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UNIVERSITY OF LOUISVILLE

THE EFFECT OF POTASSIUM-IODIDE ON THE
EMBRYOLOGICAL DEVELOPMENT OF THE CHICK;
WITH SPECIAL REFERENCE TO THE GROWTH
OF THE LONG BONES

A Dissertation

Submitted to the Faculty

Of the Graduate School of the University of Louisville

In Partial Fulfillment of the

Requirements for the Degree

Of Master of Science

Department of Biology

By

David M. Polot

1931

My deep gratitude is due Dr. Austin R. Middleton,
Director of the Biological Laboratories of the University
of Louisville, for his gracious guidance and constructive
criticism of the work.

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HISTORICAL

Since 1820 iodine has been used more or less in the treatment of thyroid diseases, but it was not until 1895 that it became known that the gland usually contains iodine.

Baumann ('95)¹ discovered that iodine was a constituent of the normal thyroid gland. By treating the glands of sheep with a strong acid he was able to destroy most of the complex materials present and to separate a mixture of substances which contains about nine per cent iodine. This work was closely followed by an investigation of the iodine content of normal and diseased thyroid glands and it became evident that the activity of the thyroid was closely related to the amount of iodine the gland contained. It has been shown in a number of papers that the administration of iodine, in the inorganic and organic form, produced a marked effect upon the normal physiological development. This effect may result in a change of normal body metabolism or body growth, and when this abnormality occurs, it is called hyperthyroidism.

Terry ('18)² found that the removal of the thyroid gland greatly retarded, if not completely stopped the process of ossification and the process of growth in the bones of the hind legs of *Rana pipiens*.

Hofmeister ('96)³ showed that the extirpation of the

thyroid of young rabbits, was followed by a remarkable retardation of the long bones, to the extent of at least a third of their proper length. "The most constant symptom," says V. Bruins,* "in man as well as in animals is a remarkable disturbance of growth. The arrest of development produces a dwarf-like appearance of the individual due to improper endochondral ossification."

Allen('18)⁴ states: "It is thus clear that both hind limbs and fore limbs can start to develop in the total absence of the thyroid gland of *Rana pipiens*, but their development ceases at an early stage. In case of the hind legs, this is readily seen."

Swingle('19)⁵ shows that iodine and its compounds when fed to the larvae of *Rana pipiens* and *Bufo lentiginosus* stimulate metamorphosis in these animals. Inorganic iodine when fed to the thyroidless larvae of *Bufo lentiginosus* brings about metamorphosis in an abnormally short time.

Iodine appears to function within the organism as a hormone itself without the intermediation of the gland. Swingle suggests that the extraction of iodine from the blood and its storage is the chief function of the thyroid gland. In this connection he also mentions tests made to determine the solubility of iodine in normal blood serums in amphibia and mammals (rats). The serum of the latter at thirty-seven degrees Centigrade acts as a

*Quoted From Biedle; Internal Secretory Organs.

solvent for finely ground iodine crystals to the extent of 0.00075 grams per cubic centimeter when stirred vigorously.

Kendall('15)⁶ has reported that the relation of iodine to the physiological activity of the thyroid is very close; in fact, the activity of the gland appears to depend upon its iodine content.

Lenhart('15)⁷ fed thyroid tissue to tadpoles and observed that the higher the iodine content of the gland fed, the more rapid the body metabolism.

Swingle('19)⁸ in comparing the thyroid gland of iodine fed and normal frog larvae, showed that the follicles of the glands of such iodine fed larvae contain a much greater colloid mass than the follicles of the controls. He also showed that the solutions of iodine brought about metamorphosis in both normal and thyroidless tadpoles in a short time. Iodine was much more active in accelerating metamorphosis than any of its compounds. Next in the order of activity were iodoform and potassium-iodide. The iodates of potassium, he claimed, appeared to have no effect.

Dye and Maughan('29)⁹ deduced from their analysis that in thyroidectomy there is a noticeable retardation of growth with a more or less marked deposit of adipose tissue, and that in the modification of growth in the bones their length was mainly affected.

Etkin('30)¹⁰ in his work on the growth of the thyroid

gland, showed a relationship between the physiological activity of the gland and the growth of the hind limbs of *Rana pipiens* during the stages of metamorphosis. He states that, "The amount of thyroid (cell number or colloid volume) per unit volume of tissue increases but slightly if at all until the hind legs start growing actively."

Cretinism and myxedema have been produced experimentally in swine by Cayor and Schlotthauer ('26)¹¹.

Landauer ('29)¹² observed thyrogenous dwarfism (myxedema infantiles) in the domestic fowl, which he attributes to a hyperthyroid gland.

Speidel ('29)¹³ has shown that the regeneration of the amputated tail of a thyroid treated amphibian larvae was hindered by the thyroid hormone, especially if the thyroid treatment was begun before regenerative process was well established. If the thyroid treatment was administered more than two days before the operation no regeneration took place, although there was some tail regulation. The removal of the thyroid gland (Allen '18)⁴ affects in no way the earlier phases of development, but at a certain definite stage, further development of the soma is dependant upon its presence. Thyroid administration to thyroidless tadpoles brought about a resumption of development even four months after it had ceased.

Hanan ('28)¹⁴ produced an abnormal physiological condition in the hen's egg by the introduction into the

air sack of 1/40,000 mg. of thyroxin, at the eight day of incubation, and produced an increase of CO₂ extending through the following three days. This was followed by a period of depression below normal, lasting eight days. Sheard and Higgins' ('30)¹⁵ investigation have shown that irradiation with an air-cooled quartz-mercury arc tends to increase the rate of metabolism. By removing part of the egg shell and replacing the removed shell with a cover glass they firmly established the fact that incubated eggs can be operated upon to produce definite results.

Willier ('24)¹⁶ grafted thyroid upon the vascular chorio-allantoic membrane of the developing chick embryo for the purpose of analyzing the function of the thyroid gland in the development of the chick.

The results he records are: that the best thyroid grafts produce the following typical modifications in the host embryo. (1) The body was quite emaciated and considerably smaller than the control, one third smaller in some cases. (2) Shortening and emaciation of the segments of the wings and particularly of the legs occurred. (3) The amount of fat stored was less than in controls.

He regards such modifications as reduction in size and emaciation of the body as hyperthyroid symptoms. These conditions he interprets to mean that metabolism was increased; more particularly there was an acceleration of

catabolism over anabolism. Willier also noted in his comparison of the results of hyperthyroid activity in the chick and frog embryos that:

- (1) Hyperthyroid symptoms occur in both.
- (2) There is a striking difference in the frog when metamorphosis is induced, while this change does not occur in the chick.

Many investigations have also shown that there is a definite relationship between the amount of iodine and the histological structure of the thyroid.

In his experiment on the hyperplastic glands of opossum, Bensley ('16)¹⁷ found that, as was true of Swingle's tadpole, follicular colloid was somewhat increased when iodine was administered daily to these animals.

In the microscopic examination of the colloid content of the glands of the two experimentals and two control cultures of frog larvae, Swingle ('19)⁸ showed a marked difference in the amount of colloid visible in the follicle. The glands of the iodine-fed animals were packed with this substance, whereas the gland of the control showed a rather scanty amount.

Gray and Rabinovitch ('29)¹⁸ have shown that the thyroid gland of the guinea pig is stimulated in all its histological phases when 0.01 gm. of potassium-iodide was fed daily.

Marine and Lenhart ('09)¹⁹ made a study of the effect

of the administration of iodine upon many different animals. They found that in such iodine treated animals the thyroid contained more colloid than in the normal glands of the controls. Yet, Claude and Blauchetiere ('10)²⁰ state that the iodine content and the amount of colloid are not related to each other, and that all degrees of disproportion exist. There may be much colloid and no iodine, or much iodine and no demonstrable colloid.

Uhlenhuth ('22)²¹ has shown that in a hyperactive thyroid gland intracellular vacuoles migrate toward the lumen and seem to be thrust into the follicle by a process of constriction. They can be seen in the acini of the epithelial lining and are markedly increased in amount in the hyperactive gland.

According to Key ('25)²² the non-staining vacuole-like bodies are not really vacuoles, but represent areas of greater fluidity in the dense colloid. These vacuoles contain the active secretion of the thyroid gland and may serve as a histological criterion of the degree of activity of the gland.

There are many different theories and conflicting opinions concerning the role the thyroid gland plays in the physiology of the body, but all investigators agree that iodine or some iodine containing compound is essential for physiological and histological changes

that occur in the animal body and in the thyroid gland. So it seems just to conclude that the arrest of growth of the skeletal organs is a typical and invariable result of abnormal thyroid function.

The present study was undertaken in the hope of securing further data which might throw more light upon the effect of iodine on the thyroid in relation to the growth of the long bones of the chick embryo, by placing potassium-iodide crystals on the egg shell membrane.

The questions with which the present investigation deals are:

- (1) Can potassium-iodide produce the same effect in chick embryos as the hormone of the thyroid grafts in the chick embryo and in the larvae of *Rana pipiens* and *Bufo lentiginosus* (Swingle '19)⁵?
- (2) Is there any relation between the histological structures of a hyperactive gland and the growth of the long bones?

MATERIALS AND TECHNIQUE

Eggs from various breeds of chickens were obtained for this experiment, although eggs of the same breed were used for each series. Special care was taken to select eggs of approximately the same size. White leghorns and Rhode Island Reds were chiefly used as they were easily obtainable in this region. It was impossible to obtain a good batch of fertile eggs in the late fall. However, fifty eggs were incubated on November 2, 1930, of which only four eggs showed signs of fertility.

The first series of good fertile eggs was incubated of February 9, 1931.

