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IMMUNOLOGICAL ASSESSMENT OF AGEING: EMERGENCE OF SCRAPIE-LIKE ANTIGENS

E. J. FIELD

MS Research Unit, Royal Victoria Infirmary, Queen Victoria Road, Newcastle upon Tyne NE1 4LP

Summary

Certain morphological similarities between scrapie—a naturally occurring disease of sheep, long looked upon as a paradigm of 'slow infections'—and the ageing process in normal animals led to a study of the development of new antigens both in scrapie-affected animals and in normal mice and humans. It was found that with advancing age new antigen(s) identical with, or similar to, those occurring in scrapie (where the time co-ordinate of ageing in the brain seems foreshortened) make their appearance both in mice and humans, and lead to special sensitization of lymphocytes in normal guinea pigs injected with old tissues so that the difference in the lymphocyte response to scrapie or normal test antigen (SND) is exaggerated. It may be that the 'new antigen(s)' depend upon molecular rearrangement of membrane structure perhaps induced by an external agent (not necessarily a virus).

Thymectomy in the new-born mouse or rat produces in many cases 'runting' which appears in many ways to be a caricature of ageing. The SND is greatly increased at a very early age by the process of thymectomy, and there is evidence that it may be held up by implantation of neonatal thymus tissue into the deprived animals.

During the first years of life there appears to be the same difference in the constitution of erythrocyte membranes which moves towards the adult type towards puberty. This may be part of a general phenomenon of significance in the physiopathology of childhood.

Finally the analogy between the changes occurring in the kuru–scrapie–CJD complex and old age, and the somewhat new antigenic materials emerging in them must *not* be taken to imply that any virus is per se concerned with the ageing process. No 'virus of old age' is known. It seems to the writer that it is much more likely that ageing (and the diseases mentioned) are associated with membrane steric rearrangement (which can be transmitted—but not necessarily by a classical virus). In the writer's opinion it is a reasonable hypothesis to entertain that as the cell 'ages' and becomes a less favourable habitat for viruses (normally built into its DNA and/or RNA) they emerge, may take on recognizable forms, and indeed may attempt to seek younger and better homes. No doubt we all carry a load of such inapparent 'viruses' which (like our fellow humans) are likely to abandon us in old age. It is a commonplace that we have an *embarras de richesse* of viruses and it may be some of these which have been 'isolated' from old tissues.

The immunological assessment of ageing here described springs, somewhat unexpectedly, from a study of scrapie in sheep, carried out some years ago in relation to the origin(s) of multiple sclerosis (MS). We may begin, therefore, with a brief account of the outstanding features of scrapie, a long known naturally occurring disease of sheep, and accepted as the paradigm of 'slow infections'—the new concept of infection introduced into our thinking by Sigurdsson (1954). An outstanding feature of such infections is that the incubation period is to be reckoned in months or years rather than days or weeks, and may indeed last one quarter, or even one third, of an animal's natural life-span (Figs. 1 and 2). Very recently indeed it has been suggested that in some breeds of mice (see below) the incubation period for the development of overt signs of infection may exceed the natural

