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THE INFLUENCE OF THE THYROID GLAND ON THE ANAPHYLACTIC REACTIONS IN ANIMALS

IV. *The Influence of Thyroidectomy in a Quantitative Schultz-Dale Test*

By

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In the anaphylactic state two essentially different types of antibodies are operating, viz. circulating and fixed antibodies. The former are carried by the blood and can be demonstrated, qualitatively as well as quantitatively, in the serum where they are precipitated when the antigen is added. The fixed antibodies are attached to the cells of the organism, and their presence is shown by *inter alia* Arthus's phenomenon and Schultz-Dale's reaction. When used with the conventional technique, the latter merely reveals whether or no antibodies are present but supplies no information as to their amounts.

In the course of the work done by one of us (Nilzén) on the part played by the thyroid gland in various hypersensitivity reactions, it was felt desirable to find a method that might be used for quantitative determination of fixed antibodies. For, as had emerged from preliminary experiments (1), isolated small intestine of thyroidectomized and sensitized guineapigs contracted after addition of antigen, though circulating antibodies were not to be demonstrated.

On the basis of Schultz-Dale's technique one of us (Ahl-

mark) developed a method for quantitative estimation of antibodies fixed in the small intestine of sensitized guineapigs. This procedure proving appropriate to shed light on the problems alluded to above, it was decided to investigate the following points:

(1) Does isolated small intestine of thyroidectomized and sensitized guineapigs contain fixed antibodies?

(2) If it does, are the antibodies reduced in amount?

MATERIALS AND METHODS

The Thyroidectomy

Male guineapigs weighing 200 to 400 g. were surgically thyroidectomized. That the thyroid had been completely removed was checked by injection of tracer doses of the isotope I^{131}, using a procedure previously reported (2). Within the week following thyroidectomy the guineapigs—as well as control animals—were sensitized with native hen's egg-white. After another 15 to 16 days antibodies fixed to the intestine were estimated with the procedure described below.

The Schultz-Dale Test

The Schultz-Dale test is carried out in the following manner. A strip of small intestine or uterus of a sensitized guineapig is suspended in normal saline to which antigen is added; if the organ contains a sufficient amount of antibody, the intestinal or uterine strip will respond with a contraction. The magnitude of this contraction is dependent upon two factors, viz. the antibody content of the organ and the amount of antigen added. Thus, when a large amount of antigen is used, a stronger contraction will result than with a smaller amount. If the guineapig is sensitized to a high degree, a given dose of the antigen will produce a more vigorous contraction than the same dose would do in an organ preparation derived from a guineapig sensitized to a lower degree.

By recording the magnitudes of the contractions and com-

paring them with contractions provoked by anyone substance acting upon the intestine, it would seem possible to achieve a certain estimation of the intensity of the antigen-antibody reaction. Seeing that histamine is liberated in the reaction, this amine was considered the substance most suitable for this comparison. However, close attention must be paid to the fact that the sensitivity of the intestinal strip to *inter alia* histamine is exceedingly variable, so that it is necessary to test the histamine sensitivity of the intestine with great care before the antigen is added. Basing the assessment of the "antigen-antibody contraction" upon comparison with a single histamine response, or with a histamine contraction provoked *after* addition of the antigen (Coulson, 1953), was not deemed adequate by the present writers. This point will be more explicitly discussed in a subsequent paper.

When appraising the "antigen-antibody contraction" the present writers proceeded as follows. Small intestine was removed from sensitized guineapigs and suspended in the Schultz-Dale bath in the same manner as for quantitative histamine determination; the essentials of the technique have been described by Magnus (3); for details of procedure see Ahlmark (4). When the spontaneous movements of the intestinal strip suspended in the bath had ceased, the histamine sensitivity of the intestine was determined. In this test it was required that the intestinal strip should respond with largely proportionate contractions to a series of different histamine doses (Fig. 1). The histamine sensitivity having thus been ascertained, antigen was added in such an amount that the contraction due to it was either identical with one of the previous histamine contractions or interjacent between two of these as to order of magnitude. The comparative value of the "antigen-antibody contraction" versus the contractions evoked by histamine was then computed by interpolation and expressed in micrograms histamine dihydrochloride per 1 g. antigen. In the experiments native hen's egg-white was used as antigen. If thus in a given instance 0.001 mg. antigen produced the same contraction as 0.1 μg. histamine dihydrochloride, this

