

PIGMENT IMPLANTS IN RATS

by

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INTRODUCTION

Proceeding the present age of experimentation the pineal body received considerable attention from the philosophers. Thus Descartes, in the seventeenth century, speculating on its possible function elevated it to the high rank of being the seat of the soul. With the advent of experimentation and particularly with our increased knowledge of several endocrine glands many investigators have tried to determine whether the pineal might have an endocrine function.

The past thirty years have seen the problem approached from almost every angle. In general, two main lines of research have been followed, that of glandular deficiency produced experimentally by pineal extirpation and that of glandular excess produced experimentally by pineal feeding, the injection of pineal extracts, and the transplantation of the pineal. Other methods of study have included clinical or pathological observations, chemical analyses, and morphological studies of the pineal. In spite of this vast study it does not seem certain that a definite function of the pineal body has yet been demonstrated.

PURPOSE

The recent success of this laboratory with pituitary implants raises the question of the effect of implants of the pineal. With this in mind the present study was begun.

REVIEW OF LITERATURE

The pineal body has been the subject of much experimentation. Investigators have attempted in many ways to find some possible function of the pineal. This work has, no doubt, been stimulated by the success of similar studies with such glands as the thyroid, adrenal and pituitary. A further incentive is supplied by clinical and pathological reports. Cases of pineal tumor in connection with precocious sexual development have led many investigators to study the pineal in regard to somatic and sexual development.

Gordon (1919) reported 70 clinical cases which have been accepted as pineal neoplasms. Bailey and Jelliffe (1911) summarized a study of 60 such cases, 17 of which were among children under 14 years of age. Of these children thirteen were boys and four were girls, disproving the theory that the condition was limited to the male sex.

The precocious sexual development was usually accompanied by advanced development of the secondary sex characters, mental development and maturity, and general body growth. Jelliffe (1922) stated that the precocious sexual syndrome is probably conditioned by the pineal body.

The effects of pineal extirpation have been studied by a number of workers. At first it presented a difficult problem of procedure in order to avoid killing the animal. Difficulty has also been encountered in securing all of the gland. This could only be determined definitely by autopsy of the animal.

Dandy (1915) performed pinealectomy on puppies from ten days to three weeks of age. Following the removal he found "no sexual precocity or indolence, no adiposity or emaciation, no somatic or mental precocity or retardation." The pineal apparently was not essential to the life of the animals nor did it seem to have any influence on their well-being.

On the contrary, Horrax (1916) in pinealectomized male guinea pigs and rats found a hastened sexual development, i.e., an increase in weight and size of the testes and seminal vesicles, as compared with the control litter mates. The histological study of the testes indicated a more advanced physiological state. Body weights were not

affected. Few differences were noted with animals which had passed maturity. There was little, if any, influence on females. Three of the guinea pigs became pregnant but later aborted.

Working with *Rana sylvatica*, Hoskins and Hoskins (1919) removed the pineals from 70 young larvae with the result that they were regenerated partially or completely and the animals developed normally.

Izawa (1923a) performed pinealectomy on 36 chickens from four to five weeks of age. Of these only three males and one female reached maturity. He reported that pinealectomized animals were precocious in growth and sexual maturity. The males crowed prematurely and when autopsied had much larger testes. The young female, likewise, showed a premature development of ovary and oviduct. From this he concluded that the pineal serves to repress the premature development of the sexual organs in both sexes.

Izawa (1923b) reported a series of studies on the growth of pinealectomized rats. The pineal was completely removed from 12 males and 10 females at 20 days of age. Eleven males and 12 females were used for controls. They were autopsied at 85 days of age. He found the operated animals had longer bodies, slightly longer tails, greater body weight and heavier brains and spinal cords than their

controls. The hypophysis, adrenals, thymus, thyroid and pancreas also were heavier in the operated animals.

Later Izava (1926) removed the pineal bodies from immature (20 day old) rats. His findings resembled those of Horrax (1916) in regard to the sexual development of the males. However, he found that the females were also influenced. Compared with the normal control, the eyes decreased 5 per cent; gonads increased about 25 per cent; and the pituitary of the females decreased about 15 per cent. This was accompanied by an acceleration in growth. It might be added that these animals were killed at 60 days so they had probably just reached maturity.

Badertscher (1924) performed pinealectomy on newly hatched chicks. His results were negative. The experiments did not disturb the chickens, which developed to maturity in a normal manner.

