Immunology, 1962, 5, 378.

Specific Inhibition of Antibody Production

II. PARALYSIS INDUCED IN ADULT MICE BY SMALL QUANTITIES OF PROTEIN ANTIGEN

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(*Received 8th November*, 1961)

Summary. A state of immunological paralysis has been induced in adult CBA mice by intraperitoneal injections of small quantities of bovine γ globulin (BGG). The minimum paralysing dose of BGG has been found to be between 50 and 200 μg. A dose as small as 2 μg. has been found to have a slight paralysing effect. The time necessary for the induction of paralysis by 50 μg. to 2 mg. of BGG in CBA mice is 3–4 days.

Paralysis is induced by only one component of BGG; this component is incapable of inducing an antibody response unless an injection of adjuvant is made at the same time or slightly before the injection of the antigen. The BGG is centrifuged at an RCF of 20,000–30,000 *g* to remove particulate matter. Failure to remove the particulate matter leads to sporadic immune responses in groups of mice injected with the protein. Mice given a paralysing injection of BGG were subsequently challenged by an injection of BGG in Freund's adjuvant. The result of this challenge was tested by an injection of radioactively-labelled antigen and the elimination of this antigen from the circulation of the challenged mice was followed for several days. 'Immune elimination' can easily be distinguished from 'non-immune elimination'. The presence of antibody to the non-paralysing components of BGG in sera from paralysed mice was confirmed using the Ouchterlony gel-diffusion technique.

INTRODUCTION

There is some argument as to whether it is more suitable to call a state of immunological unresponsiveness 'tolerance' or 'paralysis'. The term 'paralysis' will be used in this and subsequent papers. The reason for this decision will be discussed. Possible use of the term to include unresponsiveness induced by histocompatibility antigens is not discussed. 'Paralysis' seems to be a more suitable term on grounds of priority and also because of the use of the term 'tolerance' in other branches of biology.

The size of the inducing dose required is one of the differences between immunological paralysis induced by proteins and by pneumococcal polysaccharide antigens in adults. In adult rabbits and mice paralysing doses of bovine and human albumin are as high as 0.5 mg./g. body weight, injected every day for several weeks (Dixon and Maurer, 1955; Sercarz and Coons, 1959). With single injections of uncentrifuged bovine γ globulin, 100 mg. or more were required if the mice were to remain paralysed 2 months later (Dresser, 1962). With pneumococcal polysaccharide, however, paralysis may be achieved by doses

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as small as 5 μg., depending on the type of polysaccharide and the strain of mice used (Felton, 1949; Felton, Kauffmann, Prescott and Ottinger, 1955). Another difference between paralysis induced by these two classes of antigen is that paralysis induced by proteins is transient but paralysis induced by polysaccharide antigen is long lasting. This difference may be due to the different rates of catabolism of these antigens. Stark (1955) has shown that pneumococcal polysaccharide is catabolized very slowly, whereas it seems likely that protein antigens are catabolized more quickly (Campbell, 1957).

It has recently been shown that CBA mice are not immunized by saline solutions of bovine γ globulin which have been freed of all particulate matter by centrifugation at 20,000–30,000 *g* (Dresser, 1961c). Immunization can be achieved, however, if an adjuvant (Freund's adjuvant made up with saline, for instance) is injected a few days before or on the same day as the injection of centrifuged protein. It seems that at least the major component of bovine γ globulin by itself lacks the ability to initiate antibody production. It was suggested that bovine γ globulin prepared in this way lacks what was tentatively called 'adjuvanticity' in mice. It seems likely that 'adjuvanticity' is a property which is possessed by or closely associated with most antigens, because most antigens can initiate antibody production without the necessity of an adjuvant being present. A possible confirmation of this point comes from Stevens and McKenna's (1958) report of the successful initiation of antibody production *in vitro* by bovine γ globulin when the cells were taken from animals previously injected with the lipopolysaccharide of *Salmonella typhi*, but not with cells taken from uninjected control animals.

In the experiments described in this paper an investigation has been made of the effectiveness of particulate-free ('adjuvanticity' lacking) bovine γ globulin as a 'paralysitogen' (an antigen inducing immunological paralysis). It is shown that small quantities of the protein will induce a specific state of immunological paralysis in adult CBA mice. Furthermore, by varying the time at which the mice are challenged with Freund's adjuvant containing antigen, it is possible to obtain some indication of the time necessary for the induction of paralysis. In one experiment a comparison is made between paralysis induced in neonatal mice and paralysis induced in adult mice. The concept of acquired immunological paralysis as a general phenomenon including both paralysis induced in neonatal animals and paralysis induced in adult animals is briefly discussed.