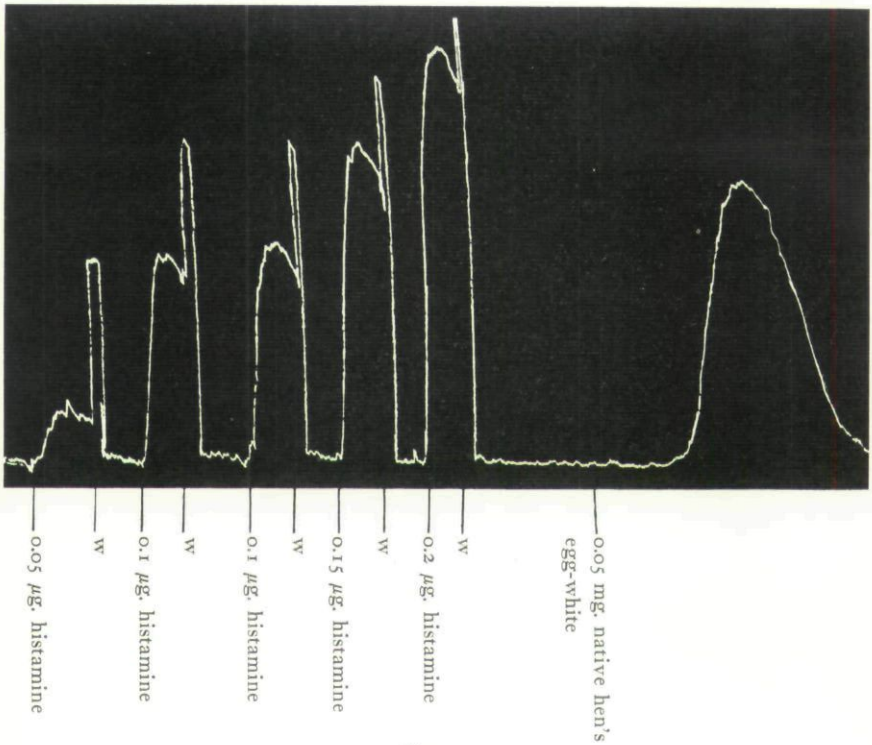

Fig. 1.

Estimation of the histamine equivalent for an antigen-antibody contraction in the guineapig intestine (cp. the text). In this instance the contraction was computed as corresponding to 0.13 μg. histamine, i.e. a histamine equivalent of 2,600. (w: change of fluid in the bath).

implied that 100,000 μg. histamine was the histamine equivalent for 1 g. antigen.

When the contraction-provoking effect of antigen was studied with intestinal strips taken from guineapigs with unknown degree of sensitization, frequently several intestinal preparations had to be tested until the appropriate amount of antigen, i.e. that which would render the contractions comparable to histamine contractions, could be accurately determined. The reason was, that the intestinal strip could be used only for one antigen estimation, the preparation by contact

with the antigen being completely or partially desensitized. As will be seen from Fig. 2, at different degrees of sensitization the comparison figures may vary up to 10^7 times.—As a rule four estimations per animal were performed, the mean of these tests being given as the animal's comparison figure.

To convey an impression of the accuracy of the method for quantitative determination of organ-fixed antibodies as described in the foregoing by the present writers, an experiment may be quoted which was carried out with guineapigs sensitized to egg-white. In Fig. 2 the comparison figures are given for "antigen-antibody contractions" in guineapigs sensitized to native egg-white with reference to the period that had elapsed since the sensitizing injection. It will be noted that a powerful Schultz-Dale effect appeared about ten days after sensitization and subsequently persisted for at least three months.