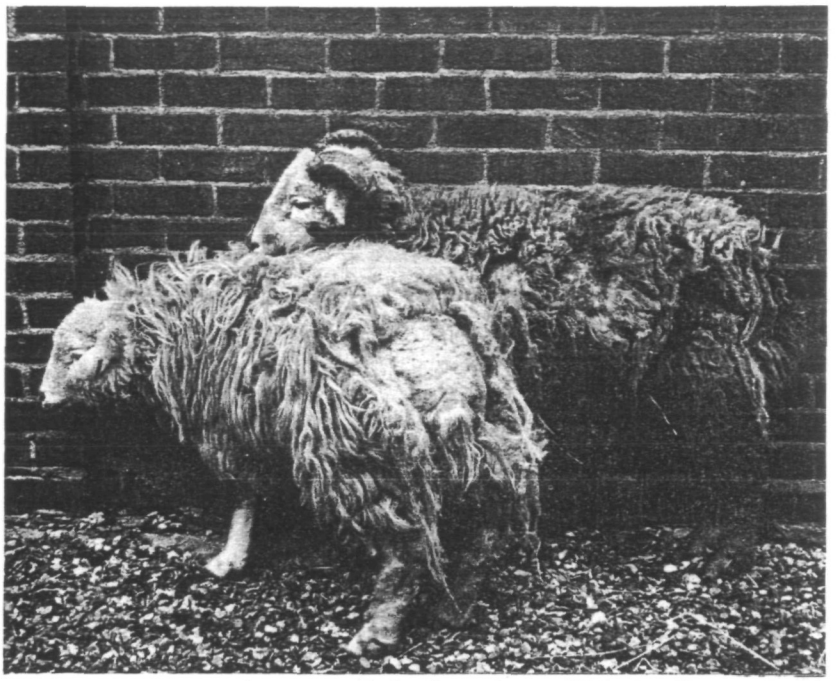

Fig. 1. Scrapie sheep. The rubbing away of the fleece from which the disease takes its name is apparent. The front animal also has adopted an unusual stance.

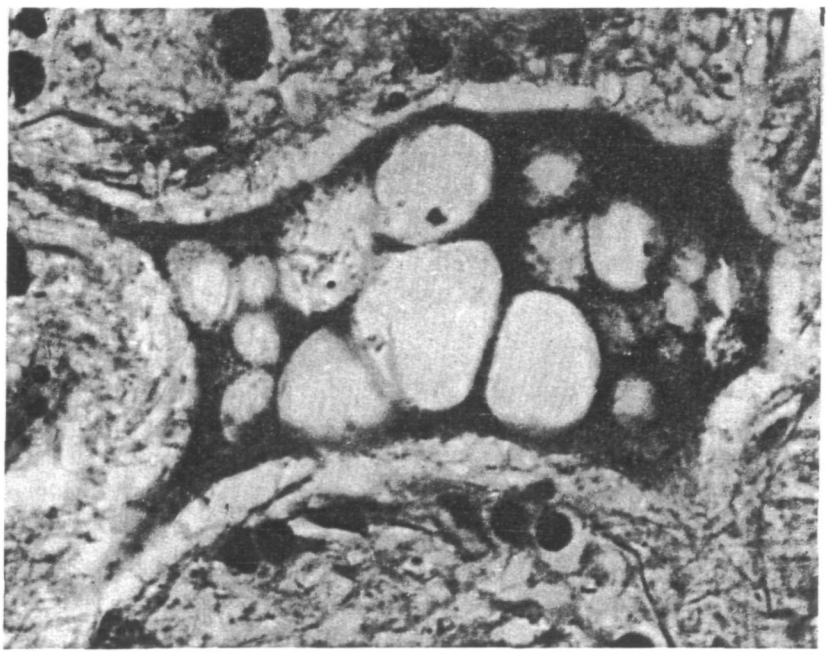

Fig. 2. Anterior horn cell of sheep with natural scrapie showing multilocular vacuolation ('bubble cell'). The Nissl substance is commonly well preserved in such cells and electron microscopy shows the vacuoles to be derived from expanded endoplasmic reticulum sacs (H & E × 800).

life-time of the breed (Dickinson et al. 1975). Certain morphological resemblances have been noted between the changes which take place in the young animal with scrapie and those found in the normal very old animal. Indeed it is almost as if the time ordinate has been compressed during the development of the disease. Chandler (1961) made a notable advance when he transferred scrapie to mice (where the incubation is six months or less instead of 2–5 years in sheep) and much subsequent work has been done in these animals [and also rats—which, too, are susceptible (Chandler & Fisher, 1963)]. All scrapie material used as antigen here described is from the mouse. Moreover, in recent years the resemblance of scrapie to kuru (the disease limited to the Fore people of the Eastern Highlands of Central New Guinea) has been recognized, and indeed since kuru (of which only some 30 cases now appear to be extant) is closely related to, if not indeed identical with, Creutzfeldt–Jakob disease (CJD), the scrapie–kuru–CJD and spongy encephalitides have become linked together as a complex (Gajdusek et al. 1977). Amongst the features of scrapie in the six-months-old scrapie mouse which are prominent in old animals are astro-gliosis (Figs. 3 and 4), amyloid bodies (occurring in clumps), orientated tubules within nerve fibres (in electron-microscopic studies of the cerebellum) and [with some strains of scrapie (Bruce & Fraser 1975)] amyloid-like plaques.

