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Recovery from Immunological Paralysis in Relation to Age and Residual Antigen

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Summary. Mice paralysed by bovine serum albumin gradually recover immunological reactivity. Recovery has been mapped at various ages by challenge with antigen in Freund's adjuvant, and comparing the resulting antigen binding capacity of the serum with that of normal controls. Recovery proceeds more rapidly in young than in old animals, irrespective of the duration of previous exposure to antigen. This is considered to confirm the importance of cell turnover in recovery. In harmony with this hypothesis, irradiation proved capable of deleting an existing state of paralysis without appreciably enhancing recovery. A cell transfer test has been used to detect antigen in paralysed animals, supplemented by measurement of radioactivity from [^{131}I]labelled antigen. Antigen can be detected throughout the lag period preceding the start of recovery in quantities probably sufficient to account for the lag.

INTRODUCTION

Recovery from immunological paralysis appears to be caused by the production of new immunologically competent cells. The alternative hypothesis, that existing cells may change their immunological status through a mechanism such as depletion of an internal store of antigen, is at variance with the following observations: (i) a long period—months or even years—may be required for recovery (Felton, Prescott, Kauffman and Ottinger, 1955; Mitchison, 1962; Humphrey, 1964); (ii) the period of recovery increases markedly with age (Mitchison, 1962); (iii) thymectomy inhibits recovery (Claman and Talmage, 1963; Taylor, 1964). The argument from age has been incomplete so far, since observations have been made only on animals which were treated with antigen from an early age where it was therefore impossible to vary the age at recovery independently of the preceding duration of treatment. The present experiments, on recovery of mice from paralysis by bovine serum albumin (BSA), fill the gap. They were undertaken with the aim of providing a comprehensive description of recovery, in a species where the prospects of investigating cell turnover by conventional methods are good, and with an antibody that can be assayed with precision. They include an examination of the effect of irradiation on recovery. The dosage needed to paralyse adult mice with BSA is already known in detail (Sercarz and Coons, 1959; Mitchison, 1964).

A latent period must intervene before the production of new reactive cells can start. During this period the concentration presumably falls, in successive stages, of (i) extracellular antigen, (ii) intracellular antigen, and (iii) hypothetical relics or messengers of antigen. The duration of stage (i) can be determined, in the present system, from the half-life of the antigen and its critical paralysing concentration. Stages (ii) and (iii) are

less accessible; an attempt has been made to measure them jointly by means of a transfer test, which measures the potential for inducing a secondary response. Measurements of [^{131}I]BSA radioactivity were carried out to provide supplementary information, of doubtful status, about intracellular antigen.

MATERIALS AND METHODS

Animals

Mice of the CBA inbred strain were used. Series for which the treatment commenced at birth included both males and females; no difference in recovery times could be detected between the sexes. Otherwise only males were used.

Induction by paralysis

A standard dose of 200 μg/g body weight of BSA in solution in 0·2 ml saline was injected intraperitoneally thrice a week, at intervals of 2 or 3 days. The dose was adjusted according to weight (by slight variation of volume) only in young mice; after reaching a weight of 25 g, mice were treated as though their weight remained constant.

Test of normality of response

The methods of immunization (Freund's adjuvant), collection of sera, assay of antibody (direct binding—the Farr test), and statistical methods are described elsewhere (Mitchison, 1964). A control group of normal mice was included in most cases, of the same age as the mice under test. Often a single group served as control for a number of test groups immunized simultaneously. A group size of eight to ten mice was aimed at; as a consequence of mortality the numbers fell finally in the range five to twelve. In young mice the dosage of Freund's adjuvant was reduced in proportion to body weight, from the 7 mg BSA in 0·2 ml administered to the standard adult 25 g mouse.

Irradiation

To test the effect of irradiation on the rate of recovery, and to provide hosts suitable for transfer of spleen cells, mice were exposed to 600 R X-irradiation at 124 kV, 5·5 mA, at a dose rate of approximately 25 R/minute.

