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An Effect of Thymectomy on Recovery from Immunological Paralysis

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Summary. Mice were immunologically paralysed with two protein antigens, and then either thymectomized or sham-operated in adult life. Recovery from paralysis was much impaired by thymectomy, but was not abolished. This result is discussed in relation to the function of the thymus, and to possible mechanisms of recovery from paralysis.

INTRODUCTION

It is generally thought that the maintenance of immunological paralysis (or tolerance) requires the persistence (or continual re-administration) of the inducing antigen. For example, paralysis induced in CBA mice with bovine serum albumin (BSA) can be maintained indefinitely by frequent injections of antigen, but if these injections are stopped the mice gradually regain responsiveness over a period of weeks. It may be assumed that the first part of this period is occupied by the degradation of extra- and intra-cellular antigen down to a critical level. The succeeding course of events is problematical. It has been supposed (Medawar, 1960) that the degradation of intracellular antigen continues until eventually the paralysed cell loses antigen from some controlling site, and thereby becomes reactive. But it might be thought that such a simple biochemical process could hardly, by itself, occupy the length of time which is required for the loss of paralysis in older animals. For example, loss of paralysis to homologous erythrocytes in adult chickens may not be complete even 1 year after elimination of extracellular antigen (Mitchison, 1962). Again, in mice, the rate of loss of paralysis to BSA varies with age, while the rate of degradation of extracellular antigen remains constant (Mitchison, 1963). It would be easier to account for these facts if degradation of antigen was not the only factor controlling the recovery from paralysis. Turnover of lymphoid cells is likely to be important, especially since in situations where this is accelerated, such as in young animals or during recovery from irradiation (Mitchison, 1962; Mäkela and Nossal, 1962), loss of paralysis is also hastened. Lymphoid cell turnover appears to be largely dependent on the thymus, since the presence of this organ is required for the growth of the lymphoid system, and the repair of this after irradiation (Miller, Marshall and White, 1962). Although thymectomy in adult life has no immediate effect on immunological responsiveness it is reasonable to expect that the thymus continues to function in the adult, even without the stimulus of irradiation. And if thymectomy in adult life slowed or prevented recovery from paralysis this expectation would be borne out and weight would be added to the argument that recovery from paralysis involves lymphoid cell turnover. To test this, paralysis was induced

in mice by injection of protein antigen alone, while the recovery of responsiveness was followed by measuring the antibody response to challenge with the antigen plus adjuvant at intervals subsequently. The rate of recovery in control mice was compared with that in mice thymectomized soon after induction of paralysis.

MATERIALS AND METHODS

CBA mice were used. The antigens were bovine serum albumin (BSA) and bovine γ-globulin (BGG), both supplied by Armour & Co. For paralysis BSA was made up to 25 mg./ml. in distilled water. BGG was made up to 2·5 mg./ml. in 0·9 per cent NaCl, and was centrifuged at 20,000 ***g*** for 30 minutes before being injected. For challenge BSA was incorporated in Freund's adjuvant (BSA Freunds) and injected as described by Dresser (1960). Sheep erythrocytes were washed three times in phosphate buffered saline and 0·1 ml. of a 10 per cent suspension injected intraperitoneally. Mice were bled 7 days later, and sera titrated as described by Makinodan, Gengozian and Congdon (1956). BGG was adsorbed on alum precipitate by the method of Proom (1943). The adjuvant effect of alum was enhanced by adding an equal volume of *Bordetella pertussis* vaccine containing 2×10^{10} bacterial cells per ml. (Burroughs Wellcome & Co.) (Dresser, 1964). The mixture was stored with 1:10,000 methiolate at 4° and 0·2 ml. injected intraperitoneally. Mice challenged with BSA Freunds were bled 20 and 40 days later; the serum was titrated for antigen binding capacity by a modification of the method of Farr (1958). The result of challenge with BGG Freunds was tested by antigen-elimination and haemagglutination techniques. In the elimination test 2 mg. of [^{131}I] BGG were injected intraperitoneally 28 days after challenge and the rate of elimination of labelled antigen from the circulation was followed as described by Dresser (1962a). The BGG was labelled by the iodine monochloride method of Macfarlane (1958). Ten days after the end of the elimination test the mice were bled again and the sera titrated by a haemagglutination method developed by Dresser (unpublished) from a suggestion by Dr. R. Ceppelini. Since commercial BGG contains minor immunizing components, besides the main paralysing component (Dresser, 1961b), the tanned cell agglutination procedure (Boyden, 1951) cannot be relied on to indicate paralysis. In the present method mouse erythrocytes were sensitized with bovine anti-mouse-erythrocyte antibody. This antibody appears to consist entirely of the paralysing component, since cells sensitized with it were not agglutinated by serum from mice paralysed with BGG. In order to prevent direct agglutination by the bovine antibody the mouse cells were first coated with a Vi antigen ('Vismigen', Istituto Sieroterapico Milanese) before being exposed to the bovine antibody. The action of such bacterial antigens as inhibitors of agglutination is described by Ceppelini and Landy (1963). For thymectomy, mice were injected first with atropine (0·01 ml./g. body weight of a 0·01 per cent solution in saline), then anaesthetized with Avertin (Winthrop Laboratories, New York) (0·01 ml./g. body weight of a 2 per cent solution in saline with 5 per cent ethanol). The thymus was removed through a median sternal incision by suction with a glass pipette.

