IODINE AND THE THYROID.

III THE SPECIFIC ACTION OF IODINE IN ACCELERATING AMPHIBIAN
METAMORPHOSIS.

BY W. W. SWINGLE.

(From the Department of Biology, Princeton University, Princeton.)

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In previous studies on the relation of iodine to the thyroid, as determined by the effects produced by feeding this substance and its compounds to larval Anurans,¹ the following conclusions were either stated or implied, all of which have a direct bearing on the present experiments. (1) Inorganic iodine and its compounds, iodoform and potassium iodide, greatly accelerate metamorphosis of tadpoles. (2) Animals from which the thyroid gland had been removed at its inception (i.e. 6 mm. larvæ), and which under normal conditions never undergo metamorphosis but grow to an abnormal size, quickly transform into frogs when fed iodine. (3) The follicles of the thyroids of tadpoles on an iodine diet show a greater colloid content than do the glands of normally fed animals. These facts led to the conclusion that iodine is essential for amphibian metamorphosis, that it is the active constituent of the thyroid glands of these animals, and, judging by its action on thyroidless tadpoles, that it exerts its action directly upon the cells and tissues of the organism without the necessity of undergoing transformation in the gland tissue; i.e., that iodine is capable of functioning as the thyroid hormone itself within the body, or else is transformed into this hormone through the activity of tissue other than that of the thyroid. (4) The chief function of the thyroid is the extraction from the blood and storage of the minute quantities of iodine taken into the organism in food and water, and the subsequent release of this sub-

stance into the general circulation under pressure of the organism's needs.

In the experiments recorded here, further evidence, indicating that the conclusions stated are essentially correct, is presented.

The problem, with which the present experiments are concerned, was suggested by Professor E. Newton Harvey, in whose laboratory the work was done, and to whom the writer is indebted for suggestions, equipment, and criticism. Briefly stated, the problem was to determine if the action of iodine upon Anuran metamorphosis is specific, or if other closely related chemical substances, such as bromine, exert a similar effect; to determine by quantitative feeding the amount of iodine, or other substances if any were found, required to produce metamorphic changes in tadpoles kept under uniform conditions; and finally to compare the histological picture presented by the thyroids of the iodine, bromine, and normally fed animals.

Throughout the work, unless otherwise stated, the larvae used were *Rana sylvatica* LeConte. All animals came from the same egg mass, and hence were of the same age. They were permitted to develop to a total length of 10 mm. before being used, though no food was fed them during this interval, nor was any needed as the larvae subsist and increase in size by utilization of the yolk in the body cells.

The food given consisted entirely of algae, practically all gathered from the same pool, and *Elodea* from the University vivarium, fed in equal quantities. The only animal matter the tadpoles received was that entangled in, or living on, the algae. The environmental factors of temperature, light, and water supply were kept as nearly uniform as possible. Too much stress cannot be laid upon these factors in any work concerned with growth and metamorphosis, as the extreme variability often observed in the growth rates of frog cultures is due largely, in the writer's experience, to slight disturbances of these environmental factors.

Each culture consisted of thirty-two larvae in 2,000 cc. of ordinary tap-water, with fairly high calcium content. The water was changed daily.

Iodine and bromine were made up in chemically equivalent stock solutions of M/10 concentration, the iodine in alcoholic, the bromine in aqueous, solution.
The fact that the iodine was in alcoholic and the bromine in aqueous solution had no effect on the results obtained. This is clearly shown elsewhere where similar results were obtained and an alcoholic solution was not used.\(^1\) Five cultures of larvae received gradually increasing amounts of iodine and bromine. Thus Bromine Culture 1 received \(\frac{1}{2}\) cc. of \(\text{m}/10\) stock solution of bromine in 2,000 cc. of \(\text{H}_2\text{O}\), yielding \(\text{m}/460,000\) solution. Iodine Culture 1 received \(\frac{1}{5}\) cc. of stock solution iodine in 2,000 cc. of \(\text{H}_2\text{O}\), making \(\text{m}/1,100,000\) solution. More drops of the alcoholic solution are required to make 1 cc. than of the aqueous solution due to the difference in surface tension of the two solutions, hence the difference in the size of the drops. Cultures 2, 3, 4, and 5 of the bromine set received respectively \(\frac{2}{5}\), \(\frac{4}{5}\), \(\frac{7}{5}\), and \(\frac{9}{5}\) cc. of the \(\text{m}/10\) solution. Cultures 2, 3, 4, and 5 of the iodine set received respectively \(\frac{3}{5}\), \(\frac{4}{5}\), \(\frac{6}{5}\), \(\frac{7}{5}\), \(\frac{9}{5}\) of the \(\text{m}/10\) solution iodine. Bromine Culture 2 compares, in regard to strength of solution used, with Iodine Culture 4, though the iodine culture is considerably the weaker. An exact chemical equivalent solution falls somewhere between Bromine Cultures 1 and 2 and Iodine Culture 4.