RESULTS

RECOVERY FROM PARALYSIS

Recovery has been followed in four series of mice, each differing in the age at which the administration of BSA ceased. The first series, shown in Fig. 1, received a single intraperitoneal injection of 5 mg BSA within 1 day of birth, and then no more until the time of test for responsiveness. This dose represents approximately $\times 25$ the standard paralysing dose; even so, the paralysis proved transient. The points shown in this and the other figures indicate the time at which the antigen, incorporated in Freund's adjuvant, was injected in order to test responsiveness. Each test involved four collections of antibody (test and control groups at 20 and 40 days after challenge), and the bars in the figures denote the mean of the four standard deviations. Newborn mice were not injected with adjuvant, and so the first series lacks a test at the start of recovery.

The second series, shown in Fig. 2, received the standard paralysing dose from within 1 day of birth for 6 weeks. The third series, shown in Fig. 3, received paralysing doses

from birth for 14 weeks. Half the mice in this series received the standard dosage, while the other half received ×5 this amount (injected at the same times). The fourth series, shown in Fig. 4, received the standard dosage for 12 weeks, commencing at 10–12 weeks of age. The four groups therefore terminated treatment respectively at 0, 6, 14 and 22–24 weeks of age; the mean body weights at termination were respectively 1·2, 19·0, 28·5 and 33·2 g.

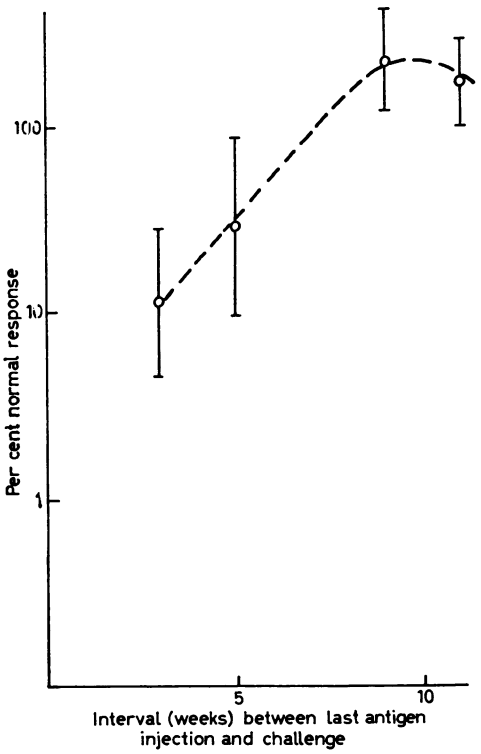

FIG. 1

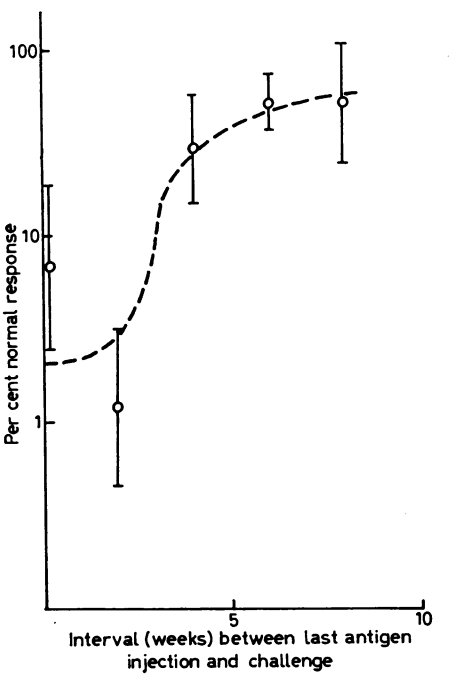

FIG. 2

FIG. 1. Recovery from paralysis, commencing within 1 day of birth.
FIG. 2. Recovery from paralysis, commencing at 6 weeks of age.