Many unsolved problems still attach to the 'enigma' of scrapie, even presenting a challenge to the Watson–Crick dogma (*The Lancet* 1967), and indeed failure to discover the causal (artificially transmissible) agent has led to suggestions that it may be a self-replicating polysaccharide (Pirie 1966, Field 1967). Pattison & Jones (1967) have pointed out that the fact that scrapie can be passed indefinitely through animals in series does not necessarily mean classical replication of a virus (see comment in *Nature* 1967). The

Fig. 3. Normal old mouse (2 years 3 months). Here the astrocytes are enlarged and stain well. The astrocytes of early scrapie stain like this, too. In the young normal mouse (up to about 9 months) astrocytes stain very faintly with thin wispy and relatively few processes (Gold chloride × 500).

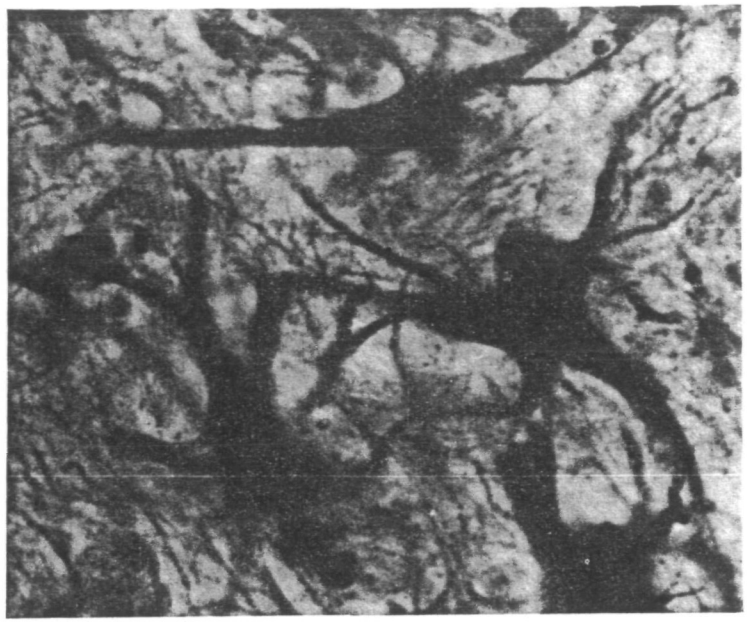

Fig. 4. Astrocytes of advanced scrapie in the rat showing well-marked hypertrophy (Gold chloride ×1024).

'agent' of scrapie is capable of withstanding enormous doses of irradiation (Alper et al. 1967), suggesting a very small target particle possibly too small to comprise an adequate self-replicating mechanism. Recently, Diener (1971, 1974) has introduced the concept of 'viroids' as much smaller agents of infectious disease—apparently short strands of RNA with a molecular weight of 75–100 000 daltons. However, the most interesting and elegant hypothesis put forward is that of Gibbons & Hunter (1967) who postulated that 'the presence of the agent of scrapie in a cell may represent an alteration in the basic three-dimensional configuration of a commonly occurring unit membrane structure. Such an alteration would not necessarily require the introduction of any new molecular component into the cell affected by scrapie'. They suggest that in scrapie there is, in effect, a steric rearrangement of the cell membrane structure. This hypothesis owes much to the model of membrane regulation by colicins proposed by Changeux & Thiéry (1967).

Amongst the many curious properties of scrapie is the repeated observation that (unlike some other slow infective agents) it does not produce antibodies (Chandler 1959, Pattison et al. 1964, Clarke & Haig 1966, Clarke 1968, Gajdusek & Gibbs 1968) though of course the limitations of method must always be borne in mind. However, Gardiner (1965) and Gardiner & Marucci (1969) recorded some collateral observations, which, in retrospect, did not receive the attention and 'follow-up' they deserved. They found that if scrapie brain materials were inoculated into rabbits, they were much better antigen producers than were the corresponding tissues from normal animals. They measured complement-fixing antibodies and went on to suggest that 'some tissue components which are poorly antigenic in the non-scrapie spleen become improved antigenically when in the scrapie spleen'.

