FURTHER STUDIES ON PINEAL IMPLANTS IN RATS

by

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INTRODUCTION

The pineal body received its name from the Latin word "pinealis", because its form resembles that of a pine cone. The pineal body is attached by a short stalk to the posterior boundary of the dorsal surface of the third ventricle The cavity of the ventricle extends for a short distance into the stalk forming the recessus pinealis, hence it may be said to form part of the posterior wall of the ventricle. This adds to the difficulty of its extirpation as any blood

resulting from the operation may flow into the open ventricle, which is quite sure to be fatal to the patient. The pineal body in the human rests on the midbrain between the two superior colliculi and is covered by pia mater. In the rat it lies just beneath the skull at the junction of the two hemispheres of the cerebrum and the cerebellem and often is attached to the skull by its upper surface. In the human it is 6 mm. long and 4 mm. broad at its maximum. Cowdry (1922) says that theoretically it is larger in women than in men and relatively larger in children than in adults. Careful studies on rats have revealed no sex differences in size and my own observation in killing 2000 donors has sustained this view. Cowdry (1922) maintains since it is a dorsal evagination of the neural tube and is never associated with the alimentary tract but invariably develops from ectoderm, that it possesses sensory rather than internal secretory potencies. It is formed of ependyma only at first but the walls thicken and mesodermal elements become included also.

Considering the pineal histologically, it consists of scattered irregular shaped ependymal cells in a framework of neuroglia and connective tissue. These are arranged in large lobules containing cyst-like structures and are marked off by septa. The nerve supply comes from the optic thalamus. Blood vessels, which are large and sinusoidal,

invade with the connective tissues. These lipoid lobules (globules) and the high vascularity are the only cytological evidences of endocrine secretion.

Galen in the second century said of the pineal body "It is a glandular substance and was devised for the same purpose as other glands of the body". However, early physicians called it a valve between the third and fourth ventricles to control the flow of cerebro-spinal fluid between the brain and spinal cord. Descartes in the seventeenth century ascribed to it the rank of being the seat of the soul. The general opinion of scientists sustains Galen's view that it is a gland. But as late a worker as Fenger (1916), after making a careful study and chemical analysis of over 8000 pineals of sheep and cattle, said, "Since the action of the pineal gland on blood pressure, pulse rate, excised heart, on uterine or intestinal muscle are insignificant in therapeutic doses and since health is not influenced by its extirpation ... it is difficult to consider the pineal body as an internal secretory organ of medicinal value." Experimental work has been more or less contradictory; so, even for those who believe it is a gland, the question still remains, what is its function? Despite the great amount of study on this subject especially in the last three decades, it is not certain that a

definite function of the pineal body has yet been proved.

Purpose

The recent success of this laboratory with pituitary implants raised the question of the effect of implants of the pineal. This work was begun last year by Mr. E. L. Lahr under the direction of Dr. G. E. Johnson. Because of the small amount of animals used the work was inconclusive. My work is therefore a continuation on a larger scale of the work done last year.

REVIEW OF LITERATURE

The function of the pineal provokes much controversy. The experiments on it are the most contradictory of any work on the endocrine glands. The fact that pineal tumors are accompanied by cachexia, adiposity and often also sexual precocity is not doubted. Bailey and Jelliffe (1911) report one case of a ten year old boy with pineal tumor who had had an unusual increase in size and weight but whose genital organs remained small and the testes very small. Horrax (1916) observes that there is no evidence of hastened sexual development in girls with pineal tumors although in boys it is usually accompanied by an early growth of genital hair, rapid growth of genital organs, and

erections and emissions of the penis at a very early age. But the question remains unanswered whether the tumors causing precocious sexual development are syndromes or adenomas. The syndrome tumors destroy the glandular tissue while the adenoma tumors are a proliferation of glandular tissue. In the former the natural function of the pineal would be the inhibition of the development of sexual organs but if adenomas, the natural function would be acceleration of sexual development. Either would agree with the findings of some experimental work and contradict other. Hence Horrax maintains that it is the syndrome which characterizes precocious adolescence. Bailey and Jelliffe (1911) suggest that the cause of adiposity may be the diminished functioning of the pituitary because of the pressure from either pineal tumors or those of nearby regions and that precocious sexual development is possibly due to adenomata of the pineal. Horrax (1916) maintains that pineal tumors occur only in children under 15 years. He says there has been only one exception to this rule. Perhaps clinical cases of adults may have begun in childhood.

