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This paper describes the use of deuteron particles to partially or completely destroy the pituitary of rats. For three decades attempts have been made to study the effects of radiation on the pituitary, not only to further our understanding of its function but also with a view to human therapy. The pituitary has been studied after irradiation by a variety of methods ranging from total body X-irradiation (guinea pigs, Strauss, 1920[1]) to a focused high-speed electron irradiation of the pituitary (rats, Mateyko, et al[2,3] 1952). The early investigators were handicapped because the available radiation, X-rays, scattered widely in tissue so that it was impossible to confine the irradiation to the pituitary alone. It must be considered that even under the most favorable circumstances a substantial part of the head was irradiated. Several investigators have reported evidence of brain damage associated with attempts to irradiate the pituitary (Selle[4], 1935 and Cicardo[5], et al, 1951). In such cases injury to the hypothalamus would complicate interpretation of specific effects on the pituitary. In investigations where the method used amounted to external head X-irradiation the dose internally was limited by the radiation tolerance of the skin. In some reported work the dose remains uncertain in terms of modern units; however, externally applied doses probably seldom exceeded those of Lawrence, et al[6], who gave 3,000 r to the rat head and produced marked retardation in the growth of the animal, similar to that seen following hypophysectomy. At such doses many investigators reported changes in function of the pituitary target organs. Both retardation and stimulation of pituitary function have been reported. Rahm[7] (1922) noted a greater weight gain in rabbits and Epifanio and Cola[8] (1932) reported accelerated body growth and increased adrenal size of rabbits after head irradiation. Martinalli[9] (1929) found an increase in the interstitial tissue of the rabbit ovary but a decrease in the number of follicles following exposure of the head. Many have reported partial or complete destruction of the anterior lobe with atrophy of all the target organs after high doses of radiation, and some describe more inhibition of one function

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of the pituitary than of others but without agreement as to which function is more easily influenced by radiation. The same lack of agreement exists concerning the histologic appearance of the cells of the anterior pituitary after irradiation. Ghilarducci[10] (1922) and Epifanio[8] (1932) using rabbits, and Lawrence, et al[6], (1937) using rats found a decrease in the number of acidophiles and in dwarfism, whereas Podljaschuk[11] (1927) found an increase in acidophiles of rabbit pituitaries following irradiation. Brunner[12], using cats and dogs (1920), and Podljaschuk[11], using rabbits (1927), found a reduction in the number of basophiles in the anterior lobe after irradiation. As pituitary stains were not in common use at the time many of these papers were published, it is probable that not too much emphasis should be placed on the contradictory claims of specific cell changes.

Truly localized irradiation of the pituitary was first accomplished by Lacassagne and Nyka[13,14,15,16] (1934), who surgically implanted a capsule of radon in the center of the pituitary of rabbits. All anterior lobe cells were equally damaged. Recently Mateyko, et al[2,3] (1952) used high-speed electrons from a Van der Graaf machine to study the immediate postirradiation effects on the pituitaries of rats. The electrons can be brought to a focus within the head. The changes in pituitary hormone content in the first 24 hours after irradiation were studied.

Accelerated high-energy nuclei (protons, deuterons and alpha particles) have definite advantages for localized radiation of small volumes of tissue within the body. 190-Mev deuterons penetrate approximately 15 cm. into tissue and they travel in approximately a straight line. The scatter is considerably less than that of any other radiation used at present in clinical radiology. Collimated beams of these particles penetrate tissue like a straight pencil and the edge of the radiation field produces sharp lines of demarcation of radiation effects, somewhat like a surgical knife. The radiological properties of these particles have recently been described[17].

The present study was designed to demonstrate deuteron-radiation destruction of a tissue lying deep within the body. The pituitary was chosen because of its central position within the head, its well defined volume and the multiple criteria by which the functional state of the pituitary can be estimated in the living animal during the postirradiation period as well as at autopsy. Because the pituitary can be removed surgically in the rat, satisfactory controls could be provided. It was also hoped that these experiments would shed some light on the radiation sensitivity of the pituitary and perhaps show a specificity in reaction of certain cell types.