MATERIALS AND METHODS

CBA mice were used in all but one experiment, for which the mutant co-isogenic strain CBA p/Cam was used. Both stocks are maintained by sib matings. The CBA p/Cam mice were a gift from Dr. D. Michie of the Department of Surgical Science, University of Edinburgh. The mice were never younger than 14 weeks old when given the first injection of centrifuged bovine γ globulin. In some experiments this first injection was followed by a course of injections, details of which are given in the results section.

The antigen used was bovine γ globulin (BGG) (Armour & Co., batch Nos. CH 2470 and DC 1473). The BGG was stored in a desiccator at 0° and protein solutions were made up (w/v) in 0.9 per cent saline (NaCl). The BGG solution was centrifuged at 20,000–30,000 *g* for 20–30 minutes* (the longer time being used for the lower centrifugal force),

* The pellet formed during centrifugation has been fixed in osmium tetroxide, sectioned and examined with the electron microscope. No bacteria were seen in any of the sections examined. Centrifuged and uncentrifuged BGG behave identically in Ouchterlony gel-diffusion tests.

before the protein was injected. The mice were challenged at various times after the paralysis-inducing injection by a subcutaneous injection of BGG in Freund's adjuvant (Freund's BGG), made up and administered as previously described (Dresser, 1960). The result of the challenge with Freund's BGG was tested using an antigen-elimination technique. The BGG was labelled with ^{131}I by the method of Wormall as previously described (Dresser, 1960). In the antigen-elimination test, 2 mg. of BGG-^{131}I were injected intraperitoneally and the rate of elimination of the labelled antigen from the circulation of the mice was followed in the manner previously described (Dresser, 1962).

The Ouchterlony gel-diffusion test was carried out as previously described (Dresser, 1961a), except that Oxoid Ionagar, No. 2 was used thus avoiding the necessity for any clarification procedure.

RESULTS

It is possible to categorize arbitrarily the different antigen-elimination responses obtained in mice. This has already been done for the responses obtained in mice injected with antigen at birth (Dresser, 1961a). Although these categories have been used in the interpretation of the results of the experiments described in this paper, the nomenclature of the categories is changed to facilitate the classification of the results, in particular in Table 5. The categories of response are illustrated in Fig. 1; this figure also shows a typical

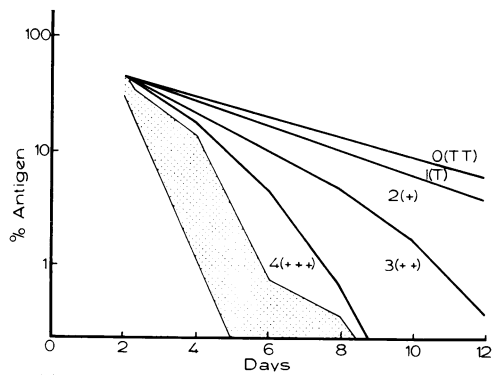


FIG. 1. This diagram illustrates the different antigen-elimination responses obtained when mice injected with Freund's adjuvant containing BGG were subsequently injected with 2 mg. BGG-^{131}I. The categories are arbitrary. The symbols in brackets represent the nomenclature used in earlier papers. The block in category 4 represents the elimination of antigen in five CBA mice which were untreated before challenge with Freund's adjuvant containing BGG.

response in previously untreated CBA mice challenged with Freund's BGG and 6 weeks later tested by an injection of 2 mg. of BGG-^{131}I. There are five categories ranging from a complete absence of response (0) to a full immune response (4). By comparison with passive immunization experiments (Dresser, 1961b) it can be seen that the difference in the rate of antibody production between category 0 and 4 must be at least 50-fold.

The result of injecting amounts of centrifuged BGG similar in proportion to body weight into newborn and adult (3-month-old, male) CBA mice is shown in Table 1. An initial intraperitoneal injection of 0.42–0.47 mg. BGG/g. body weight was made into the mice of each of the two groups. Subsequently one-tenth of the amount injected on the first

day was injected each week for 8 weeks. On the ninth week the mice were challenged with Freund's BGG and the result of this challenge subsequently tested using the antigen-elimination technique. Four of five mice in each group were completely paralysed, the fifth mouse in each group showed a partially inhibited immune response.