It is probably not necessary that iodine or bromine obtain entrance to the organism through the alimentary tract; they will probably be absorbed and reach the vascular system almost as rapidly through the integument.\(^2\)

**Observations.**

### Experiment 1. Iodine Feeding

The tadpoles, fasting since their emergence from the egg jelly, measured 10 mm. in length, and showed no indications of limb buds when first fed iodine. There were six cultures, one serving as control for the other five. There was no change during the first 32 hours in Cultures 1, 2, and 3, some mortality in Cultures 4 and 5, especially in 5. At the end of 60 hours the larvae in Cultures 4 and 5 showed, on microscopic examination, considerable fraying of the delicate tail-fin and extravasation of blood.

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from the capillary vessels. Ten animals in Culture 5 had died, two in Culture 4, none in the remainder of the cultures.

86 hours after the first iodine was fed the larvae were again examined. All of the iodine-fed animals were more deeply pigmented than the controls. Cultures 1 and 2 showed practically no tail shrinkage, whereas this showed clearly in Cultures 3, 4, and 5. The tadpoles in the stronger solutions also had well developed epithelial hind limb buds. In the controls and in Cultures 1 and 2 only the largest animals revealed any indications of such limb anlage. As is well known, the hind limbs of the Anura develop from the epithelium, and in the writer's experience, appear very early; but they remain mere formless buds for some time, usually until the animal has attained a length of 24 or 25 mm. and sometimes even larger. The limb buds then develop the two primary leg divisions and later the toes differentiate. Iodine feeding causes rapid development of the limb rudiments.

March 29, 1919. 9 days from date of first feeding. Controls: average total length 13.5 mm. Hind limb buds not visible without the aid of the microscope. Pigmentation normal. Animals active and well nourished.

Culture 1.—Average length 13 mm. Hind limb buds but slightly larger than those of the controls, pigmentation normal. Animals well nourished.

Culture 2.—Average length 13.5 mm. Hind limb buds large. Easily visible without microscope; some measured over 1 mm. in length. Pigmentation dark. Slight emaciation of the body and some shrinkage of the tail-fin. It was observed for the first time on this date that the tadpoles in all cultures except No. 1 remain near the surface of the water. This continued throughout the experiment, the animals descending to the bottom of the container only to obtain food.


Culture 5.—Average length 12 mm. Tiny hind legs present. Great emaciation and tail shrinkage present. Animals darkly pigmented. Considerable mortality.

April 4, 1919. Controls: average length 14.5 mm. Hind limb buds just visible without the microscope in animals over 15 mm. in length.

Culture 1.—Length 14.5 mm. Identical in development with the controls except for slightly larger limb buds.
Culture 2.—Length 15 mm. Hind limbs larger than those of animals in Culture 1. Pigmentation dark. Previous emaciation has disappeared. Atrophy of tail-fin gradually disappearing.

Culture 3.—Length 13 mm. Limbs larger and better developed than those of animals in Cultures 1 and 2. Slight emaciation present, also tail-fin atrophy. Pigmentation lighter than controls.

Culture 4.—Length 13.5 mm. Limbs large and well developed. Some emaciation. Tail-fin atrophied. Animals sluggish. Pigmentation dark.

Culture 5.—Abandoned on this date. The mortality rate was too high to continue the experiment. It was observed that though the death rate was high during the first week of iodine feeding in quantities such as were given here, the animals which survived could stand double the dosage given previously.

April 13, 1919. Controls: Length 21 mm. Hind limbs tiny epithelial buds with no differentiation into primary divisions or toes.

Culture 1.—Length 21.5 mm. Hind limbs larger and better differentiated than controls. Otherwise no difference in the animals of the two cultures.

Culture 2.—Length 22 mm. Hind limbs fully differentiated. No emaciation. No tail-fin atrophy, the atrophy apparent on previous examination had disappeared and did not occur again. Pigmentation dark. Animals remarkably uniform in size, something rather unusual in a culture of frog larvae.

Culture 3.—Length 18.5 mm. Legs fully formed and large. Pigmentation lighter than controls. Slight emaciation and tail shrinkage present. Animals sluggish.