Two definite courses in research have been followed in an effort to solve the problem of the normal function of the pineal. One is glandular deficiency produced experimentally by pinealectomy and the other is glandular excess produced

by pineal feeding, injection of pineal extracts or implantations of the pineal. Castration and transplantation have also been tried in further efforts to ascertain its function.

It has been very difficult to get the original writings of the earlier workers on the pineal. I have gathered much of this material from the reviews of other authors, hence in such cases I am giving the name of the author and year of publication of his review in parenthesis after the name of the original author.

Sarteschi (Gordon, 1919) in 1911 pinealectomized guinea-pigs and puppies. Of the few that lived about half showed acceleration in growth and sexual development. The testes were found larger at autopsy. Foa (Krabbe, 1923) in 1912 had three cocks and twelve hens survive the extirpation. The hens showed no differences from the controls. The males showed hypertrophy of combs and testes over their controls. They crowed and showed sexual instinct 42 to 79 days earlier than their controls. He repeated the experiment two years later with two cocks and five hens surviving. The results confirmed those of his former experiment. Foa (Cowdry, 1922) in the same year, 1914, performed pinealectomy on very young rats. There was no appreciable effect in the females. The males were stimulated to a more rapid

somatic and testicular development for 26 to 30 days. Then the controls began to gain until the forty-eighth day following the operation when there was no difference either sexually or somatically. At the maximal point of difference, histological study showed a uniform advanced development in all the tissues of the male experimentals' gonads.

Sarteschi (Krabbe, 1923) in 1915 repeated his former experiment on several rabbits and puppies. The results confirmed his earlier work. All males showed hypertrophy of testes and no effect was noted in the females. Horrax (1916) extirpated the pineal in guinea-pigs and rats. There was no effect on the 20 extirpated female guinea-pigs unless there was a tendency to breed earlier. The males showed greater size and weight of testes and seminal vesicles. The few surviving rats gave the same results. He suggests that the changes may be due to secondary conditions acting on the pituitary but doubts this. The males showed histological differences but the females did not.

Contradictory results were obtained by other workers with extirpation. Christea (Krabbe, 1923) pinealectomized 12 cocks which resulted in a marked retardation in the development of the comb, spurs, voice and feathers and also a marked atrophy of the testicles as compared with the controls.

Adler (Krabbe, 1923) in 1914 destroyed the pineal by thermocauterization in 350 tadpoles. In the few surviving ones no abnormality of sexual development was noted. Most of them developed edema and died from its effects in a few days. Dandy (1915) performed pinealectomy on puppies 10 to 21 days old and on grown dogs. He concluded the pineal was not essential to life as they showed no sexual or somatic differences and no proof of the lack of endocrine hormones was evident. Boese and Exner (Gordon, 1919) in 1912 successfully extirpated the pineal in six rabbits by cautery. The results were negative as were also those of Biedl (1912) on dogs. Fenger (1916) found the extirpation of the pineal had no effect on the health or development of cattle and sheep. Badertscher (1924) got negative results on extirpation in newly hatched chickens.

However, Izawa (1923) found that chickens pinealectomized when one month old showed an increase in both male and female gonads over controls. This would be significant if larger numbers were considered but only one female and three males survived for any length of time. Izawa also pinealectomized rats 20 days of age in 1923 and 1926. There was greater somatic development and heavier thymi in the operated animals. The increased size of genital organs was about what would be expected considering the increased body

size. Hofman (1925) got negative results on five pinealectomized rats both in regard to somatic and sexual development except that the operated animals had larger seminal vesicles. The effect of castration on the pineal has also brought contradictory results according to the literature reported by Krabbe and others.

