did not materially change, no matter whether the tissue with which the guinea pig had been injected (brain, liver, spleen, kidney) came from a young or old mouse thymectomized or not. For each tissue there was its own level of response to normal tissue test antigen. (b) The sensitization of guinea pig lymphocytes, when tested with scrapie brain (or spleen) rose sharply with the age of the thymectomized mouse from which the injected tissue (brain, spleen, liver, kidney) was taken. (c) This rise for thymectomized mice increased with age of the mouse but at different rates for different tissues.

## *Discussion*

It has already been found that both in the case of normal Webster-Swiss mice and humans the SND obtained when tissues are injected into guinea pigs rises with the age of the mouse or human from which the tissues were taken [FIELD and SHENTON, 1973a, b]. There is thus a direct relation between the SND shown by the test guinea pig lymphocytes and the chronological age of the injected tissue. Much the same is shown by the present work. The

effect of thymectomy on the reactivity of the guinea pig lymphocytes exactly parallels that of ageing. It is, however, a remarkably accelerated 'ageing'. Thus the SND found when brain from a 203-day-old normal mouse was injected was 2.6 %. This figure was already exceeded (3.1 %) in the 14-day-old thymectomized animal. And at 840 days the SND for both brain and spleen was less than that from the 48-day-old thymectomized animal. Clearly, thymectomy led to a precocious development of an antigen in the tissues with the same properties as that of normally old tissues and that produced in young animals with scrapie [FIELD and SHENTON, 1972]. This raises the important question of the relation of scrapie (a fascinating enigma of modern comparative medicine) [editorial, Lancet 1967, 1972; editorial Nature, 1969] to the ageing process.

However, there are certain quantitative differences. Whilst in the human the ageing change is greater in spleen than in kidney, in the mouse it is greatest in liver and least in kidney [FIELD and SHENTON, 1973a, b]. This relation obtains, too, in the thymectomized mouse where the SND develops to a greater degree and earlier for liver than other tissues. There have been many suggestions that ageing may be linked with immunological disturbances [BURNETT, 1959; WALFORD, 1969] and there is evidence that different organs and physiological systems show wide variation in the time during which they use their 'quota of cellular programme' [FABRIS *et al.*, 1972]. The latter authors emphasize the possible importance of the thymus as a biological clock and draw attention to the possible importance of lymphocytes in prolonging life and preventing ageing. If, as would appear to be the case, the appearance of a scrapie like antigen is a parameter of ageing, then the thymus is indeed important in ageing and different organs age at different rates.

From time to time it has been suggested that quite apart from its function as an integral part of the immunological apparatus of the body the thymus may serve as an important source of lymphocytes with a nutritional purpose (trephocytes) thus providing building blocks for growth and regulation of cells [LOUTIT, 1962]. It is conceivable that removal of the thymus at birth prevents the tissues from carrying through their 'quota of cellular programme' so that senescence sets in prematurely. Neonatal thymectomy in mice leads to an increased mortality depending upon the strain, about 50 % dying between the third and fifth month. The mean survival rate of mice dying after 6 months is also shorter than that of normal mice of the same strain so that there is a bimodal distribution of the time of death [HOWIE and HELYER, 1966]. However, post-mortem examination of those animals which survived 6 months showed no incidence of any specific disease and this sug-

gests simple accelerated ageing [WALFORD, 1969]. Curiously, there have on the other hand, been reports of enlarged thymus in human infantile progeria [GABR *et al.*, 1961].

The appearance of some antigen(s) in old mouse or human tissue and precociously in thymectomized mice either identical with the new antigen(s) in scrapie [FIELD and SHENTON, 1972] or sufficiently close to result in marked cross-reaction is interesting in view of the morphological changes in scrapie of mice which bear certain resemblances to those found in normal old age [FIELD, 1967]. Indeed it appears, so far as the brain is concerned (the only organ examined specifically for the purpose), that the changes commonly associated with old age are brought forward to a much earlier age by the scrapie process both in mouse and rat. To these morphological changes must now be added the altered antigenicity of the tissues when injected into a guinea pig. In scrapie [FIELD and SHENTON, 1972] and in old age [FIELD and SHENTON, 1973a, b] there develops a new antigenicity in the tissues. This has now been shown to be similar to that which develops precociously in thymectomized mice.

Whilst at this point there is no firm basis for speculation as to the nature of the tissue change, other work in this unit has shown that the new scrapie type antigenicity develops in all tissues of the mouse 50 days after intercerebral inoculation of scrapie agent [FIELD and SHENTON, 1973c] at a time when astrocytic enlargement – the first morphological sign of scrapie infection – is still some 2 or 3 weeks away. It is tempting to suppose that the scrapie agent which is recognized as being intimately associated with cellular membrane structures [GIBBONS and HUNTER, 1967] brings about progressive physicochemical alteration in the organization of membranes and confers upon them this new antigenicity. If so then it may be a progressive transformation for which all membrane is destined and the scrapie agent 'catalyses' the inevitable process. Attempts should be made to isolate 'scrapie agent' from really old tissues. Meanwhile the relation between scrapie agent and the new antigenic material appearing in old age and prematurely in thymectomized mice is being further studied by a combination of cell affinity chromatography and the MEM test in the same way as has already been done for encephalitogenic factor and measles virus [FIELD and MCDERMOTT, 1973].

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