In every series the expected recovery from paralysis took place, and normal reactivity was eventually attained. The oldest animals took longest to recover, and a general trend towards slower recovery with increasing age can be detected. The 30 per cent recovery points for the 0, 6, 14 and 22–24 week old series are respectively 4, 4, 6 and 16 weeks; slightly different numerical values could be arranged by choosing a different end-point, but the general trend is not in doubt. The trend cannot be accounted for by the duration of paralysing treatment, for the oldest group had, in fact, received slightly shorter treatment than the next oldest.

The recoveries display a certain pattern, but the data do not warrant any attempt to fit a defined curve; the curves shown in the figures have been drawn by eye. Except in the 0-week series, where the degree of initial paralysis is in any case doubtful, about a month appears to elapse before any significant recovery can be detected. This interval can be prolonged somewhat by increasing the dosage of antigen (compare the high and low dose

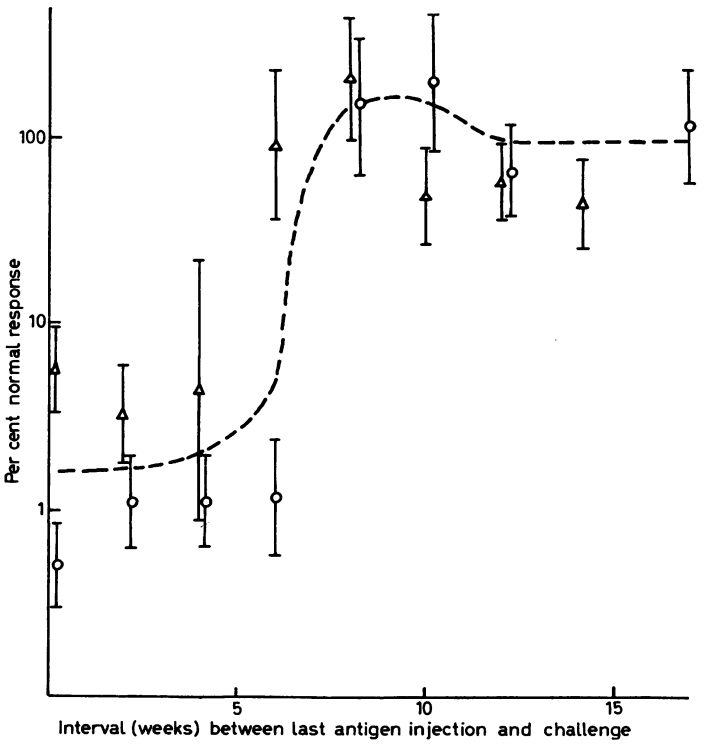

FIG. 3. Recovery from paralysis, commencing at 14 weeks of age. Normal paralysis, △; high dose (×5) paralysis, ○.