TABLE 1

A COMPARISON BETWEEN PARALYSIS INDUCED IN NEWBORN MICE AND PARALYSIS INDUCED IN ADULT CBA MICE CHALLENGED WITH FREUND'S BGG 2 MONTHS AFTER A FIRST PARALYSING INJECTION (0.42–0.47 MG. BGG/G. BODY WEIGHT)

Injection	*Category of response*				
	0	*1*	*2*	*3*	*4*
12-week-old males, injected 11 mg. BGG followed by 1 mg. BGG at weekly intervals for 2 months	4/5	—	1/5	—	—
Litter of CBA mice, injected 500 µg. at birth, and 50 µg. at weekly intervals thereafter for 2 months	4/5 (1 ♀) (3 ♂)	—	1/5 (1 ♂)	—	—

TABLE 2

THE EFFECT OF INJECTING BGG AT WEEKLY INTERVALS FOR FROM 2 TO 8 WEEKS BEFORE CHALLENGE WITH FREUND'S BGG

First injection	*Subsequent weekly injections*		*Category of response*				
	Amount	*No. of times*	*0*	*1*	*2*	*3*	*4*
2 mg.	2 mg.	7	1/5	3/5	1/5	—	—
0.5 mg.	0.5 mg.	7	1/6	2/5	2/5	—	—
12.5 mg.	0.5 mg.	7	1/5	4/5	—	—	—
0.5 mg.	0.5 mg.	3	—	2/5	2/5	1/5	—
12.5 mg.	0.5 mg.	3	2/5	2/5	1/5	—	—
0.5 mg.	0.5 mg.	1	1/5	—	3/5	—	1/5
12.5 mg.	0.5 mg.	1	2/5	—	1/5	2/5	—
Nil	Nil	—	—	—	—	—	5/5

Table 2 presents the results of an experiment designed to show the paralytic effect of different courses of injection of the antigen, continued for different lengths of time. CBA male mice were used and they were 3 months old when the first injections were made. It can be seen that all the different doses of BGG used in this experiment paralysed the mice either partially or completely. There does seem to be a slight tendency for the degree of paralysis induced in a group to be positively correlated with the length of time during which the injections were made.

An experiment using 3–4-month-old CBA male mice, was carried out to determine the time necessary for the induction of paralysis by 2 mg. of BGG. Five groups of five mice in each group were all injected with 2 mg. of BGG. At intervals of 1 hour, 3, 5, 8 and 12 days

one group after another was challenged with Freund's BGG. Five weeks after the first injection of BGG, the antigen-elimination test showed that paralysis is non-existent in the group challenged only 1 hour after the injection of 2 mg. of BGG, that there is partial paralysis in some mice challenged 3 days after the paralysing injection and almost complete paralysis in the other three groups. The results are presented in greater detail in Table 3.

TABLE 3

THE EFFECT OF VARYING THE TIME BETWEEN A PARALYSING INJECTION OF 2 MG. BGG INTO CBA MALE MICE (3 MONTHS OLD) AND SUBSEQUENT CHALLENGE WITH FREUND'S BGG

Time interval	*Category of response*				
	0	*1*	*2*	*3*	*4*
1 hour	—	—	—	—	5/5
3 days	—	1/5	1/5	1/5	2/5
5 days	4/5	1/5	—	—	—
8 days	4/5	1/5	—	—	—
12 days	5/5	—	—	—	—

TABLE 4

THE PARALYSING EFFECT OF DIFFERENT AMOUNTS OF BGG INJECTED INTO CBA MALES 12 DAYS BEFORE CHALLENGE WITH FREUND'S BGG

Amount of BGG	*Approx. no. molecules*	*Category of response*				
		0	*1*	*2*	*3*	*4*
2000 μg.*	10^{16}	5/5	—	—	—	—
200 μg.	10^{15}	2/3	—	—	—	1/3
2 μg.	10^{13}	—	—	—	2/3	1/3
0.02 μg.	10^{11}	—	—	—	—	3/3

* Mice aged 3–4 months. In all the other groups the mice were aged 6–8 months.