Injection of pineal extract prepared in various ways brought contradictory results also. Jordon and Eyster (1911), Ott and Scott (Krabbe, 1923) in 1910-1911, and Horrax (1916) found a lowered blood pressure with its intravenous injection while DeCyon (Krabbe, 1923) in 1903, Dixon and Halliburton (1909) and Cushing (Gordon, 1919) in 1912 received negative results only. The work of Howell (Krabbe, 1923) in 1898 and McCord (Cowdry, 1922) in 1915 gave fluctuating results throughout. Hoskins (1933) says the injection of extract of various tissues may produce lowered blood pressure hence if this experiment were one-hundred per cent successful it would not prove anything.
Dana and Berkeley (Gordon, 1919) found that intraperitoneal injections of young bovine pineal extract augmented the size and weight of young guinea-pigs, rabbits and kittens. Prior (Krabbe, 1923) in 1915 injecting rabbits found exactly opposite results. Weinberg and Doyle (1931) injected an extract of mature bovine pineals into white mice beginning

at 21 days of age. No evidence of retardation or acceleration of sexual maturity or somatic development was noticed. In the same year Weinberg and Fletcher (1931) repeated the experiment with an extract of calf pineals. This only confirmed the results of Weinberg and Doyle's experiment.

Other workers approached this problem from the angle of pineal feeding. McCord (1914, 1915) used 393 animals. Most of them were guinea-pigs but young and old dogs and chickens were also included. He found acceleration of growth in the experimentals. Only a moderate amount of sexual and mental precocity was observed. The experimental females gave birth to young about two weeks before their controls did. Calf pineals were more effective than mature bovine were. McCord (Cowdry, 1922) in 1917 found that paramecia fed pineals reproduced more than twice as fast as those in pure hay infusions or in those mixed with muscle tissue. McCord and Allen (1917) fed bovine pineals extracted by acetone to tadpoles which caused transparency but did not greatly influence growth. McCord (Addair and Chidester, 1928) in 1917 found that tadpoles fed for two weeks on pineal tissue were twice the size of the controls and show no signs of differentiation. Dohrn and Holmeg (1929) confirmed McCord's work.

Addair and Chidester (1928) fed pineals to tadpoles but got hastened metamorphosis and a greater loss of weight than in the controls. Hoskins (1916) found that pineal fed rats agreed closely with the controls in weight. The decrease in the weight of testes and ovaries he considered of doubtful significance because of the great variations. Sisson and Finney (1920) fed calf pineals to rats and concluded that the results were negative although the testicular weights were slightly retarded in comparison with the controls.

Implantation likewise failed of concordance in the hands of different investigators. The first implantation work was done in this laboratory beginning in 1931 by E. L. Lahr under the direction of Dr. G. E. Johnson. Wistar albino rats were used both as donors and as experimentals and controls. Forty-two rats were autopsied but some had not reached sexual maturity. There was no effect in regard to growth. In the male experimental he found lighter weight testes, greater age at which scrotality appears, delayed spermatogenesis and smaller size of tubules in the testes. In the female he found lighter ovaries in the experimental and a greater age at which the vagina opens (Lahr, 1932). The last conclusion was based on only a few animals. This could be attributed to natural variation. Kozelka (1932), working

on chickens, found no effect on body weight or on the growth of comb; no other comparisons were made. He implanted pineals from embryo or one day old chicks into half grown male and female Leghorn fowls. He also implanted two infant chicks with pineals from adult fowls.

