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Rate of Antigen Catabolism and Immunogenicity of [^{131}I]BGG in Mice

II. IMMUNOGENICITY OF [^{131}I]BGG AND ADJUVANT ACTION AFTER ALTERATION OF THE METABOLIC RATE BY VARIOUS MEANS

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Summary. Elimination of [^{131}I]BGG was observed after attempts had been made to accelerate protein catabolism by thyroxine at three dose levels. Immune-type elimination did not result during primary exposure but treatment with the hormone predisposed to immune elimination on second and tertiary exposure to antigen, even at dosages of the hormone not obviously accelerating protein catabolism.

Vitamin A did not require (thyroid) accelerated catabolism to initiate an immune response. It did not itself influence the preimmune rate of elimination of antigen.

Raised ambient temperature or a high carbohydrate diet slowed down catabolism. Mice with slow catabolism formed less antibody against BGG after the use of mycobacterial adjuvants than did controls. These adjuvants are maximally effective when antigen is being catabolized rapidly.

INTRODUCTION

Subcutaneous mycobacterial adjuvants accelerate in mice the catabolism of intravenously administered antigen in the phase before antibody is released into the circulation: the adjuvants are less effective immunologically when the rate of protein catabolism is slow (Stark, 1970). These findings have been made in the system described by Dresser (1960) with radiolabelled bovine γ-globulin as antigen. The elimination of this antigen in CBA mice is sufficiently prolonged as to allow observation of rapidly increased antigen elimination, usually at the 7th day or later, due to the entry of antibody into the circulation and the removal therefrom of the subsequently formed antigen–antibody complexes. The period before the onset of immune elimination is referred to here as the preimmune phase.

The experiments reported in this paper examine how the immunogenicity of [^{131}I]BGG and the performance of adjuvants may be affected by alterations in the metabolic rate.

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MATERIALS AND METHODS

Animals, antigen, injection procedure, mycobacterial adjuvants and counting methods were as previously reported (Stark, 1970).

Thyroid diets

Thyroxine (Eltroxin, Glaxo) was incorporated in the following amounts:

Thyroid diet A: 0·2 mg thyroxine in 600 g diet
Thyroid diet B: 0·6 mg thyroxine in 300 g diet
Thyroid diet C: 3·0 mg thyroxine in 300 g diet

For convenience and thoroughness of mixing the Eltroxin tablets were first ground down with 20 g sucrose and then mixed with pulverized diet. Amounts of the mixture were made up into a wet mash which was placed daily in a dish in each cage. Mice were placed on these diets for 1 week before and 2 weeks after the primary injection of antigen.

High carbohydrate diet

Mice in one experiment were given brown bread (about 85 per cent carbohydrate) in place of diet 41 in the 4 weeks before and 2 weeks after the administration of the antigen.

Raising of ambient temperature

The mice were kept in cages under infrared lamps so that the temperature at the top of the cages was 30°. Mice were kept under these conditions for 3 days before injection of antigen and 2 weeks thereafter.

Vitamin A

'Avoleum' (British Drug Houses) was used, containing 30,000 i.u. vitamin A/g. Mice were given 0·3 ml by stomach tube on the 3 days up to and including the day of intravenous injection of antigen.

RESULTS

EFFECT OF THYROID DIETS ON ELIMINATION OF [^{131}I]BGG

Diets A and B

Two groups of five mice were maintained on thyroid diets A and B during the 7 days before and 14 days after primary injection. Their elimination rates of [^{131}I]BGG were observed on two occasions at an interval of 2 months.

In the preimmune period of the primary elimination the rates of elimination in the thyroid groups were not significantly increased over that of the control group (Table 1). There was no superimposed immune-type acceleration after the 7th day.

The secondary exposure to antigen resulted in immune acceleration of elimination by the 6th day in all mice in the thyroid groups over the rate prevailing in the period day 2 to day 4, the final mean half-lives being not more than 2·42 days. In the control group only one mouse showed immune acceleration (to a half-life of 0·8 day on day 4).