EXPERIMENTS AND RESULTS

RECOVERY FROM PARALYSIS TO BOVINE SERUM ALBUMIN (BSA)

CBA male mice were paralysed by intraperitoneal injections of BSA at 250 μg./g. body weight, three times a week from the day of birth, for a total of 14 weeks. This

induced virtually complete paralysis, as shown by the production of less than 1 per cent of the normal quantity of antibody after challenge with BSA Freunds. Half of these mice were thymectomized 21–23 days after the last injection. Groups consisting of six thymectomized and six control mice were challenged at the intervals 0, 4, 12, 17, 20 and 24 weeks after the last paralysing injection. In addition, one group which had been neither paralysed

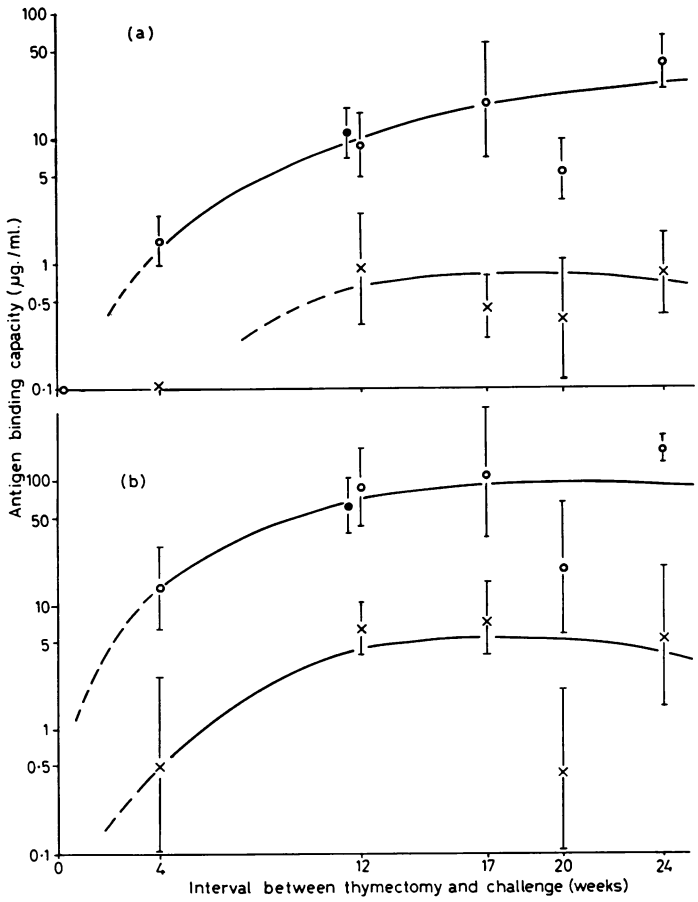

FIG. 1. These graphs illustrate the effect of thymectomy in retarding recovery from paralysis to BSA. Each point shows the mean antigen binding capacity, with Standard Deviation, of the sera from a group of five to eight mice, bled 20 days after challenge (a), and 40 days after challenge (b). Mice were first paralysed by multiple injections of BSA, then half of them were thymectomized. Recovery from paralysis was then followed by challenge with BSA Freunds at intervals up to 24 weeks after thymectomy. ×, Paralysed, thymectomized; ○, paralysed, non-thymectomized; ●, non-paralysed, non-thymectomized.

