THE INFLUENCE OF THE THYROID GLAND ON ANTIBODY FORMATION

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THE INFLUENCE OF THE THYROID GLAND ON ANTIBODY FORMATION

Wesley T. Pommerenke.

Introductory.

During recent years, that phase of biology which is concerned with the phenomena occurring in response to the injection into one animal of organic constituents coming from another animal has been given considerable experimental attention. The attitude toward these phenomena has undergone complete alteration since the time of their discovery when it was considered that they possessed only scientific interest; now they are being looked upon with still more interest because of their practical application to many clinical questions as well as to many other problems, notably the diagnosis of a large number of micro-organic diseases.

Belfanti and Carbone (1898) showed that the serum of horses that had been injected with red blood cells of rabbits possessed marked toxicity for rabbits. Shortly afterward, Bordet, one of the earliest investigators of immunologic reactions, published an account of experiments in which he showed that when a guinea pig is
repeatedly injected with washed rabbit erythrocytes its serum acquires the property of rapidly and effectively destroying and agglutinating, in a test tube, the erythrocytes of rabbits with the diffusion of the haemoglobin into the medium in which it is suspended, while the serum of normal guinea pigs is incapable of accomplishing this reaction. He further demonstrated that this reaction results from the combined activity of two substances on the red blood cells, viz., one, the sensitizer or amboceptor, which is produced in response to the injection of red blood cells and is capable of withstanding heating at 56 degrees C., the other, the alexin or complement, which is destroyed by a heat of 56 degrees. The former is present only in the specific serum while the latter is present not only in the normal specific serum but also in the serum of normal untreated animals. The antigen alone, or the antibody alone, can not neutralize the effect of the complement which is necessary to produce hemolysis. This reaction is of great importance as it is the basis of the Bordet Gengou phenomenon of complement fixation which is used in the identification of such diseases as cholera, dysentery, tuberculosis, and enteric fever (1). The reaction depends upon the fact that antigen in the presence of its specific immunebody will fix, or render inactive, the complement, so that red
blood cells sensitized by their corresponding immune serum will fail to be laked when they are added to the antigen and complement because of the absence of the available or free complement. The Wassermann test for syphilis is the classic example of the complement fixation reaction.

The discovery of the hemolytic reaction suggested the use of other body cells for the development of antibodies and experiments quoted by Zinsser (2) tell of spermatoxins, leucotoxins, pancreas cytotoxins, neurotoxins, adrenal cytotoxins, and corpus luteum cytotoxins. However subsequent investigations have shown that the cytotoxins or cytolysins for the structures named above are not entirely specific since they are not limited to their respective organs alone. The use of erythrocytes, as antigens, in demonstrating cytolysis, is a very desirable one, since the result, the laking of the blood with the liberation of haemoglobin, is easily visible to the naked eye.

Durham and Gruber discovered that when a small quantity of serum coming from an animal immunized against bacillus typhosus was added to a suspension of typhoid bacteria, the bacteria, which were previously distributed throughout the medium and gave it a uniform appearance collected into small clumpy masses, and gradually, as the agglutination progressed, settled to the bottom of the
tube in which they were contained, leaving the supernatant fluid almost entirely clear. In a control tube, containing normal serum and bacterial suspension, on the contrary, no clumping occurred and the fluid retained its cloudy appearance. This reaction is characteristic whenever a bacterial suspension is brought in contact with its specific anti-serum. Diagnostically, the reaction of agglutination is a matter of undisputed importance, especially in the early positive detection of typhoid fever, since the reaction appears at a comparatively early stage in the disease and can therefore be used as a criterion for the diagnosis. Making reservations for group-agglutinins, which, however, can be readily eliminated by using high dilutions of anti-serum, it may be said that agglutinins are specific.

The previous experiments have been concerned with the results obtained by the injection of cellular elements of one organism into another and these studies have suggested other experiments in which dissolved albuminous bodies were used as antigens. Tchisowitzch (1899), who injected horse and eel serum into rabbits, was one of the first to follow this line of investigation. He found that on withdrawing some of the blood of the rabbits so treated, and mixing its serum with that of a horse of eel, the mixture became cloudy and the reaction ceased when
part of the albuminous content of the horse or eel serum had precipitated into the bottom of the tube. Bordet was able to duplicate the same reaction using the serum of rabbits treated with chicken serum. The precipitating serum is, however, not entirely specific and is relative rather than absolute, since the serum obtained by the immunization of one animal against human serum will develop an antihuman precipitating serum which will not only precipitate human serum, but also in relatively higher concentrations, the sera of some of the other primates. Uhlenhuth (1909), basing his conclusions on long experience, states that there will be no minor or group precipitins in dilutions of antigens amounting to 1:100 or more and that by the method of sufficiently diluting the antigen it is possible to entirely eliminate such non-specific partial reactions. Nuttall (3), employing this phenomenon of precipitation has contributed greatly to a further method for the determination of zoological species. By making some 15000 precipitin tests on over 900 species he not only substantiated many of the accepted zoological classifications but also added significant information on certain disputed points. Running the precipitin upon monkeys, he showed that the reactions became feebler as the species examined is farther separated from man zoologically. It will be seen that as we read the left hand column down from man to the Ateles the
amount of the precipitate gradually decreases. When
Nuttall used other than blood of the primates, it gave
but slight or no reaction with anti-human precipitating
serum.

Nuttall's (3) Tests with Anti-human Serum

<table>
<thead>
<tr>
<th>Antihuman precipitating serum used against:</th>
<th>Precipitate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Man</td>
<td>100%</td>
</tr>
<tr>
<td>Chimpanzee (loose precip.)</td>
<td>100%</td>
</tr>
<tr>
<td>Gorilla</td>
<td>64%</td>
</tr>
<tr>
<td>Cynocephalus mormon</td>
<td>42%</td>
</tr>
<tr>
<td>Ourang</td>
<td>42%</td>
</tr>
<tr>
<td>Cynocephalus sphinx</td>
<td>29%</td>
</tr>
<tr>
<td>Ateles</td>
<td>29%</td>
</tr>
</tbody>
</table>

The percentages in the above table refer to the percentage
of precipitation after a given time, using that amount of
precipitation resulting from the use of the precipitating
serum with its specific antigen as 100%. The antigen
dilutions are equal throughout.