A "Lectro-hatch" type of incubator with a thermostatic control and holding approximately fifty eggs was used. Care was taken in controlling the incubator so that a constant temperature 39 degrees Centigrade and proper moisture were maintained. The eggs were not disturbed during the first three days of incubation, but there after they were turned daily. On the third day each egg was candled over a seventy-five watt electric light in order to eliminate those eggs which showed no sign of fertility. (Waite '28)²³

On the tenth day each egg was again candled, and the area of the shell showing the greatest capillary network was marked with a pencil on the egg shell. Each egg was weighed, and the weight recorded on the

egg shell. They were then arranged in a series so that each experimental egg weighed approximately the same as the control eggs, for it has been found that there is quite a consistent relation between the weight of egg used for hatching and the weight and size of the chick at hatching time. (Halbersleben and Mussehl '22)²⁴ One egg at a time was removed from the incubator on the eleventh day of incubation to be prepared for the introduction of the potassium-iodide crystals. The egg was placed on a pad of cotton, with the marked surface facing upward and with some absorbent cotton moistened in distilled water the marked area was washed clean and then sterilized with cotton moistened in eighty-five per cent. alcohol.

A fine coping saw that had been carefully sterilized by heating in an open flame and submerged in eighty-five per cent. alcohol was used to cut out a rectangular piece of the shell in the region of the marked area. Great care was taken to avoid sawing too deep for fear of breaking the underlying vascular membrane. Bleeding occurs when the allantoic membrane is torn.

With a sterilized forceps, a crystal of potassium-iodide(U.S.P.), weighing not more than 0.05 gram or less than 0.01 gram, was introduced on the egg shell membrane (Danchakoff '17)²⁵. The rectangular window was then replaced and sealed in place by applying melted paraffine in the grooves with a hot sterilized scalpel. The egg

was returned to the incubator with the operated surface up.* Windows were also cut in fifty per cent. of the control eggs in each series while the remaining control eggs were permitted to hatch normally. (Fig. 1 and 2)

Several other methods to introduce potassium-iodide in the eggs were attempted. They were as follows:

Series I

A solution of one per cent. potassium-iodide was introduced into the egg by dropping 0.1 cubic centimeter of the solution on the egg shell membrane by means of a ten cubic centimeter pipette. One egg was used at a time to prevent chilling. The egg was returned to the incubator with its open surface upward and allowed to remain in that position for approximately fifteen minutes in order to allow the solution to be absorbed through the membrane. Each egg was removed from the incubator, the window replaced and sealed with a strip of adhesive tape large enough to cover the groove. This method was continued each day until the twentieth day of incubation. This method proved unsatisfactory, because the adhesive tape broke the egg shell when it was removed each day for the introduction of the potassium-iodide solution. However, the experiment was carried through with the eggs that withstood the practice.

* The method as described above was worked out in part by Willier('24)¹⁶

Series II

In this experiment we decided to use the potassium-iodide crystal method; by placing a crystal of potassium-iodide on the shell membrane and replacing and sealing the window in place with paraffine. This method was found more satisfactory.

Series III

In this experiment an attempt was made to pierce the shell membrane and introduce the potassium-iodide crystal on the vascular chorio-allantoic membrane. Only five eggs from a group of fifty were used for this method. The potassium-iodide crystals immediately dissolved and went into solution within the blood in the capillaries. This method, however, proved fatal to the embryos. An attempt was also made to inject one cubic centimeter of one per cent. potassium-iodide solution through the shell membrane by means of a hypodermic needle. This method also proved fatal. The remaining eggs of this series were subjected to the same method used in Series II.

Series IV

A group of twelve, three day old chicks were experimented with by feeding one cubic centimeter of a one per cent. potassium-iodide solution daily for thirteen days in order to determine the histological effect of the potassium-iodide on their thyroid glands. Six chicks were subjected to the treatment while six were

used for controls.

The solution was administered orally with a one cubic centimeter pipette. Both the experimental and control chicks were raised under the same laboratory conditions.

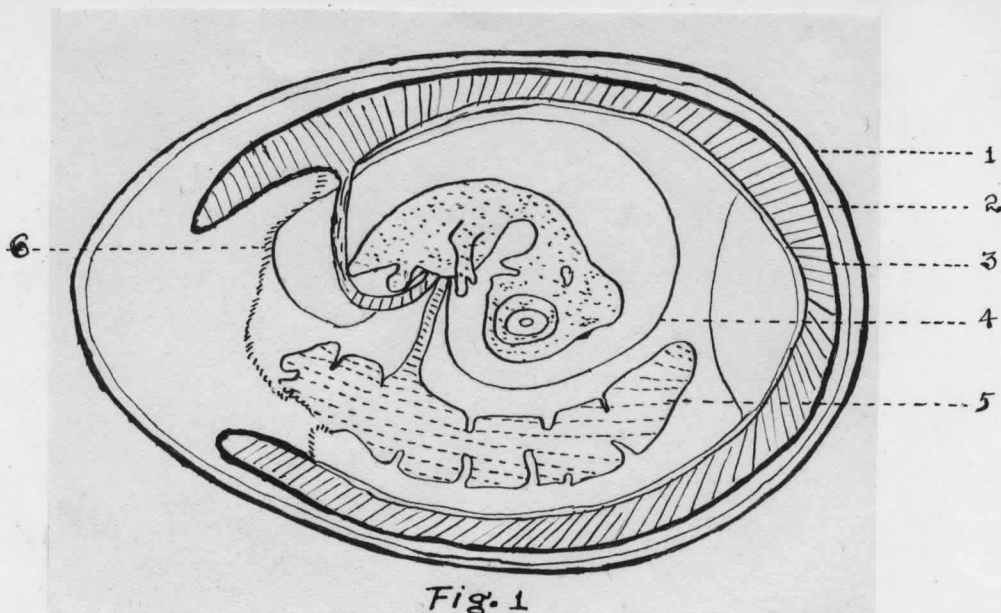


Fig. 1

Diagram of a ten day old chick embryo showing relationship of the important membranes (after Lillie).

1. Egg shell; 2. egg shell membrane; 3. allantoic membrane (highly vascular); 4. amnionic membrane; 5. yolk sac; 6. chorionic membrane.

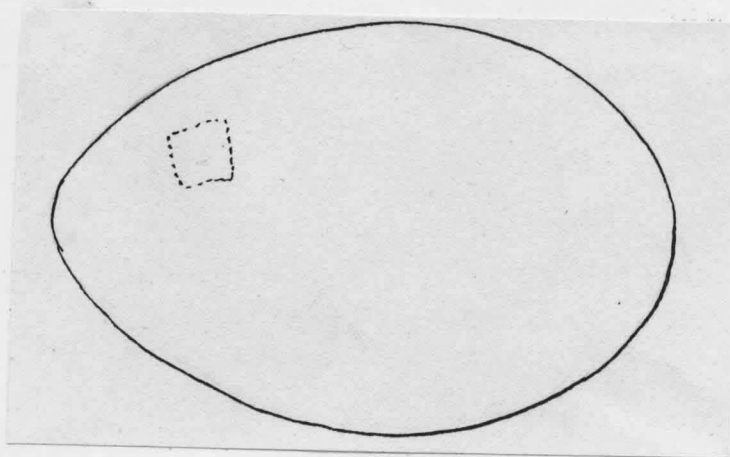


Fig. 2.

Egg, showing position where window was removed.

RESULTS

The results of this work are based upon the study of twenty-six experimental and forty one control chick embryos.

Potassium-iodide was used, because the iodide of potassium has been found to be one of the most diffusible inorganic substances (Barthelow '03)²⁶. It passes into the blood with great rapidity. In the blood, the iodide of potassium becomes an iodide of sodium, and undergoes no further change, and so far as it is known it does not modify the composition of the blood. The diffusion of potassium-iodide into and out of the blood takes place with such rapidity that in fifteen minutes it may be detected in the saliva and urine of human beings.

When the potassium-iodide was placed upon the egg-shell membrane in the area of the vascular chorio-allantoic membrane, by gradual diffusion it passed through the shell membrane into the vitelline blood vessels or the chorio-allantoic membrane.

By a study of a series of allantoises of the chicks between the sixth and twenty-first day of incubation, Danchakoff ('17)²⁵ has shown that at the beginning of their development all the vessels are subepithelial; soon, however, a rich capillary net grows and develops inside of the epithelium and expands freely above the surface of the ectoderm immediately under the egg-shell

membrane. At the thirteenth to fifteenth day of incubation the capillary net is situated above the ectoderm. The meshes in earlier stages surrounded by ectodermal epithelial cells first come at least by one of their surfaces, into immediate contact with the egg-shell membrane. The capillary net situated immediately under the egg-shell membrane is connected with numerous vessels in the deeper mesodermal layers of the allantois. The capillary net actually traverses the ectodermal membrane and expands above it in the form of a rich plexus.

Experiment Series I

Only twenty-eight of the fifty incubated eggs showed signs of fertility of the tenth day. The remaining eggs were rejected.

Seven experimental and twelve control embryos were alive when removed from the shells on the twentieth day of incubation. Five of the experimental embryos, treated with potassium-iodide solution, showed typical signs of retarded growth, as seen in Figure 3.

Their legs and wings were disarticulated from the pelvic and pectoral girdles and prepared for study by the potash method(Lee '28)²⁷.

The bones of the legs and wings become visible and are held intact by the transparent tissue when prepared by the potash method. The legs and wings were then

pinned in position on a board and extended horizontal to a centimeter ruler and measured. The leg measurements were taken from the proximal tip of the femur to the distal tip of the third digit, while the wing measurements were taken from the proximal tip of the humerus to the distal tip of the third digit.(Fig. 4)



Fig. 3. Comparison of KI-modified chick embryos, E 92 and E 82 with a control embryo, C61. C61 embryo is the control embryo for embryo E 82.