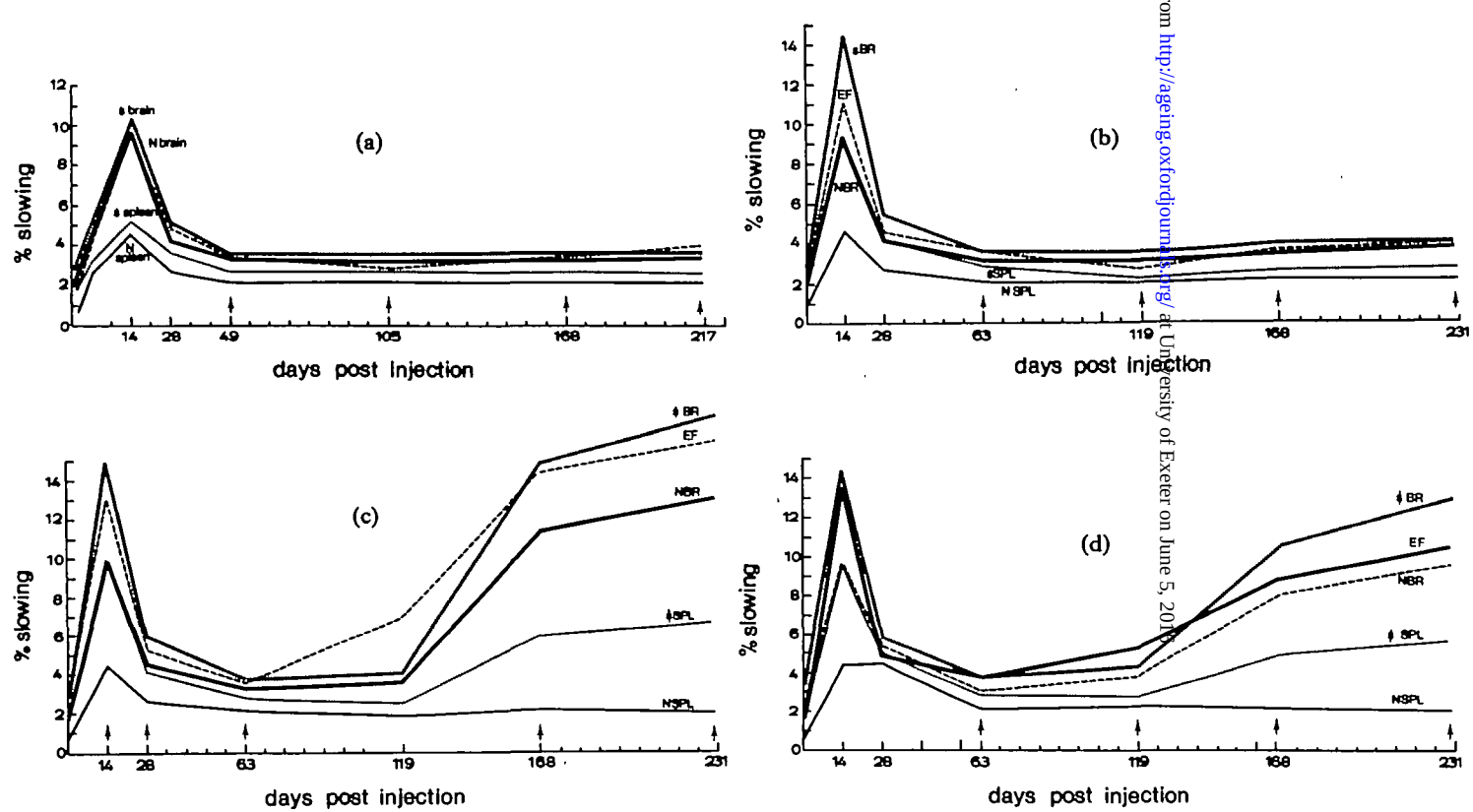

Fig. 5A. Lymphocytes removed from chimpanzee at intervals shown and tested for sensitization against normal brain, scrapie brain (both of mouse), normal and scrapie spleen (mouse) and encephalitogenic factor (EF) made from human brain. S = scrapie. (a) Andy. Normal brain injected into chimpanzee. Note the initial response in which SND (scrapie–normal difference) is small on testing with either brain or spleen. There is no later secondary response as occurs when kuru or Creutzfeldt-Jakob disease establishes itself. (b) Peter. Multiple sclerosis brain injected into chimpanzee (the same multiple sclerosis material as that from which scrapie emerged in Iceland). Note the initial response in which there is a large SND for both brain and spleen antigens. There is also a considerable EF response. There is no secondary response because no infection of slow type has established itself. The large initial SND is seen also in guinea pigs injected with multiple sclerosis brain. (c) Tim. Kuru brain injected into chimpanzee. Note the initial response in which there is a high SND for both brain and spleen as well as an EF response (since destroyed brain was injected). Note the secondary