Culture 4.—Length 19.5 mm. Legs large for body, and well formed, average 6 mm. in length. Pigmentation dark. Tail shrinkage apparent. When examined under the microscope, the fore limbs were found pressing against the skin of the pectoral region where later they burst through.

It will be noted that the tadpoles were increasing rapidly in size. Until this date, the controls were the largest animals, the iodine cultures varying but slightly in this respect. It is interesting to note that larvae of a similar age, left to develop under more natural conditions than prevail in the laboratory, had just hatched, and were not over 7.5 mm. long. At the time the eggs were collected, some of them were placed in a small, deep pool of clear water, plentifully stocked with food, in a sheltered spot not far from the laboratory, where their development was closely followed. The laboratory animals averaged over 20 mm. in length before the larvae in the pool hatched. This difference in growth rate was due to the uniform heat of the laboratory, and to the unseasonably chilly weather.
April 20, 1919. Controls: Length 25 mm. Limbs tiny epithelial buds as yet undifferentiated.

**Culture 1.**—Length 26 mm. Legs small but perfectly developed. Pigmentation dark. Animals well nourished.

**Culture 2.**—Length 29 mm. Very large, well nourished animals. Limbs large. Fore limbs just visible under the skin. Pigmentation dark.

**Culture 3.**—Length 23 mm. Legs 7 mm. long. Pigmentation light. Animals very sluggish. Fore limbs easily visible under the skin.

**Culture 4.**—Length 26 mm. Hind legs 9 to 10 mm. long. Tail shrinkage apparent. Fore legs through skin on side in three animals. Pigmentation dark. Animals sluggish.

On this date the larvae left in the pool were examined again. The average length was 13.5 mm. No limb buds were visible. Pigmentation slightly lighter than that of the laboratory controls.

April 27, 1919. Controls: Length 26 mm. Otherwise no change.

**Culture 1.**—Length 28 mm. Limbs larger and better developed than those of controls.

**Culture 2.**—Length 31 mm. Legs well developed otherwise no change.

**Culture 3.**—Length 23 mm. No change, except an increase in the size of the legs.

**Culture 4.**—Length 27 mm. Fore legs through skin in several animals. Hind legs very large.

Animals in the pool were again measured. The average length was 15 mm. Tiny epithelial hind limb buds present. No differentiation yet. Lightly pigmented. Animals well nourished and active. The experiment was abandoned May 1, 1 month and 10 days from the date of the first iodine feeding.

**Discussion of Experiment 1.**

**Metamorphosis.**—The effects upon metamorphosis of feeding known quantities of iodine in progressively stronger concentrations is obvious from this experiment and needs only brief mention here. It is clearly shown that the rate of metamorphic change in Anuran larvae depends upon the amount of iodine the animals receive; the stronger the concentration, the more rapid the change from larval to adult condition. Iodine even in such minute quantities as Culture 1

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Though no iodine was fed after May 1, and but little food given all the animals of Cultures 3 and 4 completely metamorphosed by May 17. Seven larvae of Culture 2 had transformed by June 1. Two animals from Culture 1 metamorphosed June 10. None of the control tadpoles has yet completed metamorphosis.
received \((m/1,100,000\) solution) soon showed the effect of its administration. It is safe to assume that an absolutely iodine-free diet fed to frog larvae reared in iodine-free water would inhibit metamorphosis indefinitely. The writer is conducting experiments along this line, but the data are not yet ready for publication. As stated elsewhere, the function of the thyroid appears to be that of collecting, storing, and passing into the circulation at the proper time such iodine as finds its way into the organism by means of food and water. Probably most if not all organic matter contains very minute traces of iodine in some form; most surface water contains traces of it, though iodine is said not to occur in deep fresh water springs. Lack of iodine in the environment may possibly explain why *Amblystoma trigrinum* fails to undergo metamorphosis and permanently retains its larval form in certain localities such as Lakes Xochimilco and Chalco 8 miles southeast of the City of Mexico. This phenomenon is not unknown elsewhere; for instance, in certain pools and districts in Lombardy the larvae of Triton are said to fail to metamorphose and may grow to an immense size. It seems reasonable to suppose, in the light of these iodine studies, that the failure of the Axolotl and Triton larvae to metamorphose is due to lack of iodine in the environment or else the thyroids of these animals are defective and unable to collect sufficient iodine for the organism's needs. Just as there are certain districts where cretinism and myxedema are endemic, so there are certain districts where amphibians fail to undergo metamorphosis. In both instances lack of sufficient iodine in the food and water is probably the causal factor.