Material and Methods

The position of the pituitary within the skull of the rat is such that if the beam is directed laterally through the pituitary, little or no brain tissue is irradiated. If the beam is limited by an aperture of proper size, it passes through the tympanic bulla and the third, fifth and sixth cranial nerves. Fig. 1 shows the path of the deuteron beam superimposed on a frontal section through the pituitary to illustrate the structures through which it passes. The median sagittal section of the pituitary of a rat of the age used (28 days) is a triangle of base 2.0 mm and height 1.0 mm (Fig. 1). The deuteron beam was collimated to conform to the shape and dimensions of the pituitary by being passed through a brass aperture of triangular shape slightly larger than the pituitary to allow for slight errors in placing the beam (0.4 mm greater in each direction). Fig. 2 is a roentgenogram showing the relation of the pituitary to the base of the skull. The constant position of the pituitary over the occipitosphenoid synchondrosis makes it possible to direct the deuteron beam correctly by relating it to this landmark in a roentgenogram.

To hold the rat's head in position during the irradiation, a head holder was built which was slightly modified from the type described by Asling[18]. The rats were anaesthetized with nembutal during the procedure. The animal's head was held rigidly in the horizontal plane, anteriorly by a pin between the incisor teeth and posteriorly by tapered plugs which fit into the angle between the mandible and the tympanic bulla (Fig. 3). Screw adjustments were provided for moving the animal horizontally or vertically relative to the base of the holder to an accuracy of 0.1 mm. Correct alignment of the head in proper position in the path of the deuteron beam was accomplished with the aid of roentgenograms. For this purpose two film holders were attached to the base of the apparatus, one 5 cm. and one 12.4 cm. behind the rat's head (Fig. 3). A set of crossed wires was also mounted to the base so that its image would be superimposed on the roentgenographic image of the rat's skull. The steps in the procedure of the alignment of the pituitary in the path of the deuteron beam are explained in four parts in Fig. 4. The crossed wires were adjusted in such a way that the deuteron beam passed through the intersection when the holder was placed in proper position in the radiation chamber. Fig. 4, a and b, show the deuteron beam superimposed on the shadow of the crossed wires on both the near and distant roentgenograms. These films were developed after exposure to both X-rays and deuterons. The superimposed images on near and distant plates eliminate error through parallax. After a rat was placed in the holder a roentgenogram was taken to show the position of the skull in relation to the crossed wires. On the basis of the film obtained the animal was moved with the adjusting screws until the occipitosphenoid synchondrosis was in the desired relation to the crossed wires (Fig. 4c). A new film was then exposed to X-rays and the animal holder was moved to the deuteron radiation bench. By short exposure the deuteron beam was superimposed on the X-ray image of the skull and crossed wires (Fig. 4d). This picture shows the alignment of the skull to the crossed wires and the deuteron beam. Once the alignment procedure was found to be satisfactory each new animal had only to be aligned to the crossed wires in the roentgenogram.

Dose Measurements

The average dose delivered by the deuteron particles was recorded and measured by a parallel-plate ionization chamber. This ion chamber was placed in front of the beam-limiting aperture and the measured dose in roentgen equivalent physical (1 rep = 93 ergs/g) units represented the average dose before the particles were scattered by the walls of the aperture, the air molecules and the animal tissues. Owing to the scattering phenomena the edge of the beam becomes somewhat blurred. This is seen schematically in Fig. 1.

To obtain information on the actual dose received by the pituitary gland and the surrounding tissues, two types of measurements were performed. First, the dose distribution was measured in a lucite phantom substituted for the head of the animal. A small cadmium sulphide crystal detector* was used

*Made available through the courtesy of the General Electric X-Ray Corporation.