The cells, tissues, or organs, in the body, which are
the seats of antibody production do not seem to have been
definitely located. Studies of natural and acquired im-
munity as measured by antibody formation have been made
before and after the removal of some of the more import-
ant with the hope of either localizing some organ or
organs which have an undisputed relation to the function
of antibody formation or substantiating the contention
that no particular structure in the body is alone respon-
sible for the presence of the immune bodies.
Zinsser (2) found that guinea pigs deprived of their spleens showed no difference from normal animals in regard to their susceptibility to tuberculosis. According to Wassermann (2a), the spleen is particularly rich in antibody content in animals that have been treated with bacillus typhosus. He also found that the thymus, bone marrow, and lymph nodes contained more immune substances than were present in the blood at an early stage of immunization. Wassermann and Citron also found that animals that had been inoculated intravenously with bacillus typhosus had the highest titered anti-typhosus serum present in the blood stream, while the anti-typhoid exudates of the peritoneum and pleura were more cytolytic to their respective antigens than was the blood serum. Such experiments as these may indicate that antibodies are formed in the region where they gain admittance into the body. However, according to Topley (4a), the contention that antibodies are formed in the region where they are inoculated has been disproved. Gates (4b) reports negative results on the formation of anti-hen hemolysins following partial adrenalectomy in guinea pigs. Elliot Cutler (4), experimenting with partially hypophysectomized guinea pigs, concludes that either the hypophysis plays no important role in the development of antibodies, more specifically the typhoid agglutinins, the haemoagglutinins, and the haemolysins, or that that portion of the hypophysis which
remained intact and was necessary to support life may be able to maintain adequately the function which the whole gland may conceivably possess. From these and other experiments, we may conclude that perhaps any or all cells are capable of producing immune substances and that no special single organ is an independent and sole laboratory for the formation of antibodies.

However, the considerable impetus that has been given to the study of the endocrine organs, and more particularly the thyroid gland, because of their prominent role in development, growth, and function of the individual and because of the very evident influence of these organs upon metabolism and their relation to the generative system, the liver, the pancreas, the adrenals, the pituitary, and the thymus is due to the idea that they may be associated functionally with the antibody-producing mechanism by which the body protects itself against infections.

Our knowledge concerning the embryology, anatomy, chemistry, and pathology of the thyroid gland is perhaps as complete as that of any other organ within the body. No attempt will be made to review or to refer to all the literature relative to thyroid physiology that has been written during the past decade since this alone would fill volumes. Yet a brief survey of some of the outstand-
ing experiments on the thyroid gland which indicate its affect on the whole system may suggest a possible correlation between thyroid function and antibody development.

A diminution of all vital processes within the body is the normal result of the insufficiency of the thyroid function and in extreme cases this reduction of fundamental exchanges in metabolism amounts to 58%. If thyroidectomy is accomplished in young animals they remain apathetic and dejected and one of the later symptoms of thyroid insufficiency shows itself in myxedema, an ailment resulting in the gradual swelling of the face, especially noticeable in the lips, eyelids, and nose. The skin becomes dry and rough. There is also a marked decrease in temperature. The genital organs remain infantile, and as a rule, the testes do not descend. The animals become weak and development of the disease progresses until death occurs. The clinical picture following thyroidectomy is similar in the adult to that in the young rabbits but the symptoms are less intense. Metabolism is decreased, but as they have reached maturity, the osseous system does not become affected. Nutritional disturbances following thyroidectomy are very pronounced. The thyroid is considered to be one of the regulators of the accumulation of fat and it is well known that thyroid-
feeding and hyperthyroidism results in a diminution in
the amount of adipose tissue while the exact opposite
is true in the case of thyroidectomy and consequent hypo-
thyroidism. Wendal reports a distinct increase of
nitrogen in the urine with a corresponding decrease in
weight, indicating nitrogen metabolism following the
administration of thyroid material. Marine (5) claims
that there is a direct relation between some constituent
of the gonads, especially in the female, and the thyroid.
This is shown by the crude manifestations of thyroid en-
largement occurring regularly at intervals of the menstrual
cycle. Degener (6) reports an enlargement, as much as
400% in extreme cases, of the anterior lobe of the hypoph-
ysis and especially of the pars intermedia, following
thyroidectomy in rabbits. Similar results were ob-
tained by Rogourtusch and Hafmeister in 1892. According
to Schöefer (1895) the intravenous injection of thyroid
extract results in the reduction of blood pressure and a
marked dilation in the peripheral blood vessels. Soon
afterwards this was found to be correct by many invest-
igators. Following thyroidectomy the red corpuscles dim-
inish in number and leucocytosis takes place and the amount
of oxygen carried in the circulatory system may be reduced
50%. Marine (5) believes that reactions to infection
as shown by a diminution in iodine store and a tendency to
hypertrophy clearly indicate that the thyroid gland is
an important factor in resistance to infection and that the increased production of heat is to some extent dependent upon the thyroid gland. This same author found that the treatment with iodides removes certain disorders which produce symptoms similar to those present in Grave's Disease. The iodine content of the thyroid gland is unusually high since, according to Justus (5b), in proportion to its weight, it contains from 8 to 10 times more iodine than the general bodily average does. Hill recommends that a Gram's Iodine suspension of bacteria be injected into an animal, since, as he claims, this will result in a higher titered anti-serum. The thyroid is extremely well vascularized, receiving as it does, in proportion to its size, about five times as much blood as do the kidneys. The quantity of the blood supply to the thyroid gland has been estimated by Knowlton and others to amount to from 3.5 to 5 cc. per gram per minute.

Mlle Louise Fassin, a French worker, believes that the thyroid gland is directly connected with production of alexin. She found that feeding or injecting thyroid material resulted in a rapid increase in alexin, both haemolytic and bactericidal. When the thyroid gland was removed from the rabbits, a reduction in the alexin resulted.