Diet C

The amount of thyroid hormone in the previously used diets had not produced a significantly increased catabolic rate detectable in the preimmune period. Accordingly, a

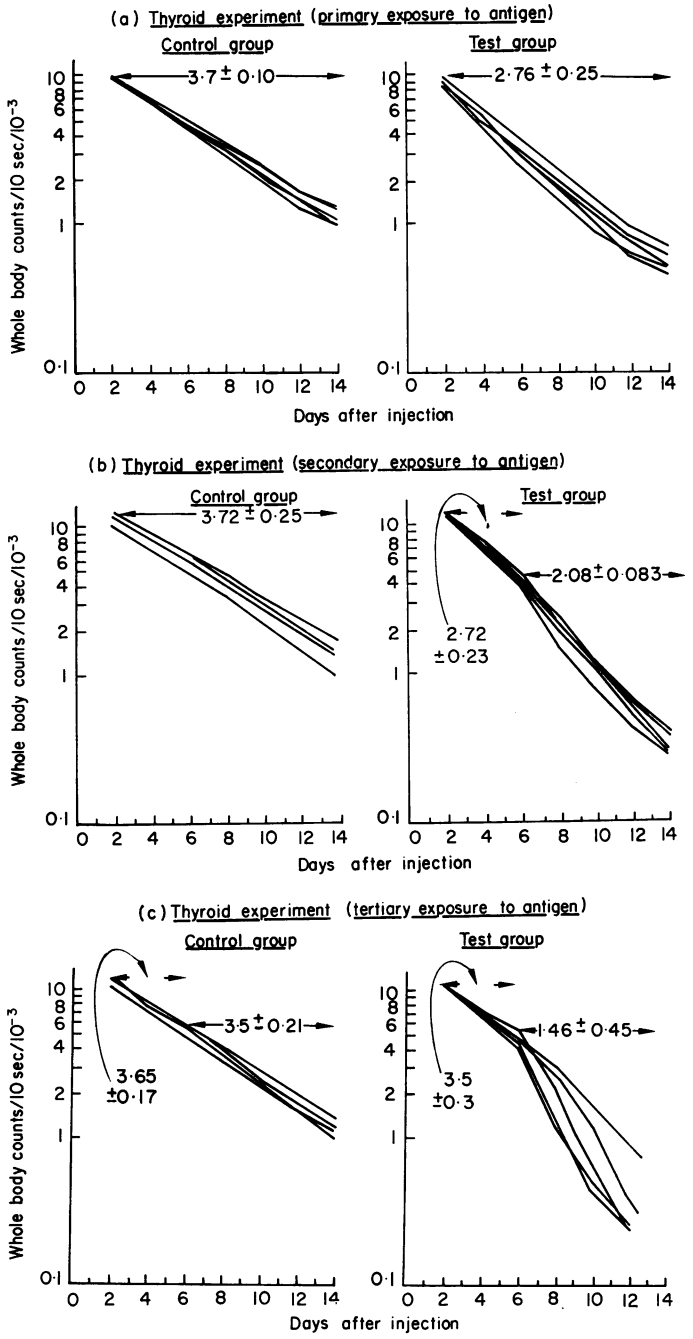

FIG. 1. Elimination of [^{131}I]BGG in CBA mice. The mice in the test group were fed on a diet containing 3 mg thyroxine per 300 g food for 7 days before and 14 days after the primary exposure to antigen. (a) Primary exposure to antigen in control and test groups; (b) secondary exposure to antigen in control and test groups 10 weeks later; (c) tertiary exposure to antigen a further 6 months later. The numbers quoted in the diagram represent the mean biological half-lives ± SD (in days) of the [^{131}I]BGG obtained graphically over the periods indicated.