Hofmann (1925) secured complete removal of the pineal in five out of 27 operated animals. The loss of the pineal seemed in no way to affect their condition of health, body growth, formation of fat, or metabolism. There was no indication of an influence on sex maturity other than the fact that the seminal vesicles were abnormally large. This fact alone would be important if larger numbers had been considered.

The effects of feeding pineal substance has been studied by McCord, Hoskins, Goddard, Sisson and Finney, and Addair and Chidester.

McCord (1914-15) reported experiments where the pineal or a pineal extract was fed to 393 animals. These were mainly guinea pigs but puppies, adult dogs, and chicks were included. Young bovine pineals or their extracts were used. Some of the conditions resulting from the feeding were like those which had been considered as accompanying pineal insufficiency as evidenced by pineal tumor. In all cases the experimental animals were stimulated to more rapid body growth but did not become larger than the normal animal. McCord also considered there was precocious mental and sexual development.

McCord and Allen (1917) demonstrated the presence of an active substance in the pineal which affected the pigmentation of pineal fed tadpoles and caused them to become very pale. This substance was extracted by acetone. This extract was even more effective than the pineal feeding.

Hoskins (1916) however, in feeding the pineal bodies to rats got very different results from those of McCord. He found the pineal fed group agreed very closely with the controls both in weights of the body and of the various organs. Instead of the increased size of the sex organs

he reported a trend toward decreased size of sex organs. This was consistent but slight in the case of the ovaries. With the testes it was not so consistent. He considered the numbers so small and the variations so slight as to make their significance doubtful. In conclusion he states that possibly pineal feeding retards the growth of the ovaries and testes.

Goddard (1917) reported the feeding of pineal extract to mentally deficient children. Only in one case, that of an eight year old girl, did he find any improvement and he concluded that feeding of pineal extract was not effective in these cases.

A powder prepared from the desiccated pineals of young calves was used by Sisson and Finney (1920). This powder was fed to 14 rats. Ten control animals were kept. The animals were autopsied and the reproductive organs studied. No histological differences were noted. The authors concluded that the feeding of desiccated pineal failed to produce any effect on the early development of the rats. It is noted, however, that the controls generally had slightly heavier testes than the corresponding experimental animals.

Addair and Chidester (1928) found that feeding pineals to tadpoles hastened metamorphosis. Contrary to

McCord's (1917) results the animals lost weight rapidly while the controls remained constant. Development responded directly to breaks in the pineal feeding.

Dohrn and Holweg (1929) report that the findings of their laboratories substantiate the results obtained by McCord.

Dixon and Halliburton (1909) experimented with pineal extract. Sheep pineals were dried in vacuo and extracts were made with (1) cold Ringer's Solution, (2) boiled Ringer's Solution, (3) 1 per cent hydrochloric acid, and (4) filtered, evaporated residue was dissolved in Ringer's and again filtered. These were injected into the external jugular veins of rabbits. In general, their results were negative.

Jordan and Eyster (1911) made intravenous injections of pineal extracts. This resulted in a fall of blood pressure and vasodilation of the intestines. It also had a slight effect on the urine. The effects, however, were so slight that they concluded if the pineal contained any hormone it must be small in amount and unimportant.

More recently Weinberg and Doyle (1931) made subcutaneous injections of extracts of mature bovine pineals into white mice. The injections of extracts were begun at 21 days of age and continued for 24 days. No evidence

of retardation or acceleration of sexual maturity could be found.

Weinberg and Fletcher (1931) duplicated the work with the exception that they used an extract prepared from the fresh pineal glands of calves. The mice again were apparently not affected by the injections. The weights of the testes of the injected mice were found to be neither increased nor decreased over the weights of the testes of the control animals.

Fenger (1916) made a study of the chemical composition of the glands of certain mammals. He collected and analyzed 2344 pineals from cattle, 1348 from sheep and 5062 from lambs. He noted the long glands in cattle and the plump glands in sheep but failed to find anything on the analysis that would point to a medicinal value. The glands of older animals seemed to contain more phosphorus and less nitrogen than those of younger animals.

Efforts to transplant the pineal have not proven successful. Hoskins and Hoskins (1919) transplanted the anlage of the pineals from the larvae of *Rana sylvatica* into 19 other young larvae. The transplants failed to grow.

