

A COMPARISON OF THE EFFECTS OF THE GONADOTROPIC HORMONE OF  
THE ANTERIOR LOBE OF THE PITUITARY AND THE ANTERIOR  
PITUITARY-LIKE HORMONE OF PREGNANCY URINE IN  
THE DOMESTIC FOWL (GALLUS DOMESTICUS LINNAEUS)

by

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## INTRODUCTION

Since the discovery in 1926 by Zondek and Aschheim, and shortly afterward by P. E. Smith (1927), that the anterior pituitary gland secreted a substance essential to the functioning of the gonads, there have been many investigations substantiating this interrelationship. Among these investigations was the search by Zondek and Aschheim (1928) for a similar chemical substance in body fluids. Such a compound they found to be abundant, along with theelin, in both the blood and urine of pregnant women, and assigned to it the name, "Prolan". This discovery led to much discussion as to whether the substance is identical with the anterior lobe product, and, if identical, whether it is produced by the anterior lobe of the pituitary, the placenta, or elsewhere.

Inasmuch as a large quantity of theelin is also present in urine and crude urine extracts, an important step toward the clarification of these problems was the discovery by Zondek and Aschheim (1928a) that the hormone could be separated from theelin by an alcohol-ether extraction process. Many methods of extraction of the anterior pituitary-like hormone have since been proposed by various workers, each based upon a procedure of adsorption and elution or precipitation and extraction of the hormone by different chemicals.

Space does not permit a discussion of each procedure; however, a method comparable to that of Katsman and Doisy (1932) has been used with success by the writer in preparing a potent extract for injection into birds. This procedure will be discussed under another heading.

Research leading toward establishing the status of these two hormones, the one from the gland and the other from pregnancy urine, has followed two trends: First, that of collating the results of the injected hormones into normal animals, and second, a like collation of results of injections into hypophysectomized animals. A study of these investigations reveals various points of controversy regarding the physiological action of both the hormone of the anterior lobe and the hormone of the urine of pregnancy. The research on various mammals will be considered in the following paragraphs and compared with similar investigations on birds.

#### REVIEW OF LITERATURE

##### Investigations on the Mammal

It was reported by Smith and Engle (1927) that implantation of fresh pituitary substance into young rats and mice produced an increase in the weight of the male reproductive

organs, exclusive of the testes, proportionately greater than that of the testes. This same effect was produced by injections of bovine pituitary extract into rats and mice (Johnson and Sayles, 1929). A growth of reproductive organs, exclusive of the testes, was produced in rats by the injection of whole pregnancy urine (Engle, 1929; Bourg, 1931). Tubule proliferation in the testes of the ground squirrel following pituitary implants (Johnson and co-workers, 1934), and in the rat following pituitary extract injections (Engle, 1932), was not produced in the mouse by the injection of anterior pituitary extract (Johnson and Hill, 1930). Extracts of pregnancy urine injected into rats (Engle, 1932), and monkeys (Engle, 1932; Aberle and Jenkins, 1934), and sexually inactive ground squirrels (Baker and Johnson, 1936) brought about tubular proliferation.

Injections of whole pregnancy urine, or extracts from it, into rats (Bourg, 1931; Engle, 1929, 1932) and into rats and monkeys (Aberle and Jenkins, 1934) produced increases in the interstitial cell mass of the testes.

Results concerning the effect of the two extracts and implantation of the pituitary gland on the production of spermatogenesis are controversial. Johnson and his co-workers (1934) reported spermatogenesis following heteropitui-

tary implants in ground squirrels, an effect not obtained on the albino mouse (Johnson and Hill, 1930), nor on the rat and monkey (Engle, 1932). Stages of spermatogenesis, excepting the formation of mature spermatozoa, were produced by the injection of pregnancy urine into rats (Boeters, 1930, 1931). This was not confirmed by Engle (1929, 1932) and Aberle and Jenkins (1934).

In general, the conclusions may be drawn from this work on the male mammal that both the anterior pituitary sex-stimulating hormone and the similar hormone from pregnancy urine produce an increase in weight of the reproductive organs exclusive of the testes, tubule and interstitial cell proliferation in the testes, and, in some cases, phases of spermatogenesis.