*Personal communication from L.C. Hill, Director, Mid-West Research Laboratories.*
The relation of the thyroid gland to the development of immune substances has received considerable attention, but because of confusing or even contradictory results, the question still remains an open one. Fjedstad (7) reports negative results on agglutination phenomena following thyroidectomy in 18 rabbits. Hekteen and Curtis (8), basing their conclusions on experiments with thyroidectomized dogs, found that thyroidectomy produces no noteworthy variation in the amount of hemolysin produced. However, Frechlin (9b) reports a reduction of hemolysin production in thyroidectomized dogs. Launcy (9a) did not observe any difference in resistance to spirochete infection in chickens that had been thyroidectomized. Garibaldi (9c) found that thyroidectomized rabbits' hemolytic serum of a high titer. Také (9) studied the effect of thyroidectomy, controlled by respiratory exchange measurements, on antibody production in rabbits and concluded that the thyroid gland is not a vital factor in antibody formation. Ecker and Goldblatt (10) found that the injection of red blood cells into thyroidectomized rabbits produced a higher titered serum, in haemolytic content, than was present in the serum of control animals. According to Koopman (11), feeding desiccated thyroid material is conducive to high titered haemolysin production.
In general, it has been found that haemolysin and also agglutinin formation is higher in thyroidectomized than in control rabbits.

Problem and Methods.

The following studies were undertaken to determine whether or not the thyroid gland is directly concerned in the formation of antibodies in rabbits. Three types of animals were used, viz., thyroidectomized, thyroid-fed, and controls. Aside from the removal of thyroids from some, and the feeding of thyroid material to others, all rabbits within a series were given the same treatment, one series being injected with human red blood cells, another, with bacillus typhosus, the other, with human serum. These experiments were completed in the three following series:

Series I. The Influence of the Thyroid Gland on the Production of Hemolysins. This series consisted of 21 rabbits, 6 of which were thyroidectomized, 8 of them were thyroid fed, while the remaining 7 were used as controls. All members of this series were similarly immunized to human red blood cells. Subsequent titers of the anti-serum indicated the degree of immunity produced.
Series II. The Influence of the Thyroid Gland on the Formation of Agglutinins. This series contained 9 thyroidectomized, 7 thyroid fed, and 6 control rabbits, all of which were immunized to bacillus typhosus. Their anti-sera were titrated after the last injections and the relative immunity of the three types of animals was studied.

Series III. The Influence of the Thyroid Gland on the Development of Precipitins. This group of animals was also composed of thyroidectomized, thyroid fed, and control rabbits, all the 22 individuals of this series being immunized against human serum. The titers of the precipitating sera of the different types of animals of the series were studied and compared.

Anatomy.

As is the case in most animals, in the rabbit, the thyroid gland is composed of two lateral lobes joined by an isthmus. The lateral lobes lie on either side of the trachea and extend cephalad from the sides of the trachea at the level of the second or third tracheal rings below the cricoid cartilage. In some of the cases, the tips of the lateral lobes extend cephalad and dorsal so that they come to lie on the lateral surfaces of the
esophagus. The isthmus is a thin, ribbon-like, mass of tissue which connects the two lateral lobes at their bases and passes over the trachea immediately below the cricoid cartilage. The general position and morphological relations of the thyroid gland is a matter of common anatomical knowledge and is too well known to call for any detailed description. Usually two pairs of parathyroids are present, an upper pair and a lower pair. They are small oval or spherical bodies, having in most cases, a faintly lighter tint than the thyroid tissue. Their position may be very variable, not only within a species, but also in different members of a single species. The position of the upper pair of parathyroids are not constant and may be situated either at the sides, front, or back of the body of the thyroid while they may be even within the capsule surrounding the thyroid gland. The lower parathyroids have a more constant position lying as they do caudal to the thyroid gland and beneath the sternothyroid muscle. The upper parathyroids, especially if they are incorporated within the capsule of the thyroid, possess no notable characteristic, sufficient to insure absolute identification by the naked eye and the effort to excavate an almost invisible body, particularly in the event of hemorrhage, is, except in unusual cases, an impossibility. It is useless to say that the operation* performed under ether anesthesia.
for the removal of the thyroid gland was done with all possible precautions to keep the wound aseptic. Special pains were taken to remove every bit of the thyroid tissue from the trachea as thyroid tissue is very prone to replace itself. The parathyroids were, as far as possible, left in situ so that their function would not be hindered. Three fatalities occurred early in the course of the experiments due to faulty technique in the removal of both the thyroid and the parathyroids; however when this was corrected the rabbits withstood the operation without any noteworthy effects other than symptoms of general indisposition for the first few days following the operation.

Two types of controls were kept. The one type consisted of rabbits which were perfectly normal up to the time when they were given injections. These controls will herein be designated merely as 'controls'. The other type consisted of individuals in which incisions were made for the removal of the gland, the neck being deliberatley lacerated, to simulate as nearly as possible the surgical effects encountered in actual removal of the thyroid. However in these cases, no tissue was removed. The individuals of this type will herein be designated as 'operation controls'. Dr. Stoland performed the thyroidectomies of series I while the writer performed all the other operations including those of the 'operation controls'.
Technique of Titrations.