Hoskins and Hoskins (1919) transplanted the pineals of young larvae of Rana sylvatica into 19 other larvae of the same species. The transplants failed to grow. Dubouik (1932) transplanted the pineals of rabbits three days old or less into rabbits of the same age. He fails to state whether the transplants became attached and grew. He believes that the pineal transplants produce acceleration of growth in young animals but halts their development if transplanted into older animals. The best results of the pineal transplants (as well as those of the pituitary) were obtained in the cavity of the spinal cord or in the cerebral cavity. More intense growth was obtained if parathyroid transplants were made also five weeks later. His methods were questionable, however, as he often used the larger animals for the ones to receive the transplants. The few animals used makes the work inconclusive.

Cornell and Goddard (1914) fed pineals to aments and delinquents. They seemed to show a mental increase but their controls showed greater physical growth. It was

concluded that mental defect in children is not due either to a deficiency in the pineal secretion or to apinealism. Goddard (1917) carried the experiment further with one of the most promising cases, a girl of eight years. He decided the effect of pineal on mental development was nil.

Krabbe (1923) quotes and agrees with the statement of Adler (1914) that we must expect different symptoms of suppression in different animal species following pinealectomy. This, if true, may account for some of the contraditions but does not account for those in which the same or closely similar species were used. Perhaps the main cause for contradiction has been the few animals used by the majority of workers. Small numbers do not give the opportunity of checking up on what is really constant in the experiment and what is due merely to natural variation.

The pineal is believed to grow until a child is about seven years of age. At this time involution begins. The basis for this belief is the calcareous deposits found in the pineal at eight years of age and older but seldom found in a normal child of less than eight years. Others would place the retrogressive change at puberty. McCord, Krabbe and others believe that the pineal still functions in the adult. Krabbe (Gordon, 1919) says that involution is no more complete at 90 than at 14 years. Others, however, still

deny that the pineal has any endocrine function. At the present, at least, only the old Scotch verdict "not proved" must be rendered.

METHODS

Young albino rats were used for this experiment. The majority of them were from inbred stock. Two of the same sex and as near the same weight as possible were chosen from a litter. One was used for the pineal implants and is hereafter called the "experimental", while the other received an equal amount of brain tissue and is referred to hereafter as the "control". The initial age varied from 14 to 23 days. Implantation periods varied from 18 to 43 days with the exception of one pair of males which were implanted only eight days. The experimentals received one pineal daily and the controls received an equal amount of cerebral cortex daily. The pineals for Groups XI and XIII were taken from sexually immature rats; all other groups received pineals from mature donors. Both sexes were used as donors indiscriminately. The donor rats were killed by striking them across the anterior portion of the spine; the skull was quickly opened and the pineal and a small amount of cerebral cortex were removed and placed in separate vials containing warm Locke's solution. An ordinary hypodermic syringe was used for the

implantation process. The gland or brain tissue was picked up in the barrel, the needle inserted under the skin and as the plunger was pressed gently the gland or brain tissue was placed subcutaneously. By this process the gland was broken open so that it was ready for immediate absorption. Merely the absorption of the daily implant was anticipated; it was not expected that the gland would become ingrafted. The work was done as quickly as possible and care was taken to do the work under aseptic conditions.

The experimental and the control animals were usually kept in the same cage. In a few cases they were in adjoining cages with identical conditions of food, water, light and temperature. A balanced ration containing vitamins A, B, D and E was kept continuously before them; while vitamin C was supplied by green feed more irregularly. They were kept in a steam heated building where a fairly uniform temperature was maintained. Each animal was weighed daily and the weight recorded preceding the implantation. Simultaneously they were observed for any evidence of sexual maturity. The descent of the testes and the opening of the vagina were the criteria employed. All animals were autopsied at the end of the implantation period. Measurements were made of the length of the body (from nose to anus), the length of the tail and the length of the right hind leg. The gonads

were dissected from as much of the attached tissue as possible, carefully weighed and placed in Bouin's fixative. The thymi were also weighed. The pineal, pituitary, and adrenal glands of all animals and also the Cowper's and the prostate glands of the male were examined and compared with those of the litter mate. The right horn of the uterus was carefully measured in the females. Serial sections of all tissues were made at 10 microns. Haematin and eosin bluish were used as stains.

