# THE INFLUENCE OF THE THYROID GLAND ON THE PRODUCTION OF ANTITOXIN IN THE GUINEA-PIG.

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PROLONGED treatment with thyroxine increases sensitivity to tuberculin in guinea-pigs (Long and Miles, 1950), whereas a single injection of thyrotrophic hormone, of thyroxine, or of tri-iodo-thyronine is without significant effect (Long, unpublished). Prolonged treatment with thyroxine induces hypertrophy of the islets of Langerhans in rats (Houssay, Foglia and Martinez, 1946) and in guinea-pigs (Long and Shewell, unpublished). Partial pancreatectomy abolishes and insulin restores thyroxine-induced hypersensitivity (Long and Shewell, 1954). It is concluded that thyroxine increases sensitivity to tuberculin in guinea-pigs by inducing hyperinsulinism (Long and Shewell, 1954).

In the present paper, the influence of the thyroid gland on the production of diphtheria antitoxin is considered. There is a close analogy between the effect of the thyroid gland on sensitivity to tuberculin and on synthesis of diphtheria antitoxin. Preliminary experiments showed that treatment of guinea-pigs with thyroxine greatly increased both the level of circulating antitoxin and associated immunity to intradermally injected diphtheria toxin. Indeed, the latter provided a measure of the former in thyrotoxic, as in non-thyrotoxic, guinea-pigs (Hartley, 1934 ; Miles, 1949 ; Long, 1950). The increase in immune response was greatest when treatment with thyroxine was prolonged and the dose repeatedly adjusted so that the animals continuously gained a little weight. An association was noted between a high level of circulating antitoxin and hypertrophy of both the adrenal cortex and the islets of Langerhans. Of the two, adrenocortical hypertrophy was considered to be more likely to cause hyperimmunity. Thyrotoxicosis is associated in man with lymphoid hyperplasia. In addition, at the time of these experiments, it had recently been suggested that cortisone induced lympholysis with liberation of antibody (Dougherty and White, 1947).

These ideas were combined in a single hypothesis, that under the influence of the thyroid and adrenocortical hormones together there was a high rate of lymphoid hyperplasia and dissolution with an increased rate of liberation of antibody. A series of experiments completely destroyed this hypothesis by breaking every link in the argument. These proved conclusively that thyroxine-induced hyperimmunity was not mediated through the adrenal cortex, and indeed that in the guinea-pig the activity of the adrenal cortex had little influence on antitoxin production (Long, unpublished). It was therefore decided to investigate the alternative relationship postulated, namely, that between the thyroid gland and the pancreas.

In the present paper, partial pancreatectomy carried out at the end of a course of thyroxine treatment and immediately before the injection of antigen is shown

to prevent increased production of diphtheria antitoxin. It is concluded that thyroxine not only influences sensitivity to tuberculin (bacterial allergy) *viâ* the pancreas, but also antitoxic immunity.

## METHODS.

In each of the experiments described, all treated and control groups consisted of 15 albino guinea-pigs (approx : 350 g. weight) of the Hampstead strain, which were assigned at random to each group. Animals were fed upon Bruce and Parkes (1947) Diet 18, hay and unlimited cabbage. Whenever one group was operated upon, all groups, operated or not, received a daily supplement of milk for the rest of the experiment. In these, as in all experiments carried out in this laboratory, only International or Laboratory Standard antigens or antitoxins were used. By such means results obtained in different experiments can be compared, and the doses of immunising antigen and test toxin likely to yield maximal accuracy employed.

The immunising antigen was a Laboratory Standard alum-precipitated diphtheria toxoid (Ba 536). Under the conditions of these experiments, 0·5 LF in 1 ml. of physiological saline injected into the adductor muscles of the right hind leg, followed, at an interval of not less than 28 days, by an injection into the same site of a second similar dose of antigen, produces, after a further interval of 10 days, a sub-maximal antitoxin response falling on the steepest part of the curve. In Experiments I and II, the first dose of antigen (the primary stimulus) was given 4 weeks, and in Experiment III it was given 15 weeks, before the second dose of antigen (the secondary stimulus).

Test toxin T.P. 2776, a Laboratory Standard that has maintained constant potency (as judged by LR/10,000 and M.R.D. estimations) since 1948, was used for all multiple Schick tests and for direct assay of antitoxin. The International Standard for Diphtheria Antitoxin and, in addition, a Laboratory Standard for guinea-pig antitoxin (to avoid confusion over problems of differing avidity) were used to determine potency of sera. In all cases the titres of sera were assayed against both standards by two methods—by a modification of the classic Römer and Sames technique (1909) and Miles' (1949 ; 1954) indirect neutralisation technique. Miles (1949) showed that the response obtained when the inflammatory lesion diameter at 24 hr. is plotted against the log. dose of toxin in passively immunised guinea-pigs is linear ; different dose-response curves are substantially parallel, and the shift in the dose-response curve is proportional to the antitoxin content of the blood. By fitting regression lines to the dose-response curves so obtained, it is possible to detect relatively small differences in the antitoxic immunity in different groups of animals ; by analysis of variance, the errors of the test and the significance of differences in the various experimental groups can be accurately determined.