Hemolysing -- For the determination of the hemolytic titer of the serum from a rabbit immunized against human blood cells, the following directions are followed: Collect about 4 cc. of immunized rabbit's blood aseptically into a hemolytic test tube by allowing it to drop from the punctured marginal ear vein, then set aside in the ice-box to clot, or if the time is pressing, defibrinate, and then place into the centrifuge until all the cellular elements are precipitated into the bottom of the tube. The serum is then withdrawn from the cells and inactivated by placing it in a 55 degree water bath for 30 minutes for the destruction of the complement. In the meantime complement is obtained by removing the serum from the blood obtained by cardiac puncture of a guinea pig. A suspension, consisting of 10% of washed human red blood cells, suspended in physiological salt solution, was previously prepared, and the following protocol set up: Set up a series of test tubes so that each tube contains successive dilutions of the sensitizer as follows: 1/10, 1/20, 1/25, 1/30, 1/35, 1/40, and so on up until the last tube has a dilution of 1/200. Place the following substances in the test-tubes and mix them well:

1 cc. of each dilution of sensitizer,
5 cc. of a 1/10 dilution of complement (excess),
1 cc. of a 1/10 suspension of human blood cells,
3 cc. of physiological salt solution.
The mixtures are now placed in the 37 degree C. water-bath for 30 minutes. That dilution of amboceptor or sensitizing which is the highest that will produce complete laking of the blood within 30 minutes is considered the the unit since it is that amount of amboceptor which will take a unit (.1 cc. of a 1:10 suspension of blood) in the presence of an excess of complement, in a total volume of 1 cc. within 30 minutes. This unit is however only approximate since an undetermined amount of complement was used in the 'excess'. The next step is to determine the accurate amount of complement in a unit (of complement), i.e. that amount that will take a unit of red blood cells in the presence of a unit of amboceptor within 30 minutes. This unit is determined by titration against amboceptor of known titer. Having obtained this, an accurate titration is made of the sensitizer and this dilution being recorded as being the unit of amboceptor since it is the highest dilution of sensitizer in a given volume that will produce hemolysis under standard conditions. This highest dilution of the amboceptor is known as the titer. Control tests were also used to determine the reliability of the results.

**Agglutinins** — To ascertain the titer of the agglutinating serum, the highest dilution of the anti-serum that will produce agglutination of bacteria is determined by
the following method: Prepare a suspension of typhoid bacilli by adding 8 cc. of physiological salt solution to a heavy 24 hour agar slant culture of the bacteria and gently rub the growth from the surface of the agar slant. Suspend the bacteria by shaking the contents of the tube. Filter the bacterial suspension through a moist filter paper and place in a 50 degree C, water-bath for 30 minutes. Next prepare the following dilutions of typhoid immune serum in test tubes: 1:10, 1:25, 1:50, 1:100, 1:150, 1:200, 1:250, 1:300, and so on to 1:5000. Now place .5 cc. of each dilution of the immune serum in a series of agglutinating tubes (3/8 in. in diameter), and in an extra tube add .5 cc. of salt solution for control. .5 cc. of the killed bacterial suspension is now added to the tubes of the above series. This, of course, doubles the dilutions of the antiserum. The tubes are now shaken to mix the fluids and thus bring about the distribution of the bacteria throughout the entire suspension. The tubes are now placed in a 37 degree C, water-bath for sixty minutes and then put aside in the ice-box for an additional 3 hours. The tubes are now examined for agglutination by using transmitted light. The use of a hand lens for the accurate determination of the limit of agglutination proved to be of value and was

* The same strain of bacteria that had been used for the titrations had also been used for immunization.
used in all agglutination titrations. The highest dilution of the immune serum that produced agglutination was recorded as the titer of the serum.

Precipitating—The titer of precipitating serum is obtained by finding the highest dilution of antigen, in this case inactivated human serum, that will produce flocculation with its specific anti-serum within a given time. The following method was used: Prepare the following dilutions of inactivated human serum: 1:20, 1:50, 1:100, 1:150, 1:200, 1:250, 1:300, and so on up to 1:8000 (further if necessary). In all precipitation titrations, 1 cc. of the anti-human serum is first placed in the bottom of small 'precipitin tubes'. 1 cc. of each dilution of human serum is then added in such a manner that the latter is layered over the former to form a ring where the two fluids unite. The ring test, showing the precipitate as a white line appears at the point where the fluids come in contact with one another. The tubes are now thoroughly shaken and placed in the 37 degree C. water-bath for an hour and then set aside in the ice-box for three more hours. With the aid of a hand-lens, the end-point of flocculation is determined and the highest dilution of antigen producing precipitation within that time is considered the titer.

* Corresponding to the kind injected into the rabbits.
Experimental.

All the animals used for the experiments herein described were young healthy rabbits, averaging about 150 grams in weight. As far as it is known, none of them had ever been previously employed for experimental purposes.

Series I: The Influence of the Thyroid Gland on the Production of Hemolysins. This series of animals was immunized to human blood cells and consisted of 21 rabbits, divided into three experimental units. The first unit contained 6 thyroidectomized rabbits; the second, 8 thyroid fed rabbits; and the third, 7 control rabbits. Three of the latter rabbits were operated upon for the exposure of the thyroid gland but no tissue was removed from these 'operation controls'. There were three fatalities among the thyroidectomized rabbits, but the experiment was completed on the number of animals indicated above. The normal anti-human hemolysins in every rabbit of the entire series was first determined but in no case was complete hemolysis observed in the tube containing such a low dilution of anti-human serum as 1:10, using .1 cc. of inactivated rabbit serum, .1 cc. of a 1:10 suspension of washed human blood cells, and 2 units of complement (from a guinea pig). The animals on which thyroid-
ECTOMY had been performed were bled two days after the operation and their sera again titrated for the presence of hemolysins. No changes in the hemolytic titer of the sera could be noticed. Seven days after the operation, the thyroidectomized rabbits were given intraperitoneal injections of 3 cc. of a 100% (original volume) suspension of washed human red blood cells. After five days the animals were again intraperitoneally injected with 3 cc. of washed human blood cell suspension, while at two more successive intervals of five days each, they were given intraperitoneal injections of 5 cc. of human blood cell suspension. The injections into the thyroidectomized rabbits were begun one week following the operation in order to utilize the period of the development of thyroid insufficiency, thereby avoiding possible stages of recovery or severe myxodema. According to Marine (5), "In rabbits this decrease (in metabolism) begins usually in from 6 to 8 days after thyroidectomy and reaches its lowest level between the 20th and 30th day". In general, the more toxic or severe the injection, provided it is not sufficient to materially weaken the animal, the more rapid will be the elimination of the antigen and the more rapid and powerful the antibody formation. Larger amounts of antigen were not used and injections were not more frequently given since these might have interfered with.* After complete recovery.
the well being of the animals and because it was not desired to get a particularly high titered anti-serum, as the primary object of these experiments was to compare the titers of thyroidectomized, thyroid fed, and control rabbits, all of which were similarly injected with antigen. Following the recommendation of Zinsser (12) the rabbits were bled on the 9th, 11th, and 13th, days after the last injection of the cell suspension and their anti-sera titrated for the hemolytic content, as the high point of the antibody curve may be expected to lie within that interval of time. In all titrations for the determination of the hemolytic titer the following reagents were used: .1 cc. of each dilution of inactivated immune serum, .1 cc. of a 1:10 suspension of washed human blood cells, 2 units of complement*, and enough salt solution to bring the total volume up to 1 cc. The highest dilution of the sensitizer which would produce complete hemolysis of the blood cells, after an incubation of 30 minutes, was considered the titer of the hemolytic serum. A comparison of the titers of the anti-sera of the different animals in this unit shows considerable variation as will be seen by examining parts 'A' in the Text-figs. 2, 3, and 4. Individual variations between members in a group, similarly treated, as shown in Table I, amounted in

