to a minimum in the interspindle lulls and reached a maximum during the spindles. When synchronized sleep was replaced by the desynchronized phase, the peaks in the pyramidal record disappeared; and the corticospinal activity became stabilized to a level similar to that reached during the interspindle lulls, provided the REM were absent. As soon as these ocular phenomena occurred, the pyramidal activity increased phasically and reached a level much higher than that seen during the spindle trains (Figure 1). These experiments, together with the demonstration that the ablation of both the motor and sensory areas of the cortex prevented the appearance of changes in pyramidal activity related with the different states of sleep and wakefulness, indicate that during desynchronized sleep there is a phasic increase in the corticofugal discharge which also originates from the somatosensory areas of the cortex (S_I and S_{II}).

In the decerebrate cats stimulation of the pyramidal tract as long as 49 days after ablation of the motor cortex, i.e. when the corticofugal fibres originating from the motor cortex had degenerated and could no longer be excited, produced a ventral root discharge which was strikingly reduced with respect to controls taken in the intact animal. The dorsal root potentials elicited by pyramidal stimulation, on the other hand, resembled those obtained in the control experiment in both size and shape (Figure 2). The fact that outbursts of pyramidal activity still occur synchronously with the REM after degeneration of the corticospinal fibres arising in the motor cortex, suggests that presynaptic inhibitory volleys originate from the sensory areas S_I and S_{II} during the REM periods of desynchronized sleep. They may at least contribute to the phasic depression of the polysynaptic reflex that occurs at the time of the bursts of REM[7].

Riassunto. Nel sonno desincronizzato si osserva un aumento della scarica piramidale originante dalle aree somato-sensoriali della corteccia in corrispondenza dei REM. Queste scariche piramidali depolarizzano le fibre afferenti primarie della via riflessa flessoria e verosimilmente contribuiscono alla depressione fasica dei riflessi polisinaptici che si manifesta durante i REM.

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[7] M. M. Gassel, P. L. Marchiafava, and O. Pompeiano, Rend. Accad. naz. Lincei, Cl. Sci. fis., mat. nat., Ser. VIII, *36*, 543 (1964); Arch. ital. Biol. *102*, 471 (1964).

On the Possibility that Thymus-Mediated Alloantigenic Stimulation Results in Tolerance Response

It now seems firmly established that thymus occupies a privileged position among other lymphoid organs (Miller et al.[1]). The various hypotheses as to whether thymus is an essential source of cells for other lymphoid organs or provides a humoral factor or a local environment required for functional maturation of cells originating elsewhere[2–7] may be complementary rather than contradictory.

One of the features distinguishing thymus from other lymphoid organs is the absence of antibody formation and of morphological symptoms of activation[8–10] which characterizes the response to antigenic stimulation in spleen and lymph nodes. This might simply be due to some sort of afferent blood-thymus bar which prevents extrinsic antigens from entry into the intact organ[11,12]. Especially, this characteristic of thymus led Burnet[2] to place in it a hypothetical mechanism which would ensure immunological tolerance to 'self-antigens'. It was assumed that immunological tolerance results from a collision of an immature lymphoid cell with the appropriate antigen[13,14]; if the 'vulnerable' phase of development of lymphoid cells coincided with their sojourn in thymus, where only autoantigens seem to be available, the potentially self-reactive clones might there be automatically filtered off and prevented from further development to reactivity. Pathological changes of an autoimmune nature following thymectomy[15,16], and some data on the role of thymus in autoimmune conditions[17,18], may be interpreted in favour of this concept even though a different explanation of some of the findings may be preferred[19].