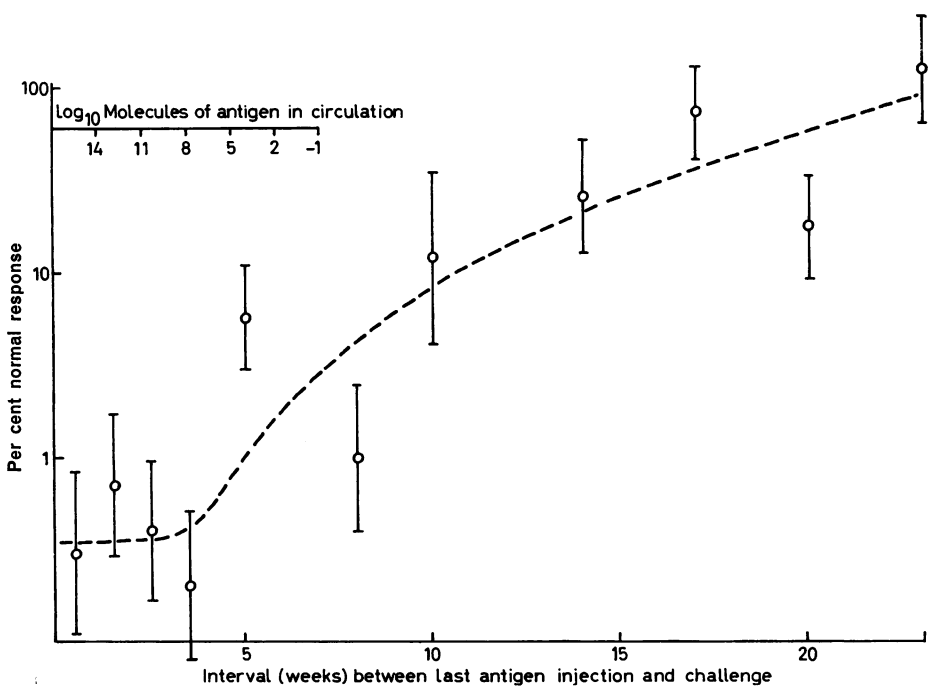

FIG. 4. Recovery from paralysis, commencing at 22–24 weeks of age.

groups in the 14-week series), but is little affected by age (compare the 14 and 22–24 week series). Once recovery has begun, however, age exerts a strong influence.

Overshoot, the tendency of once-paralysed animals to become immune, has been detected. The effect is displayed most directly when antibody could be found in sera collected prior to immunization with adjuvant, i.e. antibody elicited by the paralysing course alone. None of the groups of mice in which paralysing doses were continued beyond 6 weeks of age ever afterwards produced mean binding capacities over 1 μg/BSA/ml serum unless stimulated with adjuvant. The younger animals, however, did do so. Between 2 and 7 weeks after stopping the paralysing doses both the 0 and the 6 weeks series produced antibody spontaneously, with mean titres in the range 1–5 μg/ml. The titres, though unimpressive, were consistent. Further evidence of immunization can be found in the tendency for the response to challenge with adjuvant to exceed the control value (Figs. 1 and 3), before eventually returning to normal.

Acceleration of recovery after irradiation

Irradiation might be expected to speed recovery by encouraging the production of new immunologically competent cells. Mäkelä and Nossal (1962) found that irradiation had

TABLE 1

RESPONSE TO IMMUNIZATION WITH ANTIGEN IN ADJUVANT IN MICE RECOVERING FROM PARALYSIS

(Mean antigen binding capacities in groups of eight mice; mean coefficient of variation = 1·11.)

Weeks of recovery	Normal		Irradiated (600 R)	
	Paralysed	Controls	Paralysed	Controls
0	0·8	13·9	0·6	3·4
2	0·2	4·5	0·3	2·8
4	1·1	24·8	1·1	3·9
6	8·7	6·5	5·2	6·1
8	59·0	28·2	37·5	24·6
14	11·4	24·0	19·1	19·9

the expected effect on partially tolerant animals, although no effect had been observed in previous tests (Denhardt and Owen, 1960). The question has been examined in the present system by irradiating thoroughly paralysed mice.

Considered in isolation, irradiation appeared to have no effect on recovery: mice which were irradiated shortly after they had been paralysed subsequently made about the same amount of antibody as similarly paralysed but non-irradiated mice, whatever the time of challenge during the recovery period. Table 1 illustrates this; the mice in this experiment were 14 weeks old at the end of their paralysis-inducing treatment, and were irradiated with 600 R 5 days later. Other experiments on older mice yielded essentially similar results. Nevertheless, to conclude that irradiation has no effect would be misleading. In the non-paralysed controls this dose of radiation transiently but drastically reduced responsiveness. If, therefore, the paralysed and irradiated mice are compared with their non-paralysed controls, the irradiation does appear to accelerate recovery. Thus, at 4 weeks of recovery the response among paralysed non-irradiated mice has reached only 4 per cent of their control value (1·1/24·8), whereas paralysed irradiated mice have reached 28 per cent of their control value (1·1/3·9). The conclusion may be drawn that irradiation

does little to encourage the production of fresh immunologically competent cells (a weak stimulatory effect may possibly have gone undetected), but that by inactivating the current cellular population it tends to eliminate an existing state of paralysis.