The nature of the pineal body itself has been questioned. Is the pineal of a glandular nature or is it mere-

ly a rudimentary remnant? This question involves morphological studies. According to Tilney (1917) and Krabbe (1923) the pineal is decidedly glandular. If it were merely the remains of an organ which in an earlier phylogenetic stage had a function then we would expect to find it most highly developed in the embryonic stages of the higher animals. This they considered is not the case.

Tilney and Warren (1919) in their monograph on the pineal stated conclusively that it is a gland. Also that "as a gland, it may in some cases contribute its secretion to the cerebrospinal fluid, but in the higher vertebrates, as in birds and mammals, it is an endocrine organ contributing the products of its secretion to the blood stream".

They further state that in no way can the pineal of mammals be considered a residuum of the parietal eye of lower forms. Both represent specialization along different lines. They also originate from different areas of the epiphyseal complex.

Jordan (1921) made a cytological study of the pineal of sheep of from four to eight months of age. He reported an abundance of small spherical mitochondria and a variable number of larger lipid globules. These globules he considered as the only cytological evidence of secretory activity. In this connection however, Krabbe (1923)

pointed out that the pineals of cattle and sheep are composed of relatively larger amounts of connective tissue and less glandular tissue than most other mammals.

Herring (1927) studied the morphology and histology of the pineal region of the mammal brain. Pineals from man, child, monkey, cat, sheep, and rat were examined. We reported the bulk of the body and its functional elements had a nervous origin. The rat pineal is comparatively isolated and consists largely of compact parenchymatous cells. These cells he considered as functional secretory cells.

METHODS

Immature albino rats were used for this experiment. In most cases they were from inbred Wistar litters. Those receiving the pineal implants are hereafter referred to as "experimentals." Each experimental had a litter mate of the same sex and as near the same weight as possible for a control. It received implants of equal amounts of brain tissue. The initial ages and the lengths of the implantation periods were intentionally varied in the different groups. Initial ages ranged from 18 to 36 days. Implantation periods varied from 18 to 41 days. The experimentals of one group received implants of two pineals

daily. The experimentals of the other groups received one pineal daily. The pineals were taken from mature rats. These donor animals were killed, the skull opened, the pineal and a small amount of brain tissue removed and placed in separate vials containing warm Locke's solution. The gland or brain tissue was then picked up in a cannula with a small amount of the Locke's solution and subcutaneously injected into the proper animal. The work was done as rapidly as possible and every precaution was taken to prevent infection. In using the implants it was not expected that the gland would become ingrafted. Merely the absorption of the daily implant was desired.

The control and experimental animals of the smaller groups were kept in the same cage. In the larger groups they were separated and kept in adjoining cages. Care was taken to have the food, water, light, and temperature conditions alike in both cages.

A balanced ration containing cod-liver oil was before the animals at all times. They were kept in a steam heated building where a fairly uniform temperature was maintained.

Each day, before injecting the implant, each animal was weighed and the weight recorded for later growth studies. At this time they were carefully observed for evi-

dences of sexual maturity. The criterion used in this case was the descent of the testes into the scrotum, i.e., scrotality, of the males and the opening of the vagina of the females.

All animals were autopsied at the end of the implantation period. Measurements were taken of the length of the body, the tail, and the right hind leg. The pineal, pituitary, thymus, and adrenal glands were attached to labels and placed in Bouin's Fixative.

At autopsy of the male the prostate, Cowpers glands, and testes were fixed. The epididymis was dissected from each testis and the latter carefully weighed. Corresponding cross-sections of the right testis and long sections of the left testis were fixed.

At autopsy of the female sections of the uterus and of the vagina were saved. The ovaries were dissected from as much of the surrounding tissue and oviduct as possible and separately weighed. They were then placed in the fixative.

Serial sections at 20 microns were made of the ovaries. All other tissues were sectioned at 10 microns. Haematin and eosin bluish were used as stains.

RESULTS

Effect on Body Weight

The effect of pineal implants on body growth was one of the phases of the experiment to be considered. The daily record of body weights was used to make the growth curves shown in Figures 1 and 2. Figure 1 illustrates the growth in weight for the animals of groups I, II, and III throughout their entire implantation periods. The solid lines represent the controls and the broken lines the experimentals. The males of each group are shown on the left chart and the females of the corresponding group on the right. Figure 2 gives the same data for groups IV, V, and VI. It can readily be seen that the increases in weight of the control and experimental animals of each group are quite parallel. Therefore it is evident that the pineal implants had no effect on body weights.