Because the effect of pituitary substances is more pronounced in the female than in the male, a greater portion of the comparative work on these two gonadotropic substances has been done on the female mammal. General ovarian growth in the rat has been recorded as a result of intraperitoneal injections of anterior lobe substance (Evans and Long, 1921, 1922). Apparently no workers have reported a similar action from injections of whole or extracted pregnancy urine.

Follicle stimulation as a result of implantation of whole gland into immature mice (Engle, 1929), rats (Evans

and Simpson, 1929), and rabbits (Hill and Parkes, 1931) has also been obtained by injections of pregnancy urine into mice (Engle, 1929), rats (Evans and Simpson, 1929), and rabbits (Siegmond, 1930; Zondek, 1931; Friedman, 1930). The effect was less in rats injected with pregnancy urine than in those receiving implants of anterior lobe, however. Loeb (1932) reports rapid atresia of a majority of the follicles in guinea pigs as a result of injecting anterior lobe substance from cattle.

Ovulation in female mice has been produced by anterior lobe implants (Engle, 1929), and similarly in rabbits by use of injections (Bellerby, 1929; Jares, 1930; Hill and Parkes, 1931; Snyder and Wislochi, 1931). A like response was elicited by the use of pregnancy urine extracts in the ferret (Hill and Parkes, 1930) and the rabbit (Friedman, 1929; Winter, 1931; Hill and Parkes, 1931; Snyder and Wislochi, 1931; Wolfe and Ellison, 1932). Failure to produce ovulation by the use of the pregnancy urine factor was noted in three cases: The first, in mice (Engle, 1929); the second, in guinea pigs (Jares, 1931); and the third, in rabbits (Friedman, 1930). Workers have also recorded luteinization in the albino rat (Johnson and Sayles, 1929), the albino mouse (Johnson and Hill, 1930), and the rabbit (Hertz and Hisaw, 1934) by injecting anterior pituitary extract. Sim-

ilarly, luteinization as a result of pregnancy urine injections has been recorded in the mouse (Engle, 1929) and the rabbit (Winter, 1931; Wolfe and Ellison, 1932). "Pseudo-lutea" formation as a result of the action of each hormone on the guinea pig has been reported (Loeb, 1932).

In drawing general conclusions regarding the research work on female mammals, it might be stated that both hormones produce follicle stimulation, ovulation, and the formation of corpora lutea in most mammals used experimentally.

#### Investigations on the Bird

The similarity of action of the anterior pituitary hormone and the pregnancy urine derivative on mammals is not extended to avian forms according to the findings of various workers. Administration of anterior lobe substance has brought about a great increase in testis growth in immature ring doves (Riddle and Fleming, 1928; Riddle and Polhemus, 1931), in immature domestic ducks (Schockcart, 1931), and in cockerels (Schockcart, 1933). Failure to produce this effect with the use of pregnancy urine extract has been reported in immature ring doves (Riddle and Polhemus, 1931), in immature domestic ducks (Schockcart, 1931), and in cockerels (Schockcart, 1933). Riddle and Polhemus (1931) reported a regression of the testes of doves upon the ad-



ministration of the pregnancy urine hormone.

Contrary to its action on mammals, anterior pituitary substance failed to give any increase in the interstitial cell mass in ducks (Schockcart, 1931) and cockerels (Schockcart, 1933). Pregnancy urine extract was likewise inactive. Spermatogenesis in both the duck and domestic fowl has been stimulated by anterior lobe substance but has been reported to receive a similar stimulation by the use of pregnancy urine factor only in finches (Witchi, 1935). Stimulation of accessory structures of the cockerel has been noted as resulting from anterior pituitary substance (Domm, 1933; Schockcart, 1933) but not from the pregnancy urine fraction (Riddle and Polhemus, 1931; Schockcart, 1933).

Summarizing the work done on the male bird, it might be stated that while the anterior lobe produces testicular growth, spermatogenesis, and accessory structure growth, the related pregnancy urine compound apparently does not bring about any of these manifestations, with the possible exception of spermatogenesis in finches.

While several workers have reported growth of the ovaries of immature hens as a result of injections of anterior pituitary substance, (Riddle and Fleming, 1928; Schockcart, 1931; Riddle and Polhemus, 1931) failure of the extract to effect growth of the pigeon ovary (Riddle and

Polhemus, 1931), and hen ovary (Pearl and Surface, 1915) has been noted. Follicle stimulation by injections of this glandular extract has been recorded in the duck (Schockert, 1931) and the cockerel (Schockert, 1933). Clark (1915) reported induction of ovulation by the feeding of anterior pituitary gland to fowls. This work has been questioned by Pearl (1916) and ovulation has been found to be inhibited by the injection of this material into fowls by Walker (1925) and Noether (1930).