CATABOLISM OF ANTIGEN

The rate at which the antigen is removed from the circulation during the first few days after injection, in the present strain of mouse, is known from measurements on BSA trace-labelled with [^{131}I]. A value for the half-life of 19 hours (Mitchison, 1964) has been used to calculate the level shown in Fig. 4. The mice referred to in this figure remained paralysed to a significant extent for some months after loss of the last molecule of free antigen, as judged from this calculation. Free antigen, therefore, is not essential for the maintenance of paralysis. The same conclusion has already been drawn from experiments on cellular (Mitchison, 1962) and purified protein antigens (Dietrich and Weigle, 1963; Humphrey, 1964); the present data are particularly convincing because they combine a rapidly catabolized antigen with old, and therefore slow-recovering animals.

The point at which brisk recovery commenced in all the present experiments corresponds to a level of about 10^{10} molecules of free antigen, but this number need have no special importance. It is true that mice which received high doses of antigen remained paralysed for slightly longer than usual (Fig. 3), but only one group—that tested at 6 weeks of recovery—contributes significant evidence to this effect, and it is in any case open to question whether free antigen is responsible.

IMMUNOLOGICALLY STIMULATORY MATERIAL

Antigen, or its imprint, can be detected by biological methods which do not rely on labelling. These test methods have a respectable history (reviewed by Humphrey, 1960), and one which is particularly applicable to BSA in mice has been described (Mitchison, 1963). A more extensive application of this method has been made in the experiments referred to in Table 2. The method employs sensitive cells, obtained from immunized donors, to detect stimulatory material in the hosts into which they are transferred.

In each of the four repetitions of the experiment, cells were harvested from the spleens of mice which had been immunized with BSA in Freund's adjuvant 2–4 months earlier. The cells were pooled and then transferred intraperitoneally to hosts which had received 600 R 1 day previously (one to two donors per host). The numerous groups of hosts in each experiment received cells from the same pool, and were subsequently bled on the same day. Details of the method of transfer and further data on the dose–response relationship for BSA are given by Mäkelä and Mitchison (1965a, b). Each experiment included controls which received, intraperitoneally a few hours after cell transfer, small known amounts of BSA (Table 2, section 1). These controls served to measure the sensitivity of the transferred cells to stimulation. A second group of controls received large amounts of BSA at the same time as the test groups, but no cell transfer (Table 2, section 2). Their own cells produced some antibody, but not in quantity large enough to confuse the test. The test groups included mice injected on a single occasion with BSA (Table 2, section 3), and mice paralysed by the standard course, commencing at 10–12 weeks of age and continued for 10 weeks (Table 2, section 4).

The presence of stimulatory material in the hosts can be inferred, for as long as the experimental groups make more antibody than the sum of that made in (i) the unstimu-

lated, transferred controls (Table 2, line 1), and (ii) the non-transferred controls. As judged by this criterion, both types of test group contain stimulatory material. The titre elicited declined as the interval available for loss of stimulatory material increased, in accordance with expectation. In the most favourable case (experiment 2) stimulatory material could still be detected 10 weeks after injection of 100 mg BSA. The question naturally arises whether the stimulatory material could be free BSA in the circulation. Against this possibility three arguments can be advanced: (i) calculation from the half-life,