Table 4 shows a small scale-experiment in which a variation was made in the amount of BGG used to paralyse CBA male mice which were challenged 12 days afterwards. Unfortunately all the mice were not of the same age group, some being 3–4 months old and the others 6–8 months old. The details are shown in Table 4. Complete paralysis was obtained in two mice injected with 200 μg. BGG and a slight partial paralysis in two mice injected with 2 μg. BGG.

An attempt, using CBA p/Cam mice, was made to investigate simultaneously the induction time and the amount of BGG required for the induction of paralysis. Unfortunately, the symmetry of the experiment is spoiled by the introduction of an additional variable: there are two groups of mice, females aged 3–5 months and males aged 5–7

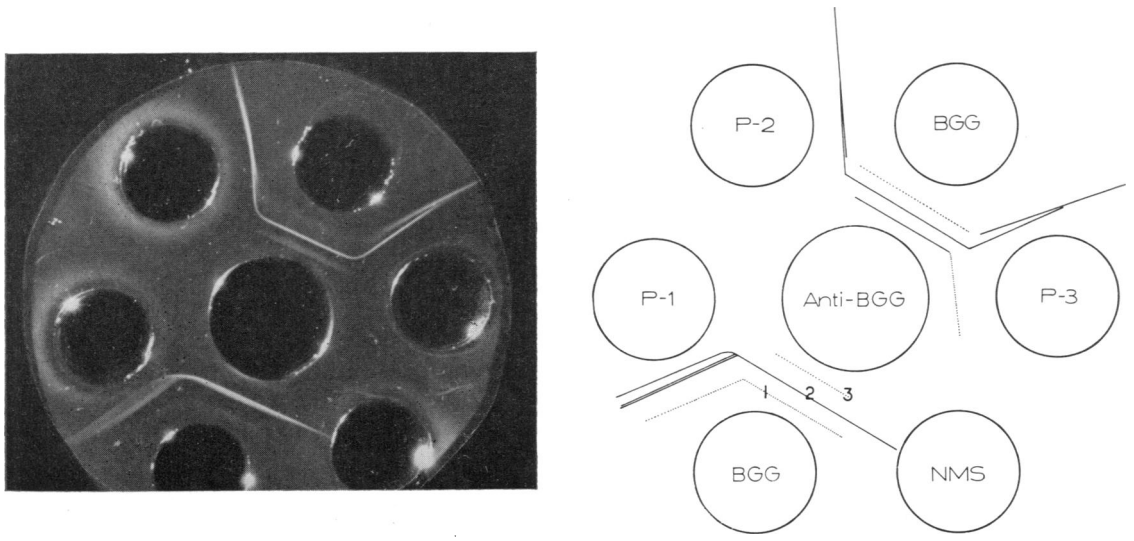
months. In Table 5 each figure representing a response is based on four or five mice and it is the mean of the categories of response for each group. It can be seen that there is no paralysis when the mice are challenged 2 days after the paralysing injection. After 3 days two of the groups exhibit some paralysis, one or two mice in each group showing a significantly lower immune response. After 4 days the mice injected with 500 μg. and 50μg. of BGG show a considerable degree of paralysis, some mice in these groups being completely

TABLE 5

AN EXPERIMENT WITH CBA P/CAM MICE TO INVESTIGATE THE RELATIONSHIP BETWEEN THE AMOUNT OF ANTIGEN NECESSARY TO INDUCE PARALYSIS AND THE INDUCTION TIME OF PARALYSIS

Sex and age	*Time between paralysis injection and challenge with Freund's BGG*	*Mean response* in group of four or five mice*					
Female, 3–5 months	2 days	4.0	4.0	4.0	4.0	4.0	—
Female, 3–5 months	3 days	—	3.8	3.6	4.0	4.0	—
Female, 3–5 months	4 days	—	2.8	1.6	4.0	4.0	4.0
Male, 5–7 months	6 days	—	2.8	3.0	4.0	4.0	4.0
Amount of BGG injected (serial dilution made up in 1 per cent normal mouse serum; the mouse serum also having been centrifuged at 30,000 *g*)		5,000 μg.	500 μg.	50 μg.	5 μg.	0.5 μg.	0.05 μg.

* Based on the category of response used in Tables 1–4. A response of 4.0 would mean that all mice came in category 4.0, and 0.0 would mean that all mice came in category 0 (completely paralysed).



FIGS. 2 and 3. Photograph and diagram of gel-diffusion experiment.