rise in EF response after 63 days—indicative of new brain destruction with auto-sensitization. Note the secondary rise in sensitization to normal and scrapie brain with high SND as infection becomes established. The animals were yet clinically normal. Note that the EF response occurs before the special scrapie response, suggesting that brain destruction has preceded the glial response. (d) Butch. Jakob-Creutzfeldt brain injected into chimpanzee. Note same phenomena as with Tim.

In the case of Tim and Butch, i.e. injection of kuru or Creutzfeldt-Jakob material, infection has established itself with immunological changes. This has not been so with the multiple sclerosis brain injection.

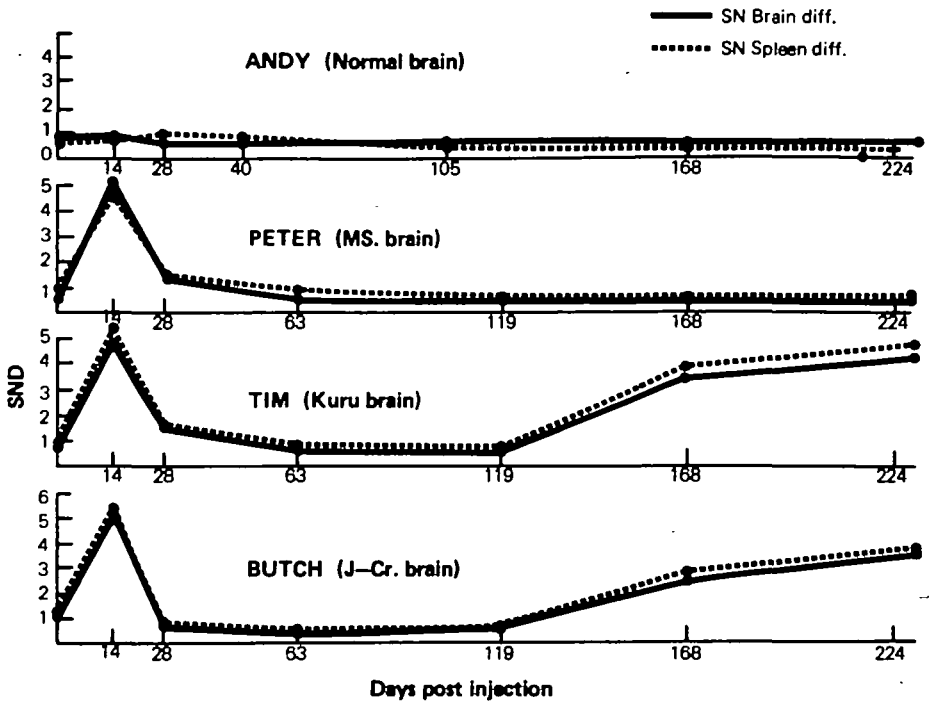

Fig. 5B. Scrapie normal difference in degree of lymphocyte sensitization shown for both brain and spleen at intervals after the original injection. Animals as in Fig. 5A.

Note no initial peak of SND in Andy (injected with normal brain). The initial peak in Peter injected with multiple sclerosis brain is characteristic. The secondary peaks denote the establishment of infection.

All measurements of lymphocyte sensitization throughout these experiments were carried out by Dr B. K. Shenton.

