THE ANTAGONISM BETWEEN THYROID AND PARATHYROID GLANDS.

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(Received for publication, July 16, 1918.)

INTRODUCTION.

It is well known that the extirpation of the parathyroids in mammals is followed by tetanic convulsions. This led to the conclusion that certain tetany-producing toxins are formed in the body, which in normal animals must be either removed or turned into non-toxic substances by the parathyroid glands. The origin of these toxic substances, however, is unknown. Though tetanic convulsions and other symptoms of tetany have been reported repeatedly to occur after injection of thymus extracts, in the frog as well as in mammals, no attention has been paid to this fact as possibly containing the solution of the problem in which organ the tetany-producing substances might be manufactured. The experiments presented in this article seem to indicate that they are contained in and produced by the thymus gland. This harmonizes with the well known fact that tetany is a disease of infants.

In the spring of 1916, about thirty salamander larvae of the species *Ambystoma maculatum* were fed exclusively on calf's thymus. Each single larva after some time began to suffer from severe tetanic attacks. Since the larvae of salamanders do not possess parathyroids this observation seemed to be of considerable interest, and, in the fall of 1916, calf's thymus was fed to larvae of another species of sala-

1 See Biedl, i, 279, 301 ff.
2 In the terminology to be employed in the classification of amphibians the nomenclature as worked out by Stejneger and Barbour in their new check list was applied here. According to this the old species *Ambystoma punctatum* corresponds to the new species *Ambystoma maculatum*.

The Journal of General Physiology
manders, Ambystoma opacum; again each single individual after a certain time came down with tetanic attacks. In 1917, the experiments were repeated with the same result on both species. It is, therefore, evident that the thymus gland contains a substance which produces tetanic convulsions in the larvæ of Ambystoma maculatum and opacum.

**Tetanic Symptoms.**

The tetanic symptoms exhibited by salamander larvæ fed with thymus closely resemble the symptoms produced by parathyroidectomy in mammals. The first attacks in the larvæ are confined to the hind portions of the body. In general, this part of the body suffers more than any other during the entire tetanic period, and when the acute attacks become less and less frequent and severe it is again the hind portions of the body which alone are attacked. Within several days after the onset of acute tetany the entire muscular system exhibits severe clonic convulsions. Each single attack begins at the tail and spreads toward the head; the tremors are severest in the legs and in the muscles of the lower jaw. During each attack the mouth is thrown widely open. When the attack begins the animals are thrown on one side. Besides the clonic convulsions a tonic spasm of the entire body is observed; the legs are stretched out and the body is bent with its concavity towards the back. During the attack the animals discharge much air and the vessels of the skin become very red.

In the early stages of the disease the convulsions are induced only upon stimulation which is best effected by removing the larvæ from the water, placing them on filter paper, and pinching the legs or the tail slightly. Each attack lasts only a short time, the larvæ recovering after from 2 to 3 minutes. Later any attempt of the larvæ to swim or to snap at a piece of food suffices to induce an attack and some individuals may float for several days on one side, being rigid from tonic spasm.

8 to 14 days after the first attack the hind legs begin to show signs of a permanent tonic spasm; the legs are stretched backward and become twisted around their longitudinal axis with the inner surface pointing upward; the feet follow this movement and finally the fore legs undergo a similar change.

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3 See Biedl, i, 79 ff.
Relation between Tetany and Development.

Tetany cannot be produced by thymus feeding before a certain developmental stage is reached by the larva; namely, that at which probably the secretive power of the animal's own thymus glands is established. This stage of development corresponds to that of the full development of the toes of the larvae.

Six larvae of *Ambystoma opacum* (opacum, 1917, B), from North Carolina, hatched in the laboratory from eggs of the same female, were kept at approximately 25°C. and fed on calf's thymus from the 19th day after hatching. The animals were tested for tetany regularly on a definite day every week, for the purpose of recording the percentage of animals suffering from tetany; clonic convulsions of the entire body as well as of the hind portion only were considered as indication of tetany. No animal suffered from tetany when examined the first time after the commencement of the thymus feeding. With the progress of development (as indicated by the development of the toes) the percentage of animals suffering from tetanic convulsions increased and reached a first maximum at the time when all toes were developed. No animal had convulsions until all the toes of the fore legs and at least the third toe of the hind limbs were developed.

In a second series of experiments, with eight *Ambystoma opacum* larvae (opacum, 1916, B) hatched from eggs of the same female and kept at approximately 25°C., thymus feeding was started on the 14th day after hatching. None of the larvae of this series showed any signs of tetany before the full number of toes had developed. In a third series (Ambystoma opacum, 1916, D) consisting of eight larvae from eggs of the same female and of the same age as the larvae of the previous series, this becomes still more evident. Though the larvae of Series D, 1916, were fed on thymus simultaneously with Series B, 1916, convulsions developed 4 weeks later than in Series B, 1916. Development was retarded by keeping the larvae in low temperature (15°C. approximately).