nor thymectomized was challenged with the 12-week group. The mice were bled at 20 and again at 40 days after challenge, and the sera titrated for BSA-binding capacity. The results are shown in Fig. 1. The non-thymectomized mice were beginning to lose paralysis at 4 weeks, and had regained normal responsiveness by 12 weeks after the last paralysing injection. By contrast, recovery in the thymectomized mice was much slower,

and had ceased by 12 weeks, the titres remaining thereafter at about 3 per cent of the control levels.

Finally, the specificity of paralysis was tested in three of the groups (17, 20 and 24 week groups) by challenge with sheep blood cells. The responses, assessed by agglutination, were not significantly different as between thymectomized and control mice (Table 1). Although a non-paralysed thymectomized group was not included in this experiment, in other experiments mice thymectomized when 3 months old have retained almost normal responsiveness to BSA for at least 12 weeks (Taylor, unpublished data), indicating that the poor responses obtained in the thymectomized groups were not due to non-specific unresponsiveness such as is seen after thymectomy in early life.

TABLE 1

THE EFFECT OF CHALLENGE WITH SHEEP RED CELLS 31 WEEKS AFTER THE LAST PARALYSING INJECTION OF BSA

	No. of mice in group	*No. of mice producing serum agglutinating to the following titres:*				
		1/160	1/320	1/640	1/1280	1/2560
Thymectomized	13	1	1	5	4	2
Non-thymectomized	16	—	—	4	8	4

RECOVERY FROM PARALYSIS TO BOVINE γ-GLOBULIN (BGG)

To induce paralysis with this antigen it is not necessary to begin injections at birth, since a single injection of BGG (freed from particulate matter) is sufficient to paralyse an adult mouse (Dresser, 1962b). Forty-eight 3-months-old CBA male mice were each injected with 250 μg. of centrifuged BGG and a further thirty-two were left as non-paralysed controls. Half of the paralysed and half of the control mice were thymectomized 12–15 days after this injection. Equal numbers of thymectomized and control mice, among the paralysed group, were challenged at 3, 12 and 24 weeks after the paralysing injection, while the non-paralysed controls were challenged at 3 and 24 weeks only. The first challenge, with BGG Freunds, resulted in erratic responses in the non-paralysed controls. For this reason alum-precipitated BGG+*B. pertussis* was used in the groups challenged subsequently.

The elimination response obtained by injecting 2 mg. of [^{131}I] BGG into CBA mice has been calibrated by Dresser (1961a). He gave regular injections of immune mouse serum into non-immunized mice in order to simulate different rates of antibody production. The elimination curves which he obtained (Fig. 2—categories 0–4) represent five arbitrary categories of response. The interval between each category represents a four-fold increase in the rate of administration of antiserum. Two additional uncalibrated categories have been added here to cover the highest responses.

The results of the eliminations have been classified according to this scheme (Table 2). Unfortunately, since BGG Freunds was used for the first challenge at 3 weeks after thymectomy, the results of this are not strictly comparable to the others, in which alum-precipitated BGG+*B. pertussis* was used. The results shown in Fig. 3 and Table 2 agree, in general, with those obtained with BSA as the antigen. On closer comparison it may be