We wished to check this hypothesis by assuming that the respective mechanism might be 'misused' for non-self-antigens if these were artificially introduced into the thymus of adult animals. However, the cell population in an adult thymus seems to involve also a proportion of functionally mature cells, as indicated by the findings of antibody formation in thymus injected with various

[1] J. F. A. P. Miller, Adv. Immunol. *2*, 111 (1962).
[2] F. M. Burnet, Aust. Ann. Med. *11*, 79 (1962).
[3] J. F. A. P. Miller, Proc. Roy. Soc. B, *156*, 415 (1962).
[4] D. Osoba and J. F. A. P. Miller, Nature *199*, 653 (1963).
[5] R. H. Levey, N. Trainin, and L. W. Law, J. Nat. Cancer Inst. *31*, 199 (1963).
[6] C. E. Ford, and H. S. Micklem, Lancet *1963 i*, 359.
[7] J. E. Harris, C. E. Ford, D. W. H. Barnes, and E. P. Evans, Nature *201*, 884 (1964).
[8] M. Björneboe, H. Gormsen, and F. Lundquist, J. Immunol. *55*, 121 (1947).
[9] A. Fagraeus, Acta med. scand. *130*, Suppl. 204, 3 (1948).
[10] T. N. Harris, J. Rhoads, and J. Stokes, J. Immunol. *58*, 27 (1948).
[11] A. H. E. Marshall and R. G. White, Brit. J. exp. Path. *42*, 379 (1961).
[12] S. L. Clark jr., Am. J. Anat. *112*, 1 (1963).
[13] F. M. Burnet, *The Clonal Selection Theory of Acquired Immunity* (Cambridge University Press, 1959).
[14] J. Lederberg, Science *129*, 1649 (1959).
[15] J. de Vries, L. M. van Putten, H. Balner, and D. W. van Bekkum, Rev. Franç. Étud. Clin. Biol. *9*, 381 (1964).
[16] D. E. R. Sutherland, O. K. Archer, R. D. A. Peterson, E. Eckert, and R. A. Good, Lancet *1965 i*, 130.
[17] F. M. Burnet and M. C. Holmes, Nature *194*, 146 (1962).
[18] M. Vojtíšková and Z. Pokorná, Lancet *1964 i*, 644.
[19] I. F. Loutit, Brit. J. Radiol. *36*, 785 (1963).

antigens[11,20]. Detection of the masked tolerance response would thus require some ancillary measure. We used sublethal irradiation whose function might be double: to facilitate the maintenance of a necessary level of the tolerance inducing antigen while the replacement of the reactive population takes place[21], and to render the adult recipient more thymus-dependent than normal[19,22].

As it is extremely difficult (if not impossible) to inject an exact volume of antigen (spleen cell suspension) into mouse thymus in situ, it was inoculated into an excised isogenic thymus which was then grafted under the skin in the thymic region of thymectomized recipients of the same age. Recipients (male mice of the inbred strain B10.LP differing only at the H-3 locus from the donor strain C57BL10/ScSn, further only B10) were submitted to some or all of the following procedures: thymectomy in 4 weeks, exposure to 400 R of Co-60 from Theratron B (5 weeks), grafting thymus, whose donor was either non-irradiated or irradiated at the same time as recipient, and antigen administration ($2 \cdot 10^7$ of B10 spleen cells) in 6 weeks and skin grafting test in 13 weeks. The results are presented in the Table. A long-lasting tolerance was induced in most of the irradiated animals when antigen was introduced into the preirradiated (group 1) or non-irradiated thymic isograft (group 2), but also when the antigen was given subcutaneously outside the irradiated thymic isograft (group 3). This result seems to depend on two main factors, neither of them being sufficient alone (groups 5, 6, 7): irradiation of the recipient (but not necessarily of thymus donor) and specific antigenic pretreatment. (Non-specific skin grafts B10.BY differing from the antigenic inoculum in two weak antigens of the H-1 and H-3 locus were rejected at a normal rate in 11 mice out of 12. Prolonged retention of one non-specific graft might be due to the failure to take of the thymic isograft in the given mouse.) It does not appear to be critical whether the antigenic spleen cell suspension is given inside or outside thymus. The lower incidence of tolerant animals in group 4 seems to indicate that the manipulation with the thymic isograft in the first three groups might have facilitated the effect of antigen. This point remains to be clarified.