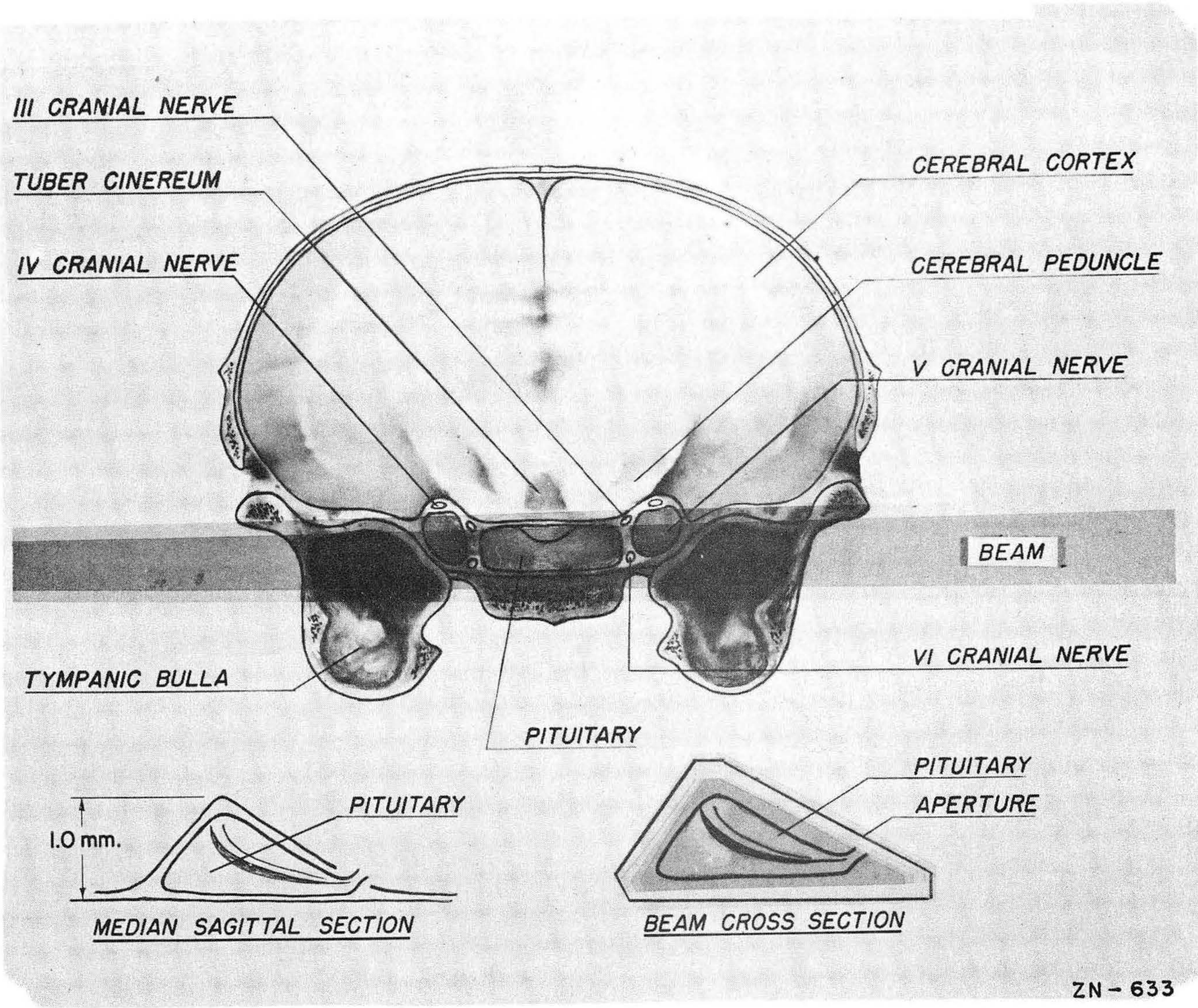

Fig. 1

The path of the deuteron beam superimposed on a frontal section through the pituitary of a 28-day-old rat to illustrate the structures through which it passes. Lower left shows median saggital section of pituitary. Lower right shows the cross section of the beam superimposed on the saggital section of pituitary. It represents the size of the aperture in relation to the pituitary and also represents the intensity and scatter of the beam.