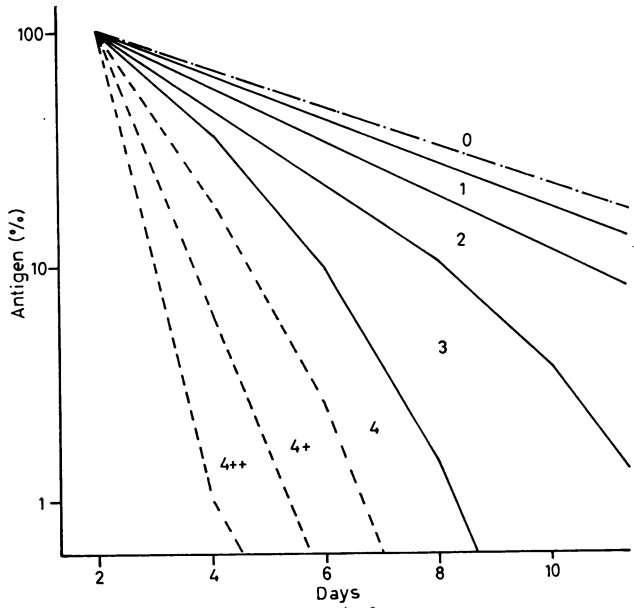

FIG. 2. This diagram illustrates the seven categories of antigen-elimination response which have been used to classify the experimental results (see Table 2). ———, Lines separating categories obtained by Dresser's passive immunization experiments; - - - - -, lines separating additional arbitrary categories; —·—·—, mean rate of antigen elimination in fully paralysed mice, for comparison.

TABLE 2

ANTIGEN ELIMINATION RESPONSES ILLUSTRATING THE EFFECT OF THYMECTOMY IN RETARDING RECOVERY FROM PARALYSIS TO BGG

Interval between thymectomy and challenge (weeks)			*No. in group*	*No. of mice responding within each of the following categories:*						
				0	1	2	3	4	4+	4++
3	Paralysed	Tx*	7	7	—	—	—	—	—	—
		Sh	7	3	4	—	—	—	—	—
	Not paralysed	Tx	6	—	—	2	2	2	—	—
		Sh	6	—	—	1	3	2	—	—
12	Paralysed	Tx	7	—	4	3	—	—	—	—
		Sh	6	—	—	3	2	—	1	—
24	Paralysed	Tx	8	—	—	—	3	4	1	—
		Sh	7	—	—	—	—	2	5	—
	Not paralysed	Tx	5	—	—	—	—	—	—	5
		Sh	6	—	—	—	—	—	—	6

Tx* = thymectomized; Sh = sham-operated.

noted in the BGG experiment that recovery from paralysis in the non-thymectomized mice was much slower, while the differences between the responses of these and the thymectomized groups appeared to be less than in the BSA experiment. The results of the haemagglutination (Table 3), however, indicate much less recovery on the part of the thymectomized mice than would appear from the elimination results. They emphasize the fact that recovery from BGG paralysis, even with the thymus intact, is far from complete at 24 weeks. The responses of the non-paralysed controls, challenged either at 3 or 24 weeks (Fig. 3 a and c) show that thymectomy alone has only a marginally depressive effect on the response.

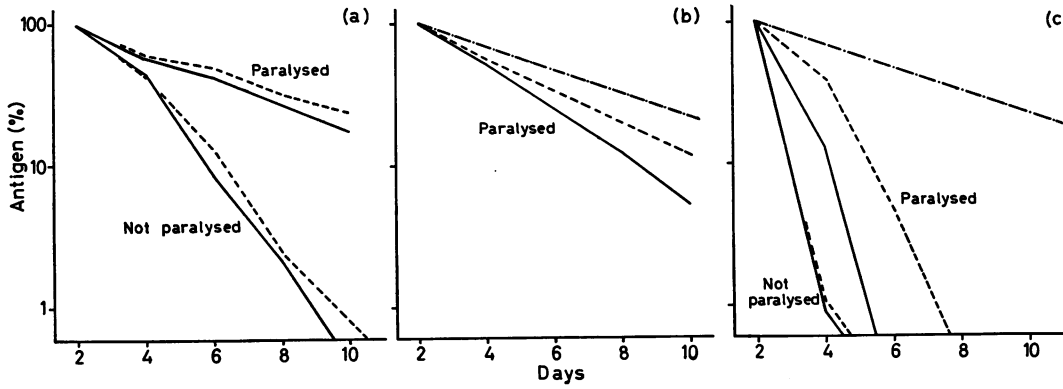

FIG. 3. These graphs illustrate the elimination responses listed in Table 2. Each line represents the mean elimination response for a group of five to eight mice. The graphs show the results of challenge at 3 weeks (a), 12 weeks (b), and 24 weeks (c) after thymectomy. - - - - -, Thymectomized groups; ———, non-thymectomized groups; —·—·—, mean rate of elimination in fully paralysed mice, for comparison.