Following the introduction of a new and highly sensitive method of measuring lymphocyte sensitization—the macrophage electrophoretic mobility (MEM) test (Field & Caspary 1970, Caspary & Field 1971, Shenton & Field 1975)—it was decided to test for lymphocyte sensitization to scrapie as opposed to normal mouse brain when normal guinea pigs were injected with emulsions of either, since it had been found that the method possessed exquisite discriminating powers between antigens differing minimally in amino acid make-up (Carnegie et al. 1973).

It was found that if normal brain (or spleen) were used for immunizing the guinea pigs, then the response of the guinea-pig lymphocyte after about 10 days was greater to scrapie brain used as antigen than it was to normal brain—that is, there was a positive scrapie–normal difference (SND). If scrapie brain had been used for immunizing the guinea pigs, then the degree of lymphocyte sensitization to scrapie brain was much greater whilst remaining much the same to normal brain used as test antigen, i.e. the SND was markedly increased. On this basis it was possible to evolve a method of titrating rapidly the scrapie 'content' of mouse or sheep brain in place of the tedious biological titrations with serial dilutions previously necessary (Field & Shenton 1972, 1973*a*, 1974). It was

apparent that as scrapie developed [as a 'slow infection' in Sigurdsson's original (1954) sense], something new appeared in the brain, either scrapie 'agent' or new antigenic material readily recognized by the guinea pigs inoculated with it.

In Newcastle at about this time we had chimpanzees showing the first transmission outside the U.S.A. of kuru and Creutzfeldt-Jakob disease (CJD). Because of their resemblance to scrapie, their lymphocytes were tested with scrapie and normal brain (or spleen). They, too, showed a well marked SND (Fig. 5A and B). Control studies with OND (other neurological diseases) including MS, neurosyphilis, glioma, etc., showed no such increase in SND. A common pathological feature of the scrapie–kuru–CJD is precocious astro-gliosis, and it has been suggested that a new antigenic material makes its appearance in association with the hypertrophied astrocytes (Field & Shenton, 1973*b*), although in other organs there are no special microscopic changes to be seen.

Because of the general resemblance between the scrapie–kuru–CJD on the one hand and the ageing process on the other, attempts were made to see if similar new antigenic material emerged in the tissues during *normal* ageing—as opposed to the seemingly contracted time scale operating in the young animal with scrapie.

Preliminary experiments showed that lymphocytes derived from normal old people showed no increased SND. If new antigens were emerging in the tissues, then they were being recognized as 'self' and no special sensitization to them was occurring. However, it might be different if a guinea pig were injected with aged tissue and challenged to detect a difference between young and old tissue by testing its lymphocytes for SND. The guinea pig, that is, might be able to detect a difference between the antigenic properties of young and old normal tissues, whilst the normal ageing person might not. This, in fact, turned out to be just the case.

Experiments were carried out with both mice and humans. Fig. 6 shows the results in normal mice. It will be seen that with different tissues from mice of increasing age the

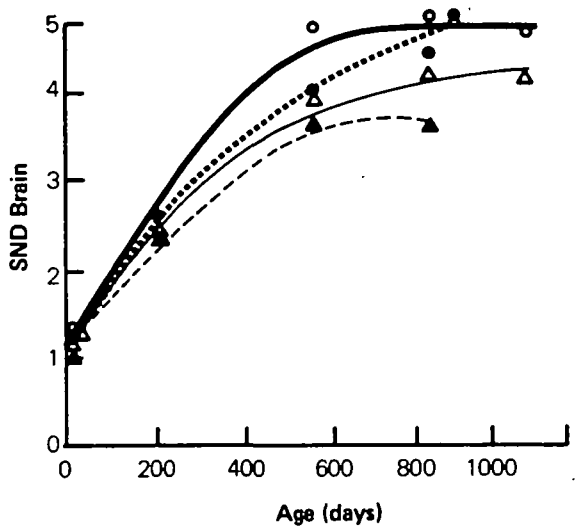

Fig. 6. Guinea pig lymphocyte sensitization to scrapie and normal mouse brain injected with mouse tissues of different ages. – – –=mouse liver injected ●; ———=mouse spleen injected ○; ———=mouse brain injected △; – – – – –=mouse kidney injected ▲.