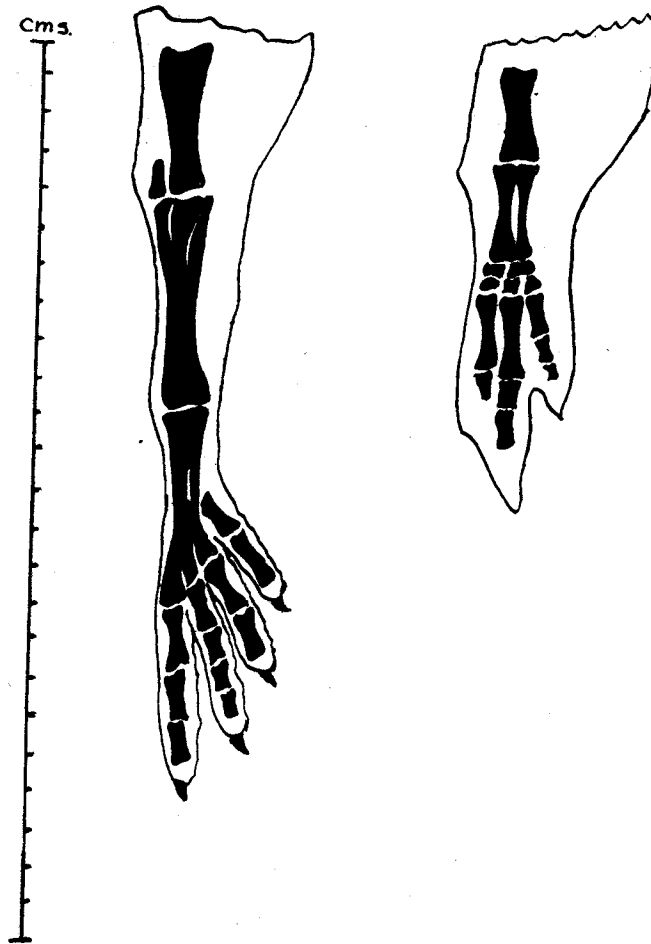


Figure 4.

Diagrammatic drawing of a typical extended leg and wing of a 20 day old chick embryo, after treated by the potash method. The bones of both leg and wing are actual size and drawn to scale in centimeters.

Difference in Growth of the Long Bones

Comparison of the bones of the experimental embryos with those of their controls brought to light marked differences.

The average length of the bones of the experimentals was one-fifth shorter and of less diameter than their control embryos. The bones of several of the experimentals were at least one-fourth shorter than their controls, as shown in Figure 3; Table I. In most cases the bodies of the experimental embryos were small and emaciated.

In only two cases E87 and E95, were these modifications apparently absent; that is, the growth of these two experimental animals was similar to their controls. As seen in Table I, the length of the legs of E87 and E95 were 8.9 cms. and 9.0 cms. respectively, and their control's legs measured 9.0 cms. and 9.1 cms. respectively. Wing-length of these two experimentals measured 4.0 cms. and 4.0 cms. in comparison to 4.9 cms. and 3.9 cms. for their controls.

The average length of the bones of the legs of the experimental embryos was 7.61 cms., while that of their controls averaged 9.15 cms. The wing length of the experimentals averaged 3.4 cms. and those of their controls, 4.11 cms.

The average length of the legs and wings of the five experimental embryos that showed typical modifications

Table I
Size of Legs and Wings of Chick Embryos Series 1
20 Days Incubation

	<u>Experimental</u>			<u>Controls</u>	
	Length of Legs in cms.	Length of Wings in cms.		Length of Legs in cms.	Length of Wings in cms.
E82	6.9	3.1	C61	9.2	4.2
E84	7.1	3.3	C65	9.5	4.3
E85	7.3	3.3	C67	9.0	4.0
E87	8.9	4.0	C68	9.0	4.0
E90	7.1	3.3	C70	9.1	3.9
E92	7.0	3.2	C71	9.3	4.2
E95	9.0	4.0	C72	9.1	3.9
			C73	8.9	3.9
			C75	9.4	4.1
			C76	9.1	4.0
			C79	9.4	4.2
			C80	8.9	3.9
AV.	7.61	3.47	AV.	9.15	4.11

was 7.08 cms., and 3.24 cms., respectively. Figure 5 and 5A illustrate graphically the growth of both legs and wings of the experimental and control embryos.

As Table I indicates, there was a range in the experimental embryos of 6.9 cms. to 9.0 cms. for leg length, while their controls varied from 8.9 cms. to 9.5 cms. The wings of the experimentals varied from 3.1 cms. to 4.0 cms. in comparison to a range of 3.9 cms. to 4.3 cms. for their control embryos.

The width of the legs and wings of the potassium-iodide modified embryos were less in diameter, especially the legs, and the scales on their legs were less well developed than on their controls. (Fig. 3)

Experiment Series 2

Of the fifty eggs placed in the incubator, thirty-two were fertile when candled ten days later. Nineteen experimental embryos were subjected to the treatment of potassium iodide by placing a crystal of potassium-iodide weighing not more than 0.05 gm. or less than 0.01 gm. on the egg shell membrane near the area of the greatest vascularity; while nineteen were used as controls. Only ten experimental and fifteen control embryos were alive when removed from their shells on the twentieth day of incubation.

Three experimental embryos, A37, A44, and A48 showed no marked differentiation in body development

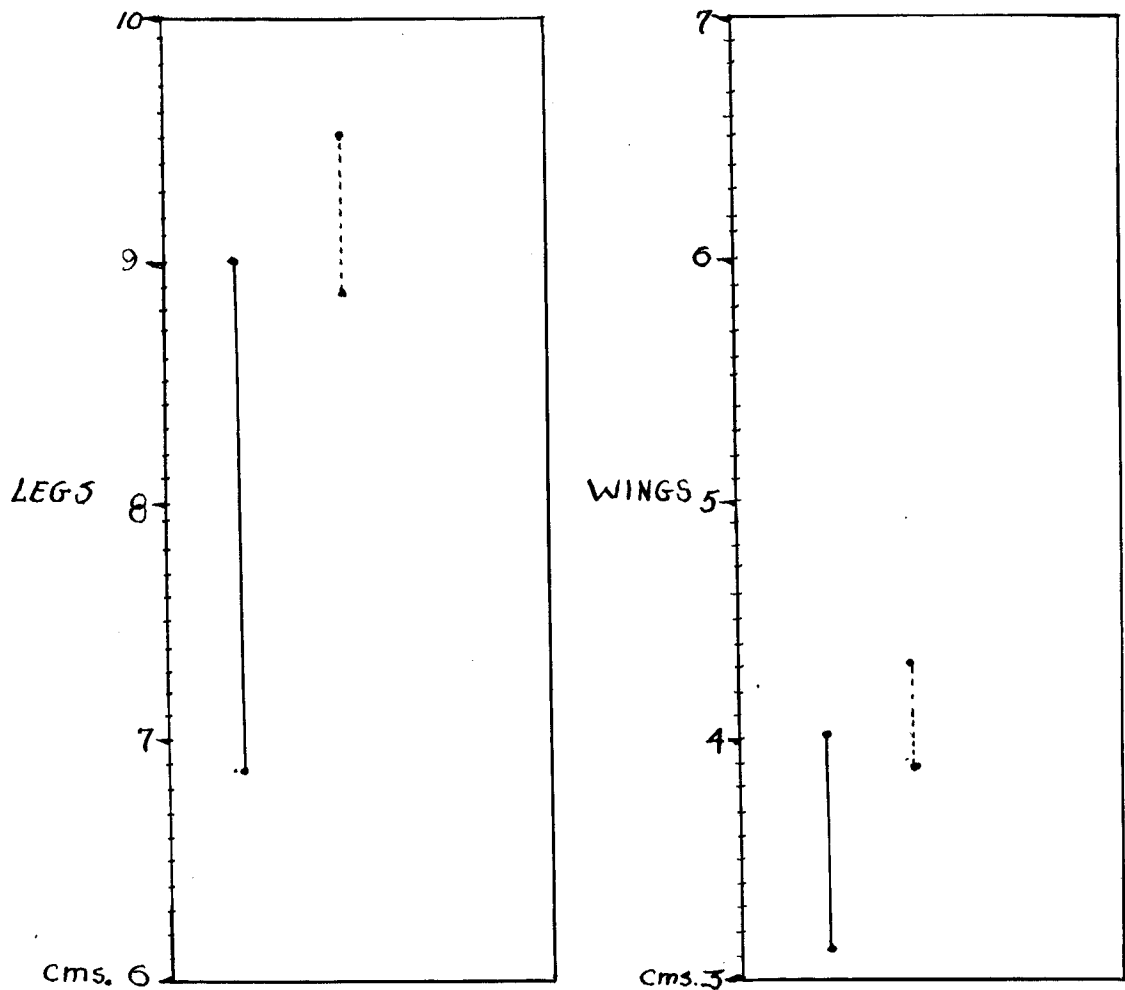


Fig. 5 Graph of distribution of leg and wing-length of the twelve controls and seven experimental chicks embryos of Series I. The broken line shows the range of the controls; the solid line, that of the experimentals. The measurements are made in centimeters. The leg-length of the experimentals range from 6.9 cms. to 9.0 cms. Those of their controls range from 8.9 cms. to 9.5 cms. The wing-length of the experimentals range from 3.1 cms. to 4.0 cms. while those of their controls range from 3.9 cms. to 4.3 cms.