TABLE 3

SERUM ANTI-BGG AGGLUTINATION TITRES FROM THE GROUP OF MICE CHALLENGED 24 WEEKS AFTER THYMECTOMY (SEE TABLE 2)

		No. of mice in group	*No. of mice producing serum agglutinating to the following titres:*								
			>1/8	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
Paralysed	Tx*	8	3	4	1	—	—	—	—	—	—
	Sh	7	—	—	—	2	1	2	1	1	—
Not paralysed	Tx	5	—	—	—	—	—	—	1	3	1
	Sh	6	—	—	—	—	—	—	3	—	3

*Tx = thymectomized; Sh = sham-operated.

One possible explanation of the discrepancy between the elimination and haemagglutination results is that thymectomy itself may influence the rate of non-immune catabolism of BGG. This possibility was suggested by the finding that six out of seven neonatally-thymectomized mice catabolized mouse γ-myeloma protein nearly twice as fast as did normal controls (Humphrey, Parrott and East, 1964). In order to test this, twelve CBA mice, six of which had been thymectomized at 2 months old were each injected at 11

months old with 2 mg. of [^{131}I] BGG without prior immunization. The rate of elimination in the thymectomized group was slightly higher (mean half-life = 4·18 days) than in the controls (mean half-life = 5·29 days). While this difference may not be sufficient to account for the discrepancy just mentioned, it does indicate that caution must be used in the interpretation of results based on antigen elimination in thymectomized animals.

DISCUSSION

Results similar to those obtained here for BGG have recently been reported by Calman and Talmage (1963). They are entirely in agreement in showing that the thymus is necessary for normal recovery from immunological paralysis in the adult. Taking this with the evidence for its influence on recovery from irradiation (Miller, Doak and Cross, 1963), there is now little doubt that the thymus continues to function in adult life. As already mentioned in the introduction, the kinetics of recovery from paralysis suggest that this process depends on lymphoid cell turnover, which in turn is at least partly dependent on the presence of the thymus. If so, it is of interest to enquire where the new competent lymphoid cells come from. They could arise by division of pre-existing competent lymphoid cells, or they could be recruited from stem cells. Present evidence is in favour of the latter as being the chief mechanism. Ford and Micklem (1963) have provided circumstantial evidence that the progenitors of the cells which recolonize the lymph nodes and spleen after irradiation originate mainly in the bone marrow, and then pass via the thymus to reach the other lymphoid tissues. Other evidence obtained by the use of thymus grafts in thymectomized (Miller, 1963) or normal (Wakonig-Vaartaja and Metcalf, 1963) animals confirms that host cells enter the thymus graft, while cells from the graft pass to the lymph nodes and spleen. Finally, it has been shown that cells in the lymph nodes and spleen which have come from a thymus graft are immunologically competent (Taylor, 1963). The process suggested by these experiments is that stem cells pass from the bone marrow to the thymus, divide and mature therein, and finally leave to become competent cells. In addition, some stem cells may be able to mature without actually entering the thymus (Osoba and Miller, 1963; Levey, Trainin and Law, 1963).

Although this process could well provide the principal means for recovery from paralysis —and the effect of thymectomy observed in the experiments described here is compatible with this hypothesis—it cannot be excluded that mature paralysed cells may be able to recover from paralysis. Indeed, the fact that some recovery occurred in spite of thymectomy could be interpreted in this way.

Another possibility is that maturation of lymphoid cells from stem cells is only partially dependent on the thymus and can therefore continue at a slow rate even after thymectomy. An argument against this is that no recovery of immune response is observed for at least 10 weeks after irradiation, using skin allografts or sheep red cells (Miller *et al.*, 1963) or BSA (Taylor, unpublished) as the antigen. It could be, on the other hand, that some influence of the thymus persists after thymectomy—perhaps as a hormone, or as a population of immature cells which are capable of maturing in the absence of the thymus.

Whatever the mechanism may be, it is evident that the tempo of recovery, in both thymectomized and control animals, depends on the antigen. Of the antigens used here BGG has the longer half-life and induces the more lasting paralysis. Although cell-turnover evidently plays a part in recovery from paralysis, the time taken for degradation of antigen may still be a controlling factor.

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REFERENCES

BOYDEN, S. V. (1951). 'The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera.' *J. exp. Med.*, **93**, 107.