SND detected in the guinea pig lymphocytes increased. The same increase in SND with age of the tissue injected was found in humans (Table I). For practical purposes a simple human 'biopsy' is an ordinary sample of venous blood. Either red cells (Field & Shenton 1973*c*) or pure lymphocytes (Fig. 7) may be used to inoculate the guinea pigs whose lymphocytes are tested for SND about 10 days later.

Table I. Guinea pig lymphocyte sensitivity to scrapie and normal mouse brain and spleen after immunization with different human female tissues

Age years	*Normal brain*	*Scrapie brain*	*Normal spleen*	*Scrapie spleen*	*Brain SND*	*Spleen SND*
Normal brain injected						
20	9.2	10.8	4.5	5.4	0.9	1.1
30	9.3	10.3	3.9	5.3	1.4	1.0
52	9.1	12.9	5.8	9.4	3.8	3.6
72	9.6	14.3	5.7	11.1	4.7	5.4
91	9.2	13.5	6.0	10.9	4.3	4.9
Spleen injected						
30	4.3	5.3	9.8	10.4	1.0	0.6
91	3.9	10.5	9.3	14.8	6.6	5.5
Heart injected						
30	4.3	5.1	4.3	5.2	0.8	0.9
91	4.2	9.0	4.1	8.4	4.8	4.3
Liver injected						
30	4.3	5.1	4.0	5.0	0.8	1.0
91	4.5	10.2	4.4	10.3	5.7	5.9
Kidney injected						
30	4.2	5.2	4.2	5.0	1.0	0 8
91	3.5	8.8	4.4	9.1	5.3	4.7

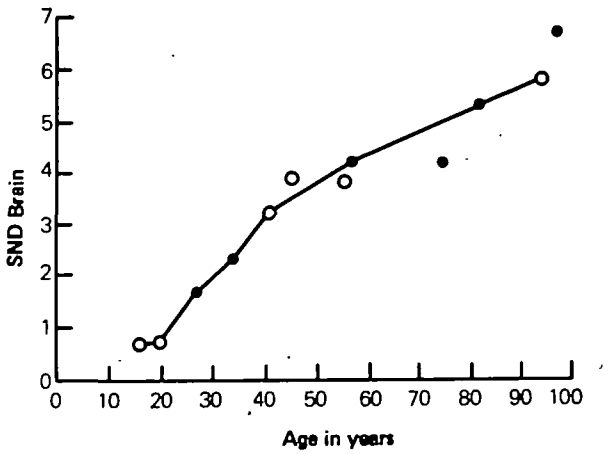

Fig. 7. Guinea pig lymphocyte sensitivity to scrapie and normal mouse brain after immunization with human peripheral lymphocytes. Female lymphocytes ○, male lymphocytes ●.

It may be concluded that with advancing age new antigens appear in both guinea pigs and humans which are similar (or sufficiently similar) to those appearing in young mice with scrapie. These give a cross-reaction which can be quantitated and used as a parameter of ageing. Clearly it would be of great interest to correlate such studies with the results of other ageing assessments and to study examples of alleged extreme old age (e.g. in Soviet Georgia or Ecuador) and factors which might be alleged to 'age' people.

As to the nature of the new scrapie-like antigen appearing as the subject ages, it might be studied by differential absorption on Sepharose–Biogel beads as used by McDermott et al. (1974) for establishing a relation between encephalitogenic factor (EF) and measles virus. It is possible that the 'antigen' will (perhaps like the scrapie agent itself) turn out to be a biochemical–biophysical rearrangement of the surface composition of cells. We know that the common cancer antigen found in all human tumours which exhibit malignant properties (no matter whether the pathologist calls them carcinoma, sarcoma, embryoma, leukaemia, etc.) resides on the *surface* of the malignant cell and not in its endoplasmic reticulum or nuclear membrane (Dickinson et al. 1972). Like cancer, the ageing problem may well turn out to be a cell surface membrane problem, a topic on which ideas are currently very fluid (Chapman 1975).