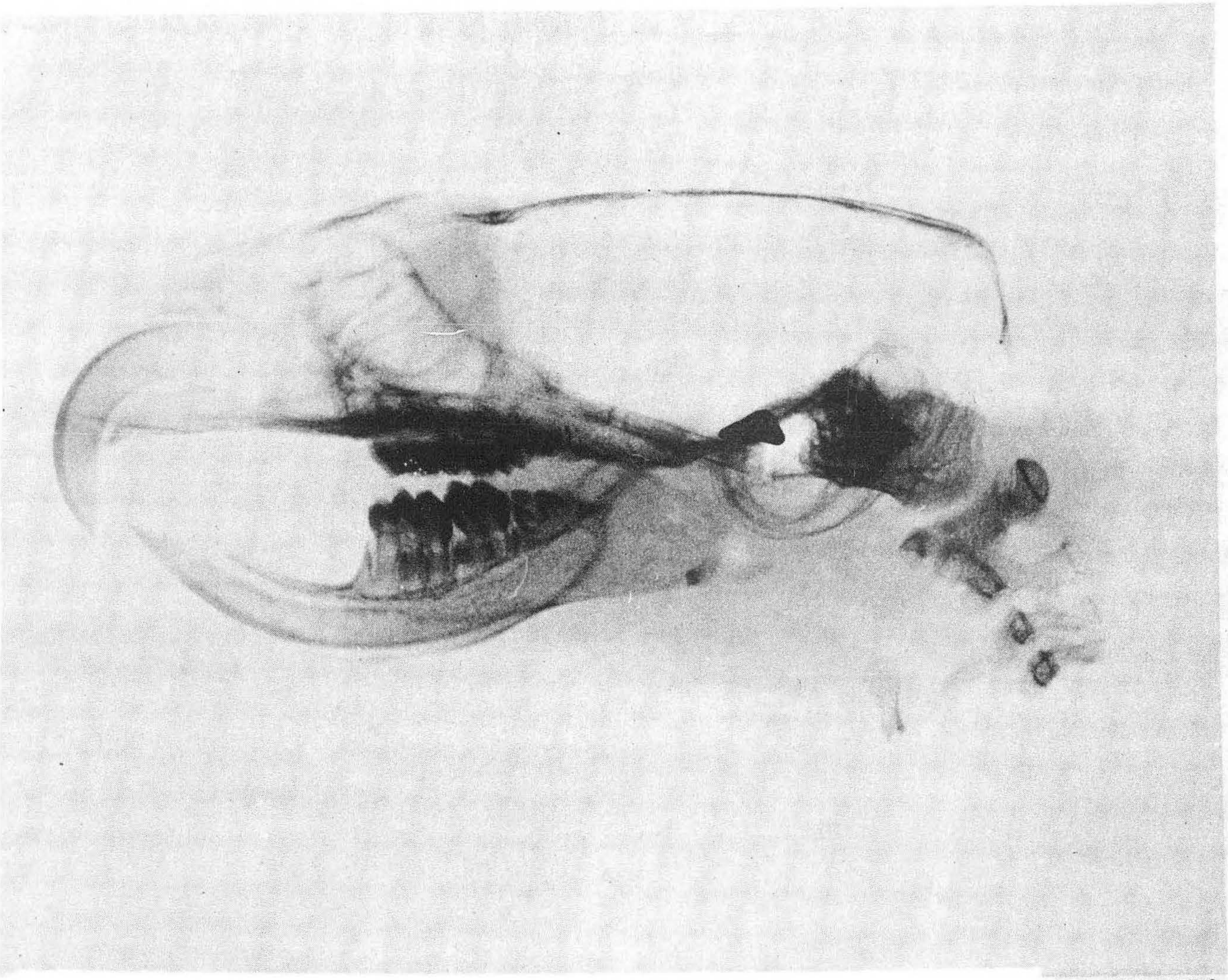

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Fig. 2

Roentgenogram showing the relation of the pituitary to the base of the skull. The block triangle represents a lead block of the exact size and shape of the pituitary gland, which was placed in the position the pituitary usually occupies. The pituitary consistently lies 3/4 anterior and 1/4 posterior to the occipitosphenoid synchondrosis.