CALMAN, H. N. and TALMAGE, D. W. (1963). 'Thymectomy: prolongation of immunological tolerance in the mouse.' *Science*, **141**, 1193.

CEPPELINI, R. and LANDY, M. (1963). 'Suppression of blood group agglutinability of human erythrocytes by certain bacterial polysaccharides.' *J. exp. Med.*, **117**, 321.

DRESSER, D. W. (1960). 'Elimination of ^{131}I-labelled protein antigens from the circulation of the mouse.' *Immunology*, **3**, 289.

DRESSER, D. W. (1961a). 'A study of the adoptive secondary response to a protein antigen in mice.' *Proc. roy. Soc.*, B, **154**, 398.

DRESSER, D. W. (1961b). 'Acquired immunological tolerance to a fraction of bovine gamma globulin.' *Immunology*, **4**, 13.

DRESSER, D. W. (1962a). 'Specific inhibition of antibody production. I. Protein-overloading paralysis.' *Immunology*, **5**, 161.

DRESSER, D. W. (1962b). 'Specific inhibition of antibody production. II. Paralysis induced in adult mice by small quantities of protein antigen.' *Immunology*, **5**, 378.

DRESSER, D. W. (1964). In press.

FARR, R. S. (1958). 'A quantitative immunochemical measure of the primary interaction between ^{131}I-BSA and antibody.' *J. infect. Dis.*, **103**, 239.

FORD, C. E. and MICKLEM, H. S. (1963). 'The thymus and lymph nodes in radiation chimaeras.' *Lancet*, **i**, 359.

HUMPHREY, J. H., PARROTT, D. M. V. and EAST, J. (1964). 'Studies on globulin and antibody production in mice thymectomized at birth.' *Immunology*, **7**, 419.

LEVEY, R. H., TRAININ, N. and LAW, L. W. (1963). 'Evidence for the function of thymus tissue in diffusion chambers implanted in neonatally thymectomised mice.' *J. nat. Cancer Inst.*, **31**, 199.

MACFARLANE, A. S. (1958). 'Efficient trace labelling of proteins with iodine.' *Nature (Lond.)*, **182**, 53.

MÄKELA, O. and NOSSAL, G. V. (1962). 'Accelerated breakdown of immunological tolerance following whole body irradiation.' *J. Immunol.*, **88**, 613.

MAKINODAN, T., GENGOZIAN, N. and CONGDON, G. C. (1956). 'Agglutinin production in normal, sublethally irradiated, and lethally irradiated mice treated with mouse bone marrow.' *J. Immunol.*, **77**, 250.

MEDAWAR, P. B. (1960). 'Theories of immunological tolerance.' *Ciba Foundation Symposium on Cellular Aspects of Immunity*, p. 134.

MILLER, J. F. A. P. (1963). In *The Thymus in Immunobiology* (Ed. by R. A. Good). Harper and Rowe, New York.

MILLER, J. F. A. P., DOAK, S. M. A. and CROSS, A. M. (1963). 'Role of the thymus in recovery of the immune mechanism in the irradiated adult mouse.' *Proc. Soc. exp. Biol. (N.Y.)*, **112**, 785.

MILLER, J. F. A. P., MARSHALL, A. H. E. and WHITE, R. G. (1962). 'The immunological significance of the thymus.' *Advanc. Immunol.*, **2**, 111.

MITCHISON, N. A. (1962). 'Tolerance of erythrocytes in poultry: loss and abolition.' *Immunology*, **5**, 359.

MITCHISON, N. A. (1963). 'Suppression of induced tolerance by treatment of the whole animal.' *Coll. int. Centre National de la Recherche Scientifique*, No. 116, 199.

OSOBA, D. and MILLER, J. F. A. P. (1963). 'Evidence for a humoral thymus factor responsible for the maturation of the immunological faculty.' *Nature (Lond.)*, **199**, 653.

PROOM, H. (1943). 'Preparation of precipitating sera for the identification of animal species.' *J. Path. Bact.*, **55**, 419.

TAYLOR, R. B. (1963). 'Immunological competence of thymus cells after transfer to thymectomised recipients.' *Nature (Lond.)*, **199**, 873.

WAKONIG-VAARTAJA, R. and METCALF, D. (1963). 'The origins of mitotic cells in natural thymus grafts.' *Lancet*, **i**, 1303.