TABLE 2

ANTIGEN BINDING CAPACITY OF HOST SERUM (μg BSA/ml), AT INTERVALS AFTER TRANSFER

	Experiment 1	Experiment 2		Experiment 3		Experiment 4	
	10 days	10 days	20 days	10 days	20 days	10 days	20 days
1. CONTROLS							
BSA injected after transfer (μg)							
0·0	0·9	0·2	1·8	0·2	0·4	0·0	0·3
0·01	—	1·4	6·4	12·4	97	0·1	1·2
0·1	153	2·3	4·7	8·4	25	0·3	1·7
1 0	320	3·2	4·5	40	155	0·6	2·7
10·0	331	—	—	11·2	28	1·1	5·2
2. NO TRANSFER							
Single injection BSA (100 mg)							
2 weeks before transfer date	3·1	0·7	0·4	—	—	—	—
4 weeks before transfer date	0·8	0·4	1·4	1·5	1·6	—	—
6 weeks before transfer date	—	0·1	0·3	—	—	—	—
3. EXPERIMENTAL GROUPS							
Single injection BSA (5 mg)							
2 weeks before transfer						0·4	0·8
4 weeks before transfer						0·5	1·3
6 weeks before transfer						0·4	0·3
8 weeks before transfer						—	—
10 weeks before transfer						—	—
Single injection BSA (100 mg)							
2 weeks before transfer	17·4	73	212	32	61		
4 weeks before transfer	6·7	10·1	44	1·7	6·8		
6 weeks before transfer	—	2·3	32	1·5	6·3		
8 weeks before transfer	—	0·4	16·6	—	—		
10 weeks before transfer	—	0·1	8·5	—	—		
4. PARALYSED, LAST INJECTION							
BSA (5 mg)							
2 weeks before transfer	—	47	133	20	227	2·3	6·7
4 weeks before transfer	—	—	—	5·2	29	0·6	2·0
6 weeks before transfer	—	—	—	6·6	36	0·0	0·8
8 weeks before transfer	—	—	—	—	—	0·2	0·5
Mean No. of mice per group	8	5		6		8	
Coefficient of variation	1·19	1·33	1·31	1·38	1·47	1·29	1·47

and comparison with the titres elicited by microgram stimulation post-transfer, indicate that not enough BSA would be retained to account for the degree of stimulation observed; (ii) single large doses of BSA elicit enough antibody to cause immune clearance of free antigen; (iii) the time course of response is incompatible in experiment 2—comparing the 10 and 20-day titres, free BSA elicits an earlier response than does the hypothetical stimulatory material. Stimulatory material is retained to about the same extent after full paralysis as after a single injection of BSA; when material left from the earlier injections of BSA is allowed for, the titres shown in the last two sections of Table 2 are similar. The

comparison lacks force, however, because the effect of even a single large dose of BSA is chiefly paralytic (Mitchison, 1964).

The limitations of this method of test can be seen in Table 2. The peak response varies from one cell pool to another, and the dose–response curve is flat and irregular. Equally seriously, in one experiment, the cells seem to be much more susceptible to stimulation by retained material (experiment 2), but in the others the relationship is more equal.

RETENTION OF RADIOACTIVITY

Supplementary measurements of retention have been made from radioactivity. BSA was labelled with [^{131}I] from iodine monochloride at >1 atom/molecule, and then injected intraperitoneally into adult mice. The radioactivity retained in blood and certain organs was then measured at intervals by counting in a well crystal. The mice drank only iodide solution from 1 week prior to injection (0·005 per cent NaI in 0·45 per cent NaCl), but this did not completely block uptake in the thyroid.

TABLE 3

RETENTION OF RADIOACTIVITY AFTER INJECTION OF 5 mg [^{131}I]LABELLED BSA INTO 3-MONTH-OLD MICE

Days	Fraction retained × 10^{-5}			
	Blood (0·8 ml)	Liver (0·9–1·5 g)	Spleen (50–90 mg)	Thyroid (3·5 mg)
17	10·1	3·7	0·77	455
30	2·8	3·4	0·41	194
42	0·99	1·8	0·29	141

In measurements of the type concerned here, where retention is under study over a period of weeks, it might be an advantage to use [^{125}I]. However, the status of measurements is so much in doubt that only the cheaper isotope was employed. Furthermore, problems of geometry do not arise in measuring radioactivity of whole organs when [^{131}I] is used.

No great difficulty or hazard was experienced in administering sufficient radioactivity in the form of [^{131}I] to give a measurable number of counts. For example, in a typical experiment, five mice received an initial 40 μc and were autopsied 30 days later. From the liver 428 ±151 counts per 10 minutes were obtained with a background of 117 ±28 counts per 10 minutes (standard deviation between mice). For measurements at intervals of over 30 days 60–160 μc of initial radioactivity were administered.