RESULTS

Effect on Body Growth

The effect of pineal implants on body growth was one of the phases of the experiment to be considered. Every fifth one of the daily records of body weight was used to make the growth curves shown in Figures 1 and 2. Figure 1 illustrates the growth in weight for the male animals. Those implanted with pineals from mature donors are on the left (A) and those implanted with pineals from immature donors are on the right (B). The heavy lines represent the controls and the light lines, the experimentals. Figure 2 gives the same data for the female animals. The gain in body weight was greater in the experimental males in seven of thirteen comparisons with their controls. The experi-

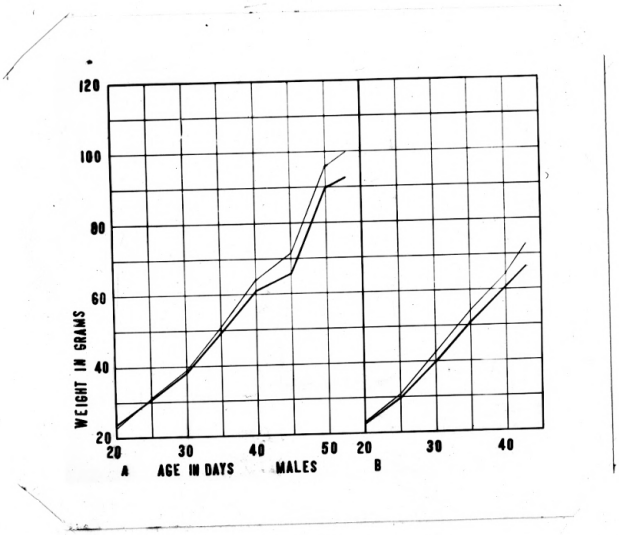

Fig. 1. Growth curves of males. A represents those receiving pineals from mature donors and B represents those receiving pineals from immature donors.

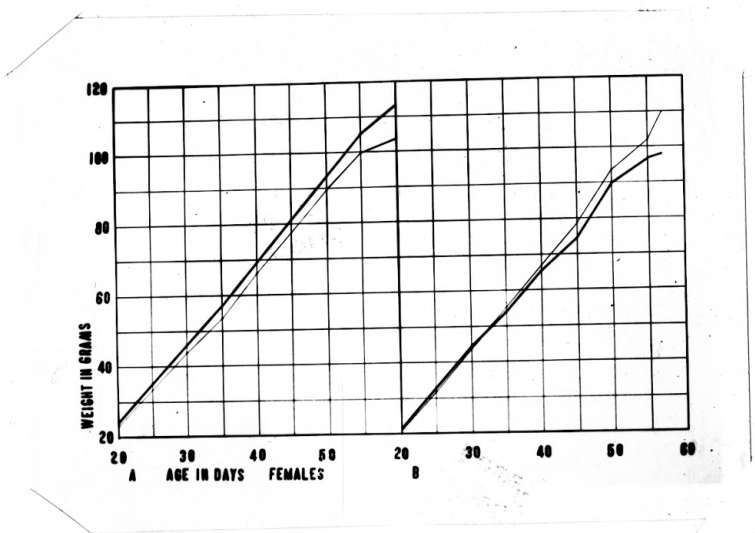

Fig. 2. Growth curves of females. A represents those receiving pineals from mature donors and B represents those receiving pineals from immature donors.

mental females showed a greater gain in somatic weight in ten of thirteen comparisons with their controls. It can be readily seen from the growth curves that the differences in the weights of the control and experimental animals are not significant. It is evident that the pineal implants have had no effect on body weights.