Effect on Growth

Weight may be governed by the amount of fat deposited. Therefore, by itself, weight would not be a good measure of body growth if the animals showed a tendency to become fat. The autopsy records give also the length of the

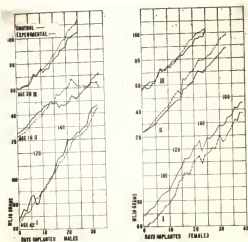


Figure 1. Growth curves of males and females of groups I, II and XII.

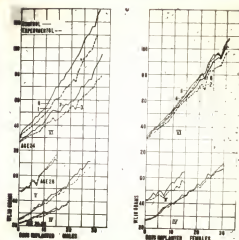


Figure 2. Growth curves of males and females of groups IV, V and VI.

right hind leg, the length of the body from anus to nose and the length of the tail. These figures together with the weight of the thymus are tabulated in Tables I and II. No significant influence on the growth of bone as indicated by length is found. Generally, the heavier animals have longer bodies, tails and legs, although where the animals are nearly equal in body weight there are variations.

A study of weights of the thymus shows the experimental female having a heavier thymus than the control in seven out of eight comparisons. This was not true for the males, however, for there they were almost equally divided. The thymus was heavier in the control in five of nine comparisons.

Development of the Testes

Table III gives the weights of both testes for the control and experimental animal as they were recorded at the time of autopsy. The last column shows the difference in total weights (C-E). It will be noted that the experimental animal had smaller or lighter testes than its control in seven out of nine comparisons. The differences in weight range from 64 milligrams to 319 milligrams in favor of the controls.

Table I. Comparison of Experimental and Control
Males as to Body Growth

Group : Animal:	Length of body: tail to anus	Length : of tail:	Length of rt.: hind leg	Weight of thymus. mgm.
II-1Ea	141	143	86	120
II-3C	156	161	83	250
III-1E	166	170	92	250
III-3C	156	168	90	245
IV-5E	119	108	70	87
IV-6C	115	116	70	82
IV-1E	136	144	78	170
IV-7C	134	141	80	270
V-1E	135	147	78	200
V-5C	145	143	77	180
VI-1E	152	170	85	375
VI-5C	148	173	90	350
VI-3E	142	143	81	230
VI-7C	144	146	77	282
VII-3E	146	144	77	270
VII-7C	151	154	82	315
VII-1E	158	153	80	293
VII-5C	149	152	78	311

E, Experimental (pineal implanted); C, Control (brain implanted). Lengths are given in millimeters

Attention should be called to the two instances where the experimental male had the heavier testes (Table III, Groups III and V). The control animal V-3C was apparently abnormal. The weight of its testes was 91 milligrams which is much lower than that of any other normal animal

Table II. Comparison of Experimental and Control
Females as to Body Growth

Group :	Length of body:	Length :	Length of rt.:	Weight of
animal:	tail to anus	: of tail:	hind leg	: thymus, mgm
I-2E*	178	171	96	490
I-4C	191	173	98	485
II-2E	156	157	85	310
II-4C	155	156	88	300
III-2E	162	160	87	
III-4C	162	156	87	200
IV-2E	133	141	79	277
IV-10C	136	bob	78	200
V-2E	136	131	73	205
V-4C	133	141	71	107
VI-2E	147	166	84	400
VI-6C	149	169	85	320
VI-4E	153	155	79	335
VI-8C	150	154	77	292
VII-2E	148	153	79	314
VII-6C	152	148	77	327
VII-4E	157	152	78	307
VII-8C	154	146	78	323

*E, Experimental (pineal implanted); C, Control (brain implanted). Lengths are given in millimeters.

studied. Animal IV-6C was three days younger yet its testes weighed 311 milligrams. In the case of the animals of group III, the males were 62 days of age and had reached full maturity. It is possible that this added age had given the experimentals time to compensate for an earlier

Table III. Comparison of Experimental and Control Males as to Weights of Body and Testes