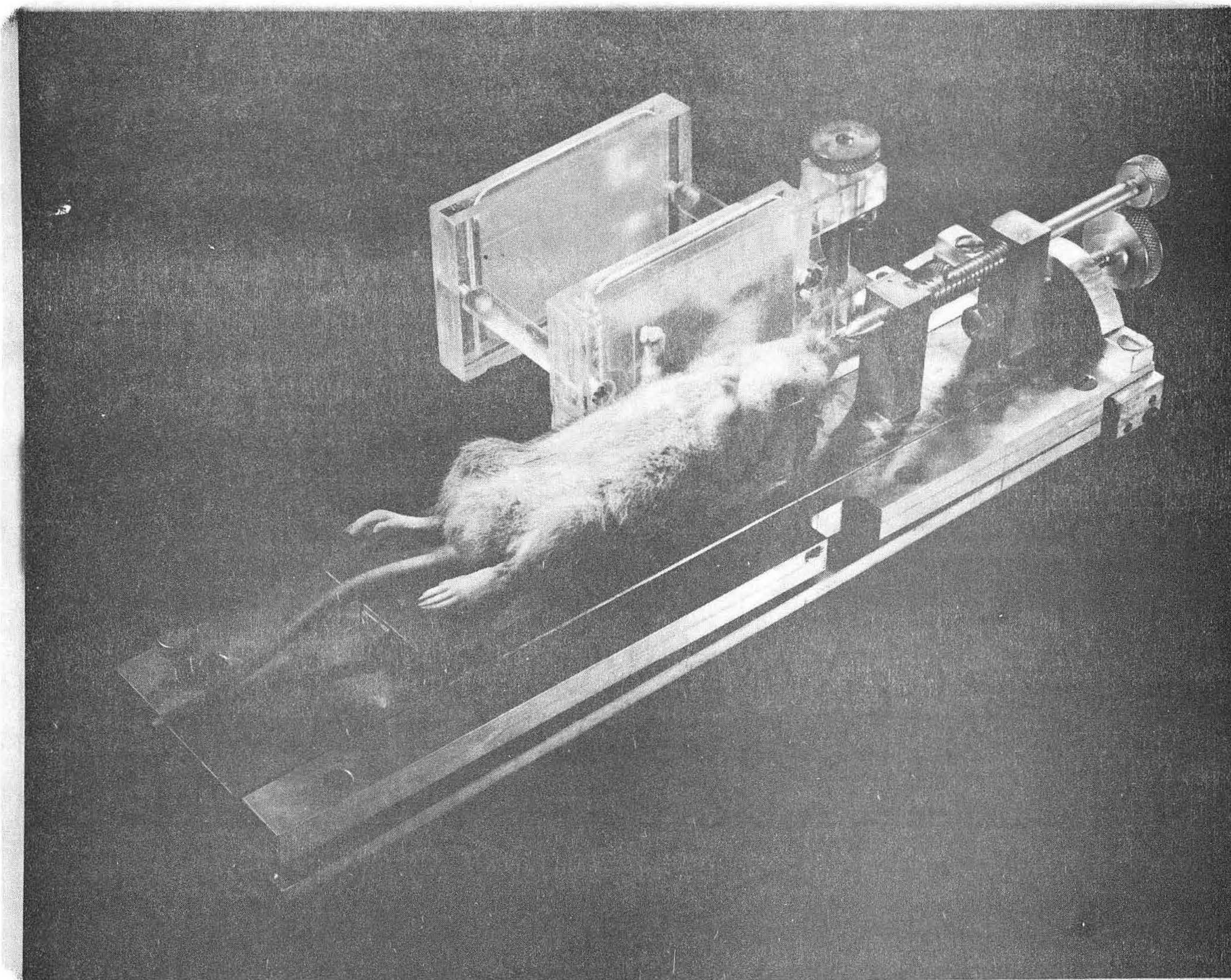

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Fig. 3

Rat mounted in the head holder used during the irradiations. The figure illustrates the three-point suspension of the head, the screw adjustments, and the two film holders used for correct alignment of the head. Note the adjusting screws for horizontal (H) and vertical (V) movement of the animal, and of the crossed wires (C).