TABLE 1

RATES OF ELIMINATION OF [^{131}I]BGG IN MICE RECEIVING ORAL THYROXINE DURING PRIMARY EXPOSURE TO ANTIGEN

Group	Elimination rate (half-life in days)	No. of mice showing alteration of elimination rate*		
	Days 2–4	Day 4	Day 6	Day 8
Control (five mice)				
Primary exposure	3·58 ± 0·15	–	–	–
Secondary exposure	3·7 ± 0·36	1 (0·8d)	–	–
Thyroid diet A (five mice)				
Primary exposure	3·5 ± 0·25	–	–	–
Secondary exposure	3·24 ± 0·41	1 (1·0d)	4 (2·25 ± 0·34d)	–
Thyroid diet B (five mice)				
Primary exposure	3·74 ± 0·15	–	–	–
Secondary exposure	3·2 ± 0·23	1 (1·5d)	4 (2·42 ± 0·19d)	–

* The altered half-lives are shown in parentheses below the No. of mice.

group of mice was maintained on thyroid diet C with a higher thyroxine content during the 7 days before and 14 days after the primary injection of antigen. Antigen elimination tests were performed also 10 weeks later, and again 6 months after the secondary exposure (Fig. 1).

During the primary exposure to antigen the mean half-life of the trace-labelled protein in the control group was 3·7 days: in the thyroxine treated group the mean half life was 2·76 days with no evidence of immune elimination on or after the 6th day. Ten weeks later, however, on the 6th day after a second dose of antigen, there was significant immune type acceleration in the thyroxine treated group, the mean half-life increasing from 2·72 to 2·08 days at that time. A third exposure 6 months after the second showed a further accentuated immune reaction in this group (Fig. 1, 3B). Control mice gave no response on the secondary exposure to antigen (one mouse had died in this group). On the tertiary exposure, one control mouse gave a mild response (half-life accelerating from 3·8 to 3·3 days after the 6th day).

EFFECT OF THYROID DIET AND ORAL VITAMIN A ON ELIMINATION OF [^{131}I]BGG

The increased antigen catabolism produced by thyroid diet alone had not resulted in the clear-cut primary immune responses which followed the use of mycobacterial adjuvants. Mice on thyroid diet C were also given oral vitamin A, a putatively adjuvant substance which had so far failed in the context of delayed-type hypersensitivity (White, 1968). Four groups of five mice were set up for the elimination experiment as follows:

Group 1 Normal diet
Group 2 Thyroid diet C
Group 3 Normal diet and vitamin A orally
Group 4 Thyroid diet and vitamin A orally

Immune type elimination was observed in the groups receiving vitamin A with or without thyroid diet (Fig. 2). There was no evidence that vitamin A had accelerated the

protein catabolism during the preimmune phase, the elimination rates over this time being (in days):

Group 1 3·6 ±0·22
Group 2 2·76±0·35
Group 3 3·56±0·38
Group 4 2·86±0·25 (one mouse died in this group)

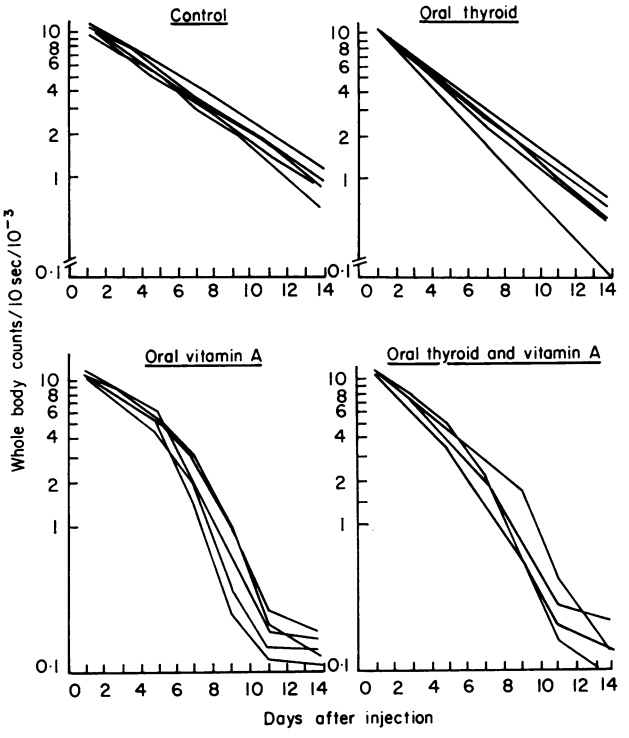

FIG. 2. Influence of thyroid hormone and vitamin A on the elimination of a 1 mg i.v. dose of [^{131}I]BGG. The mice in the different groups shown have received: (top left) (a) Normal diet; (top right) (b) Diet incorporating 3 mg thyroxine per 300 g feed in the 7 days before and 14 days after injection of [^{131}I] BGG; (bottom left) (c) 10,000 i.u. vitamin A orally on the 3 days before injection of [^{131}I]BGG; (bottom right) (d) a combination of (b) and (c).