Further experiments (Long, 1950 ; Miles, 1954) showed that the same relations held in actively immunised guinea-pigs (cf. Hartley, 1934), so that substantially the same method was used for measuring active immunity and immunity passively conveyed with serum to previously non-immune guinea-pigs. The latter provides a true measure of circulating antitoxin for neutralisation of injected toxin is in this case independent of hormonal effects. In practice, results obtained in actively or passively immunised animals were identical.

Thus, in all experiments, the immune response was measured by the intradermal injection of graded doses of diphtheria toxin, using this modification of the classic multiple Schick technique.

### *Experiment I.*

Two groups of 15 guinea-pigs were used. Both groups were given a first dose of antigen 4 weeks before the secondary stimulus. Two weeks before the secondary stimulus one group was treated with thyroxine. Sodium thyroxine solution, 0·20 mg./kg. body wt. was injected subcutaneously three times a week for 2 weeks, so that at the time of the secondary stimulus a severe thyrotoxicosis of 2 weeks' duration had been produced.

### *Experiment II.*

To study the effect of prolonged mild thyrotoxicosis on antitoxin formation one group of 15 guinea-pigs was injected subcutaneously with sodium thyroxine solution, 0·10 mg./kg. body wt., twice weekly for 18 weeks. A second group of animals was given 50 mg./kg. body wt. propylthiouracil orally three times a week for the same period. After 14 weeks'

treatment these and two untreated groups were given a first dose of antigen, followed 4 weeks later by the secondary stimulus.

*Experiment III.*

To study the effects of partial pancreatectomy and thyrotoxicosis on antitoxin production in the guinea-pig, 4 groups of 15 animals were given a first dose of antigen. After 5 weeks two groups were given 0·10 mg./kg. body wt. sodium thyroxine solution subcutaneously once a week for 10 weeks. Five to 8 days before the secondary stimulus was given, 15 weeks after the first stimulus, the guinea-pigs from one of the thyroxine-treated groups and from one of the untreated groups were partially pancreatectomised under ether anaesthesia. The other two groups of animals were sham-operated under ether anaesthesia at the same time. Approximately $\frac{2}{3}$ of the pancreas was removed, the head of the pancreas being left intact in order to avoid damaging the pancreatic ducts. Post-mortem examination showed that in each case an adequate amount of functional pancreas remained.

Electrophoretic analysis of sera in each experiment failed to show a significant difference between groups.

## RESULTS.

*Experiment I.*

Severe thyrotoxicosis of 2 weeks' duration caused a significant ($P < 0.001$) 2·4-fold increase in immunity to intradermally injected diphtheria toxin. This was associated with a corresponding increase in circulating antitoxin.

*Experiment II.*

Mild thyrotoxicosis of 18 weeks' duration caused a significant ($P < 0.001$) 12·8-fold increase in immunity to intradermally injected diphtheria toxin (Table I).

TABLE I.—*Effect of Mild Thyrotoxicosis of* 18 *Weeks' Duration on the Secondary Response to Diphtheria Toxoid. Comparison of Individual Treatment Effects with the Effect in Controls.*

| Treatment. | Dose. | Secondary response. | F. | P. |
|---|---|---|---|---|
| Sodium thyroxine | 0·1 mg. twice weekly for 18 weeks | 12·8-fold increase | 16·5 | <0·001 |
| Propylthiouracil | 50 mg./kg. thrice weekly for 18 weeks | 1·5-fold decrease | <1 | — |

Comparison of antitoxin values obtained by different techniques.

| | Modified multiple Schick method (Long, 1950) (u./ml.). | Indirect neutralisation method (Miles, 1949) (u./ml.). | Römer and Sames method (1909) (u./ml.). |
|---|---|---|---|
| *Nil* | 4 | 4 | 4 |
| Sodium thyroxine | 71·2 | 60 | 70 |

F = ratio of variance due to the difference between treatments to animal variance. P = probability that the corresponding value of F could occur by chance.

*Degrees of freedom.*—Sodium thyroxine 1,27. Propylthiouracil 1,27.

This was associated with a corresponding increase in circulating antitoxin. Treatment with propylthiouracil for 18 weeks decreased the immune response, but the effect was not significant. This was associated with a corresponding decrease in circulating antitoxin. Similar experiments have consistently shown a decrease in immunity of approximately 1·5-fold, but with a group of this size animal variation has always been too great for the difference to be shown to be

significant. The effect is probably real; propylthiouracil tends, if anything, to depress antitoxin synthesis in guinea-pigs.

*Experiment III.*

Very mild thyrotoxicosis (B) of 10 weeks' duration caused a significant increase in immunity to intradermally injected diphtheria toxin (Table II). Partial pancreatectomy (A) caused a slight but not significant decrease in immunity. Partial pancreatectomy carried out in thyrotoxic guinea-pigs (A + B) immediately before the second injection of antigen (the secondary stimulus) caused a slight but not significant decrease in immunity to intradermally injected diphtheria toxin. In these, as in all experiments quoted, immunity to diphtheria toxin was associated with comparable changes in the level of circulating antitoxin.