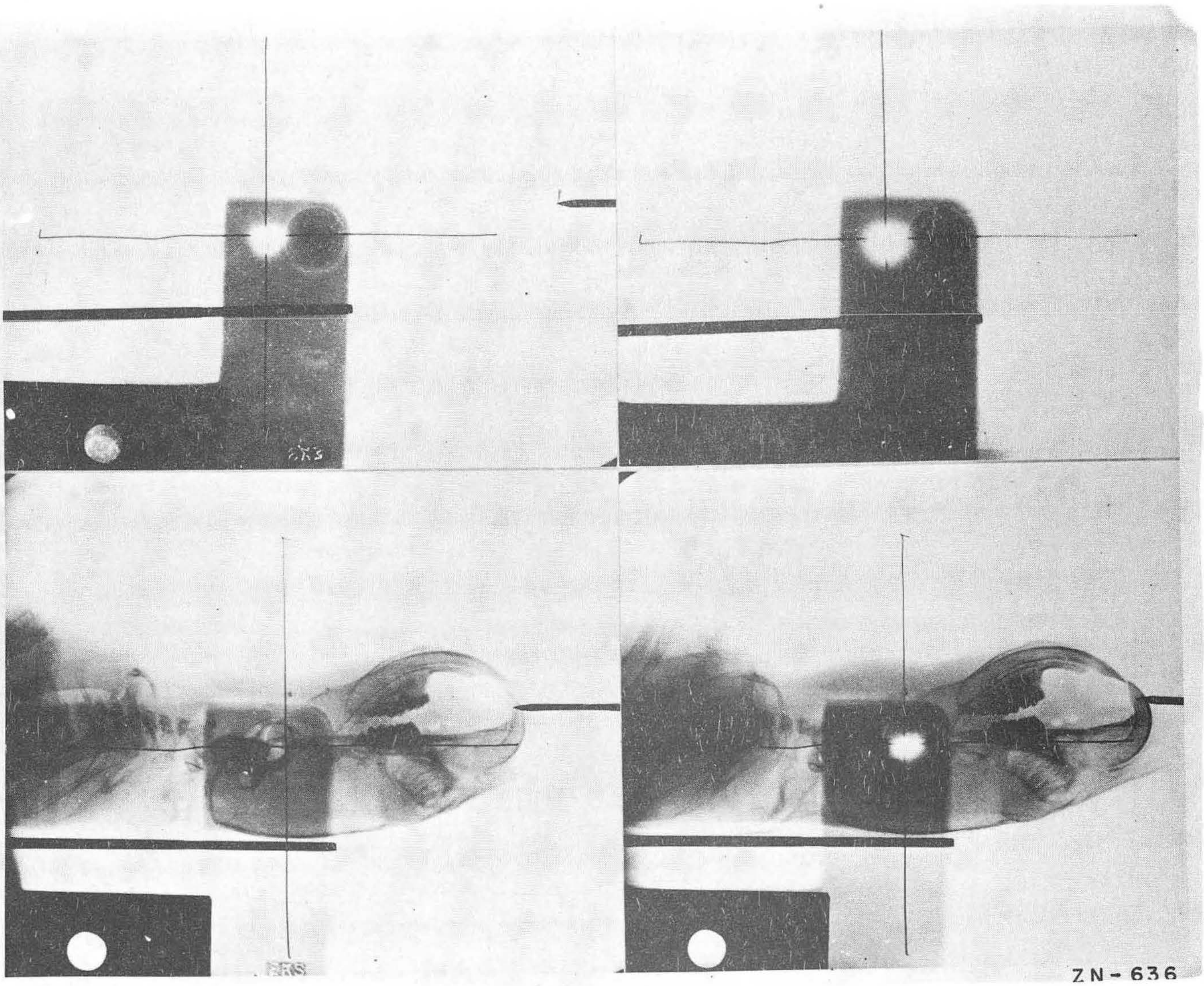

Fig. 4

The four steps in the procedure of aligning the pituitary in the path of the deuteron beam: (a) shows the deuteron beam superimposed on the shadow of the crossed wires on the near roentgenogram; (b) shows the deuteron beam superimposed on the shadow of the crossed wires on the distant roentgenogram; (c) shows the crossed wires in proper position to the occipito-sphenoidal synchondrosis; (d) shows the alignment of the skull to the crossed wires and the deuteron beam.

to scan across the beam[19]. The electric impedance of the crystal decreases as radiation impinges on it. The dose distribution in the horizontal plane obtained by this method is plotted in Fig. 5. The figure shows that the dose is not uniform for the entire area irradiated. It falls off near the edge of the pituitary and some irradiation reaches the immediately surrounding area. It is well known from surgical hypophysectomy that a very small fragment of gland will produce sufficient hormone to partially maintain the target organs (Smith[20], 1932). Therefore, after damage to the pituitary by irradiation any remaining biological activity is determined by that part of the pituitary which received the least dose. The average doses stated in this paper therefore do not represent the actual minimum doses that are able to destroy individual anterior pituitary cells but rather the doses which must be given to allow for the inaccuracies of the method.

Because of the scattering and the lack of perfect alignment of the beam one expects fluctuations in the actual amount of radiation received by the pituitaries of the individual animals. To obtain an approximate figure for these variations the overall dose received by the pituitary has been measured by assaying the radioactivity induced in the gland itself[21,22]. The deuteron particles induce activity in the atomic nuclei of the tissue. With no limiting aperture in the path of the beam (whole-head irradiation), there is no problem of positioning, and scattering can be neglected, so that the dose recorded in the monitoring ionization chamber is the dose delivered to the pituitary. Immediately after exposure of several animal heads to 10,000 rep doses of deuterons with no limiting aperture in the path of the beam the pituitary was removed, weighed, and then counted in a scintillation counter. A typical postirradiation decay curve for the pituitary is shown in Fig. 6. This curve illustrates three components with different half lives. The C^{11} activity is induced chiefly from the irradiation of C^{12} isotope by deuterons and has a 20.5-minute half life.