Pregnancy urine, either whole or extracted, inhibited the growth of the dove ovary (Riddle and Polhemus, 1931). If excessive amounts are injected, the ovaries react but slightly and mainly by degenerative processes in the sparrow (Witschi, 1935). No effect of pregnancy urine on the ovaries of hens has been reported other than inhibition of ovulation similar to that of anterior lobe substance (Noether, 1930).

In the female bird it may thus be seen that according to some workers, the differences in effect of these two substances are most marked, the anterior lobe hormone producing growth and follicle stimulation in the ovaries of most birds tested, while the pregnancy urine hormone was almost totally inactive in these respects.

It is the purpose of this thesis to set forth the re-

sults of the preparation and assay of a potent pregnancy urine extract of the anterior pituitary-like hormone; to compare the effects of this hormone with those of the anterior pituitary, gonadotropic hormone when injected into immature male and female domestic fowls; and to present a technique of hypophysectomy in the fowl with preliminary results of that operation.

#### THE PREPARATION AND ASSAY OF A PREGNANCY URINE EXTRACT

The preparation of an active extract of pregnancy urine is based upon the benzoic acid adsorption method of Katzman and Doisy (1932). Seven liters of the urine, collected over a period of a week, were preserved by the addition of a little chloroform and kept at a temperature of from eight to ten degrees Centigrade in a refrigerator. After making slightly acid to litmus paper, the urine was vigorously stirred with a mechanical stirrer while 350 cc. of a saturated solution of benzoic acid in acetone were added. The material was then allowed to remain in the refrigerator over night. By means of a suction filter, the precipitated material was filtered and dissolved in a volume of acetone equal to that originally added (350 cc.). The solution was then centrifuged and the small amount of acetone-insoluble material (containing the active principle) was washed

thoroughly with acetone to remove traces of benzoic acid and theelin. Distilled water was then added to the material, the mixture thoroughly shaken, and then centrifuged. This process was repeated several times, the supernatant liquid being placed in a sterile bottle to a volume of approximately 150 cc. With 0.5 per cent phenol added as a preservative, the extract was kept at a low temperature in the refrigerator.

The potency of the extract thus obtained was determined by the method of Katzman and Doisy (1932), in which one rat unit is defined as the minimum quantity of material which, administered subcutaneously to 21-day-old rats in six equal portions during the course of three days, causes opening of the vagina and oestrus by the 27th day. In two 21-day-old female rats injected with 0.1 cc. twice daily for three days, the vaginas opened and cornified cells were present in vaginal smears by the 26th day. Four females were then injected twice daily for three days with 0.1 cc. of a one to ten dilution of the extract, and, in all but one, the vaginas opened and cornified cells were present in vaginal smears on the 27th day. Controls for both groups were negative. In the assay, 0.1 cc. of the one to ten dilution was taken as the minimum quantity. From this the extract was calculated to contain  $16 \frac{2}{3}$  rat units per cubic centimeter.

## HORMONAL INJECTIONS INTO CHICKS

As a preliminary study, six, 21-day-old White Leghorn chicks, three males and three females, were injected intraperitoneally with the extract (prepared as previously described), one cc. daily for a period of 11 days. A similar group of two-day-old chicks also received daily, one cc. injections for a period of 11 days. Both groups with controls were killed on the second day following cessation of the injections. At the time of sacrificing, all birds were apparently in good health. Appearance, body weight and comb growth of the experimental birds did not differ from those of the controls. Histological examination of the gonads revealed no noticeable differences between the experimentals of each group and their respective controls. Likewise no great difference in gonad size was noted. There were no visible evidences of toxicity of the extract. The low potency of the extract might account for the lack of stimulation.