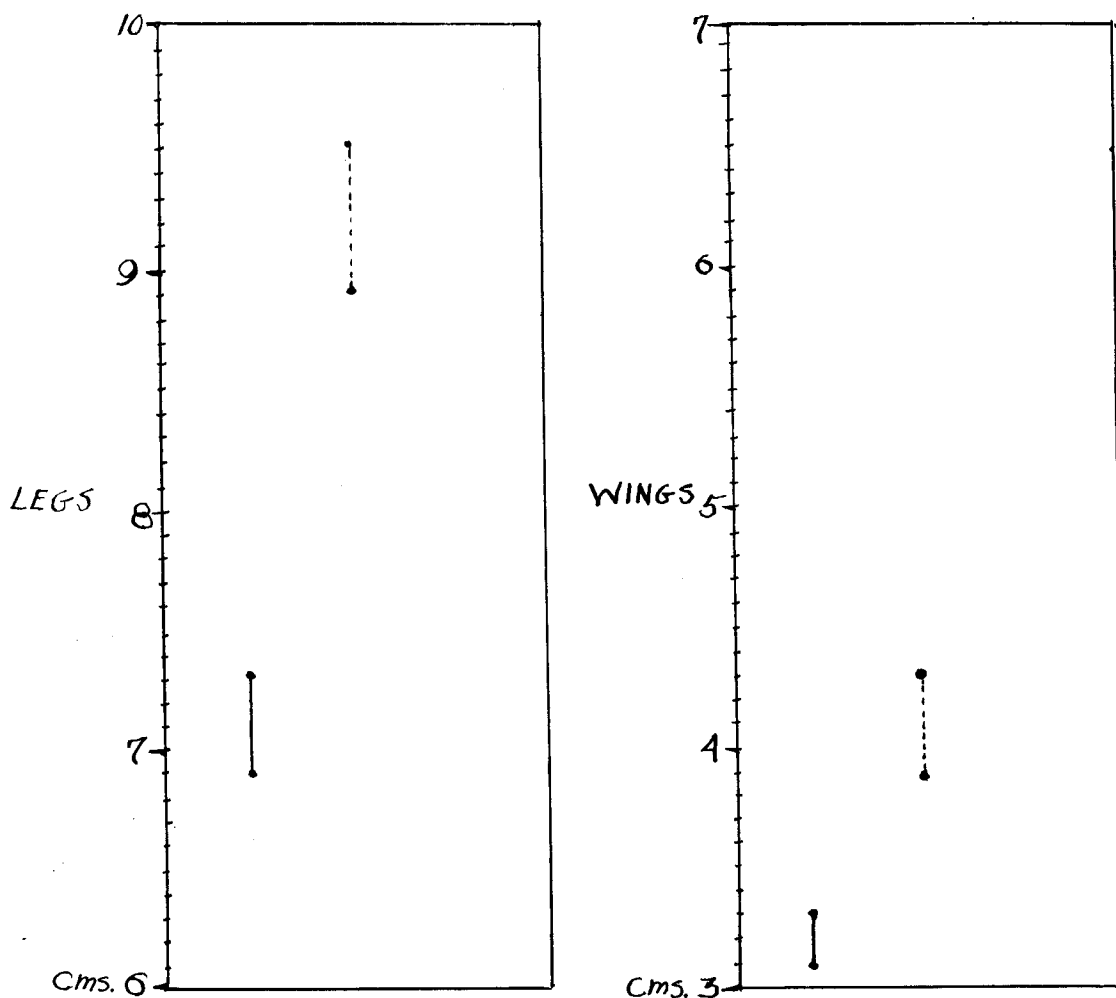


Fig. 5A Graph of distribution of leg and wing-length of the twelve controls and 5 experimental chicks embryos, omitting the two normal sized experimentals, Series I. The broken line shows the range of the controls; the solid line, that of the experimentals. The measurements are made in centimeters. The leg length of the experimentals range from 6.9 cms. to 7.3 cms. while those of the controls range from 8.9 cms. to 9.5 cms. The wing-length of the experimentals range from 3.1 cms. to 3.3 cms; while those of their controls range from 3.9 cms. to 4.3 cms.

in comparison to their controls. Yet A40 and A47 were highly modified especially effecting their legs and wings, as seen in Table II and Figure 6.

The average length of the legs of the experimentals was 8.1 cms.; that of their controls 9.2 cms. The wings length of the experimentals averaged 3.6 cms. to that of 4.1 cms. for the control embryos. The legs and wings of the experimental embryos were less in diameter than their controls. In most cases the bodies of the iodide-treated embryos were small and emaciated.

The average leg-length for the seven experimental embryos that showed typical modifications was 7.48 cms. and their wing-length averaged 3.35 cms. Three experimental embryos were typically normal in body development. Figures 7 and 7A illustrate in graphic form the differences in rate of development of the experimental embryos to that of the controls.

Embryos A37, A44, and A48, although treated with potassium-iodide crystals developed normally. They showed no symptoms of modifications. Yet A40 and A47 showed marked effects, for their bodies were rather small and emaciated. Their beaks were especially small compared to those of their controls.

As seen in Table II and Figure 6 the length of the legs of A40 and A47 were 6.9 cms. and 6.8 cms. respectively, compared to 8.9 cms. and 9.4 cms. for their

Table II
 Size of Legs and Wings of Chick Embryos Series 2
 20 Days Incubation

	<u>Experimental</u>			<u>Controls</u>	
	Length of Legs in cms.	Length of Wings in cms.		Length of Legs in cms.	Length of Wings in cms.
A21	8.1	3.7	C70	9.3	4.2
A25	7.6	3.4	C71	9.0	4.1
A31	7.9	3.5	C73	9.6	4.4
A37	9.3	4.2	C74	9.3	4.2
A39	7.8	3.6	C75	9.5	4.3
A40	6.9	3.0	C79	8.9	4.0
A44	9.1	4.2	C80	9.0	4.0
A47	6.8	3.0	C82	9.4	4.2
A48	9.3	4.2	C83	9.2	4.0
A50	7.3	3.3	C84	9.2	4.1
			C85	9.0	3.9
			C89	9.3	4.1
			C93	9.1	4.0
			C94	8.9	4.0
			C95	9.3	4.1
AV.	8.1	3.6	AV.	9.2	4.1



Fig.6. Comparison of KI-modified chick embryos ,A40 and A47 with their respective controls, C79 and C 81.

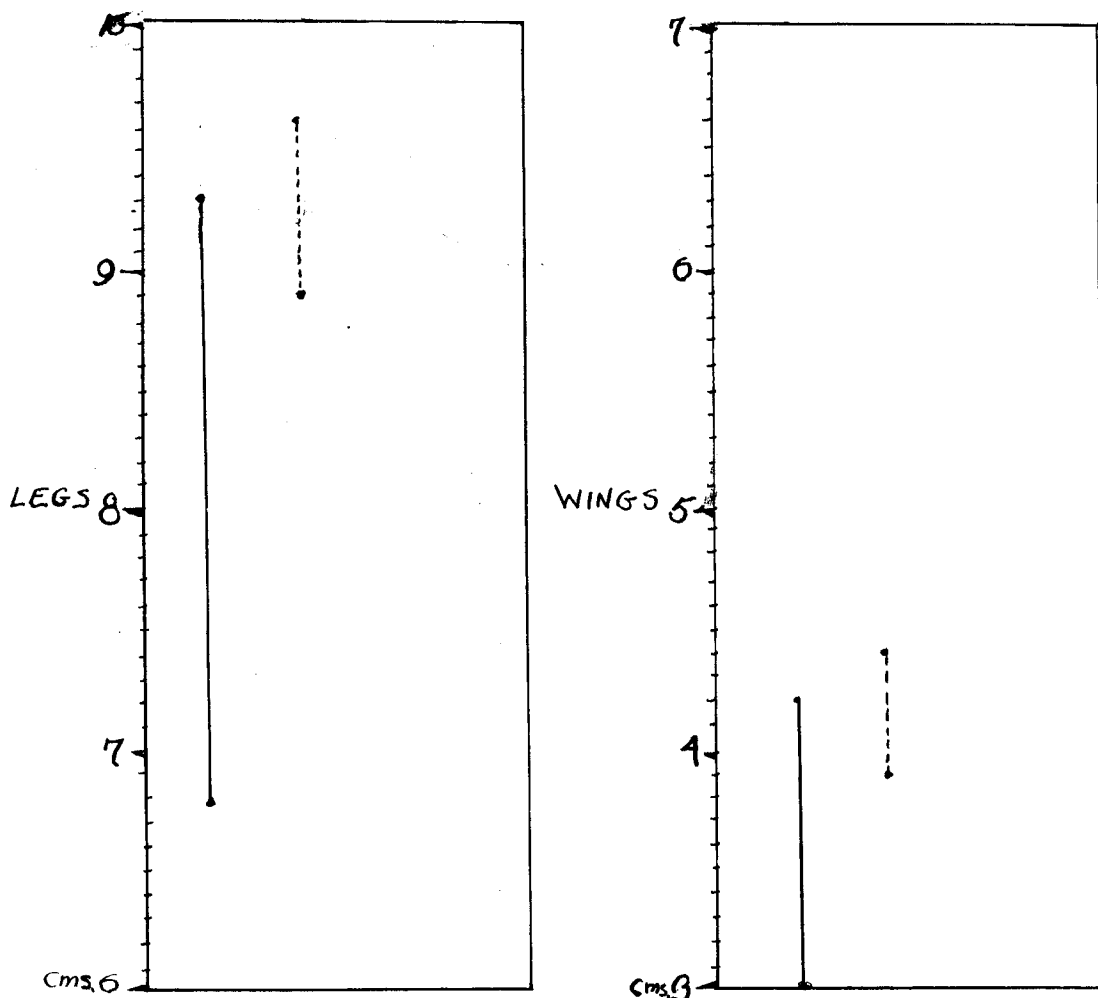


Fig. 7 Graph of distribution of leg and wing-length of 15 controls and 10 experimental chick embryos of Series II. The broken line shows the range of the controls; the solid line, that of the experimentals. The measurements are made in centimeters. The leg-length of the experimentals range from 6.8 cms. to 9.3 cms. The wing-length of the experimentals range from 3.0 cms. to 4.2 cms. while those of the controls range from 3.9 cms. to 4.4 cms.