EFFECT OF INCREASED AMBIENT TEMPERATURE ON ELIMINATION OF [^{131}I]BGG WITH AND WITHOUT ADJUVANT (*M. tuberculosis* IN OIL)

Mice were kept under warm conditions as described in the 'Materials and methods' section in order to slow down their metabolic rate (Pennycuik, 1967) and possibly alter the ability of *M. tuberculosis* in oil to induce an immune response. A group of mice at 18° and a group at 30° were injected with adjuvant; a second group at each temperature acted as controls.

Control mice (Table 2) show a significantly faster rate of elimination of the antigen (mean half-life 3·48 days) than do the warm mice (mean half-life 3·84 days, $P<0·01$, by the *t*-test). Both groups receiving adjuvants show evidence of a primary immune response but

the rate of immune elimination in the warm mice is significantly slower (mean half-life 1·7 days) than the control group (1·175 days: $P = 0{\cdot}05$).

The ratio of the protein catabolic rates (30° : 18°) is 3·84 : 3·48 in the non-adjuvant groups, i.e. 1·1 : 1·0. The ratio of the protein catabolic rates in the preimmune phase of the adjuvant groups is 3·1 : 2·67, i.e. 1·17 : 1·0. The ratio of the immune rates of elimination in the adjuvant groups is 1·7 : 1·17, i.e. 1·45 : 1·0. That is to say that the immune elimination in the mice at 18° is faster compared to the group at 30° than might be expected from their metabolic rates alone.

TABLE 2

EFFECT OF RAISED AMBIENT TEMPERATURE ON ELIMINATION RATES OF [^{131}I]BGG IN CBA MICE

Other treatment		Elimination rates (half-life in days) at ambient temperatures of:	
		18°	30°
Nil (five mice)		3·48 ±0·08	3·84±0·173
M. tuberculosis in Bayol 55 (five mice)	Days 2–8	2·67 ±0·264	3·1 ±0·56
	Days 8–14	1·175±0·34	1·7 ±0·31

EFFECT OF HIGH CARBOHYDRATE DIET ON ELIMINATION OF [^{131}I]BGG WITH AND WITHOUT ADJUVANT (WAX D IN OIL)

Test groups were given a high carbohydrate diet as described in the 'Materials and methods' section to reduce the specific dynamic action of dietary protein and thereby effect a significant slowing in the metabolic rate. One group of mice on normal and one on high carbohydrate diet were given Wax D in oil as adjuvant. A second group on each diet acted as controls.

The metabolic rates were slowed in the mice receiving the carbohydrate diet (mean half life of 3·98–3·22 days, $P<0{\cdot}01$): the rates of immune elimination were also significantly slower in the adjuvant-treated mice (2·2 as compared with 1·58 days).

TABLE 3

EFFECT OF HIGH CARBOHYDRATE DIET ON ELIMINATION OF [^{131}I]BGG IN CBA MICE

Other treatment	Elimination rates of mice	
	On normal diet	On carbohydrate diet
Nil (five mice)	3·22±0·19	3·98±0·054
Wax D—in oil s.c. (four mice)		
Days 2–7	2·72±0·47	3·27±0·09
Days 7–14	1·58±0·38	2·2 ±0·52

The ratio of the catabolic rates is 3·98 : 3·22, i.e. 1·25 : 1·0. The ratio of the preimmune rates of elimination in the adjuvant groups is 3·27–2·72 days, i.e. 1·2 : 1·0. The ratio of the immune rates of elimination in the adjuvant groups is 2·2 : 1·50, i.e. 1·41 : 1·0. That is to say, the rate of immune elimination is faster in the group receiving normal diet than would be expected from the different rates of metabolism alone.