In order to determine the effects of a more potent pregnancy urine extract in the young chick and to compare these effects with those elicited by the anterior lobe substance, a second study was made. In this group 26 White Leghorn chicks, all of the same brood (many of them bro-

thers and sisters), 37 days of age were used. Eight birds, five males and three females, were given ten, daily intramuscular injections of 0.5 cc. "Antuitrin", a Parke Davis and Company product. This is a soluble extract of the anterior lobe of the pituitary, each cubic centimeter of which represents 1.2 gm of fresh gland. Another group of six males and four females was given a similar daily dosage, over the same period of time of "Antuitrin-S" (Parke Davis and Company), an extract of pregnancy urine assaying 100 R.U. per cubic centimeter. Controls, five males and three females, were injected daily with 0.5 cc. physiological salt solution.

Body weights and comb measurements were taken at the beginning of the injection period. On the 13th day following the first injection, the birds were killed. Their body weights and comb measurements were again recorded; their gonads removed, weighed and sectioned.

#### Results of Injection

The testis weights of birds injected with Antuitrin ranged from 38.1 mgm. to 115.9 mgm. with the average at 55.6. The ovarian weight of the females in this group varied between 41.3 mgm. and 158.1 mgm. with the average at 102.2. Testis weights of the birds injected with Antuitrin "S"

ranged from 20.4 mgm. to 160.64 mgm., the average falling at 60.056. Ovarian weights were from 73.5 to 171.7 mgm. with the average at 109.1. In the control group, testis weights fell between 32.1 and 109.0 mgm., averaging 34.575, while ovarian weights fluctuated between 34.7 and 118.2 mgm., averaging 79.9.

It may thus be shown that the average testis weights were higher in the Antuitrin and Antuitrin "S" groups, 55.6 mgm. and 60.056 mgm. respectively, than in the saline injected controls, at 34.565 mgm. Likewise, in the females the extract-injected birds showed higher average ovarian weights, 102.2 mgm. for the Antuitrin and 109.1 mgm. for the Antuitrin "S" groups, than the controls at 79.9 mgm. Of the 42 gonad weights determined, six weights were discarded as obvious errors in technique.

These average gonad weights were not correlated with average gains in body weights. In the males of the Antuitrin group there was an average gain of 97.2 gm. over the 13-day period; in the females, an average of 105.16 gm. In the Antuitrin "S" group there was an average increase in body weight of 104.63 gm. for the males, and 63.75 gm. for the females. The control group males gained an average of 95.86 gm.; the females, 82.1 gm.

Follicles in representative sections of ovaries were counted and the least diameter of each was measured. In the Antuitrin group from 36.8 to 39.1 per cent of the follicles had diameters of 100  $\mu$  or over. In the Antuitrin "S" group from 15.6 to 20.0 per cent of the follicles had diameters of 100  $\mu$  or over. From 8.9 to 15.0 per cent of the follicles in the control group were of this diameter. These percentage differences were even more pronounced in follicles of 200  $\mu$  in diameter or over. In the Antuitrin group there were from 17.0 to 20.2 per cent of them 200  $\mu$  or over; in the Antuitrin "S" group, from 5.0 to 7.9 per cent; and in the controls, from 0.0 to 1.4 per cent.

A similarity between the relationship of these figures and the gonad weights may be noted.

To determine if there was a like correlation in the testes of the male birds, the lumina of 100 tubules in representative sections of testes were measured. In the Antuitrin group, the average lumen diameter was 8.36  $\mu$ ; in the Antuitrin "S" group, 7.88  $\mu$ ; and in the controls, 3.75  $\mu$ .

It is concluded from these observations that the anterior pituitary-like hormone, when injected into immature male and female chicks, has a gonadotropic action similar to that of the anterior pituitary gonad-stimulating hormone.



These results are not in agreement with those of other workers on this question. The difference may be due to the potency or the amount of the extracts administered. Several investigators of this problem have not stated the potency of the extracts used, but it is believed that the amounts and potency described in this thesis are relatively high. In view of this fact, the failure of the extract to elicit response in the preliminary experiment may be due to the low potency.

#### HYPOPHYSECTOMY IN THE FOWL

As an approach to a future study of differences in anterior pituitary extracts and anterior pituitary-like material, a technique for hypophysectomy in the fowl was developed. While the technique of hypophysectomy is now performed readily on many mammals and amphibians (Camus and Roussay, 1920; Aschner, 1912; Smith, 1930; Houssay, Giustiani and Tascano-Gonzales, 1923; White, 1933; Crowe, Cushing and Homans, 1910), it has been comparatively recent that such an operation has been successfully accomplished on birds. Fischera (1905) attempted cauterization of the pituitary body in immature fowls and reported success on but four birds.