Moreover, it seems likely that in the experiments recorded in the literature where frog larvae restricted to certain diets, for example thymus gland tissue, apparently fail to undergo metamorphosis at the proper time, or not at all, the cause of the delayed metamorphosis is lack of sufficient iodine. Thymus tissue is said not to contain iodine, but in the mammals it is not at all uncommon to find thyroid tissue embedded in the thymus. The discordant results obtained by investigators who have fed thymus tissue to frog larvae may be explained on this hypothesis, although the writer believes that certain

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other environmental factors also play a part which may lead to misinterpretation of results.\textsuperscript{5}

In a recent number of this \textit{Journal}, Uhlenhuth, while accepting the conclusions stated regarding the relation of iodine to amphibian metamorphosis, thinks that still another substance is needed to cause the thyroid gland to excrete the iodine necessary for metamorphosis. This hypothetical factor he terms an excretor substance\textsuperscript{6} and thinks that it is evolved during the growth processes of the organism.

The assumption of an excretor substance obscures rather than clarifies the already sufficiently complicated problem of amphibian metamorphosis by the introduction of an unknown and purely hypothetical agent. That iodine is essential for metamorphosis we have seen, but it is equally certain that iodine is not the sole factor involved, or else Anuran larvae of the different species, having different periods of larval life, should if reared together and fed equal amounts of iodine undergo metamorphosis simultaneously. This they will not do. The writer believes that the long larval life of \textit{Rana catesbiana} is related to slow thyroid development in that species and the consequent inability of the organism to utilize iodine except in large amounts; conversely, that the short larval life of \textit{Bufo} is due to very rapid thyroid differentiation, hence greater powers of iodine assimilation. There is no experimental proof to substantiate this view and it is to be regarded merely as a suggestion.

Amphibian metamorphosis is an extremely complicated phenomenon, and in the last analysis will probably be found to depend, not on a single factor, but on a group of factors both extrinsic and intrinsic, the cooperation of which is necessary to complete the process.

\textit{Growth.---}The data on growth recorded for the iodine-fed tadpoles showed that the size of the larvae varied considerably for each individual culture; at a given time some of the animals would be 10 to 12 mm. longer than others of the same culture, but for the experiment as a whole there was a continuous upward trend of the growth curve


\textsuperscript{6} Uhlenhuth, E., Relation between thyroid gland, metamorphosis, and growth \textit{J. Gen. Physiol.}, 1918–19, i, 473.
of the animals in each of the cultures. There was no continuous upward or downward growth curve depending upon the iodine concentration. Thus, the animals of Culture 2 outgrew both controls and the animals in stronger concentrations; the animals in Culture 3 were smaller than those of Cultures 2 or 4. Strong concentrations of iodine bring about cessation of growth very quickly, and differentiation is stimulated, whereas very weak solutions such as those of Cultures 1 and 2 tend to stimulate both growth and differentiation. Rapid differentiation apparently does not occur along with rapid growth; an overdose of thyroid extract or iodine to frog larvae immediately starts the mechanism of differentiation. If the larvae are very young, not only does growth cease, but differentiation does not proceed very far before death of the organism supervenes due to the extreme height to which metabolic activities rise. The organism literally withers away.

The work of Janney is of interest in this connection. He has shown that an overdose of the thyroid hormone leads to a loss in weight and size of the organism; conversely, a certain minimum dose results in a gain in both size and weight. This is very well shown in the present experiment, the animals of Cultures 1 and 2, receiving minimum doses of iodine, outgrew the controls and the animals of the stronger concentrations. As shown elsewhere by the writer, overdosage with iodine or thyroid extract leads to decrease in size and great emaciation of the animals. The conclusion in regard to growth is that iodine in minimum doses stimulates growth, though not to any great degree, and in larger doses it leads to cessation of growth and tissue disintegration or absorption. Iodine feeding has but little effect on pigmentation. If fed in sufficiently strong concentrations the animals appear darker than the controls.