* Titer determined by previous titration.
some cases to almost 200%. That some rabbits do not produce a very high titered anti-serum, while the anti-serum of other rabbits, treated similarly however, is comparatively high titered is known to all who have used rabbits for antibody production. The effect of the individual variations is overcome, however, by taking group averages.

During the course of these experiments, the work on each series consumed about 40 days and in this time the thyroidectomized animals showed no marked differences from the thyroid fed or control individuals. In other words, the thyroidectomized rabbits developed no very noticeable symptoms during the course of these experiments beyond a slight dryness and thickening of the skin, most apparent about the face, and a lowering in bodily temperature. These animals were comparatively less active and gradually took on weight. As one of the indices of the general condition of the rabbits, they were weighed from time to time throughout the course of the experiments and the results of these weighings are shown graphically in Text-figs. 12, 13, and 14.

As one might naturally expect, individual differences in symptoms were noticeable in the thyroidectomized individuals in all three series. However, the above, are general statements concerning the average conditions of
the 23 thyroidectomized rabbits that were used throughout the tests. In some of the animals the myxodemic symptoms became more and more pronounced after the experiments were completed, altho they, no doubt, had already started during the progress of the experiments but were at that time less noticeable. Death was the result in these (4) cases. At present, some 90 days after the operation of thyroid removal, a number (2) of rabbits are developing marked symptoms of insufficiency of the thyroid function, while still others (2) can not be easily distinguished from normal animals. A study of the clinical symptoms following thyroidectomy and thyroid feeding might give interesting and valuable results but lack of time and facilities precluded the inclusion of these added, detailed, and continued observations with those of the experiments proper as the rabbits were disposed of as soon as the titers of their immune-sera had been determined in order to provide space for another series of animals.

Along with the work on the members of the first experimental unit, a second group of 8 individuals of the second experimental unit (rabbits that were thyroid fed) were immunized against human red blood cells, receiving injections in every way comparable to those given to the

* Perhaps regeneration of the thyroid gland has occurred in these cases. However all visible thyroid tissue was removed in the operation.
thyroidectomized rabbits. Likewise they were bledd on
the 8th, 11th, and 13th days respectively following the
last immunizing injection of the cell suspension to de-
termine the hemolytic strengths of their anti-sera. The
results of these titrations are summarized in Table 1
and in Text-figs. 2, 3, and 4. However before they
were given any injections of human red corpuscles, and
before thyroid feeding had commenced, tests were made
for the presence, in their blood, of normal hemolysins
for human blood cells, and as was the case in the first
experimental unit of this series, no complete hemolysis
was present in the tube having .1 cc. of a 1:10 dilution
of the inactivated rabbit serum. From then on, until
the time of the final titration of their anti-sera, all
the rabbits in this unit were fed daily, on thyroid
material* equivalent to six grains of fresh thyroid con-
stituents. After five days of thyroid feeding, but
before any injections of the blood suspension was given,
the sera of the rabbits were again examined for their
hemolytic content but no change could be observed. The
rabbits were then intraperitoneally injected with 3 cc.
of washed human blood cells suspended in saline solution
up to the original volume. This injection was followed
after five days with another injection one of 3 cc. Two
intraperitoneal injections of 5 cc. each followed at inter-

* Obtained in tablet form from Parke Davis & Co.
vals of five days each. The titers of the hemolytic sera developed in response to the injection of human blood cells were determined by titrating according to the previously described method and are represented in parts 'B' in Text-figs., 2, 3, and 4. The extreme, i.e., the maximum and minimum, titers, as well as the averages are shown in Table I.

Simultaneously, with the two previous experimental units, a third unit, composed of 7 rabbits, was immunized against human blood cells. These animals were used as controls and were of two types, viz., 4 controls, i.e., those that were given no experimental treatment other than the repeated injections of human erythrocytes, and 3 'operation controls' which were operated upon for the exposure, but not removal, of the thyroid gland. The purpose of using a double system of controls throughout the experiments on the three series of rabbits was to ascertain that any facts noted with reference to the thyroidectomized rabbits were not due merely to the physical or surgical effects of the operation; and since these 'operation controls' behave and react like the controls rather than like the thyroidectomized rabbits, they are classed with the former. So any statement concerning changes in thyroidectomized animals is made with the justifiable assurance that these results are due to
conditions caused by the removal of the thyroid gland. All the members of this third experimental unit were bled, their sera being tested for the presence of normal hemolysins, but, as was the case in the first two experimental units, no complete hemolysis was present in the tube containing 0.1 cc. of a 1:10 dilution of inactivated serum from the rabbit. At intervals of five days each, the rabbits were given intraperitoneal injections of washed human blood cells, suspended in salt solution up to the original volume, as follows: first injection, 3 cc.; second injection, 3 cc.; third injection, 5 cc.; fourth injection, 5 cc. On the 9th, 11th, and 13th days after the last injection, the animals were bled, their sera being titrated, following the previously described method, for the determination of their hemolytic activity. The results of these titrations are shown graphically in parts 'G' in Text Figs. 2, 3, and 4, and numerically in Table I.