In 1927 Ogata and Nishimura reported successful re-

removal of the gland in pigeons. Mitchell in 1929 extirpated the anterior lobe in 162 immature fowls. Later, Martins (1933) reported partial success in hypophysectomy in pigeons and chickens utilizing a technique similar to that of Ogata and Nishimura (1917). Hill and Parkes (1934) accomplished removal, by suction, of the gland from White Leghorn chickens. The transbuccal route of entry used by Hill and Parkes combined with the actual destruction of the gland by cauterization, attempted by Fichera, will be discussed.

The bird is given an intraperitoneal injection of 1/75 grain of atropine sulfate in order to control the mucous secretion in the mouth. Anesthesia was produced by an intravenous injection of Nembutal (0.2 cc. per pound of body weight), supplemented with ether. The head of the bird is securely fastened toward the operator and braced vertically in position by a suitably shaped block on each side. It is held down by an elastic band over the beak, a slit having been cut between the blocks to allow for the comb (Fig. 1). After plucking the area between the wattles and the ventral surface of the neck, a U-shaped tracheal cannula is inserted about two inches cephalad from the sternum. A piece of rubber tubing is then attached to the free end of the cannula and extended back over the breast to the anesthetist. While

the normal air passage is not completely closed, the use of a tracheal cannula is a highly desirable method of administering the anesthetic.

Starting about 0.5 cm. behind the anterior margin of the wattles and midway between them, an incision 2.5 cm. long is made through the skin and platysma, the edges of the incision being retracted by hooks attached to elastic. The anterior part of the hyoid apparatus and the muscles covering the floor of the mouth are plainly visible (Fig. 2). A strip of the floor of the mouth free from muscles is brought into view by forcing the hyoid apparatus to the left. An anterior-posterior incision 2 cm. long is then made in this area which is between the myohyoid and the stylohyoid muscles. The hooks, mentioned before, are then used to retract the tongue and hyoid apparatus to the left, and to retract both the right hand margin and the posterior end of the incision. The roof of the mouth is thus brought into view. It is deeply cleft from the rostrum posteriorly to the basitemporal bone, with the exception of a bridge of soft tissue connecting the two sides at the point above the articulation of the pterygoids and palatines with the rostrum (Fig. 3).

This bridge may be severed with small scissors and the cleft posteriorly deepened by blunt dissection. The two

sides of this deepened cleft are separated by a spring retractor, and the exposed area cauterized to free it of connective tissue and periosteum (Fig. 4). A rounded hole about five millimeters in diameter is then drilled at the angle of about 45 degrees with the level of the head, so that just a portion of the basi-temporal bone is included. For this, a  $3/32$  inch dental burr inserted in a small, motor-driven, hand drill is used (Fig. 5). Care must be exercised not only in ascertaining that the hole is in the mid-line, but also that the pituitary capsule is not drilled into directly. When the pressure of the drill causes the base of the hole to "give" slightly, drilling should cease and the remaining thin layer of bone be picked out with a small recurving probe. The vascular pituitary will then lie exposed (Fig. 6), the posterior lobe being seen first due to the angle of approach. The hole may be cautiously deepened anteriorly, exposing the remainder of the pituitary which, although comparatively large in area, two millimeters by four millimeters, is quite thin. Lateral enlarging of the hole may lead to serious hemorrhage as the gland is surrounded by blood vessels.

By the use of a negative pressure cannula, excess blood may be removed from the hole, and the preheated cautery tip inserted into the bulging capsule (Fig. 7). In-

section of the cautery tip to a depth greater than two millimeters may lead to brain injury. Complete destruction of the gland must be accomplished in as short a time as possible, due to the rapid accumulation of blood which renders the cautery ineffective. Fatal hemorrhage may be prevented by plugging the drill hole with bone wax immediately after completing the cauterization. Upon removal of the spring retractor, the two halves of the palate slip back over the drill hole sufficiently to heal without suturing (Fig. 8). The floor of the mouth is then sutured (Fig. 9), the cannula removed, and the opening to the trachea sutured. Two cubic centimeters of 50 per cent glucose solution is administered intramuscularly after removal of the bird from the operating table, in order to reduce shock and temporarily to relieve the upset in sugar metabolism which may result from hypophysectomy. Six of the seven birds operated upon were on their feet and apparently normal by two hours following the operation. Six birds lived from 24 hours to 15 days. Death, as observed in five of the seven birds, was preceded by a period of droopiness of about one hour, followed by vigorous tetany lasting for one minute or more. One of the other two birds died unobserved. A seventh bird is still alive at the 19th day. Postmortem examination revealed complete hypophysectomy without visible brain injury in but

one bird, hypophysectomy and brain injury in three birds, and partial hypophysectomy in two birds.