In *Ambystoma maculatum* similar results were obtained. In one instance (Ambystoma punctatum, Series K, 1917) six larvae, all hatched from eggs of the same female and kept at approximately 25°C., were fed on thymus exclusively from the 13th day after hatching. The first acute attacks were observed 5 weeks after hatching.
Similarly it was found in *Ambystoma opacum* as well as in *Ambystoma maculatum* that the acute tetanic attacks disappear entirely after a certain stage is reached, though the animals continued to be fed exclusively on thymus gland. When the larvae reach a stage in which they resemble normal larvae ready for metamorphosis, it becomes more and more difficult to induce an acute tetanic attack and no animal ever showed such attacks after it had metamorphosed, in spite of thymus feeding.

**Thymus Gland Contains a Tetany-Producing Substance.**

The question arises: Why does the thymus gland when fed to salamander larvae cause tetany only during a certain developmental period, and why is it ineffective before and after this period? In order to give an answer to this question it was necessary to make a histological examination of the larvae.

As regards the beginning of tetany several larvae which had been fed thymus were examined histologically just before and after the beginning of tetany. In a number of *Ambystoma opacum* larvae of the same age and hatched from eggs of the same female (*Ambystoma opacum*, 1917) tetany was observed in a few individuals as early as 4 weeks after hatching; in these few individuals, however, it did not reappear until 6 to 7 weeks after hatching, and at this time the majority of the larvae developed tetany. At this time larvae of this set with and without tetany were killed and preserved for examination to find in which point they differed. A preliminary microscopical study of the anatomy and histology of one tetanic and two pretetanic larvae demonstrated a noteworthy difference in regard to the developmental state of the thymus glands, but in no other respect.

It was found that the larvae which had not yet developed tetany had smaller thymus glands than the larva which had already fallen a victim to tetany. The thymus glands of the latter larva are more than twice as large as those of the pretetanic larvae, though the difference in length of the entire animals is only 33 per cent, the tetanic specimens being the larger ones. This is shown in Fig. 1 in which the three left thymus glands of a pretetanic larva (left vertical row)
Fig. 1. Comparison between size of the primordial thymus of a pretetanic larva of *Ambystoma opacum* (left vertical row) and a larva of the same species shortly after the beginning of acute tetanic convulsions (right vertical row). It will be seen at a glance that the thymus glands of the latter animal are much larger than those of the non-tetanic larva. The drawings were made with a camera lucida at 490 linear magnification from the largest cross-section through each of the glands.
and of the tetanic larva (right vertical row) are drawn at the same magnification.  

Still more significant are the differences in structure. In the pretetanic larvae the thymus glands represent small accumulations of purely epithelial cells. They do not show any kind of differentiation. Their epithelial character is indicated also by the presence of considerable quantities of pigment granules such as are found in the epithelium of the pharyngeal pouches from which the glands have separated. In most of the glands there are no mitoses to be found at all, while in some there are one to two mitoses present. No membrana propria is formed.

Compared with the thymus of the pretetanic larvae, in which none of the characters of a true thymus has developed, five of the glands of the tetanic larva show some of the aspects of a real thymus in as far as they contain elements which are characteristic of that organ. Again no membrana propria is formed, but the glands consist of a variety of cells. Leaving aside the question as to whether or not the newly developed cell elements are of mesenchymal origin, it is sufficient to notice that several kinds of cells now constitute these glands; two of them are recognized clearly as the main elements making up the fully developed thymus of an Ambystoma opacum larva.

The first and second pairs of the thymus glands have disappeared already in this stage from both the pretetanic and tetanic larvae, and only the third, fourth, and fifth pairs are present. According to Maximow and to Baldwin the first and second pairs are found absent already in larvae of from 11.5 to 13.0 mm. in length.

Concerning the development of the amphibian thymus glands and the origin of their cell elements see the papers by Maximow, by Dustin, and by Baldwin. It should be mentioned that the development of the thymus glands in Ambystoma opacum was found by the writer to correspond in the main with the description as given by Maximow and Baldwin for Ambystoma tigrinum and Ambystoma maculatum respectively. The size of the larvae, however, corresponding to the various developmental stages of the glands was found to be much more variable and in general larger. In a normal stock animal of Ambystoma opacum, for instance, the right gland of the third pair was found to be still connected by an epithelial stalk with the branco-pharyngeal epithelium and of purely epithelial character, though the larva has a length of over 37 mm., while Maximow and Baldwin found the glands fully separated in larvae of from 10 to 12 mm.; Maximow saw the commencement of mesenchymal immigration in larvae of about 10 mm. in length, and Baldwin in larvae of 19 to 20 mm. in length.
Correspondingly the epithelial character of the glands is lost; this finds its expression also in the fact that pigment granules have disappeared altogether. In addition a considerable number of mitoses may be found, which mostly seem to be cell divisions of the newly arrived elements. Of particular importance is the fact that in one of the glands of the fifth pair, which I found to be frequently ahead of the third and fourth pairs in development, the cell plasma of a number of cells stains pink, and here one nucleus which also is stained pinkish exhibits a swollen appearance, characters which are ascribed by Maximow to the formation of the medulla of the thymus.