The decimal dilutions of the average titers are purely hypothetical in nature. Since the dilutions were increased by 5 in every tube, the final titers can only be accurate within the limits of 5. However, the decimals were used to show a more exact average and to distinguish between sera being almost equally titered.
Text-fig. 1. Showing the great variability in titers of anti-sera of rabbits that have been given the same laboratory treatment. The circles on the curves represent the titers of the anti-typhoid sera of all the members of the three experimental units in Series II. The figures on right side represent titers and were obtained from the titrations of the 9th day following the last injection of the bacterial suspension.

Thyroidectomized rabbits.

Thyroid fed rabbits.

Control rabbits.
Table I.

Influence of the thyroid gland on production of hemolysins.

<table>
<thead>
<tr>
<th>Experimental unit</th>
<th>Titration No.</th>
<th>Maximum</th>
<th>Average</th>
<th>Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Six Thyroidectomized Rabbits</td>
<td>1</td>
<td>1:110</td>
<td>1:79.16</td>
<td>1:40</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1:90</td>
<td>1:91.6</td>
<td>1:60</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1:105</td>
<td>1:82.5</td>
<td>1:50</td>
</tr>
<tr>
<td>Eight Thyroid Fed Rabbits</td>
<td>1</td>
<td>1:96</td>
<td>1:70</td>
<td>1:40</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1:90</td>
<td>1:87.5</td>
<td>1:35</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1:85</td>
<td>1:79.8</td>
<td>1:35</td>
</tr>
<tr>
<td>Seven Control Rabbits</td>
<td>1</td>
<td>1:105</td>
<td>1:83.5</td>
<td>1:60</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1:105</td>
<td>1:77.9</td>
<td>1:50</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1:105</td>
<td>1:79.4</td>
<td>1:65</td>
</tr>
</tbody>
</table>

An examination of the graphic records of these titrations (Text figs. 1, 2, 3, and 4) shows great variations. Again referring to Table I, it will be noted that there is more variation in the titers of the immune sera among different individuals of a single experimental unit than is present between the averages of the different experimental units. That is, differences is the titers of the immune sera among members of a single unit may amount to about 300%, since the maximum titer in the case of one the thyroidectomized rabbits was 1:110 while the minimum titer in the same unit was 1:40, while the averages of the different units in this series did not vary more than 15%.
Text-fig. 1. The extent of the horizontal lines indicates the titers of the hemolytic sera coming from the rabbits of Series I. These rabbits were immunized against human blood cells. The heavier horizontal lines represent the average titers of all the sera in the three experimental units. The vertical lines extending from the heavy 'average' lines serve to facilitate a comparison of the three units. The titers represented in this figure were obtained by titrating the immune serum on the 7th day following the last injection of human blood cells.

A = Thyroidectomized rabbits.
B = Thyroid fed rabbits.
C = Control rabbits.
Text-fig. 3. The same as Text-fig. 2, only, in this case, the titers as represented in the figure, were obtained from the titrations on the n-th day following the last injection of the washed human blood cell suspension.

A = Thyroidectomized rabbits.
B = Thyroid fed rabbits.
C = Control rabbits.
Text-fig. 4. Showing the titers of the hemolytic sera developed in rabbits by the 13th day following the last injection of the human cells.

A = Thyroidectomized rabbits
B = Thyroid fed rabbits.
C = Control rabbits.
from one another. The highest titered serum in the first unit was higher than the highest titered serum in either the second or third units. The lowest titer in the control unit was higher than the lowest titer in the thyroidectomized or thyroid fed units. However, it will be seen upon examination of the graph for Series I in Text-fig. 11 that the total average of all the titrations in the case of thyroidectomized rabbits is greater than the total average of the titrations of either the thyroid-fed or of the control rabbits, while the average titers of the thyroid fed animals are lower than the those of either the thyroidectomized or control animals. The average titers of the thyroidectomized and the control units are almost equal and easily lie within the limits of experimental error, so no general statement can be made concerning the effect of thyroidectomy on the formation of Hemolysins. However, on the face of it, one might conclude from the experiments in this series that thyroidectomy is conducive to the development of high titered anti-serum while thyroid feeding tends to cause a reduction in the titer of the immune serum.

Series II. The Influence of the Thyroid Gland on the Formation of Agglutinins. This study was completed with 22 rabbits which were immunized against bacillus
Text-fig 5. A comparison of the titers of the anti-sera against bacillus typhosus in the sera of the members of the three experimental units of Series II. The titers recorded in the figure are those obtained by titrating on the 7 day after the final inoculation of the bacterial suspension. Here again, the heavier lines represent group averages.

A = Thyroidectomized rabbits.
B = Thyroid fed rabbits.
C = Control rabbits.
typhosus. Three experimental units were used as follows: the first unit consisted of 9 thyroidectomized rabbits; the second unit, of 7 thyroid fed rabbits; the third unit, of 6 control rabbits. The controls were of two types—three normal controls, and three operation controls. Tests for the presence of natural agglutinins were first made on all the rabbits of the entire series but in no case was noticeable agglutination present in tubes in which 1:50 dilutions of the rabbits' serum were mixed with typhoid suspension. On the second day following the operation, the thyroidectomized rabbits were again tested for the presence of agglutinins for typhoid bacilli in their sera. The operation controls, as well as the rabbits of the second experimental unit, which had been fed daily on six grains of thyroid material for six days, also were again tested for normal agglutinins, but not until after the injections of the typhoid bacilli into all of the rabbits of the entire series, were noticeable agglutinins present in their sera. All the animals in this series were now given four immunizing injections of bacillus typhosus at intervals of five days each. The 'Laboratory Strain # 57' ** was used. It had been grown on artificial media for several years and was carried along

* Six days after the operation in the thyroidectomized unit and six days thyroid feeding had commenced in the thyroid fed unit.

** Obtained from the Department of Bacteriology of the University of Kansas.
Text-fig. 6. Same as in Text-fig. 5, only in this case, the titrations for the determination of the titer of the agglutinating sera were made on the 9th. day following the final injection of the typhoid suspension.