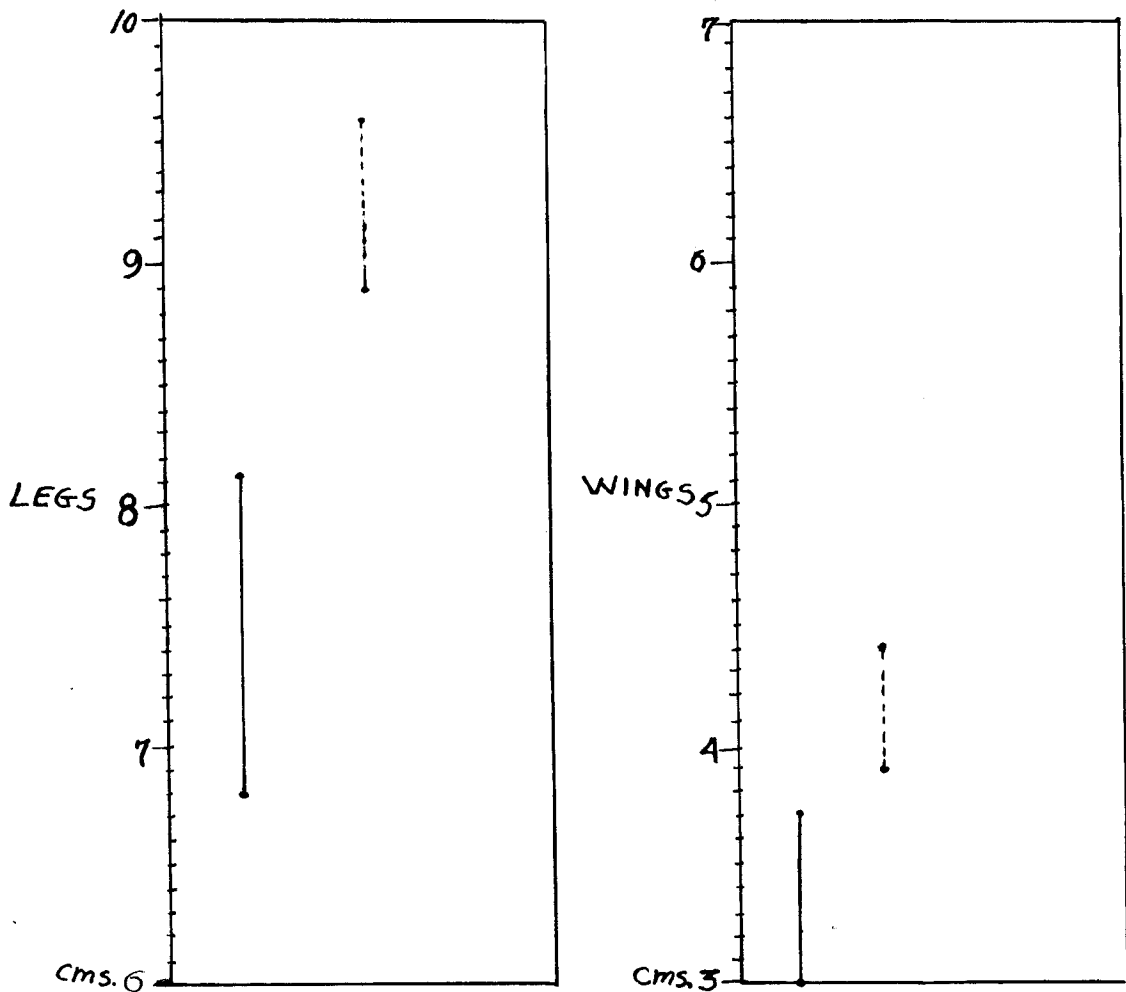


Fig. 7A Graph of distribution of leg and wing-length of 15 control and 7 experimental chick embryos omitting 3 normal sized experimentals of Series II. The broken line indicates the range of the controls; the solid line that of the experimentals. The measurements are made in centimeters. The leg-length of the experimentals range from 6.8 cms. to 8.1 cms., while those of their controls range from 8.9 cms. to 9.6 cms. The wing-length of the experimentals range from 3.0 cms. to 3.7 cms. while those of their controls range from 3.9 cms. to 4.4 cms.

respective control embryos. The wing length was 3.0 cms. for the experimentals in comparison to 4.0 cms and 4.2 cms. for their respective controls.

In the experimental embryos a variation in the development of the legs ranged from 6.8 cms. to 9.3 cms., and for their controls 8.9 cms. to 9.6 cms. The wing length in the experimental embryos varied from 3.0 cms. to 4.2 cms. while the control wing length varied from 3.9 cms. to 4.3 cms.

Experiment Series 3

In this series the experimental procedure was carried out as in Series 2. The potassium-iodide crystals produced marked effects of the experimental embryos of this series. The slight difference in the size of the legs and wings compared to Series 1 and 2 is due to a difference of one day less of incubation.

This study is based upon nine experimental and thirteen control embryos that survived from thirty-eight eggs.

An abnormal development occurred in one case, A124 was enclosed within the yolk when removed from its shell. The embryo, however, was alive.

A review of Table III shows a variation of leg length in the experimental embryos ranging from 6.9 cms. to 8.8 cms. while those of their controls vary from 8.2 cms. to 8.8 cms. The length of the wings of

Table III
 Size of Legs and Wings of Chick Embryos Series 1
 19 Days Incubation

	<u>Experimental</u>			<u>Controls</u>	
	Length of Legs in cms.	Length of Wings in cms.		Length of Legs in cms.	Length of Wings in cms.
A121	7.3	3.3	A101	8.7	3.8
A123	7.6	3.4	A102	8.4	3.6
A124	7.2	3.3	A103	8.2	3.5
A126	8.6	3.7	A104	8.8	3.8
A127	7.8	3.4	A107	8.2	3.5
A131	8.8	3.9	A108	8.7	3.8
A132	8.2	3.6	A110	8.8	3.8
A134	7.2	3.3	A111	8.4	3.6
A146	6.9	3.1	A112	8.3	3.5
			A115	8.5	3.6
			A116	8.7	3.7
			A118	8.2	3.5
			A119	8.3	3.4
AV.	7.73	3.44	AV.	8.47	3.63

the experimentals vary from 3.1 cms to 3.9 cms. and those of their controls range from between 3.4 cms. to 3.8 cms.

The average length of the legs of the experimental embryos were 7.77 cms. contrasted to 8.47 cms. for the leg-length of their controls, and an average wing-length of 3.44 cms. for the experimentals to 3.63 cms. for those of their controls.

A146 showed modifications which were quite pronounced. Its leg-length was 6.9 cms. as compared with an average leg-length of 7.75 cms. for the entire experimental group, and an average leg-length of 8.47 cms. for the entire control group. Further, the shortest leg-length for any control embryo was 8.2 cms. The body was emaciated, the legs and wings less in diameter and the head smaller than the average for the controls. (See Fig. 8 and Table III).

However, A126 and A131 showed no signs of retarded growth. Their legs measured 8.6 cms. and 8.8 cms. in length compared to 8.8 cms. and 8.7 cms. respectively for those of their controls. The wing length measured 3.7 cms. and 3.9 cms. respectively, while the wing length of their control embryos measured 3.8 cms. and 3.8 cms. respectively. Their leg and wing length was greater with respect to the average leg and wing length of the experimentals, yet it is almost equal to the average leg and wing lengths of the control embryos.



Fig. 8. Comparison of KI-modified chick embryos. A134 and A146 with a control embryo, A112. A112 embryo is the control embryo for embryo A146

The average leg and wing-lengths for the seven experimental embryos showing typical body and long bone modifications were 7.45 cms. and 3.33 cms. respectively. Fig. 9 illustrates in graphic form the leg and wing length of the experimental embryos with those of their controls.

Experiment Series 4

The purpose of this experiment was to study the effect of the potassium-iodide on the histological structure of the thyroid gland and compare the histological changes in these thyroid glands with those of the experimental and control chick embryos of the preceding series. No attempt was made to measure the legs or wings of these chicks.

The thyroid glands were removed from the experimental chicks fed daily for thirteen days with one cubic centimeter of a one per cent. solution of potassium-iodide and prepared for histological study. The same was done with the thyroid glands of their controls.

However, a physiological difference was noticed between the experimentals and their control chicks. The controls, although living under the same laboratory conditions, developed leg weakness, and before the thirteenth day, four died. All the ex-