The thymus glands of the pretetanic larvæ are therefore mere accumulations of epithelial cells and do not possess any structures to indicate that they would be capable of exerting any of the functions of a thymus, while in the tetanic larva at least some of the glands resemble a true thymus and probably have commenced to behave as such with regard to function. From these observations we may conclude that the thymus feeding does not begin to call forth tetany in salamander larvæ until the thymus glands of the larvæ themselves are able to secrete. This suggests that tetany is the effect of the combined action of the secretion of certain substances by the thymus glands of the larvæ and of substances introduced into the larva by feeding it with the thymus gland from other animals.

Prevention of Tetany by the Parathyroids.

It is well known that salamander larvæ do not possess parathyroids; these glands develop during metamorphosis. Though the end of the tetanic period coincides approximately with the development of the parathyroid glands, yet larvæ which had ceased to show convulsions for some time apparently possessed no parathyroids when examined histologically. It is, however, possible that in spite of this in such larvæ certain processes had commenced which would soon have led to the formation of these glands and that these processes were already able to check tetany.

A connection between the development of the parathyroids and the cessation of tetany seems probable on account of the fact that tetany
was never observed after metamorphosis and after the development of the parathyroids. This suggestion finds support in the fact that in the larvae of frogs and toads, which develop their parathyroids soon after hatching, thymus feeding never produces tetanic convulsions.

* A Second Mechanism to Counteract the Action of the Thymus in Salamander Larvae.

If the parathyroids are responsible for preventing tetany in *Ambystoma opacum* and *Ambystoma maculatum*, the question may be asked why the larvae of these salamanders are not normally victims of tetany, since they possess six well developed thymus glands and no parathyroids. It is necessary to assume that the larvae must possess some other mechanism capable of inhibiting the action of their own thymus glands but inadequate when an excess of thymus substance is introduced into the larva.

That such a mechanism is present becomes evident from the behavior of the larvae of a third salamander species (*Ambystoma tigrinum*) closely related to the two others. It is surprising that the larvae of *Ambystoma tigrinum* never develop tetany even if they are fed exclusively on thymus gland of which they eat enormous quantities. *Ambystoma tigrinum* larvae were fed from about the 14th day after hatching, on calf's thymus exclusively; some of them were kept at 25°C., others at 15°C. One of these larvae has not metamorphosed yet, 60 weeks after hatching; it has been fed on thymus for more than a year. None of these larvae ever showed any signs of tetany.

Yet the larvae of the species *Ambystoma tigrinum*, like those of the two other species, do not possess parathyroids. Evidently they must have some other mechanism to counteract the tetanic effect of the thymus; and this mechanism must be sufficient not only to prevent tetany from the animal's own thymus but also tetany from an excess amount of thymus when introduced with its food.

**CONCLUSIONS.**

From the facts stated in this paper it is evident that the thymus gland of mammals contains a substance which is capable of producing...
tetany when fed to the larvae of certain species of salamanders (Ambystoma opacum and Ambystoma maculatum). As long as the larvae have not developed their own thymus glands, they are able, by means of some mechanism, to counterbalance the tetanic action of the thymus substance introduced in their food. When, however, the secretion from their own thymus glands is added to the thymus material introduced with the food, this mechanism of preventing tetany becomes inadequate and tetany ensues. In the larva of a third species of salamander, Ambystoma tigrinum, this mechanism will prevent tetany even when the larvae are fed on thymus.

In mammals the parathyroids are known to prevent tetany and are supposed either to absorb the tetany-producing substance and thus prevent its action or to change it into another non-toxic substance. It is at least probable that in the amphibians the parathyroids play the same rôle. Larvae of anuran amphibians, which develop their parathyroids soon after hatching, never show tetanic convulsions if they are fed on thymus, but in certain species of salamanders, whose parathyroids develop only during metamorphosis, the larvae invariably have tetanic convulsions upon thymus feeding, while the metamorphosed animals never show tetany.

But in addition to the parathyroids the salamanders must possess still another mechanism which during the larval period inhibits the production of tetany by the animal’s own thymus glands. In the larvae of Ambystoma opacum and Ambystoma maculatum this mechanism is sufficient only to prevent tetany from the animal’s own thymus, while in the larvae of Ambystoma tigrinum it is capable of preventing tetany even when the larvae are fed with thymus.

If the thymus is the organ by whose action tetany is produced, we can understand why tetany in human beings occurs far more frequently in children than in adults, since in the latter the thymus gland is replaced, at least to a great extent, by connective tissue. The relation of thymus to tetany may also possibly explain the occurrence of tetany during pregnancy; while the parathyroids of the mother may be sufficient to prevent tetany from her largely atrophied thymus, they may not be sufficient to prevent tetany from the excess of thymus substance furnished by the fetus to the blood of the mother.
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