A = Thyroidectomized rabbits.
B = Thyroid fed rabbits.
C = Control rabbits.
during this experiment by daily transfers on agar with occasional plating to determine its purity. The suspension used for the injection was prepared in the same manner as were the suspensions used in the titrations for agglutination determination. This method has already been described, however, in addition to inactivating the suspension by heating it at 56 degrees C. for 30 minutes, a small quantity of formalin was added to retain its sterility. The suspension was prepared in lots of about 100 cc. each so that all of the rabbits could be immunized from the same stock vaccine.

The first two injections, amounting to 1 cc. and 2 cc. each respectively, were given subcutaneously, while the last two injections of 4 cc. each were administered intraperitoneally. On the 7th, 9th, and 11th days following the last injections all the animals of the three experimental units were bled and their sera titrated to determine the degree of immunity produced. The titers of the anti-typhoid sera from this series are graphically represented in Text-figs. 1, 5, 6, and 7, and numerically in Table II. Curves were drawn in Text-fig. 1 to show the great variability in the response of different animals, similarly treated, to the injection of foreign matter.
Text-fig. 7. Showing the relative degree of immunity produced on the 11th day after the last injection of the bacterial suspension. It will be noted upon examining this and other figures that the unit averages are very nearly alike.

A = Thyroidectomized rabbits.
B = Thyroid fed rabbits.
C = Control rabbits.
Table II.
The influence of the thyroid gland on the formation of agglutinins.

<table>
<thead>
<tr>
<th>Experiment unit</th>
<th>Titration No.</th>
<th>Maximum</th>
<th>Average</th>
<th>Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nine</td>
<td>1</td>
<td>1:9900</td>
<td>1:7210</td>
<td>1:5400</td>
</tr>
<tr>
<td>Thyroidectomized</td>
<td>2</td>
<td>1:8400</td>
<td>1:7300</td>
<td>1:5500</td>
</tr>
<tr>
<td>Rabbits</td>
<td>3</td>
<td>1:6800</td>
<td>1:7340</td>
<td>1:5800</td>
</tr>
<tr>
<td>Seven</td>
<td>1</td>
<td>1:8700</td>
<td>1:6325</td>
<td>1:4700</td>
</tr>
<tr>
<td>Thyroid-fed</td>
<td>2</td>
<td>1:8700</td>
<td>1:6600</td>
<td>1:4900</td>
</tr>
<tr>
<td>Rabbits</td>
<td>3</td>
<td>1:8800</td>
<td>1:7820</td>
<td>1:5200</td>
</tr>
<tr>
<td>Six Control</td>
<td>1</td>
<td>1:10300</td>
<td>1:7330</td>
<td>1:3300</td>
</tr>
<tr>
<td>Rabbits</td>
<td>2</td>
<td>1:10300</td>
<td>1:7600</td>
<td>1:3300</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1:10000</td>
<td>1:7500</td>
<td>1:3700</td>
</tr>
</tbody>
</table>

The above Table II, as well as Text-figs. 1, 5, 6, and 7, is self explanatory. It may be interesting to note, however, that in this series the averages of the titers of the control unit is greater than either that of the thyroidectomized or of the thyroid fed unit. Nevertheless, this difference in titer between the control and the thyroidectomized units is almost too small to be detected and falls within the range of experimental error. However, the difference in titer of the control unit and the thyroid fed unit is quite noticeable. The maximum as well as the minimum titered anti-serum is found in the control unit. From the results of the experiments of this series one might be led to believe that thyroidectomy has no influence on antibody formation and that thyroid feeding
results in a decrease in the development of immune substances; yet these contentions are not warranted without the consideration of the results of the experiments of Series I and Series III.

Series III. The influence of the thyroid gland on the development of precipitins. This series was immunized against human serum and was composed of 22 rabbits which were divided into the following three units: the first unit contained 8 thyroidectomized rabbits; the second unit, 7 thyroid fed rabbits; the third unit, 7 control (normal and operation) rabbits. Preliminary tests showed the absence of normal precipitins when dilutions of antigen of 1:50 were used. Precipitin tests were again made against with the sera of these rabbits before the immunizing injections were given. In addition to their regular food, the thyroid fed rabbits were given 6 grains of thyroid material daily from the day following the preliminary precipitin test to the day of the final titration of the anti-serum. At five day intervals all rabbits of the entire series were given intraperitoneal injections of inactivated human serum in amounts of

# Since these rabbits were not previously immunized against human serum no antibodies had developed.

** Corresponding to the type used in the titrations.
Text-fig. 8.

This figure shows a comparison of the titers of the precipitating serum coming from rabbits that had been given repeated intraperitoneal injections of inactivated human serum. The titers here recorded are those obtained by titrating the immune serum on the 7th day after the final injection of human serum into the rabbits of the three units of Series III.

A = Thyroidectomized rabbits.
B = Thyroid fed rabbits.
C = Control rabbits.
1 cc., 2 cc., 3 cc., and another of 3 cc. each respectively. The rabbits were bled on the 7th, 9th, and 11th days following the last injection and titers of their antiserum determined. The results of these titrations are illustrated in Table III and also in Text-figs. 8, 9, and 10.

### Table III

The influence of the thyroid gland on the development of precipitins.