If carefully controlled, this procedure is considered to be a most satisfactory method of hypophysectomy.

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#### SUMMARY

1. An extract of anterior pituitary-like hormone assaying  $16 \frac{2}{3}$  rat units per cc. was prepared from fresh pregnancy urine. It had no stimulating effect on the gonads of 21 and two-day-old chicks when injected in daily, 1 cc. doses for 11 days.

2. The commercial extracts of the pituitary gland, Antuitrin, and of pregnancy urine, Antuitrin "S", had an activating effect on the gonad weights of immature male and female chicks. The average testis weight in the Antuitrin group was 55.6 mgm.; in the Antuitrin "S" group, 60.056 mgm.; and for the controls, 34.375 mgm. The average ovary

weight in the Antuitrin group was 102.2 gm.; in the Antuitrin "S" group, 109.1 gm.; and for the controls, 79.9 gm. High gonad weights were not always found to be indicative of correspondingly high gains in body weight.

3. Follicles in representative sections of ovaries were counted and measured. In the Antuitrin group from 36.8 to 39.1 per cent of the follicles had diameters of 100  $\mu$  or over. In the Antuitrin "S" group from 15.6 to 20.0 per cent of the follicles had diameters of 100  $\mu$  or over. In the control group only 8.9 to 15.0 per cent of the follicles had this diameter. Follicles of 200  $\mu$  in diameter or larger were counted. In the Antuitrin group there were from 17.0 to 20.2 per cent of them this size; the Antuitrin "S" group, from 5.0 to 7.9 per cent; and the control group, from 0.0 to 1.4 per cent.

4. The lumina of 100 tubules in representative sections of testes were measured. In the Antuitrin group the average lumen diameter was 8.36  $\mu$ ; in the Antuitrin "S" group, 7.88  $\mu$ ; and in the controls, 3.75  $\mu$ .

It is concluded from these observations that the pregnancy urine factor, Antuitrin "S", apparently has a gonadotropic action on birds similar to that of the anterior lobe product, Antuitrin. Although little work has been done on

this problem, such a conclusion is not in agreement with the results of other workers. A possible explanation is advanced.

5. Hypophysectomy in seven birds, using a transbuccal entry and destruction of the gland by cautery, is discussed with details of the technique.

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## PLATES

**EXPLANATION OF PLATE I**



Fig. 1. Fowl's head held in place in a suitably shaped block by an elastic band. The area between the wattles has been plucked.

Fig. 2. Incision made through skin and platysma with the margins retracted by hooks and elastic. Pterygoid apparatus and the muscles of the floor of the mouth exposed. The tracheal cannula is in place.

## PLATE I



Figure 1



Figure 2

## EXPLANATION OF PLATE II

Fig. 3. Roof of the mouth exposed by retractors at the edges of the incision. Hyoid apparatus is retracted to the left. The palate cleft is in view.

Fig. 4. The posterior portion of the deepened cleft is exposed for drilling. A spring retractor is used to spread the opening.

## PLATE II



Figure 3



Figure 4

**EXPLANATION OF PLATE III**

Fig. 5. Burr in position for drilling.

Fig. 6. Drill hole shown with pituitary  
exposed for cauterization.

## PLATE III



Figure 5



Figure 6



## EXPLANATION OF PLATE IV

Fig. 7. Cautery tip in place for destruction of the gland.

Fig. 8. The retractor has been removed allowing the palate to close over the drill hole.

## PLATE IV



Figure 7



Figure 8

## EXPLANATION OF PLATE V

FIG. 9. Retractors withdrawn, and the incision through the floor of the mouth sutured.

Fig. 10. A skull in place to show position and size of the drill hole.

## PLATE V



Figure 9



Figure 10