Animal Group Number	Initial: age : days	Final: age : days	Initial: body weight	Final: body weight	Total testes weight	Difference : C - E*
II-1E : 16	53	37	23.0	60.0	1195	319
II-3C : 16	53	37	26.1	71.3	1514	
III-1E : 36	62	26	59.1	113.3	1765	210
III-3C : 36	62	26	61.7	103.4	1545	
IV-1E : 24	53	29	25.3	67.3	849	178
IV-7C : 24	53	29	24.9	71.6	1027	
IV-5E : 21	41	20	21.6	40.8	140	171
IV-6C : 21	41	20	21.6	41	311	
V-1E : 28	44	16	47.5	76.7	470	391
V-3C : 28	44	16	47.8	71.9	91	
VI-1E : 24	55	31	29.1	110.5	1072	275
VI-5C : 24	55	31	30.4	129.4	1347	
VI-3E : 24	57	33	26.4	78.7	659	146
VI-7C : 24	57	33	26.9	95.2	805	
VII-1E : 24	55	31	26.2	104.0	923	64
VII-5C : 24	55	31	26.2	96.8	987	
VII-3E : 20	53	33	20.6	83.6	931	209
VII-7C : 20	53	33	20.4	98.2	1140	

* E, Experimental (pinealectomized); C, control (brain implanted).

Body weights are given in grams.

Table IV. Comparison of Experimental and Control
Females as to Weights of Body and Ovary

Animal:	Initial:	Final:	Days	Initial:	Final:	Total ovary weight, mgm.
Group:	age	age	Im-	body	body	Difference
Number:	days	days	planted:	weight:	weight:	Control: Experimental: C - E*
I-2E ^b	40	81	41	60.2	146.2	118
I-4C	40	81	41	68.6	155.5	137 [†]
II-2E	16	50	34	24.0	105.8	54
II-4C	16	50	34	23.7	94.2	58
III-2E	36	61	25	56.7	102.8	66
III-4C	36	61	25	57.3	102.1	78
IV-2E	24	55	31	26.5	71.9	58
IV-10C	24	55	31	26.0	67.9	70
V-2E	28	44	16	41.2	56.8	18
V-4C	28	44	16	42.3	64.0	18
VI-2E	24	56	32	31.3	108.7	41
VI-6C	24	56	32	30.9	108.8	78
VI-4E	24	56	32	30.5	98.4	50
VI-8C	24	56	32	32.5	101.8	73
VII-2E	24	58	34	21.8	103.3	59
VII-6C	24	58	34	22.3	92.4	71
VII-4E	22	61	39	25.4	107.5	24
VII-8C	22	61	39	24.6	97.0	27

*E, experimental (pineaal implanted); C, control (brain implanted).

[†]Measurements instead of weights. The figure gives the approximate computed volume in cubic millimeters. Body weights are given in grams.

retardation and the difference at autopsy was merely that of normal variation.

In the other seven comparisons the differences in weight of the testes range from 64 milligrams to 319 milligrams. Omitting group V which contained an abnormal control, it is found that there is an average difference in weight of 144 milligrams. The testes of the controls averaged 1064 milligrams. While those of the experimentals averaged 940 milligrams. This means that the testes of the experimentals were 13.2 per cent lighter than those of the controls.

Development of Ovaries

If the implants had a retarding influence on the testes of the male a corresponding retardation of the sex organs of the female might be expected. Table IV gives a comparison of the combined weights of the two ovaries of each experimental and of each control. In this case measurements rather than weights were taken on the first three groups. The figures are in cubic millimeters. In each case the control animal had slightly larger ovaries. The remaining groups are compared as to weight in milligrams. Here again the control animal had the heavier gonads in most cases. In one instance they were of equal

weight. In eight other comparisons the control had larger or heavier ovaries than the experimental. It seems then, that the same retarding influence that had checked the growth of the testes in the experimental males had also affected the development of the ovaries of the experimental females.

Sexual Maturity

It might be expected that factors retarding the development of the gonads would likewise have a tendency to retard sexual maturity. The age and weight at which the animals reached sexual maturity was carefully noted in the later groups. In the first five groups only the condition at the time of autopsy was recorded although the length of the implantation periods was governed by the condition of maturity. The criterion used in determining sexual maturity was the enlargement of the testes and their descent into the developing scrotum in the males and the opening of the vaginae in the females.