The radioactivity retained in the blood, liver, spleen and thyroid is shown in Table 3. This experiment involved nine mice at autopsy in each interval, and the values shown in the table are means. The mice were bled by cardiac puncture before autopsy, and were perfused with saline through the portal vein in order to reduce the blood content of the liver. The radioactivity detected in the liver and spleen undoubtedly includes some activity from the blood, but this cannot represent the major part. Counts in the blood and organs declined steadily over the period of measurement, at a rate very much less than the initial rate of clearance of the labelled protein from the circulation (half-life = 19 hours).

The only attempt at fractionation was made on the blood. The result was not illuminating. Washed cells, the trichloracetic acid precipitable fraction of the plasma, and the supernatant contained approximately equal amounts of radioactivity. In this and all other experiments the highest amounts of radioactivity were detected in the thyroid. The liver

regularly contained five to ten times as much radioactivity as the spleen. On the assumption, possibly quite unwarranted, that the radioactivity reflects accurately the quantity of protein retained in the organs, the liver in these mice contained an amount of the order of 0·1 μg. The fraction of radioactivity retained is independent of the initial dose of protein at least over a range between 3 and 100 mg (Table 4, six mice per group). Injection into paralysed hosts does not affect the retention, as is shown in the same table. Paralysis was induced here by the standard procedure of repeated injections of 5 mg BSA. The radioactivity cannot be chased out by subsequent injections of unlabelled protein (again shown in Table 4); hence, under the same doubtful assumption, paralysed mice retain well over 0·1 μg BSA in the liver for over 7 weeks.

TABLE 4

LACK OF EFFECT OF PARALYSIS, CHASE-ANTIGEN, OR DOSAGE ON RETENTION

	Days	Fraction of radioactivity retained in liver $\times 10^{-5}$
3 mg labelled BSA into paralysed hosts	20	2·6
3 mg labelled BSA, followed by 5 mg unlabelled BSA three times a week	22	2·0
	41	1·5
100 mg BSA	16	2·9
	33	1·7

DISCUSSION

Recovery from paralysis has been found to proceed at a rate which varies markedly with age, irrespective of the duration of the preceding period of exposure to antigen; the oldest animals take longest. The rate of removal of the antigen from the serum does not alter with age to an extent large enough to account for this variation, and, in any case, antigen disappears from the circulation of older animals well before recovery is complete. The intracellular catabolism of antigen is also unlikely to vary markedly with age. Cell turnover, therefore, seems the only factor likely to account for the pattern observed. We can inquire, then, whether production of new cells will account for all the observations without recourse to the postulate that individual paralysed cells recover.

In the mouse the rate of lymphocyte production has not yet been measured by means of tritiated thymidine. In the rat small lymphocytes are produced at a rate proportional to body growth (Caffrey, Rieke and Everett, 1962), so that the absolute rate reaches a maximum at 150–250 g body weight and falls thereafter. This variation would be quite sufficient to account for the kinetics of recovery from paralysis and augurs well for any future comparison with the kinetics of lymphocyte production in the mouse.

Previous students of overshoot (Terres and Hughes, 1959; Coons, 1963; Siskind, Paterson and Thomas, 1963) have argued that the phenomenon lends support to the opposite theory, that cells lose their tolerant state through the depletion of a stock of antigen, and that when this falls below some critical level the production of antibody ensues. Overshoot was indeed observed in the present experiments, but to a smaller extent than would be expected of the level of antigen theory. Overshoot was found only over the time when immunologically stimulatory material could still be detected by the transfer test; therefore what we detect as overshoot may be no more than immunization of newly

produced cells by relics of antigen. In older mice, where new cells develop more slowly, overshoot against BSA could no longer be detected; the observations of Sercarz and Coons, it may be noted, were made on fairly young mice, and the effect was strongest in their youngest animals.