Experiment 2. Bromine Feeding.—The results obtained by feeding iodine did not, however, rule out the possibility that the accel-

8 Swingle, W. W., The acceleration of metamorphosis in frog larvae by thyroid feeding, and the effects upon the alimentary tract and sex glands, J. Exp. Zool., 1917-18, xxiv, 521.
erating effect upon metamorphosis both in normal and thyroidless frog larvae might be due to constant irritation of the body epithelium. Moreover, bromine, besides being an irritant to the epithelium, is closely related chemically to iodine, and it has been recently asserted by Kendall\(^9\) that the substitution of bromine for the iodine molecule in his substance thyroxin isolated from the thyroid gland would not alter the gross chemical nature or physiological activity of this so-called active thyroid principle. All this being true, interesting results were anticipated when bromine was fed to frog larvae. As a matter of fact, however, only negative results were obtained, even when very strong concentrations were fed over a long period of time. There was absolutely no observable effect upon growth or differentiation. It seems useless to state in detail the entire experiment, so only a brief summary will be given here. Some account is necessary because of the theoretical importance of such negative findings.

The bromine cultures were in all respects similar to the iodine cultures; method of feeding, amounts fed, and dates of examinations were also the same. It was soon observed that all the larvae in Cultures 3, 4, and 5 showed pronounced tail-fin atrophy. In several of the animals the delicate tissues were literally eaten away, the action of the bromine thus simulating in a superficial way the action of iodine and thyroid extract. None of the bromine-fed animals developed legs or limb buds any faster than the controls, whereas the tadpoles fed equivalent amounts of iodine underwent marked metamorphic changes. The cause of the pronounced tail atrophy among the larvae at once became apparent when the tails of the animals were exposed for 20 or 30 seconds over the mouth of the stock bromine solution. The fumes attacked the delicate tissues, literally shriveling them up. An animal treated in this way will generally lose within 3 or 4 days that portion of the tail so treated. This artificially induced atrophy in no way resembles true physiological atrophy and resorption. Iodine in strong concentration will have a slight caustic effect on the tail in early stages of the experiment, though the effect is slight compared with that of bromine.

During the latter part of the experiment three and four times as much bromine (chemically equivalent to the iodine) was fed as was given in the highest concentrated iodine culture, but the only effects produced on the larvae were atrophy of the tail and slight emaciation. Proper precautionary measures were taken that the bromine did not pass out of solution as fumes, thus weakening the concentration. It may be added that this experiment was tried out on two species of Anura, *Rana sylvatica* and *Rana catesbiana*, with negative results in each case.

Discussion of Bromine Feeding.

This experiment though negative has considerable theoretical interest in its bearing upon the relation of iodine to the thyroid. Kendall, who claims to have isolated and synthesized the physiologically active principle of the thyroid, lays especial emphasis upon the presence of the oxy-indol nucleus of his compound, thyroxin, and considers the presence of iodine in the combination of little importance as it acts probably as a catalyst and in no other way. He says in this regard: “The presence of iodine in the compound must exert some influence, and this influence is probably that of rendering the active groups (CO—NH) more reactive.” He thinks that if the iodine were absent from his thyroxin, it would take merely a greater “working pressure” to bring about the reaction of the thyroxin. “The substitution of iodine by hydrogen or chlorine or bromine would undoubtedly be followed by an alteration in the degree of reactivity of the substance, but its gross chemical nature and properties would not be altered thereby. That iodine breaks off from the molecule and is used as iodine *per se* for any purpose seems absolutely impossible.”

Dr. Kendall's work deserves careful consideration. However, the writer finds it impossible, in the light of the bromine experiment just recorded, to believe that this halogen can substitute in any way for iodine in its physiological activity. Split off the iodine molecule from the thyroxin complex isolated by Kendall and probably the residue would be a physiologically inert substance.

If the gross chemical nature and properties of thyroxin would not be altered by splitting off the iodine molecule, how is it that thyroidless tadpoles, which never undergo metamorphosis under normal conditions, nevertheless quickly transform into frogs if fed
inorganic iodine? It would seem that if iodine is merely an activator of the thyroxin of the thyroid gland, then every cell in the organism elaborates thyroxin or else a thyroidless tadpole could not metamorphose under the stimulus of inorganic iodine. A view that appeals to the writer is to assume that iodine when taken into the body acts upon all the cells of the organism by stimulating intracellular oxidations in each cell and tissue. It is unnecessary to assume that iodine is used \textit{per se} for any purpose, and from this viewpoint there is nothing against the idea that iodine acts as an organic catalyst.\textsuperscript{10}