Our working hypothesis might be reconciled with the present results by assuming that in this particular experimental situation immunological tolerance can be induced in adult mice by procuring the entry of antigen in thymus, either by its direct inoculation or in an indirect way. Is there any evidence to indicate that the sublethal irradiation of the recipient might be held responsible for such

[20] J. D. SHERMAN, M. M. ADNER, and W. DAMESHEK, Proc. Soc. exp. Biol. Med. *115*, 866 (1964).
[21] A. LENGEROVÁ and M. POLÁČKOVÁ, Folia biol., Prague *9*, 189 (1963).
[22] J. F. A. P. MILLER, Nature *195*, 1318 (1962).

Scheme and results of the experiment on the possible role of thymus in the induction of transplantation tolerance

Group	Pretreatment of the B10.LP recipient of skin graft				Reaction to test skin graft	
	Irradiation 400 R		B10 cell injection as to thymus	Origin of thymus	Specific (B10)	Non-specific (B10.BY)
	Host	Thymus				
1	+	+	intra	isogenic heterotopic graft	tolerance > 220 days (8 of 10)	
2	+	–	intra	isogenic heterotopic graft	tolerance > 220 days (9 of 10)	
3	+	+	extra	isogenic heterotopic graft	tolerance > 120 days (12 of 12)	normal rejection (11 of 12)[a]
4	+	+	extra	autologous (no manipulation)	normal rejection (13 of 20) tolerance > 120 days (7 of 20)	normal rejection (8 of 8)
5	–	–	intra	isogenic heterotopic graft	normal rejection (12 of 12)	
6	+	+	none	isogenic heterotopic graft	normal rejection (12 of 12)	normal rejection (12 of 12)
7	–	–	extra	autologous (no manipulation)	normal rejection within 19 days (29 of 29)[b]	
8	–	–	none	autologous (no manipulation)	normal rejection within 19 days (39 of 39)	normal rejection within 19 days (20 of 20)

[a] Prolonged survival (> 40 days) in 1 of 12 mice. [b] With a four week interval between the antigenic pretreatment and skin grafting, sensitivity was no longer detectable.

an indirect effect? We are inclined to believe that there is. The blood-thymus barrier does not interfere with the traffic of syngenic cells between, for example, bone marrow and thymus[7]. A cell of foreign origin but with a similar inclination as a syngenic stem cell might thus participate in this traffic and act at the same time as a vehicle for its tolerance-inducing antigens, if it were not detected as foreign before entering thymus. Such a condition could be met if the antigen recognition mechanism of the recipient were not yet developed or temporarily suppressed, for example by irradiation. The ease with which transplantation tolerance is induced during the perinatal period, the findings in the thymus of antigenically different cells originating from spleen cell inocula which had been used to induce either tolerance in new-born[23] or recovery in lethally irradiated adult mice[24], and the resumed thymus-dependency of irradiated adult animals[25] appear compatible with such a view even though a cause-effect relationship between the presence of cellular antigen in thymus and the tolerance pattern of host response cannot be proved so far. This question is being further studied.

Résumé. La tolérance de transplantation a été induite dans une combinaison antigéniquement faible du donneur et receveur par l'irradiation subléthale et le prétraitement antigénique spécifique chez la souris adulte. Les résultats obtenus sont compatibles avec l'idée que la fonction hypothétique du thymus lors du développement de l'autotolérance puisse jouer un rôle même dans la tolérance expérimentelle envers l'antigène étranger autant que cet antigène est permis d'entrer, sur quelque principe, dans le thymus.

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[23] J. J. TRENTIN and J. SESSION, in *Mechanisms of Immunological Tolerance* (Prague 1962), p. 31.
[24] C. E. FORD, P. L. T. ILBERRY, and J. F. LOUTIT, J. cell. comp. Physiol. *50*, 109 (1957).
[25] J. F. A. P. MILLER, S. M. A. DOAK, and A. M. CROSS, Proc. Soc. exp. Biol. Med. *112*, 785 (1963).