In the diagram BGG represents 1 per cent BGG in saline; anti-BGG is an antiserum to BGG (all components) prepared in outbred mice; NMS is serum from untreated CBA mice; and P — 1, 2, 3 represent serum from three paralysed mice. For future convenience the three major lines have been numbered 1, 2 and 3. These lines may correspond to three distinct components of BGG, which therefore could be called fractions 1–3 (F_1, F_2 and F_3).

paralysed. A direct comparison cannot be made between the groups already described and the groups challenged after 6 days as these mice are older. However, even in these groups of older mice, it can be seen that 50 μg. is the smallest amount of BGG having a detectable paralytic effect.

Figs. 2 and 3 illustrate the lines of precipitation obtained in a gel-diffusion experiment with sera from mice paralysed by 2 mg. BGG and challenged 12 days after the paralysing injection by an injection of Freund's BGG. One month later the result of this challenge was tested by the antigen-elimination technique. Sera for the gel-diffusion experiment were obtained 14 days after the injection of the labelled antigen. No precipitation lines which can be related to an antibody directed against the major component of BGG were detected in the sera from paralysed mice, although a line due to the persisting major component of the antigen can be faintly seen with only one sample of serum. In previous experiments (Dresser, 1961a) the sera were obtained 7 days after the injection of 10 mg. BGG and in this case the persisting paralysing component of BGG was sufficiently concentrated to form a line of precipitation with an antiserum directed against all the components of BGG. Lines due to antibody directed against the non-paralysing component(s) of BGG can be seen with all serum samples from the paralysed mice.

DISCUSSION

When the amount of antigen injected per g. body weight is constant, the results suggest that there is no difference between states of immunological unresponsiveness induced in neonatal and 3-month-old adult mice (Table 1). This conclusion may appear surprising if considered in relation to previous work on immunological unresponsive states, from which it can be concluded that relatively larger amounts of protein antigen are required to paralyse adults than are required to induce 'tolerance' in neonatal animals (see Hanan and Oyama, 1954; Dixon and Maurer, 1955; Cinader and Dubert, 1955, 1956; Smith and Bridges, 1958; Terres and Hughes, 1959; Sercarz and Coons, 1959). In a previous paper a similar conclusion was reached after attempts to induce a state of immunological unresponsiveness in mice aged from a few hours to about 1 month (Dresser, 1961a). These experiments were, however, carried out using BGG which had NOT been centrifuged. It has since been pointed out that centrifugation of the BGG removes small quantities of particulate matter which may act as an adjuvant enabling the major component of the BGG to induce immunity (Dresser, 1961c).

The results summarized in Table 2 seem to show that there is a tendency for the response in groups of mice which have been exposed to the antigen for the longest duration before challenge to show the greatest degree of paralysis. The results of these experiments in which the minimum dose used was 1 mg. in two injections of 0.5 mg. of BGG, do not seem to be compatible with the results which show that a single injection of microgram quantities of BGG can paralyse mice. It therefore seems that the increasing degree of paralysis observed in these experiments (Table 2) cannot be taken as evidence supporting any hypothesis which postulates a cumulative effect due to long exposure to small quantities of antigen. A possible reason for the increasing degree of paralysis observed may be related to the occurrence of mice showing large immune responses in groups which are otherwise completely paralysed. The CBA mice used are highly inbred, so the observed variation can hardly be due to genetic differences. Previous experiments have shown that various substances, including killed mycobacteria, may act as adjuvants when injected at

the same time or before the antigen (Dresser, 1961c). It is possible that subclinical bacterial infections or changes in the symbiotic flora of the mouse might be sufficient to act as an adjuvant and result, in some of the mice, in the stimulation of immunity in at least a small number of cells, rather than paralysis in all cells. As it seems likely that antibody release (synthesis?) can be inhibited in cells already induced to form antibody by very large amounts of antigen (Felton, 1949; Dixon and Maurer, 1955; Dresser, 1962), it is possible that smaller amounts of antigen might be capable of paralysing a few cells induced to form antibody. In such a situation it might be expected that a cumulative paralytic effect would become manifest in any experiment in which the paralysis-inducing injections were continued for a period of time. The hypothesis just discussed is made less likely as the sole cause of variation in the experimental groups, by the variable results obtained in those experiments in which a large initial paralysing dose of antigen was used.