Weight may be governed by the amount of fat deposited. Hence weight alone 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 right hind leg, the length of the body from nose to anus, and the length of the tail. These figures are tabulated in Tables I and II. The experimental males had longer bodies in seven of thirteen comparisons; longer tails in nine of twelve comparisons; longer right hind legs in six of thirteen comparisons with their controls. The experimental females had longer bodies in ten of eighteen comparisons; longer tails in ten of seventeen comparisons; and longer right hind legs in ten of eighteen comparisons with their controls. No significant influence on the growth of the bone, as indicated by these records, was found. Usually heavier animals had longer bodies, tails, and legs; although where the animals were equal in body weight, there were variations. In the two cases where the Roman Numerals differ in the

Table I. Comparisons of Growth and Sexual Maturity in Males

Group - Animal	Age in Days			Length in mm.			Body weight gm.		Weight, mgm.	
	Initial	Final	Scrotal	Body	Tail	Hind leg	Initial	Final	Thymus	Testes
IX -3E*	22	45	33	141	131	94	26.1	78.7	203	888
IX -7C*	22	45	32	145	136	87	25.0	87.6	268	1030
IX -4E	22	45	33	144	127	94	28.6	83.4	318	793
IX -8C	22	45	32	137	126	85	30.1	75.8	165	793
X -3E	20	48	42	138	131	87	26.3	74.1	130	195
X -7C	20	48	36	145	128	91	24.4	76.3	210	280
X -4E	20	48	37	147	132	88	19.8	75.6	270	258
X -8C	20	48	40	126	122	77	20.1	60.4	220	185
XI -1E	22	43	36	142	129	85	22.7	74.2	210	266
XI -5C	22	43	34	127	120	88	21.7	63.6	202	290
XI -2E	22	43	33	140	132	90	25.4	71.0	150	413
XI -6C	22	43	31	131	120	86	23.5	70.6	270	535
XII -1E	20	38	38	132	118	90	22.0	55.1	180	423
XII -5C	20	38	36	143	119	93	23.2	59.2	207	617
XII -4E	16	34	35	123	112	88	17.2	50.0	170	367
XII -8C	16	34	33	128	110	86	18.2	53.9	180	525
XIII-6E	15	34	28	130	109	75	15.5	53.3	240	387
XII -7C	15	34	33	128	113	89	14.9	52.1	168	422
XIV -2E	20	28	25	118	108	80	29.4	44.1	90	375
XIV -4C	20	28	24	127	98	81	29.4	47.3	135	398
XV -3E	20	53	30	147	130	89	21.3	91.8	405	610
XV -8C	20	53	29	148	Short	90	21.3	79.0	345	477
XV -4E	14	47	27	145	136	88	16.8	82.7	230	754
XV -9C	14	47	23	136	121	85	15.7	68.3	270	492
XV -5E	20	53	28	158	151	93	19.2	107.9	500	1210
XV -10C	20	53	26	156	143	94	23.3	105.3	270	1537

*E, Experimental (pineal implanted) animals; C, Control (brain implanted) animal.