With a second group of rats the usual aperture for pituitary irradiation was inserted and the animals were carefully aligned by the technique described above. The same dose was given and the pituitary was again removed immediately and counted. Table I shows that the pituitaries irradiated with the limiting aperture in place have an average of only 63 percent of the activity induced in the pituitaries after whole-head irradiation (standard deviation of 14 percent). No doubt the lesser activity induced and the greater fluctuations when the beam was limited to the pituitary are due to lack of perfect alignment and to scattering of the beam at the edge of the aperture. From the activation analysis it is assumed that the probable dose received by the pituitaries is 63 percent of the measured dose. In this paper only the probable dose will be given.

The fact that only 63 percent of the radiation is intercepted by the pituitary implies that the immediately surrounding tissues receive approximately 37 percent. It is obvious from Fig. 5 that the dose falls off rapidly immediately outside the pituitary.

Biological Technique

Male rats of the Long-Evans strain were subjected to pituitary irradiation at 28 days of age. Doses of 3,150 to 25,200 rep were given to the pituitaries. The controls consisted of normal rats which received no irradiation,

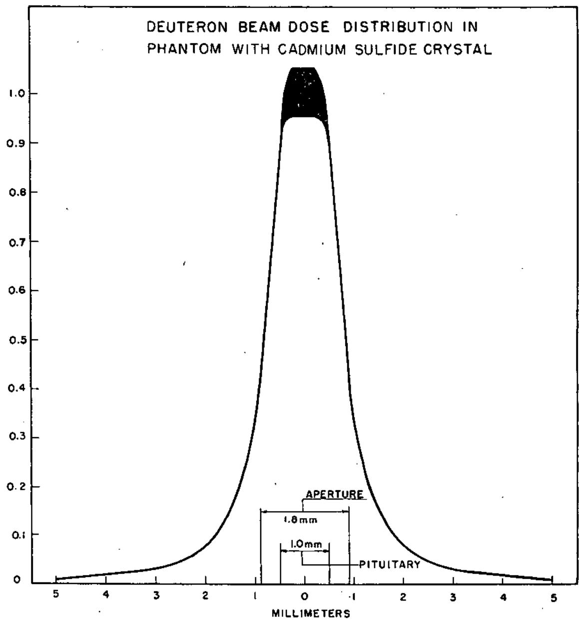

Fig. 5

Beam intensity distribution in lucite phantom measured in the position of the rat pituitary. The distribution is plotted in relative units. The measurements were carried out with a cadmium sulphide crystal.

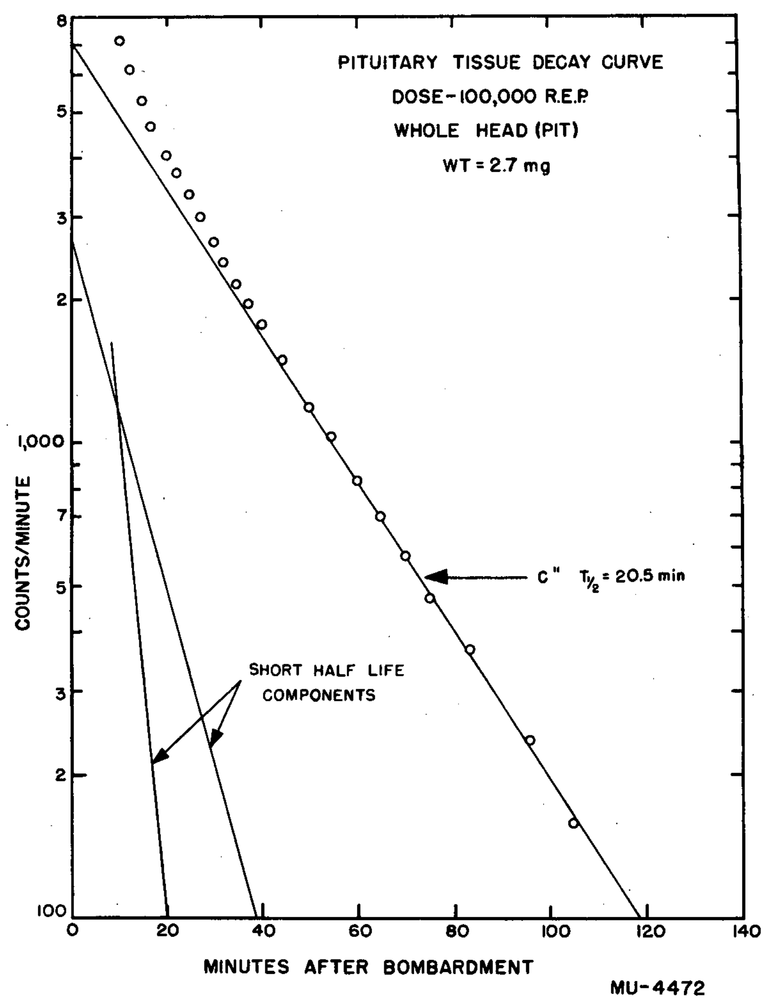

Fig. 6

The decay curve of a single pituitary irradiated with 10,000 rep deuterons to the whole head. Immediately after irradiation the pituitary was removed, weighed and counted directly in a scintillation counter.