A further and possibly more attractive alternative, which cannot at the moment be dismissed or confirmed, is that the age of the mouse may play an important part in the ease with which BGG can induce paralysis. All the mice used in this experiment (Table 2) were of the same age-group but the mice in the group injected for the first time only a fortnight before challenge were respectively 2 weeks and 6 weeks older than the other groups when they received their first paralysis-inducing injection. Although equivocal with respect to the age of the mice, the results summarized in Table 5 may well be compatible with the hypothesis that it is easier to be certain of paralysing a young mouse than an older one.

Tables 3 and 5 are based on experiments designed to indicate the time necessary for the induction of paralysis. It seems that a significant degree of paralysis is induced in 3 days and, at least in most mice, a nearly complete paralysis is induced by 4 days. This figure of 3–4 days for the induction time of paralysis is in fact the time between the injection of antigen and the subsequent injection of Freund's adjuvant containing more antigen. The injection of adjuvant will not induce immunity once a state of paralysis is achieved. The experimental facts are compatible with a hypothesis that a minimum concentration of antigen is required within a cell, possibly at a different 'site' from that associated with the induction of immunity, for a state of paralysis to be induced. The induction time of paralysis could therefore represent the time taken by the cells to concentrate sufficent antigen to exceed this minimum limiting concentration. It is possible that the adjuvant acts immediately after it is injected; if true, this means the induction time observed would be the real time necessary for the induction of paralysis. If the adjuvant does not act immediately the real induction time for paralysis could be longer than the observed time. Although the concept of an induction time for paralysis may indicate an adaptive or differentiational change at the cellular level, the mechanism itself must, for the moment, remain a matter for conjecture.

An attempt has been made to determine the minimum amount of antigen necessary for the induction of paralysis. Tables 4 and 5 show that for a state of complete paralysis the minimum dose must be about 50–200 μg. of BGG. In Table 4 it can be seen that an injection of as little as 2 μg. of BGG may very slightly inhibit subsequent antibody production to that antigen. The approximate number of protein molecules injected in each dose is indicated in Table 4. It has been shown (Dresser, 1961a, 1962) that there were 10^9 to 10^{12} molecules of BGG present in mice which were still paralysed to BGG after these mice had been made unresponsive by injections of relatively large amounts of BGG at birth or in adult life. The experiments described in this paper indicate that at least

10^{13} to 10^{15} molecules of BGG are required to initiate a state of paralysis. It therefore seems possible that for the *de novo* induction of paralysis more antigen is required than is necessary for the maintenance of a state of paralysis. Although this may be analogous to certain aspects of 'preinduction' in adaptive enzyme formation in bacteria (see Cohn, 1957), it must remain a tentative suggestion until more quantitative data is available concerning the amount of antigen required for induction and maintenance of paralysis.

THE TERMINOLOGY OF THE UNRESPONSIVE STATE

The problem whether or not the phenomenon described in this paper is paralysis or tolerance is both biological and semantic. Tolerance has been described as the state of immunological unresponsiveness induced in immunologically immature animals (Billingham, Brent and Medawar, 1956). If tolerance is related to the immunological immaturity of an animal, then the phenomenon described in this paper cannot be included in the term 'tolerance'; unless one subscribes to the suggestion that cells are immunologically immature until they come in contact with an adjuvant. If the mechanism of the state of unresponsiveness is similar when induced in both neonatal and adult animals, then a single term should be used for the phenomenon. The validity of the hypothesis that tolerance and paralysis are the same phenomenon will be briefly discussed below. As a general term, 'paralysis' has priority over 'tolerance' having been used by Felton and Bailey in 1926, and later (Felton and Ottinger, 1942). Although the term 'tolerance' is descriptive of the acceptance of homologous skin or lymphoid grafts or even the replacement of the host's immunologically competent tissue by that of the graft, it is thought that 'paralysis' is more apt for use in relation to immunological unresponsiveness induced by other than histocompatibility antigens. Another objection to the term 'tolerance' is that it is used in other branches of biology, which can lead to such confusing phrases as 'drug-induced tolerance'.