Table V summarizes the findings concerning the maturity of animals of groups VI and VII. It is seen that the testes descended into the scrotum from three to five days later in the implanted male than in the control. A similar condition is observed in the maturity of the females. The vaginae of the experimental females opened five or six days

Table V. A Study of the Age of
Sexual Maturity

Males: Group : Age at time of descent of: Weight : number: testes i.e., acrotality : in grams.		
VI-1E	39	50.5
VI-5C	34	51.3
VI-3E	43	50.7
VI-7C	40	51.5
VII-1E	33	46.1
VII-5C	35	36.3
VII-3E	34	34.5
VII-7C	31	34.8
Females: Group : Age at which : Weight : number: vagina opened: in grams.		
VI-2E	34	92.0
VI-3C	46	81.3
VI-4E	33	93.0
VI-9C	49	82.6
VII-2E	36	98.4
VII-3C	51	76.9
VII-4Ea	61	107.5
VII-8C	61	87.0

*Vagina closed at time of autopsy.

later than those of the controls.

Observations of the males of the first five groups were not recorded in enough detail to make a comprehensive study of their maturity possible. The conditions of the females of these groups at the time of autopsy affords

some interesting data. These are shown in the following table.

Table VI. Notes on the Maturity Condition of the Females of Groups I to V at the Time of Autopsy

Group Number	Age in days at autopsy	Sexual condition
I-2E	81	vagina open
I-4C	81	vagina open
II-2E	52	vagina closed
II-4C	52	vagina open
III-2E	62	vagina open
III-4C	62	vagina open
IV-2E	55	vagina closed
IV-10C	55	vagina open
V-2E	34	vagina closed
V-4C	34	vagina closed

The females of groups II and IV were killed a few days after the control animal had reached maturity. In both cases the vagina of the experimental was yet closed indicating two more instances of delayed maturity following the implantations.

As a further check on the sexual condition of the males at the time of autopsy the method used by Anderson (1931b) was applied to the males of group VII. This method involves a study of smears taken from the head and tail of the epididymis at the time they are killed. At 53 days of

age VII-3E and VII-7C were compared. Smears from the former showed spermatids in the tail and immature spermatozoa in the head of the epididymis. The control, VII-7C, had immature spermatozoa in both the head and tail of the epididymis. At 55 days of age VII-3E and VII-5C were studied. A viscous fluid extruded from both the head and tail of the epididymis of the control. Numerous motile spermatozoa were found in both head and tail. Little if any fluid extruded from the epididymis of the experimental. A smear from the head had a few motile sperm.

The opening of the vagina is generally accepted as a very accurate indication of puberty of the female. In these observations it was found to be closely correlated with ovulation as determined by the development of corpora lutea. Sections of ovaries from mature experimental and control animals showed numerous ova and follicles in various stages of maturity. In all such cases corpora lutea were present, even though the animal was autopsied within two days after the vagina opened.

The two instances shown in Table VI where the vagina of the control was open while that of the experimental was closed at the time of autopsy gave further proof of relationship. The ovaries of the former contained corpora lutea while those of the latter did not. The age of

puberty of the female was observed to be from 48 to 51 days although one control was still immature at 61 days. This agrees with the findings of Anderson (1931a,b) who reported a variation of from 36 to 95 days depending on the season of the year and the breed of rats. The mean average in the spring of the year was 47.8 days. She also reported that maturity was earlier in the spring and summer and that Wistar albino rats were earlier than other breeds studied.

Histological and Cytological Study

A brief histological study was made of sections of the pineal, pituitary, thymus, adrenal, Cowpers, prostate, testis, ovary, and walls of the uterus and vagina of a number of experimental and control animals. Except for the testes these tissues showed nothing abnormal.

Under the microscope the tubules in the testes of the experimental animals were smaller than those of a corresponding region in the control. This condition was uniform. Microphotographs of the testes (Figs. 3 to 10) give ocular evidence of this variation.

A careful cytological study showed the retardation of the testes of the experimental animals, which has been so consistently found in other comparisons, was here reflected in retarded spermatogenesis. Comparisons of slides



Figure 3. Section of the testis of the experimental animal, II-1E.



Figure 4. Section of the testis of the control animal, II-3C, showing the normal size of the tubules at 53 days of age.

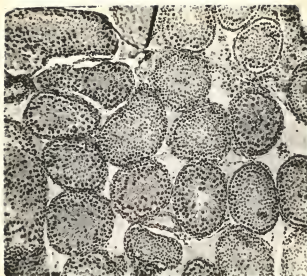


Figure 5. Section of the testis of the experimental animal, IV-1 R.