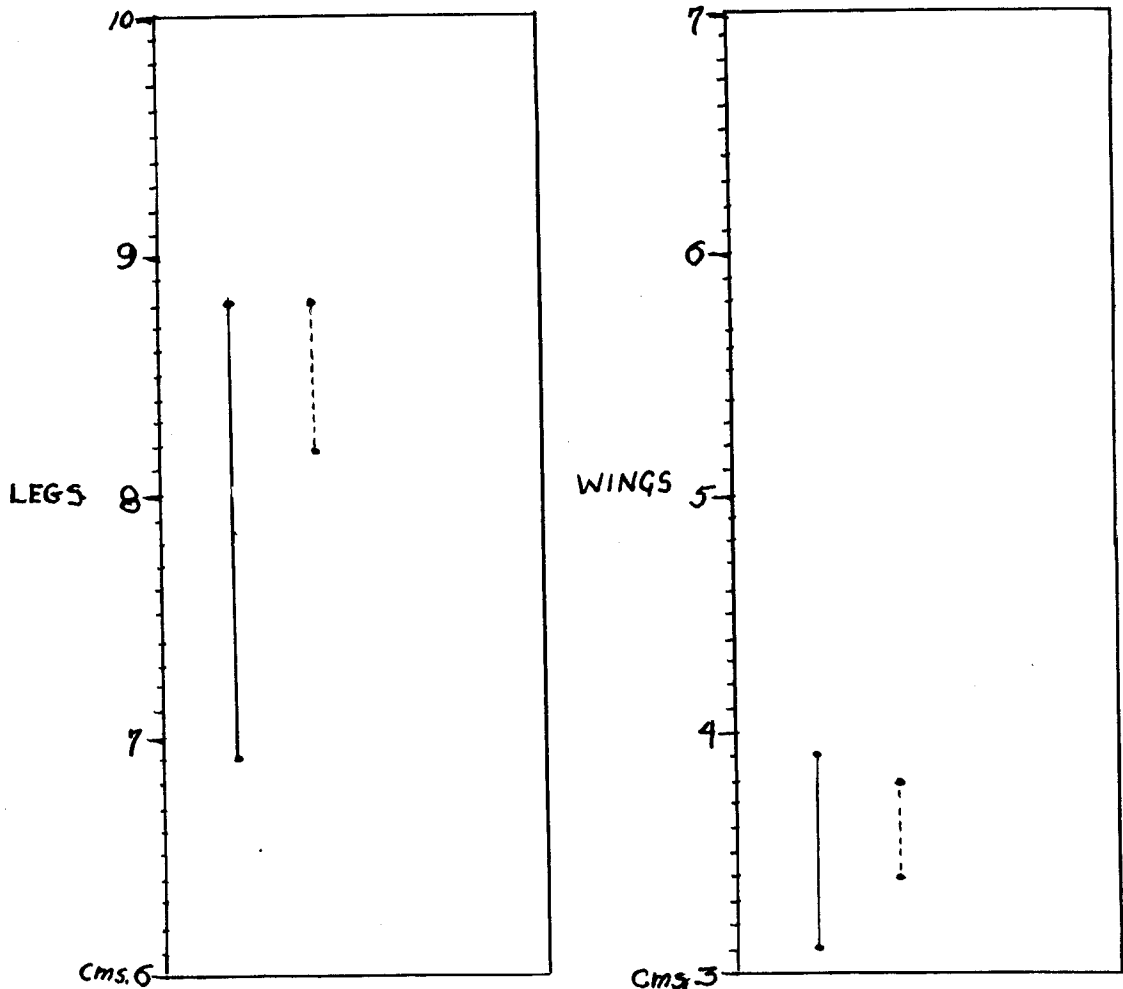


Fig. 9 Graph of distribution of leg and wing-length of 13 control and 9 experimental chicks embryos of Series III. The broken line shows the range of the controls; the solid line, that of the experimentals. The measurements are made in centimeters. The leg-length of the experimentals range from 6.9 cms. to 8.8 cms. while those of their controls range from 8.2 cms. to 8.8 cms. The wing-length of the experimentals range from 3.1 cms. to 3.9 cms., while those of their controls range from 3.4 cms. to 3.8 cms.

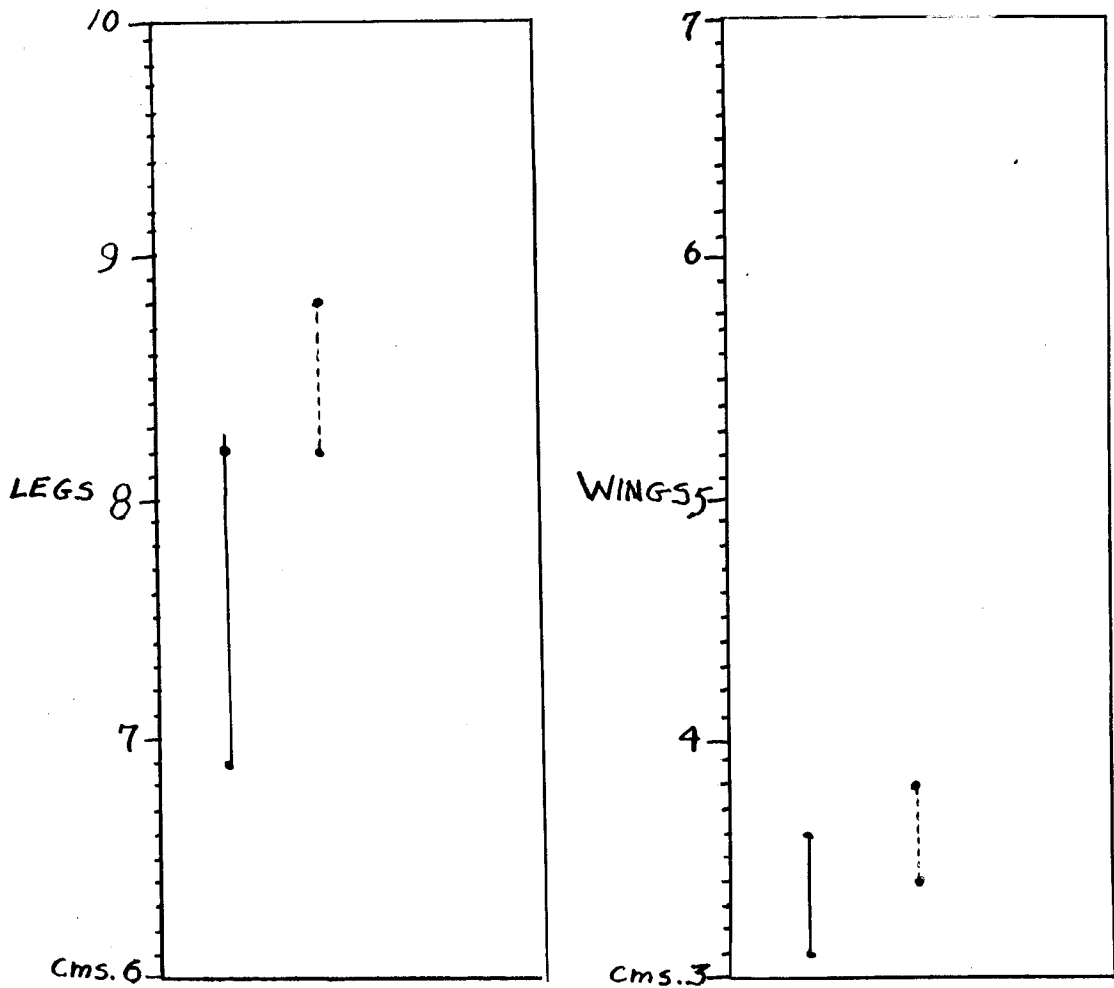


Fig. 9A Graph of distribution of leg and wing-length of 13 controls and 7 experimental chick embryos of Series III, omitting 2 normal sized experimental embryos. The broken line shows the range of the controls; the solid line, that of the experimentals. The measurements are made in centimeters. The leg-length of the experimentals range from 6.9 cms. to 8.2 cms. while those of their controls range from 8.2 cms. to 8.8 cms. The wing-length of the experimentals range from 3.1 cms. to 3.6 cms. while those of their controls range from 3.4 cms. to 3.8 cms.

perimental chicks survived the potassium iodide treatment and appeared healthy.

Histology of the Thyroid Glands Embryos

Series 1, 2, 3.

The thyroid glands were fixed in Bouin's fixative solution, stained in Daelarfield haemotoxylin and counter-stained with eosin. They were cut six microns in thickness.(Fig. 10)

Experimentals

In the thyroid of the experimental embryos, the follicles were found to be chiefly oval or rounded in outline. The cells of the follicular epithelium were approximately low columnar in form, however, many of these cells were cuboidal. The intercellular boundaries were not very definite and distinct. The cells presented a fairly uniform appearance.

The nuclei of the follicular cells were mostly spherical, yet many were ovoidal in form. The nuclear membrane was fairly distinct and the entire nucleus deeply stained. The colloid appeared typical in form, though somewhat variable in staining. In some cases it filled the entire follicular cavity, in other cases it was retracted somewhat from the follicular epithelium.

Many vacuoles appeared in the colloid, especially near the superficial portion of the gland. These