Since thymectomy in neonatal mice and rats leads in many cases to 'runting', in some respects a caricature of ageing, it seemed of interest to study the effect of the operation upon the SND described above. Tissues from animals which had been thymectomized (or had undergone 'sham' operation) were injected after the lapse of progressive intervals into guinea pigs whose lymphocytes were tested for SND after 10 days. It was found that the SND rose very rapidly after neonatal thymectomy (Fig. 8). Indeed by 14 days the SND in a thymectomized mouse was that attained at about 300 days in the normal animal (Field & Shenton, 1973*d*). The changes occurred in all the tissues though at somewhat

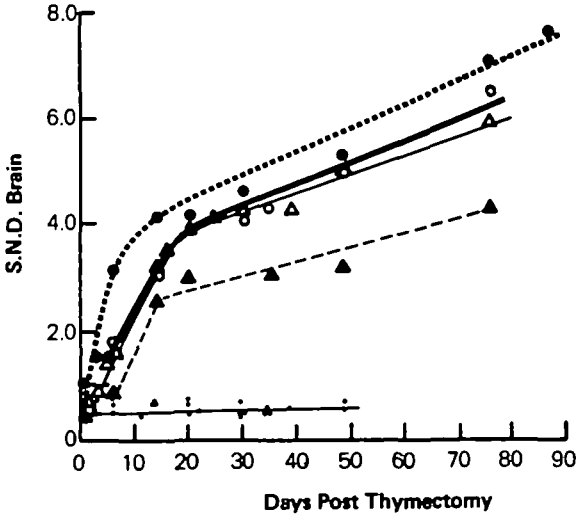

Fig. 8. Relation of SND of lymphocytes from guinea pigs inoculated 8 days previously with tissues from thymectomized mice. - - - - = mouse liver injected ●; ——— = mouse spleen injected ○; ——— = mouse brain injected △; - - - - = mouse kidney injected ▲.

differing rates; this is true of the human tissues, too, mentioned above. Intraperitoneal implants of new-born mouse thymus into neonatally thymectomized animals appeared to hold up the ageing process (Field & Shenton 1973*e*) (Table II).

There have been many suggestions that ageing may be in some way linked with immunological alterations (Burnett 1970, 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 emphasize

Table II. Ageing prevention by thymic implants in mice

Age of mouse	*Spleen injected*	*Brain injected*	*Liver injected*	*Kidney injected*
44	4.25			
85	6.7			
64*		4.3 (5.5)†	5.0 (6.1)	2.8 (3.7)
85*		4.2 (6.3)	5.1 (7.6)	

* Animals which had received thymus implants or cells.

† Figures in brackets are expected values.

Note that animals which received thymic implants have lower SND with all tissues tested than those without thymic implants.

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. Clearly this is allied to the 'trophic function' of lymphocytes, proposed long ago but much neglected of recent years (Hewson 1773, Loutit 1962, Shields 1972).

It may be noted in passing that quite apart from its function in regulating the ageing process in some way, the thymus has a more immediate endocrine activity through its secretion of 'thymosin' which 'opsonizes' T lymphocytes and enables them to recognize all manner of antigens (Field 1976).

Finally, another phenomenon has recently been discovered at the other end of the life span and again involving ageing. In a recent study of the effect of PGE_2 (an arachidonic acid-based prostaglandin), it was found that the RBC of normal children showed great increase in absolute electrophoretic mobility in the presence of minute concentrations of PGE_2 (down to 1.95 pg/ml). Umbilical cord venous blood was most responsive to PGE_2, but this responsiveness persisted throughout childhood and from about the age of 10 onwards moved towards the adult pattern—where 31.25 pg/ml causes a speeding up and 15.625 pg/ml is without effect. These observations indicate a different biochemical–biophysical make-up of RBC in children with a resulting excessive avidity for PGE_2. Not enough is known of the molecular biology of membranes (see, e.g., Weissmann & Claiborne's 1975 monograph) to attempt any fruitful explanation at this level, but it is of considerable interest that MS patients who have been on a prolonged course of gamma linolenate show the same increased mobility of their RBC in the presence of low concentrations of PGE_2. The significance of this is beyond the scope of the present paper, and we have not yet established whether normal subjects so treated similarly acquire a 'child-like' response by their RBC.

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