It may be necessary to distinguish between the paralysis of cells already induced or being induced to form antibody (overloading paralysis — Medawar, 1961) and the paralysis of cells which have not been so induced. In the former situation the cellular mechanism involved may not be a central inhibition of antibody synthesis but an inhibition of antibody release followed by the subsequent intracellular catabolism of the antibody which is being steadily synthesized. Stark (1959) has investigated such a possible mechanism in relation to pneumococcal polysaccharide paralysis. The paralysis induced by very small amounts of BGG, which has been described in this paper, seems likely to be due to a central failure to produce antibody, because of the small amounts of antigen involved and the intracellular lability of protein antigens (Campbell, 1957).

If a quantitative comparison between pneumococcal polysaccharide-induced paralysis and protein-induced paralysis is made, it becomes apparent that pneumococcal polysaccharide paralysis and protein-overloading paralysis are similar phenomena. Table 6 summarizes some relevant information. The column headed 'Ratio a/b' shows that polysaccharide paralysis and protein-overloading paralysis in the presence of an adjuvant are similar in that the minimum paralysing dose is much greater than the minimum immunizing dose. However, when the BGG lacks 'adjuvanticity', the immunizing and paralysing doses appear to be similar. An immunizing dose of pneumococcal polysaccharide will initiate antibody production without the addition of an adjuvant, so presumably this antigen has 'adjuvanticity' closely associated with it. It is thought that most antigens are

TABLE 6

Antigen	(a) *Minimum paralysing dose*			(b) *Minimum immunizing dose*		*Ratio a/b*	*Source*
	C3H	*Sw. white*	*CBA*	*C3H*	*CBA*		
Polysac. Type I	500 µg.	50 µg.	—	0.01 µg	—	50,000	Felton *et al.* (1955)
Polysac. Type II	50 µg.	5 µg.	—	0.0025 µg.	—	20,000	,,
Polysac. Type III	50 µg.	5 µg.	—	0.1 µg.	—	500	,,
Centrifuged BGG	—	—	50–200 µg.*	—	125 µg.‡	approx. 1	This paper
BBG + adjuvant	—	—	0.5–1 g.†	—	125 µg.‡	approx. 5,000	Dresser (1962)

* Challenged 12 days after the paralysis-inducing injection.
† Challenged 2 months after paralysis-inducing injection (protein overloading).
‡ Details to be published in a subsequent paper in the current series.

in this category, so that most attempts to paralyse adults are perhaps a simultaneous immunization and suppression of antibody production in the cells just induced to form antibody. This hypothesis could account for the large quantities of antigen needed to paralyse adults when serum proteins are used (Dixon and Maurer, 1955; Sercarz and Coons, 1959) or when tissue antigens are used (Shapiro, Martinez, Smith and Good, 1961). The ease with which newborn animals can be paralysed may be explained by their immaturity with respect to 'adjuvanticity', their failure to recognize foreignness or, in the terms of Burnet and Fenner (1949), the immaturity of their mechanism for the recognition of 'not-self'. When an animal is injected with antigen at birth the resulting state of 'tolerance' may in certain circumstances involve the simultaneous induction of immunity and paralysis. Thorbecke, Siskind and Goldberger (1961) have recently shown that two strains of mice injected with 1 mg. BGG at birth show an inhibited immune response when challenged 14 days later. However, they also showed that 'paralysed' mice showed secondary responses when subsequently challenged in the second month after birth. It is possible that the state of immunological unresponsiveness induced by Thorbecke *et al.* may have been of the protein-overloading type. The situation is probably complicated by the inability of neonatal mice to synthesize γ globulin (antibody) even though the inductive phase of antibody production has been initiated in a normal manner. It seems likely that the induction of paralysis ('tolerance') is not fundamentally a phenomenon associated with foetal or early life.

In conclusion, the following simplified situation is suggested:

$$\text{BGG (centrifuged)} \longrightarrow \text{CBA mouse} = \text{PARALYSIS}$$
$$\text{BGG (centrifuged)} + \text{adjuvant} \longrightarrow \text{CBA mouse} = \text{ANTIBODY PRODUCTION}$$

From the immunological point of view it will be important to know if this phenomenon is a general one. If the principle is general, then it can be predicted that paralysis can be

induced in adults by small quantities of any antigen providing any accompanying 'adjuvanticity' is removed. The action of a non-specific substance determining which of two apparently exclusive states an antigen (substrate, inducer) will induce, may be of interest from the point of view of cellular differentiation.

ACKNOWLEDGMENTS

I wish to thank Professor R. D. Owen, Dr. N. A. Mitchison and my wife for the benefit of discussion, and Miss J. Mallarkey for technical assistance.

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