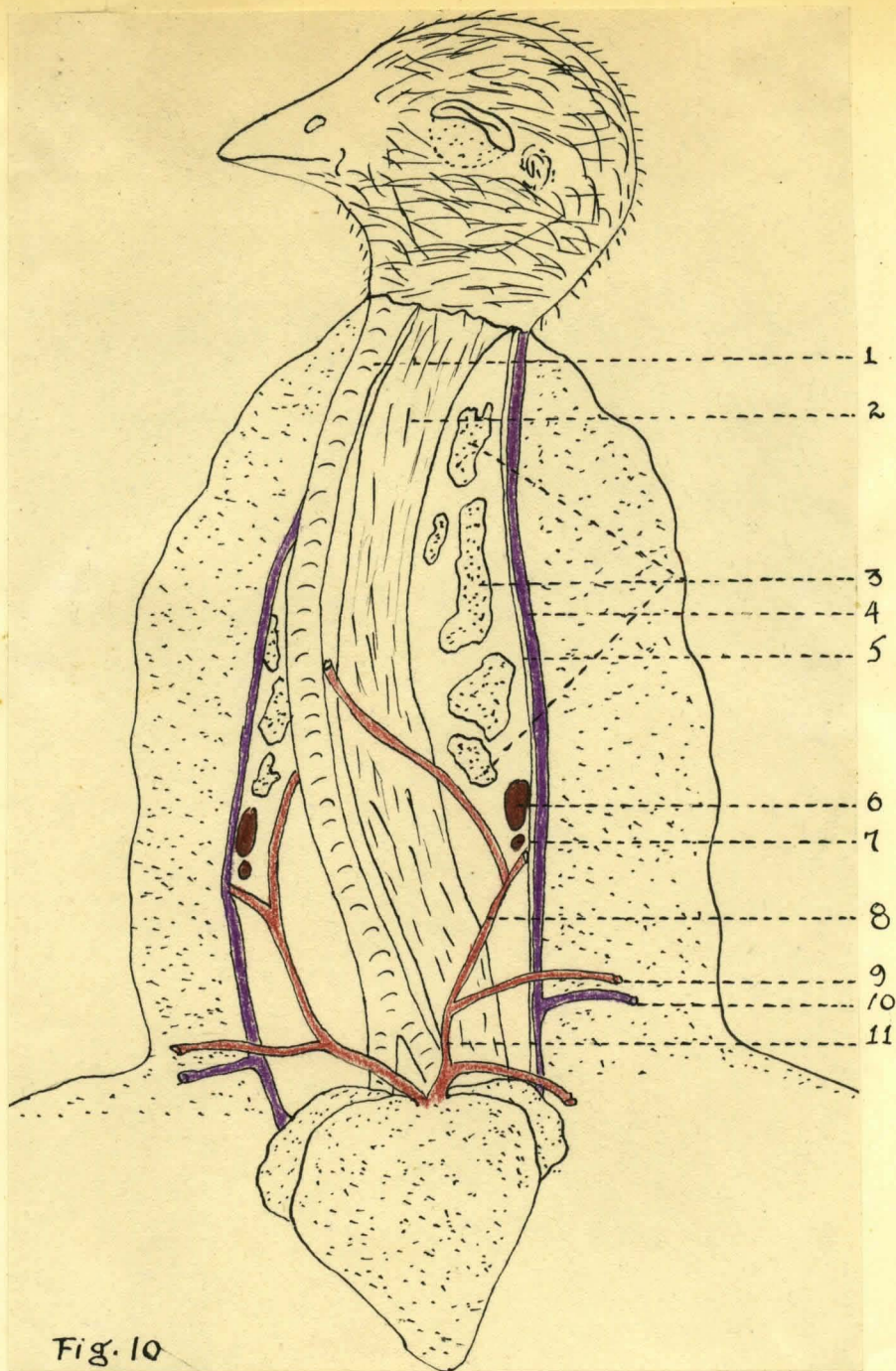


Fig. 10

Dissected neck of a 20 day chick embryo showing the position of the thyroid and parathyroid glands.

1. Trachea; 2.oesophagus; 3.left thyrmus; 4.jugular vein; 5.vagus nerve; 6.thyroid(left lobe); 7.parathyroid gland(left lobe); 8.common carotid artery; 9.brachial artery; 10.brachial vein; 11.subclavian artery.

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vacuoles were usually small, however, in some follicles they replaced at least one-half of the colloid material, as seen in Figure 11. They were also spherical in form and were most frequently found near the outer edge of the colloid, in close proximity to the follicular cells.

The interfollicular connective tissue formed a delicate fibrous stroma, typical of a normal gland.

Histologically, all the glands presented a normal picture, save for the variation in amount of the colloid and the vacuolation cited above. The variation in the amount of colloid present in the experimental thyroids and their excessive vacuolation are in no way characteristic of the normal thyroid gland.

No colloid droplets, described by Bensley('14)¹⁷ were detected in the epithelial cells of the hyperactive thyroid glands of the experimental embryos.

Controls

The thyroid glands of the control embryos studied were typically normal. The only outstanding difference between them and the experimentals was the excessive number of vacuoles found in the experimental thyroid glands. Although there were some vacuoles present in the normal glands of the controls, they were markedly small in size and very few in number.

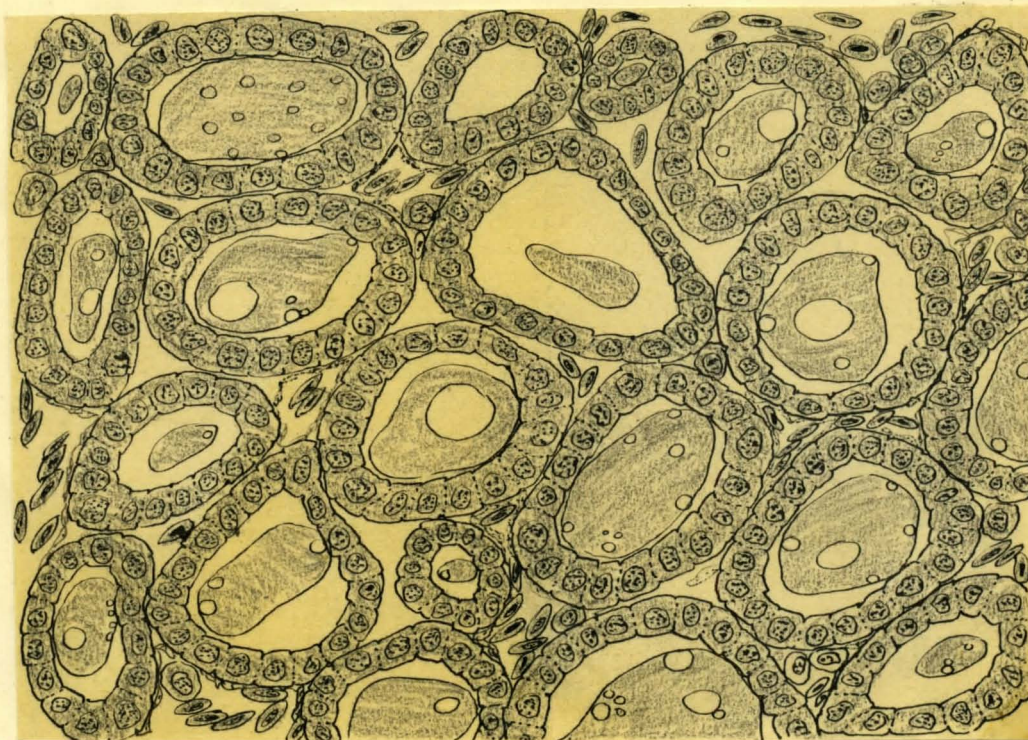


Fig. 11.

Camera-lucida drawing of the superficial portion of the thyroid gland of experimental chick embryo A40, Series 2, showing the abnormal amount of vacuoles in the colloid material. Drawn with a Leitz microscope, x475.

The follicular cells of the control thyroids were mostly low columnar. Very few of the cuboidal type of cells could be noticed. However, the follicles were smaller in size as compared with the experimentals. See Figure 12.

Histology of the Thyroid Glands of the
Adult chicks Series 4

The thyroid glands removed from the six, nineteen day old chicks, dosed with one cubic centimeter of a one per cent. potassium-iodide solution daily for thirteen days, were prepared for histological study and compared with the thyroids removed from their controls and those of the experimental and control chick embryos from the three preceding series.

The follicles in the thyroids of the experimental nineteen day old chicks were oval or rounded in outline presenting a normal appearance. The cells of the follicular epithelium were low columnar in form with very few colloidal cells present. The inter-cellular boundaries were indefinite, yet the cells presented a uniform appearance.

The nuclei of the follicular cells were either spherical or ovoidal in form depending upon the shape of its cells. They took the normal amount of staining with respect to the surrounding structures. The

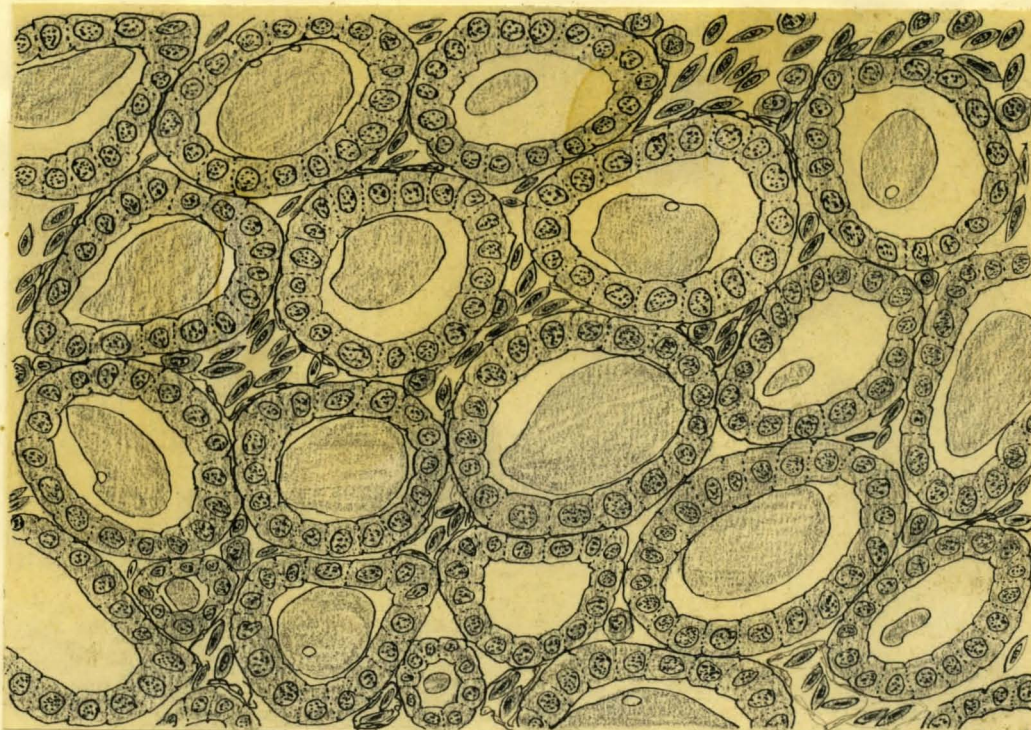


Fig. 12.

Camera-lucida drawing of a portion of the thyroid gland of control chick embryo C71, Series I; showing the normal structure of the colloid material. Drawn with a Leitz microscope, x475.

follicular colloid stained faintly , and filled, in some follicles the entire follicular cavity, and was either retracted or lacking in the other follicles.

The glands showed no striking differences from those of the controls except for the number of vacuoles present in the colloid material. These vacuoles were found throughout the gland, and varied in size and number in the different follicles. They were present in different places in the colloid material, either in the center or near their edges. The size of the follicles was slightly larger in most cases with respect to those of their controls.

Although the colloid of the thyroid glands of the experimental and control nineteen day old chicks showed typical staining properties, there was a noticeable difference in the degree of staining compared with the glands of the experimental and control chick embryos in the preceding three series. The colloid of the glands of the nineteen day old chicks stained very faintly with the Delafield haematoxylin and eosin, used in the former experiment.