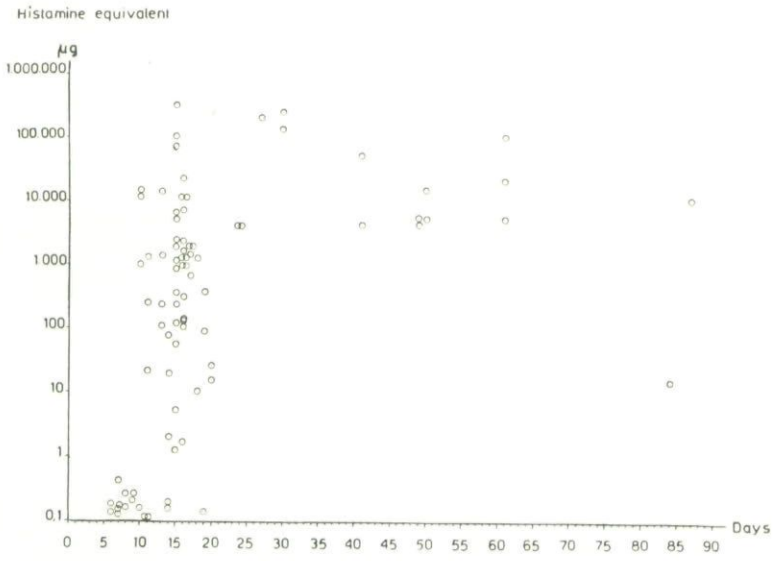

Fig. 2.

Histamine equivalents for antigen-antibody reactions in guineapig intestine with reference to the period that had elapsed since the sensitizing injection (of native hen's egg-white).

RESULTS AND DISCUSSION

In Table 1 the comparison figures are presented for 19 thyroidectomized animals and 20 control animals not subjected to surgery but sensitized simultaneously with their comparison partners.

TABLE 1

Histamine equivalents in 19 thyroidectomized guineapigs and 20 controls examined 15 to 16 days after the sensitizing injection of hen's egg-white.

Controls	Thyr. ect.
110000	22000
42000	14000
15000	7000
8000	2000
8000	900
4000	400
4000	400
4000	200
2000	200
1000	100
1000	100
900	100
600	80
400	20
200	0.8
100	0.6
80	0.5
2	0.3
0.8	

As will be seen from this table, in the thyroidectomized animals the "antigen-antibody contractions" remained at a lower level than in the controls. Hence it emerges *inter alia* that, whereas but 4 of the 20 control animals showed "histamine equivalents" of less than 200, 9 of the 19 thyroidectomized animals displayed the same value. This suggests that the amount of antibody fixed to the intestine is smaller in the thyroidectomized animals.

To judge by the results of preceding studies (1, 6, 7, 8),

free antibodies are markedly reduced in animals thyroidectomized prior to sensitization. Further, it has been observed (1, 9) that local anaphylaxis (Arthur's phenomenon) cannot be elicited if thyroidectomy precedes sensitization. The findings reported in the present paper show that even though the amount of antibodies be decreased in the cells of the small intestine, antibodies are none the less present. Hence there is a difference between, on the one hand, the amount of free antibodies and that of antibodies fixed to the small intestine, and on the other, the amounts of antibodies fixed to the skin and intestine respectively. Thyroidectomy accordingly does not seem completely to abolish antibody formation. The present study does not supply any information as to whether the antibodies demonstrated—though in smaller amounts—in the intestine had been engendered at that site of transferred from antibody-producing organs.

The response to histamine was much the same in small intestine of thyroidectomized and control animals. Also here a remarkable difference thus obtains as to the mode of reaction displayed by different organs; for, as has earlier been reported (2), thyroidectomized guineapigs withstand histamine aerosol for considerably longer periods than control animals.

SUMMARY

On the basis of Schultz-Dale's technique a quantitative method was developed for assessing antibodies fixed to guineapig intestine. With this procedure the "antibody amounts" were compared in thyroidectomized and control animals. The former showed a less powerful Schultz-Dale effect, which suggests that in these animals the amount of antibody fixed to the intestine is smaller than in guineapigs not thyroidectomized.

The histamine response was similar in intestinal preparations from thyroidectomized animals and from those not subjected to surgery.

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