Recovery from paralysis coincides surprisingly well with the loss of intracellular relics of antigen. The minimum paralysing dose of BSA, when administered three times a week is about 1 μg (Mitchison, 1964—and unpublished data). Stimulatory material falls below this level—i.e. becomes less active than 1 μg administered in a single dose—4–8 weeks after injecting 5–100 mg BSA, as judged from the transfer test (taking account of both the 10 and 20-day titres). This is approximately the time when recovery gathers momentum, after the latent period. The agreement may be no more than coincidence, but it suggests that intracellular relics of antigen are responsible for the initial delay in recovery. Measurements of antigen by retained radioactivity, doubtful as they are, fall in the same range.

The bulk of the retained radioactivity eventually localizes in the liver. One is tempted, therefore, to believe that antigen is retained in macrophages, and hence for a while continues to paralyse newly formed lymphocytes. The suggestion is hardly new, for that powerfully and long-lasting paralysing antigen, pneumococcal polysaccharide, persists almost solely in macrophages (Kaplan, Coons and Deane, 1950).

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REFERENCES

Caffrey, R. W., Rieke, W. O. and Everett, N. B. (1962). 'Radioautographic studies of small lymphocytes in the thoracic duct of the rat.' *Acta haemat. (Basel)*, **28**, 145.

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

Coons, A. H. (1963). 'Tolerance and persistence of antigen.' In *Tolérance Acquise et Tolérance Naturelle a l'Egard de Substances Antigéniques Définies* (Ed. by A. Bussard). C.N.R.S., Paris.

Denhardt, D. T. and Owen, R. D. (1960). 'The resistance of the tolerant state to X-irradiation.' *Transplant. Bull.*, **7**, 394.

Dietrich, P. M. and Weigle, W. O. (1963). 'Induction of tolerance to heterologous proteins and their catabolism in C57BL/6 mice.' *J. exp. Med.*, **117**, 621.

Felton, L. D., Prescott, B., Kauffman, G. and Ottinger, B. (1955). 'Studies on mechanism of immunological paralysis induced in mice by pneumococcal polysaccharides.' *J. Immunol.*, **74**, 17.

Humphrey, J. H. (1960). 'Methods of detecting foreign antigen in cells, and their sensitivity.' In *Mechanisms of Antibody Formation* (Ed. by M. Holub and L. Jaroskova). Prague.

Humphrey, J. H. (1964). 'Immunological unresponsiveness to protein antigens in the rabbit. I. The duration of unresponsiveness following a single injection at birth.' *Immunology*, **7**, 449.

Kaplan, M. H., Coons, A. H. and Deane, H. W. (1950). 'Localization of antigen in tissue cells. III. Cellular distribution of pneumococcal polysaccharides Types II and III in the mouse.' *J. exp. Med.*, **91**, 15.

Mäkelä, O. and Mitchison, N. A. (1965a). 'The role of cell number and source in adoptive immunity.' *Immunology*, **8**, 539.

Mäkelä, O. and Mitchison, N. A. (1965b). 'The effect of antigen dosage on the response of adoptively transferred cells.' *Immunology*, **8**, 549.

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

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.' In *La Tolérance Acquise et la Tolérance Naturelle a l'Egard de Substances Antigéniques Définies* (Ed. by A. Bussard). C.N.R.S., Paris.

Mitchison, N. A. (1964). 'Induction of immunological paralysis in two zones of dosage.' *Proc. roy. Soc.* B, **161**, 275.

Sercarz, E. and Coons, A. H. (1959). 'Specific inhibition of antibody formation during immunological paralysis and unresponsiveness.' *Nature (Lond.)*, **184**, 1080.

Siskind, G. W., Paterson, P. Y. and Thomas, L. (1963). 'Induction of unresponsiveness in newborn and adult mice with pneumococcal polysaccharide.' *J. Immunol.*, **90**, 929.

Taylor, R. B. (1964). 'An effect of thymectomy on recovery from immunological paralysis.' *Immunology*, **7**, 595.

Terres, G. and Hughes, W. L. (1959). 'Acquired immune tolerance in mice to crystalline bovine serum albumin.' *J. Immunol.*, **83**, 459.