Table II. Comparisons of Growth and Sexual Maturity in Females

Group - Animal	Age in Days: Initial	Final	Vagina open	Length in Mm.: Body	Tail	Hind leg	Body weight, gm.: Initial	Final	Weight, mgm.: Thymus	Ovaries
IX -1E*	26	56	52	160	153	99	36.4	113.5	425	72
IX -5C*	26	56	55	142	144	84	36.1	98.5	285	23
X -2E	20	62	59	162	155	100	22.2	110.6	415	103
X -6C	20	62	58	155	152	95	21.7	111.7	390	55
X -1E	20	62	62	149	147	89	20.7	94.8	308	82
X -5C	20	62	60	147	148	91	21.6	97.5	365	100
XI -3E	22	57	50	165	153	99	26.0	122.2	360	104
XI -7C	22	57	57	153	152	97	26.8	105.0	270	86
XI -4E	22	57	57	150	Short	90	25.0	99.3	365	91
XI -8C	22	57	57	144	Short	92	25.3	89.8	518	95
XIII-1E	20	53	44	155	150	104	20.2	108.0	450	88
XIII-7C	20	53	52	149	142	94	22.5	95.4	345	69
XIII-2E	20	54	49	151	143	93	21.3	97.9	435	58
XIII-8C	20	54	54	154	146	100	21.0	101.0	505	68
XIII-3E	20	55	35	147	143	97	21.7	93.6	368	56
XIII-9C	20	55	55	148	136	96	21.9	92.4	385	49
XIII-4E	18	54	51	147	141	99	16.4	88.3	290	48
XIII-10C	18	54	54	152	142	98	16.4	101.1	460	55
XII -3E	16	56	51	149	133	92	14.7	81.0	240	55
XIII-11C	16	56	55	156	147	96	15.1	106.7	522	58
XIV -1E	20	63	61	155	151	93	26.2	93.3	295	97
XIV -3C	20	63	63	167	155	105	29.1	124.8	296	68
XV -2E	14	59	54	177	152	103	16.8	123.8	510	58
XV -7C	14	59	59	171	154	101	16.1	122.0	370	57
XVI -1E	23	56	48	161	155	98	38.0	119.4	370	81
XVI -7C	23	56	50	155	146	94	39.3	118.4	410	69
XVI -2E	22	44	42	149	142	92	33.6	95.0	385	70
XVI -8C	22	44	44	151	140	94	34.2	95.5	405	53
XVI -3E	22	55	47	154	153	97	29.3	113.4	340	67
XVI -9C	22	55	52	159	151	95	29.6	113.5	409	61
XVI -4E	22	55	53	146	148	89	26.0	86.7	320	41
XVI -10C	22	55	55	151	130	95	24.6	95.2	385	61
XVI -5E	20	54	54	165	145	97	26.1	114.0	400	59
XVI -11C	20	54	47	158	146	98	26.1	111.6	410	75
XVI -6E	20	53	51	150	136	96	24.4	98.6	300	58
XVI -12C	20	53	51	148	133	89	23.4	98.6	310	54

*E, Experimental (pineal implanted) animal; C, Control (brain implanted) animal.

pair, they were not litter mates.

A study of the weights of the thymi showed that the experimental females had a lighter thymus in thirteen of eighteen comparisons with their controls. The thymi of the experimental females averaged 365 mgm. while those of their controls averaged 391 mgm. The experimental males showed a lighter thymus weight in only seven of thirteen comparisons with their controls. The thymi of the experimental males averaged 238 mgm. and those of the control males 224 mgm. These figures show that in the cases in which the controls were lighter, that they were much lighter hence have a lighter average. The differences, however, were not significant. Comparisons of the sizes of other endocrine glands and of the uterus were made at autopsy but no distinct differences were noted.

Effect on Sexual Maturity

The age at which each animal reached sexual maturity was carefully noted and given in Tables I and II. The criteria used in determining sexual maturity was the descent of the testes into the developing scrotum in the males and the opening of the vagina in the females. It can be readily seen in Table I that the testes descended earlier in the control male in eleven of thirteen comparisons with

the experimental. The average age of scrotality in the experimental male was 32.7 days while in the control it was only 31.4 days. In both of the cases in which the experimental male became scrotal first, the experimental had shown a greater acceleration of body growth over its control which in one case was very marked. The age at scrotality varied from 23 to 42 days.

The testes at autopsy were heavier in the control male in nine of thirteen comparisons with the experimental. The average weight of the testes of the control male were 583 mgm. and 534 mgm. in the experimental male. In one of the remaining four pairs, the testes were of equal weight although the experimental had shown an acceleration of growth of 20 per cent over its control. The other three pairs in which the testes of the experimental males were the heavier, the experimentals had shown an acceleration of growth over their controls of 22, 25 and 38 per cent, respectively. It is to be expected that the larger animal would usually have the larger testes.