It has been shown that patients with complete atrophy of the thyroid have basal metabolic rates 40 per cent below normal. The question arises as to what maintains energy output from 100 per cent below normal, which would mean death, up to 40 per cent below normal, the point to which basal metabolism sinks in the absence of the thyroid. Kendall attempts an answer to this question which he himself raises, by assuming that there are other chemical substances in the body possessing the same grouping that occurs in his thyroxin. Such substances according to Kendall are amino-acids and proteins, creatine and creatinine. A more probable assumption, in the light of the iodine experiment on thyroidless tadpoles, is that the basal metabolism of patients suffering from athyreosis is prevented from sinking below 40 per cent by the iodine which is taken into the body in their food and drink, and which is absorbed and used by the cells and tissues of the organism without the necessity of its undergoing transformation in the thyroid gland tissue. This is exactly what occurs when iodine is fed thyroidless tadpoles. Moreover, the basal metabolism of thyroidless frog larvae is low. Apparently, what keeps it sufficiently high for the continuance of the life processes is the minute quantities of iodine taken into the body in the food and water.

However iodine may act physiologically, the writer feels certain of his ground in asserting that bromine cannot be substituted for it.

\textsuperscript{10}The writer suggested in a recent paper\textsuperscript{4} that the thyroid gland probably did not elaborate an internal secretion. This meant that the gland did no more than the other cells and tissues of the organism could do, if they were given the appropriate conditions. Certainly the evidence obtained from the metamorphosis of thyroidless tadpoles under the stimulus of iodine feeding strongly indicates this.
Microscopic Examination of the Thyroids.

The glands of the tadpoles were preserved for study of the gross and histological structure after the method previously described. They were first studied whole, then sectioned.

Frog larvae of equal age, reared under identical environmental conditions, vary considerably in size, hence it is necessary, in order to obtain a true picture of thyroid conditions in the various cultures, to preserve animals as nearly equal in size as possible. The thyroids increase in size as the organism grows, consequently no comparison is possible between thyroids of large and small larvae, even though taken from the same culture.

Examination of the algae-fed controls and bromine-fed animals failed to reveal any differences either in size of the thyroids or in the colloid content between the two cultures. Bromine feeding apparently has no effect on the thyroid gland.

On the other hand, however, a comparison of the thyroids of animals taken from Iodine Culture 5 with those from algae-fed controls of similar size showed little if any difference in the gross size and structure, but a difference in the colloid content of the follicles of the two sets of animals. Colloid is present in the thyroid follicles of the algae-fed larvae but is rather scanty in amount and thin in consistency compared to that found in glands from animals fed iodine over long periods.

In an abstract presented recently before the Anatomical Association Allen states that "iodine feeding does not cause any marked increase in colloid deposition in the thyroid glands of pituitaryless tadpoles." This statement, though apparently contradictory to the writer's results, nevertheless is in line with them. In the first place extirpation of the pituitary gland, not only in Anura but in all species investigated, leads apparently to imperfect development of the thyroid, and it is probably because of this malformation and consequently malfunctioning of the thyroid that pituitaryless frog larvae do not normally metamorphose. Hence such animals will react to iodine feeding just

as the writer previously found thyroidless tadpoles did. Tissues and
cells, other than those of the thyroid, assimilate and use the iodine
taken into the organism. The need for iodine is great, hence the feed-
ing of iodine should result in no marked deposition of colloid in the
imperfect thyroids of pituitaryless animals.

In the second place, the iodine mixture used by Professor Allen
in his experiment (starch iodide) was obtained from the writer and
represented a 1:100 mixture of iodine and flour. The pituitaryless
animals probably had long passed their normal period of meta-
morphosis and hence required a smaller amount of iodine and flour
to enable them to undergo metamorphosis.

The writer feels certain that prolonged iodine feeding increases the
colloid content of the thyroid, both in amount and consistency of the
colloid, and that the results with pituitaryless animals are due to the
reasons stated.

CONCLUSIONS.

1. Amphibian metamorphosis depends upon the amount of iodine
secured by the larvae; the greater, the quantity the more rapid the
differentiation.

2. Bromine is physiologically inert when fed even in large quanti-
ties to frog larvae, hence it cannot be substituted for iodine. Bromine
feeding has no effect on the thyroid.

3. Iodine is the active constituent of the thyroid gland, in the
Anura at any rate, and functions within the body by stimulating
intracellular oxidations; it is apparently specific in its action.

4. The basal metabolism of patients suffering from athyreosis,
whose metabolism is 40 per cent below normal, is very likely held at
this figure and prevented from sinking lower to the death point by
the introduction of iodine into the body through food and water.

5. The thyroid gland is an organ the function of which is the ex-
traction from the circulation, storage, and supplying to the organism,
under the pressure of its needs, the small quantities of iodine taken
into the body. The chief function of this gland then is the utiliza-
tion of iodine in small quantities.