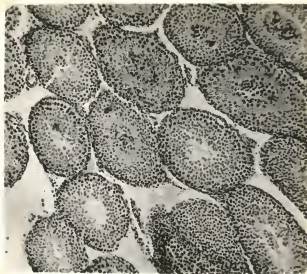


Figure 6. Section of the testis of the control animal, IV-7 C.

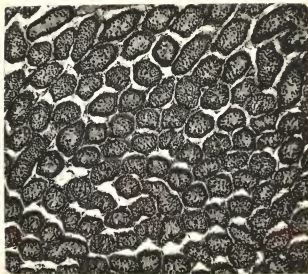


Figure 7. Section of the testis of the experimental animal, IV-5 P.

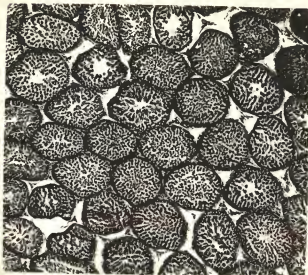


Figure 8. Section of the testis of the control animal, IV-6 C.

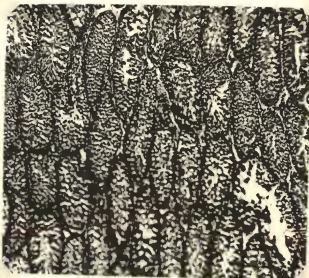


Figure 9. Section of the testis of the experimental animal, VI-3 E.

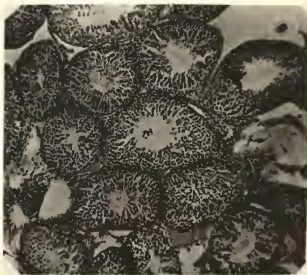


Figure 10. Section of the testis of the control animal, VI-7 C.

made from groups of different ages showed lumina of the tubules forming later in the experimentals. Furthermore their development of primary spermatocyte, spermatid and mature spermatozoa was somewhat slower.

DISCUSSION

A question might well be raised concerning the effectiveness of subcutaneous implantations. Johnson and Wade (1931) got striking results in the ground squirrel after six or eight daily implants of the pituitary. In using the implants it was not expected that the gland would become ingrafted. Merely the absorption of the daily implant was desired. Several pieces of skin where implants had been made were sectioned and studied. No evidence of the growth of the gland was found. In all cases they were gradually being absorbed.

Implantations of the pineal gland should be classed in the category of glandular excesses. The results obtained in this experiment are in accord with the slight indications reported by Hoskins (1916) and Sisson and Finney (1920) with pineal feeding, except that they are much more pronounced. Since these conditions result in inhibition of sex maturity it would seem logical that pinealectomy would result in a precocious sex maturity.

Horrax (1916) and Izawa (1933a, b, 1926) report such findings with guinea pigs, rats and chickens.

The stages of spermatogenesis reported in the control animals in this paper as well as those reported by Anderson (1931) do not agree with some of the observations of Allen (1918) concerning the maturity of the spermatozoa. He states that spermatids begin to differentiate at from seven to ten days after birth and that ripe spermatozoa appear at the time of the descent of the testes. The present observations include only a few animals. At 53 days of age only immature spermatozoa were found in the epididymis. In sections of the testes at 53 days no mature spermatozoa were found and at 43 days of age the development had not proceeded further than spermatids.

Donaldson (1915) states that "as a rule the descent of the testes occurs about the fortieth day of age or somewhat earlier." Allen (1918) gives the age of the descent of the testes as from 36 to 40 days. In this paper the descent of the testes, or scrotality, was found to occur at from 31 to 40 days of age. Where castration or autopsy is not desirable it is found to be a very satisfactory means of observing puberty in the male.

CONCLUSIONS

Observations from 40 animals indicate the following results of pineal implants in rats:

- I. That they do not influence body growth of either sex
- II. That they do have a tendency to retard sexual maturity, as indicated
 - A. In the case of the males by:
 1. Lighter weight of testes when autopsy is made under 60 days of age
 2. Greater age at which they become scrotal
 3. Smaller size of tubules in the testes, and
 4. Delayed spermatogenesis
 - B. In the case of the females by:
 1. Smaller or lighter ovaries
 2. Greater age at which the vagina opens.

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