The opening of the vagina is generally accepted as a very accurate indication of puberty in the female. Long and Evans (1922) found that ovulation took place either simultaneously with the opening of the vagina or within five days afterwards in the majority of cases of the hundreds of

albino rats studied. Referring to Table II the reader will see that the vagina opened first in the experimental female in thirteen of eighteen comparisons with their controls. Two of the remaining pairs were equal leaving only three cases in which the vagina of the control opened first. The average age at which the vagina opened was 51.1 days in the experimental females and 54.3 days in the controls. The average weight of the ovaries was greater in the experimental females in eleven of thirteen comparisons with their controls. The average weight of the ovaries of the experimental females was 72 mgm. and of the controls was 64 mgm. The age at which the vagina opened varied from 35 to 65 days. This agrees with the findings of Anderson (1931) who reported a variation of 36 to 95 days depending on the season of the year and the breed of rats. Wistar albino rats were found to open earlier than other breeds.

Histological study failed to reveal any constant differences between experimentals and controls in the gonads of either sex or in the various glands studied.

DISCUSSION

A question might well be raised concerning the effectiveness of subcutaneous implantations. Johnson and Wade (1931) got striking results in the thirteen-lined ground

squirrel after only six or eight daily implants of the pituitary. The implanted glands were not expected to become ingrafted; merely the absorption of the daily implant was anticipated. Examination at autopsy showed that the glands were being gradually absorbed. Pineal implantations may be classed as glandular excesses.

The results I have found in regard to the effect of the pineal on sexual maturity agree with Foa (Krabbe, 1923) in 1912 and 1914, Horrax (1916) and Sarteschi (Gordon, 1919) in 1911 and 1915 except that histological difference in the testes as found by Foa and Horrax were not found in this experiment. This experiment did not show any acceleration of growth in either sex or the ovary weights affected as reported by Izawa (1923, 1926) but I did find more striking differences in testicular weights than those found by him when body weight is also considered. Christea (Krabbe, 1923) in 1912 found retarded testicular development in pinealectomized cocks which is contradictory to my findings. My work agrees with Weinberg and Doyle (1931) and Weinberg and Fletcher (1931) in the finding of no effect on body growth or on the gonads of the female, but disagrees in regard to the testicular development of the males, for they found no effect there also.

The results of this experiment agree with those found in this laboratory last year by E. L. Lahr in regard to the males (except that I did not find the histological differences found by him) but disagrees with the results found in the females. Statistical treatment of the difference in testicular weights between experimentals and controls of all male rats used by Lahr and myself showed that the results were not significant (difference/probable error = 2.5).

One failure of past workers has been to entirely disregard the initial and final body weights of the experimental and control animals in the comparison of testicular weights. Heavier animals should have heavier testes and become scrotal earlier than the litter mates of lighter weight if everything else were identical. Hence taking this into consideration I have six control males which were lighter in body weight but became scrotal before their experimental litter mates as shown in Table I. The other seven control males were either lighter than their experimental and became scrotal later or heavier and became scrotal earlier than their experimental litter mates. In either case it is not significant. There was no case in which the control male was heavier than the experimental and became scrotal later than its experimental litter mate.

Hence the ratio is 6 to 0 in favor of the conclusion that pineal implants retard sexual maturity as shown by scrotality.

This experiment has also five experimental males which were heavier than their controls yet had lighter testes except in one case in which the testes were of equal weight. The other eight experimental males were either lighter than the controls with lighter testes or heavier than the controls with heavier testes which in either case would not be significant. There was no case in which an experimental was lighter than the control but had heavier testes. Hence the ratio is 5 to 0 in favor of the conclusion that pineal implants retard the growth of testes.

CONCLUSIONS

Observations on 26 males and 36 females indicate that pineal implants on rats:

1. Do not influence the body growth of either sex.
2. Do not affect the sexual maturity of the female.
3. Do suggest a retarded sexual maturity in the male indicated by:
    a. Weight of testes at autopsy.
    b. Age at which they become scrotal.

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