TABLE II.—*Effect on the Secondary Response to Diphtheria Toxoid of Very Mild Thyrotoxicosis of* 10 *Weeks' Duration in Partially Pancreatectomised, and in Intact Guinea-pigs. Comparison of the Individual Treatment Effects with the Effect in Controls.*

| Treatment. | Potency ratio. | F. | P. |
|---|---|---|---|
| A | 1·35 (0·74-fold) | <1 | — |
| B | 0·41 (2·5-fold) | 6·73 | 0·05–0·01 |
| A + B | 1·21 (0·83-fold) | <1 | — |

| | High dose toxin. | | Low dose toxin. | |
|---|---|---|---|---|
| | Mean. | Range. | Mean. | Range. |
| Controls | 24·4 | (20·5–27·0) | 18·1 | (15·0–21·0) |
| A | 25·4 | (17·5–32·0) | 19·1 | (13·0–24·25) |
| B | 21·9 | (15·0–27·0) | 15·9 | (7·0–20·0) |
| A + B | 25·0 | (15·0–30·0) | 18·5 | (7·5–23·0) |

The responses in each treatment group were compared with the control in order to assess the significance of the effect of treatment on immunity.

For example, analysis of variance of Group B *v.* controls.

| Source of variation. | Sum of squares. | D.F. | Mean square. | F. | P. |
|---|---|---|---|---|---|
| Between treatments | 82·2510 | 1 | 82·2510 | 6·73 | 0·05–0·01 |
| Regression | 562·7344 | 1 | 562·7344 | 313·22 | <0·001 |
| Departure from parallelism | 0·0844 | 1 | 0·0844 | <1 | — |
| Between animals | 342·1542 | 28 | 12·2198 | — | — |
| Residual | 46·7125 | 26* | 1·7966 | — | — |
| Total | 1033·9365 | 57* | — | — | — |

Potency = 0·41465. * 1 dead control animal.

Comparison of antitoxin values obtained by different techniques.

| Treatment. | Modified multiple Schick (Long, 1950) (u./ml.). | Indirect neutralisation method (Miles, 1949) (u./ml.). | Römer and Sames method (1909) (u./ml.). |
|---|---|---|---|
| — | 10 | 10 | 10 |
| A | 7 | >10 | >10 |
| B | 25 | 30 | 40 |
| A + B | 8 | >10 | >10 |

A = Partial pancreatectomy. B = Sodium thyroxine.

F = ratio of variance due to the difference between treatments to the animal variance, and has (1,28) degrees of freedom. P = probability that the corresponding value of F could occur by chance.

## DISCUSSION.

Since the criterion employed in the measurement of antibody is the neutralisation of toxicity, the difference in antitoxin levels cannot be attributed to a modification of the serological (as distinct from the immunological) properties of circulating antibody as a result of hormonal activity. It reflects a difference in immunity.

Thyroxine increases active immunity to intradermally injected diphtheria toxin and this is associated with a comparable increase in circulating antitoxin. The effect is increased if thyroxine is given in a manner calculated to stimulate but not to overwhelm the compensatory endocrinological response.

Partial pancreatectomy carried out towards the end of a course of treatment with thyroxine, and immediately before antigenic stimulation, prevented this increase in immunity. From this it is concluded that treatment with thyroxine causes an increase in immunity *viâ* the pancreas. Additional confirmatory evidence for this conclusion was obtained by a statistical comparison of groups A and B (thyroxine treatment and partial pancreatectomy) with group A (partial pancreatectomy), which showed that thyroxine had no effect in pancreatectomised animals.

The conclusion reached is that treatment of guinea-pigs with thyroxine increases the production of diphtheria antitoxin and that this effect is produced *viâ* the pancreas, presumably *viâ* the islets of Langerhans.

The influence of thyroid activity on antitoxic immunity in guinea-pigs is analogous to its influence on sensitivity to tuberculin. If these two immunological phenomena are representative of allergic and non-allergic responses to bacterial infection it can be claimed that thyroxine, by its effect on the pancreas, increases bacterial allergy and antitoxic immunity.

The importance of species difference in studies of this kind cannot be too strongly emphasised (Perla and Marmoston, 1941 ; Long, 1954). There is a reasonable chance that observations made in the guinea-pig will apply to man (Long, 1954).

## SUMMARY.

Using both the response to diphtheria toxin injected intradermally and the assay of circulating antitoxin in guinea-pigs immunised with diphtheria toxoid, it was found that thyroxine increases immunity and that partial pancreatectomy prevents the action of thyroxine in increasing immunity.

It is concluded that thyroxine increases immunity in guinea-pigs by inducing compensatory hypertrophy in the pancreas, presumably of the islets of Langerhans.

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