DISCUSSION

Thyroid diet experiments

The use of thyroxine in accelerating antigen catabolism produced a degree of immunity which was manifested by circulating antibody at the second stimulation with antigen. The demonstration that this phenomenon could occur with the lower doses of thyroid without increased catabolism in the early or preimmune phase implies that a generally increased catabolism is not necessarily the mechanism of action of thyroid in this system. The effect of administration of thyroid is weak compared with that of the mycobacterial adjuvants after which the primary immune response is well defined.

Although the nature of the action of the thyroid hormone in these experiments is a matter for speculation, it has been known for a long time that the hormone has an influence on the performance of the non-specific body defences (Marbé, 1910) and can indeed stimulate antibody production to diphtheria toxoid in several species including man (Long and Shewell, 1955; Long, 1957). It has also produced hyperplasia of lymphoid tissue in guinea-pigs (Ernstrom and Gyllensten, 1959; Gyllensten, 1962) and in the fowl (Hohn, 1959).

Thyroid diet and vitamin A

Vitamin A did not require accelerated catabolism produced by thyroid hormone to initiate an immune response nor did it itself accelerate the preimmune rate of elimination of antigen. Vitamin A was added to the thyroid treatment because it was thought that if accelerated catabolism were essential to adjuvant action, the previously reported delayed-type hypersensitivity experiments (White, 1968) with the vitamin as adjuvant may have failed because no such component was provided. It is clear from the present vitamin experiments that such a component is unnecessary for antibody formation in the BGG/CBA system, for there is no preimmune acceleration in the first 7 days of elimination, yet an obvious primary response follows. Dresser (1968) has also observed the effectiveness of vitamin A in this model and has outlined a mode of action based on observations of lysosomal behaviour before cellular division of lymphocyte transformation. Release of lysosomal enzymes such as ribonuclease by the membrane-active vitamin A is suggested as providing a stimulus to division crucial in antigenic stimulation, one cycle of division in the presence of antigen being enough to set off the immunological response. If the lymphoid cell is regarded as multipotent, it is difficult to see how this division can result in specific differentiation unless this allows the passage of information regarding the shape of antigen to some effector site. There must be penetration of some moiety from the outside of the cell which selects the response. The concept of antigen more readily entering cells because of altered membrane permeability suggested by Gall (1966) for the adjuvant activity of surface-active nitrogenous bases would also seem apt for the action of vitamin A.

Slowing of antigen catabolism

The two procedures which brought about a slowing of antigen catabolism, namely the warm environment and the carbohydrate diet, reduced the amount of antibody produced against the antigen on the evidence of the immune elimination rates (Dresser, 1965), even when allowance has been made for the different metabolic rates. The studies of Hardy and Rowley (1968) with Sobey mice are comparable for they showed that individuals tolerant to bovine serum albumin were ones that eliminate the antigen at a slow rate. Although the diminution of adjuvant action and antibody production is minor, the

experiments perhaps suggest how the process of keeping the concentration of protein antigens temporarily above tolerance-inducing thresholds might be aided.

Action of mycobacterial adjuvants

The increased rate of protein catabolism brought about by mycobacterial adjuvants must be regarded as a contribution favouring their adjuvant action. Persistence of undigested antigen molecules may discourage immunogenicity by continuing to eliminate relevant clones: the rapid removal of molecules would reduce the likelihood of this.

On the other hand hypercatabolism alone (as produced by thyroxine) is not sufficient to bring about an obvious primary response. The degree of immunization laid down and detectable at the time of secondary exposure could be attributed to that action of thyroxine noted at lower doses where there was no measurable effect on the half-life on the protein in the preimmune phase.

The vitamin A experiments have shown that immunization can be produced without hypercatabolism. That permeability agents present in adjuvant granulomata might produce similar effects cannot be excluded. Nevertheless it might be deduced from the mycobacterial experiments reported here and in the previous paper (Stark, 1970) that any permeability agents present are not powerful enough to overcome the depressant effects of persistent antigen. Only if antigen is being catabolized rapidly are these adjuvants maximally effective.

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