Hämatologische Untersuchungen am Spitzhörnchen *Tupaia tana* (Raffles 1821) (*Tupaiidae, Prosimiae*)

Die Spitzhörnchen (Tupaiidae), kleine in Südostasien beheimatete Säugetiere gewinnen wegen ihrer basalen Zwischenstellung zwischen Insektivoren und Halbaffen (Prosimiae) zunehmend an wissenschaftlichem Interesse. Nach der vorherrschenden Meinung werden sie als Infraordo Tupaiiformes[1] der Subordo Prosimiae zugerechnet, wobei sie den Insektenfressern, aus denen sich die Primaten entwickelt haben, noch sehr nahe stehen. Andere Autoren stellen sie als Subprimaten noch näher zu den Insektivoren, unbestritten bleibt jedoch ihre wichtige Zwischenstellung. Diese hat inzwischen zu einer Reihe von anatomischen, biologischen und ethologischen Untersuchungen geführt[2], zumeist an der Art *Tupaia glis* Diard 1820. Hämatologische Untersuchungen sind uns bei einer Literaturanalyse[2] bisher nicht bekannt geworden, es sei daher über erste diesbezügliche Befunde berichtet.

Zur Untersuchung gelangte ein Pärchen adulter Tiere von *Tupaia tana* (Raffles 1821), das über den Tierhandel in juvenilem Zustand aus Thailand bezogen worden war. Zum Vergleich wurde als Vertreter der Insektivoren ein fast erwachsenes Männchen des Igels (*Erinaceus europaeus* L.) mit herangezogen; die jeweils erhobenen Befunde wurden den Normwerten bei erwachsenen Menschen gegenüber gestellt. Die Blutentnahme erfolgte beim Spitzhörnchen aus dem caudalen Venenplexus unterseits etwa in der Mitte des Schwanzes, beim Igel an der Aussenseite des Unterschenkels der hinteren Extremität.

Beim Spitzhörnchen ist die Erythrocytenzahl im Vergleich zum Menschen relativ hoch, sie beträgt 5,5 bis 6 Millionen/mm³ (Mittel 5,8 Mill.). Dem entspricht auch der durchschnittlich über 45% liegende Hämatokritwert (Normalwert beim Menschen zwischen 42 und 45%). In diesem Zusammenhange sei erwähnt, dass nach neueren Untersuchungen[3] an Populationen von Wildsäugern bei systematisch nahestehenden Arten eine Korrelation zwischen Erythrocytenzahl und Körpergewicht besteht, wobei die Zahl der roten Blutkörperchen mit abnehmendem Körpergewicht zunimmt. Der Hämoglobingehalt des einzelnen Erythrocyten liegt bei *Tupaia tana* mit 29,3 $\gamma\gamma$ unter dem Normwert des Menschen (32,0 $\gamma\gamma$). Entsprechend finden sich bei der panoptischen Färbung nach Pappenheim häufig Hypochromasie, Polychromasie und ganz vereinzelt auch basophile Tüpfelung der Erythrocyten. Hinsichtlich Grösse und Form sind keine wesentlichen Abweichungen im Vergleich zum Menschen zu beobachten, eine erheblichere Anisocytose oder Poikilocytose besteht nicht. Makrocyten, Megalocyten oder kernhaltige Vorstufen konnten im peripheren Blutbild nicht gefunden werden.

Beim Igel beträgt die Erythrocytenzahl im Mittel 5 Millionen/mm³, das Gesamthämoglobin und damit auch der Hb-Gehalt des einzelnen Erythrocyten liegt deutlich über dem des Menschen. Auffällig ist die starke Grössenschwankung (neben Zellen mit 7 μ ⌀ relativ häufig Makro- und Megalocyten) sowie das Vorkommen von Formverschiedenheiten (Poikilocytose) bei den roten Blutkörperchen. Dabei fanden sich – ähnlich wie beim *Tupaia tana* – deutlich ausgeprägt Polychromasie und basophile Tüpfelung der Erythrocyten. Da jedoch der Igel als Insektenfresser während des Winters in einen

[1] W. FIEDLER, in *Primatologia I* (Basel-New York 1956), vol. I, p. 1. – E. THENIUS und H. HOFER, *Stammesgeschichte der Säugetiere* (Springer Verlag, Berlin-Göttingen-Heidelberg 1960).
[2] I. LEICHT, unpubl. Literaturstudie (Würzburg 1963).
[3] P. B. DUNAWAY et al., Oak Ridge National Lab. Publ. 107, ORNL-3697, 79 (1964).