<table>
<thead>
<tr>
<th>Experimental Unit</th>
<th>Titration No.</th>
<th>Maximum</th>
<th>Average</th>
<th>Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eight</td>
<td>1</td>
<td>1:11500</td>
<td>1:7800</td>
<td>1:4700</td>
</tr>
<tr>
<td>Thyroidectomized</td>
<td>2</td>
<td>1:12300</td>
<td>1:8800</td>
<td>1:4800</td>
</tr>
<tr>
<td>Rabbits</td>
<td>3</td>
<td>1:11700</td>
<td>1:8080</td>
<td>1:4800</td>
</tr>
<tr>
<td>Seven Thyroid fed Rabbits</td>
<td>1</td>
<td>1:10700</td>
<td>1:6925</td>
<td>1:3100</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1:10400</td>
<td>1:7410</td>
<td>1:4000</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1:10600</td>
<td>1:7100</td>
<td>1:3900</td>
</tr>
<tr>
<td>Seven Control Rabbits</td>
<td>1</td>
<td>1:9400</td>
<td>1:6966</td>
<td>1:4200</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1:9800</td>
<td>1:7100</td>
<td>1:4700</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1:9200</td>
<td>1:6920</td>
<td>1:4900</td>
</tr>
</tbody>
</table>

The above table shows that within a single unit variations in titers of precipitating serum may amount to more than 200%, and sometimes even as much as 300%. The average titers of the anti-serum of the rabbits of the thyroidectomized unit are quite noticeable higher than are the titers of the rabbits of both the thyroid fed and the control units. The rabbit having the highest titered anti-serum was in the thyroidectomized group while the
Text-fig. 9. - Same as text-fig. 3 with the exception that in this case the titers recorded are those obtained by titrating the agglutinating sera on the 9th day following the last injection of antigen.

A = Thyroidectomized rabbits.
B = Thyroid fed rabbits.
C = Control rabbits.
lowest titered anti-serum was found in the group of the thyroid fed animals. The titers of the thyroid fed and control units ran approximately a parallel course.

Discussion.

The averages of all the titrations of the anti-sera of all the rabbits in each of the three series are graphically recorded in Text-fig. 11. An examination of this record shows one consistent tendency throughout the entire study, and this is that the thyroid fed rabbits have, on the average, a lower titered anti-serum than have the rabbits that have submitted to thyroidectomy. The 'operation controls', because of their behavior and reactions, belong properly in the control units. The control units that had been immunized against human red blood cells and bacillus typhosus responded with the formation of their respective antibodies, the titers of which were somewhat higher than those of the thyroid fed units. However the series that had received injections of human serum, produced (taking averages of all the individuals of the three units) the lowest titered anti-serum in the control unit, this titer being slightly lower than that of the thyroid fed unit. The average titers of the thyroidectomized and of the control
Text-fig. 10.

The titers represented in this figure are those obtained by the titration of the anti-human precipitating sera developed in the rabbits by the 11 day following the final injection of human sera.

A = Thyroidectomized rabbits.

B = Thyroid fed rabbits.

C = Control rabbits.
units of the series that had been immunized against human blood cells and against typhoid bacteria respectively are so nearly equal that any differences may readily be accounted for because of experimental error. The average titers of the precipitating sera from the thyroidectomized unit is considerable higher than is the average of the control unit. On the face of it, Text-fig. 11 might give us reasons for believing that thyroidectomy, in rabbits, if the animals are subsequently immunized, is conducive to high titered antibody formation, and that thyroid feeding, on the other hand, results in a decrease in the titer of the anti-serum in animals similarly immunized. However, too much emphasis must not be placed on the observations of the above named tendencies. Significant as they may be, the data presented are not sufficient to make generalization secure. Animals, within the same unit, that have received practically identical treatment react very differently in antibody production. This diversity of reactions as evidenced by noteworthy differences in titers of immune-body content in their sera, following artificial immunization, may amount to 300% or more, even when animals of the same size, weight, age, condition, and health are used. These facts, which are known to all that are familiar with the antibody development in rabbits, make it difficult to
Text-fig. 11. A comparison of the averages of all the titers of the anti-sera of all individuals in each of the three experimental series. The figures on the left side of the page represent the dilutions or titers. The points on the curves, or graphs, indicate the averages of all the titers of all titrations, following immunization, in the nine experimental units of the three series.

\[ A = \text{Series I.} \]
\[ B = \text{Series II.} \]
\[ C = \text{Series III.} \]
establish what may be known as a typical, normal, and uniform reaction in antibody development. However, to minimize the effects of individual variation, unit averages were used as a basis for drawing conclusions. It is hoped that further study will give us a basis for a more accurate comparison of immune serum from thyroidectomized and thyroid fed animals with immune serum from otherwise normal animals.

Conclusions.

The results obtained by the experiments described above do not warrant any direct association of the thyroid gland with antibody production because:

1. Individual differences in titers of immune sera among different members of a single unit vary more than do the averages of different units. That is, the titers of immune sera of rabbits that have been thyroidectomized, thyroid fed, or used as controls, may vary more among themselves than do the averages among the thyroidectomized, thyroid fed, or control units.

2. Antibody formation is independent of the thyroid gland since:

   A. Thyroidectomy is apparently without effect on the production of hemolysins, agglutinins, and precipitins.

   B. Thyroid feeding is without noteworthy influence on the development of antibodies.
The writer takes pleasure in acknowledging the many helpful suggestions given by those who have greatly assisted in making possible the study herein described, and is not unmindful of the special thanks and credit due to Dr. H. H. Lane, Prof. E. L. Trecce, and Dr. O. O. Stoland for technical assistance, and kindly criticisms in the preparation of this paper.
Text-fig. 12. Showing the effect of thyroidectomy and thyroid feeding on the weight of rabbits. The weights here pictured may be used as an index of some disorders resulting from the improper functioning of the thyroid system. The weights of the animals of Series I are shown in the figure. The numbers on the left side represent grams.

--- Thyroidectomized rabbits.
--- Thyroid fed animals.
--- Control rabbits.

A = Date of thyroid removal.
I = Dates at which injections of antigen were given.
F = Time when thyroid feeding had started.
T = Time of titrations of immune sera.
Text-fig. 18. Showing the effect of thyroidectomy and thyroid feeding on the weight of the rabbits in Series II.

--------------- Thyroidectomized rabbits.
-._-_-. Thyroid fed rabbits.
............. Control rabbits.

I = Dates of antigen injections.
T = Dates of titrations of the immune sera.
F = Date at which thyroid feeding was started.
A = Date of removal of the thyroid glands.
Text-fig. 14. Showing the effect of thyroidectomy and thyroid feeding on the weight of the rabbits of the third series, i.e., those that were immunized against human serum. Labeling is the same in this figure as it is in Text-figs. 12, and 13.
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5b. Justus. Quoted by Cutler.
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