DISCUSSION

The results presented in this paper show that potassium-iodide, when injected as a solution or in crystalline form on the egg shell membrane of the chick embryo at the eleventh day of incubation, produces body modifications that are noticeable on the nineteenth and twentieth day of incubation.

Of the twenty-six experimental chick embryos subjected to the above treatment, nineteen showed a retarded body development. In some cases their bodies were small and emaciated, and their heads and beaks were usually smaller than those of their controls. In all nineteen experimentals, the legs and wings were at least twenty-five per cent. shorter than the corresponding lengths of their controls and both types of appendages were less in diameter than those of their controls.

These effects have been generally interpreted to mean that a hyperthyroid condition has occurred, that is, that there was an increased rate of metabolism. The small emaciated bodies and the short legs and wings seem to indicate that catabolism has increased over anabolism.

This view has been supported by many recent investigators, who have studied the effect of body development in relation to the administration of iodine or some iodine containing compound to animals

and human beings. In feeding the thyroid material in different forms to young animals, Cameron and Carmichael ('20) have shown that there is a marked diminution in size of the organs in the bodies of the animals, and the muscles of the body were of distinctly smaller size than normal. These findings suggested to them a status of hyperthyroidism. The results were explained as an abnormal tissue catabolism. Although many of the organs were affected, the thyroid gland remained normal, and was in a resting condition because of the absence of stimulation to the gland.

Willier('24)¹⁶ noticed that the legs and wings of the thyroid-grafted chick embryos was smaller in size when compared to those of their controls. He also attributed these modifications to a hyperthyroid condition.

Hammett('27)²⁸, Swingle('19)⁸, Lenhart('15)⁷, Speidel('29)¹³, Etkin ('30)¹⁰, and Allen('18)⁴ in their work on the thyroid glands of the amphibian larvae, noticed that the thyroid gland plays a major role in the development of the body. Swingle('19)⁸ stimulated metamorphosis in the larvae of *Rana pipiens* by feeding potassium-iodide, while Lenhart('15)⁷ found that the higher the iodine content of the gland fed to frog larvae, the more rapid the body metabolism. Such rapid body metabolism expresses itself in amphibia

by increased rate of metamorphosis.

Hanan('28)¹⁴, by the introduction of thyroxin in small quantities on the air sac of the chick embryos on the eighth day of incubation, increased the carbon-dioxide production. Hering('17)²⁹ fed thyroid material to young white rats and found that it tended to diminish body weight, which he also attributed to an increase in rate of metabolism.

In the experimental animals there was a wide variation in the length of the wings and also in the length of the legs, as can be noted in Tables I, II, and III; Figures 4,5, and 6. Even in the controls there was a slight variation in the length of the legs and wings. Since the same amount of iodide was used in each series one might expect to obtain the same amount of variation in each case. However, Willier('24)¹⁶ reported similar variations in his experiments on chick embryos. He attributed this to the possibility that the chick embryos themselves varied in their physiological traits, such as rate of body metabolism and degree of resistance to foreign material. This accounts for the greater degree of variation in the experimentals, since their normal physiological balance was disturbed. And it also accounts for the higher rate of mortality among the experimental embryos.

Since hyperthyroid conditions in the chick embryos were induced by the administration of the iodide, the question arises: What role did the potassium-iodide play in the production of the modifications in the chick embryos? Was the iodide of potassium utilized by the thyroid gland after entering the blood stream; or was it converted into a physiologically active substance in some of the body tissue?

The results obtained with feeding of iodide to the thyroid-ectomized tadpoles led Swingle('19)⁵ to advance the view that the function of the thyroid gland is chiefly for iodine storage rather than for the elaboration of specific hormones, and moreover that the tissues of his animals are capable of utilizing iodine directly without the intermediation of the gland.

The host embryos showed typical normal thyroid glands, according to Willier('24)¹⁶, after the thyroid graft ceased to function. He believed that the thyroid graft in the chick embryo functioned independantly of the host thyroid, and in no way was it modified histologically by the hormone of the thyroid graft.

Kendall('29)³⁰ disagrees with Swingle and Willier. He believes that the only way iodine increases metabolism in animals is through its effect on the thyroid gland and not by its action directly on the body tissues. The relation of iodine to the physio-

logical activity of the thyroid gland is very close; in fact the activity of the gland depends upon its content. Etkin('30)¹⁰ also noticed in *Rana pipiens* a relationship between the physiological activity of the gland and the growth of the hind limbs. However, Abelin('27)³¹ showed that there is no parallelism between biological activity and the amount of iodine.

On the other hand, Rabonovitch('28)³² noticed a rapid increase of proliferation of the thyroid epithelium, as evidenced by the great increase of mitoses, when guinea pigs were injected with potassium-iodide intraperitoneally. The increase was greater when large amounts of potassium-iodide was administered. Marine and Rogoff('17)³³ produced definite histological changes in the thyroid gland of tadpoles in twenty-four hours by the injection of 50 mg. of iodine. The greater the degree of stimulation the more pronounced were the changes in the thyroid gland.

The histological appearances of the thyroid glands of the iodide-treated chick embryos and the nineteen day old chicks were in closer agreement with the latter investigators. It has therefore been concluded that in these experiments, the potassium-iodide so stimulated the thyroid gland in some way, as to bring about a hyper-active condition. The thyroid glands in many of the chick embryos treated with potassium-

iodide, showed no degree of change in the amount of colloid present, or in its staining properties. The findings of various investigators in regard to thyroid changes following some form of iodine treatment, either as an inorganic salt, or the gland substance itself, have not been uniform.

Swingle('19)⁸, Marine and Lenhart('09)¹⁹ and Bensley('16)¹⁷ report that in a hyper-active gland the colloid mass is increased. Aesbacher('05)³⁴ states that the variation in staining properties of the colloid indicates a varying iodine content. Thomas and Delhougne('24)³⁵, Hewer('27)³⁶, and Marine and Lenhart('09)¹⁹ report a parallelism between the iodine content and the amount of colloid material. Yet Claude and Blauchetiere('10)²⁰ found that the iodine content and the amount of colloid present in the thyroid gland is by no means a true index of the functional activity of the gland. Colloid may be increased in conditions where the functional activity of the gland is diminished as in hypothyroidism. Such apparent discrepancies he interprets as being equally due to absorption, which he regards as being fully as important as secretion. He also believes that the amount of colloid found in a gland depends upon secretion and absorption, and the physiological activity of the

gland depends largely upon the equilibrium between them. In one instance, there may be very little colloid in an extremely active gland due to rapid absorption of the thyroid product yet on the other hand there may be much colloid in the follicles when secretion and absorption have been diminished.

Many vacuoles were found in the colloid material of the glands of the iodide-treated embryos. (Figure 10) These vacuoles were especially noticeable in the superficial portion on the glands. Uhlenhuth('23)²¹ has noticed vacuoles in hyper-active glands, while Key ('23)²² believes that these vacuoles serve as a histological criterion of the degree of activity of the thyroid. Wyss('89)³⁸ study on the effect of pilocarpine poisoning in cats and rabbits, revealed vacuoles in the acini of the follicles, which he attributed to an engorgement of the thyroid gland with blood. Because of their lack of affinity for stains he termed these vacuoles "Chromophobe" secretions. Bensley('22)³⁹ described vacuoles which occurred in the base of a normal gland. He believes that these vacuoles are the real antecedents of the secretion of the thyroid gland. He also observed (Bensley '16)¹⁷ that the hyperplastic glands in opossums fed with iodine, appeared almost identical in histological structure with the normal thyroid gland of the controls, yet differed markedly in the amount of vacuoles, and thus probably in secretory potential,

confirming the former experiment of Marine and Kendall on mammals. Even, Wen-Choa Ma ('25)⁴⁰ also described the presence of vacuoles in the thyroid gland. These vacuoles almost filled the entire follicular lumen.

The thyroid glands of the modified chick embryos in these experiments show histological features that are in agreement with Bensley('16)¹⁷, Uhlenhuth('23)²¹, von Wyss('89)³⁸, and others, that is the presence of vacuoles in the thyroid follicles of the chick embryos indicates that the iodide was utilized by the thyroid gland, as evidenced by the increased amount of secretion present in the form of vacuoles in question.

SUMMARY

1. The purpose of these experiments was to make an analysis of the effect of potassium-iodide on the embryological development of the chick, especially on the growth of the long bones (legs and wings), and also of its effect upon the histological activity of the thyroid gland.
2. Potassium-iodide, as a solution or in crystalline form was placed upon the egg shell membrane of the chick embryo in the region of the greatest vascularity. The iodide was administered on the eleventh day of incubation, and the embryos were removed from their shells on the twentieth day in Series I and II, and on the nineteenth day in Series III.
3. Nineteen of the twenty-six experimental embryos showed typical modifications. Their bodies were small and emaciated. Their legs and wings were shorter than those of their controls and the diameter of their bones were recognizably shorter.
4. The leg and wing length of the experimental embryos varied from a slight modification to a retarded growth of at least twenty-five per cent. of those of their controls.

5. The thyroid glands of the experimental chick embryos appeared normal, histologically, except for the excess amount of secretion-loaded vacuoles in the colloidal material near the superficial portion of the gland, which is considered indicative of hyper-activity by many recent investigators.
6. The thyroid glands of six, nineteen day old chicks dosed daily with one c. of a one per cent. solution of potassium-iodide for thirteen days, were studied and compared with those of the experimental chick embryos. These glands showed histological structures that were identical with those of the experimental chick embryos.
7. The modifications in body size, retarded leg and wing growth and the abnormal number of secretion-loaded vacuoles in the thyroid glands, are considered to be hyperthyroid symptoms, which may be interpreted to mean that there was an increase of catabolism over anabolism.

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