Table I

Induced C^{11} Activity of the Rat Pituitary After Exposure to 10,000 rep Measured Dose Through Large and Small Beam Apertures.

Animal No.	Type Exposure	Exposure Time (Sec.)	Measured Dose rep	Pituitary Wt. mg.	Counts per minute, mg. and measured rep	% induced radioactivity in pituitary
1	Whole Head	169.5	10,000	3.3	0.038	100
2	"	235	"	2.9	0.038	100
3	Small Aperture	249.5	"	3.4	0.018	48
4	"	199	"	3.3	0.021	56
5	"	212	"	3.2	0.025	64
6	"	145.5	"	2.5	0.032	85

Average amount received by pituitary with small aperture $63 \pm 14\%$

rats which received the same background irradiation as the experimental animals (1/2 or less of the dose delivered to the pituitary) and surgically hypophysectomized rats. Approximately 1,000 rats were used in this study. All animals were fed a complete diet*. Periodically the general appearance, weights and tail lengths were recorded. At autopsy the testes, seminal vesicles, prostrate, adrenals, thyroid, thymus and pituitary were dissected, weighed and fixed for histologic study. The testis and thyroid were fixed in Bouin's fluid and imbedded in paraffin. The adrenals were fixed in neutral formalin and sectioned by the frozen method to be stained for lipid.

In one group of rats given 12,600 rep to the pituitary the circulating red cell volumes were determined in order to demonstrate whether the anemia which characteristically follows surgical hypophysectomy[24] could be shown to develop after injury of the pituitary by irradiation. The red cell volumes were determined by the Fe^{59} labeled red cell dilution method[25].

Results

By the fifth day after irradiation there were small ulcerations on each side of the head where the beam penetrated (Fig. 7). These ulcerations healed after three weeks and in no instance was a permanent ulceration observed. The new hair in these areas was white. These patches of white hair are shown in Fig. 7 and they demonstrate the direct path of the beam. The animals receiving pituitary irradiation showed none of the effects of total body irradiation. No diarrhea, postirradiation hypothermia or transient loss of weight were observed.

Changes in production of growth hormone by the pituitaries of the irradiated rats were estimated by measurements of gain in weight and tail length. The average gains in weight and increase in tail length of groups of rats given doses of 3,150, 9,450 and 18,900 rep to the pituitary are plotted in Figs. 8 and 9. These figures show that at each dose level (from 3,150 to 18,900 rep) the weight gain and tail length attained are below normal, the retardation being progressively more at higher doses. The weight curve was modified by radiation in two distinct ways. Within a few days after irradiation there was an abrupt dose-dependent decrease in the daily rate of weight gain followed by a more gradual decrease in growth rate until it approached that of surgically hypophysectomized controls (Fig. 10). There is no evidence of recovery in growth in any of the experiments, even with the lowest doses. Some of the rats which survived the highest doses showed as complete failure to gain in weight and length as did the surgically hypophysectomized controls. Fig. 11 shows one such animal 5 months after irradiation (18,900 rep), flanked by normal and surgically hypophysectomized controls of the same age. Such irradiated animals were comparable to surgically hypophysectomized rats, with small well formed bodies without obesity and with soft infantile fur.

The pituitary target organs were studied in groups of rats given from 3,150 to 31,500 rep to the pituitary. Groups of animals were autopsied

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*Diet I consists of: Ground whole wheat, 67.5 percent; casein technical, 15 percent; skim milk powder, 7.5 percent; sodium chloride (iodized), 0.75 percent; calcium carbonate, 1.5 percent; melted fat, 6.75 percent; fish oil (vitamin A and D concentrate), 1.0 percent.

Fig. 7

Areas of white hair at the point of entry (left) and exit (right) of the deuteron beam. These patches of white hair appeared after the small ulcerations which formed at the same sites had healed.

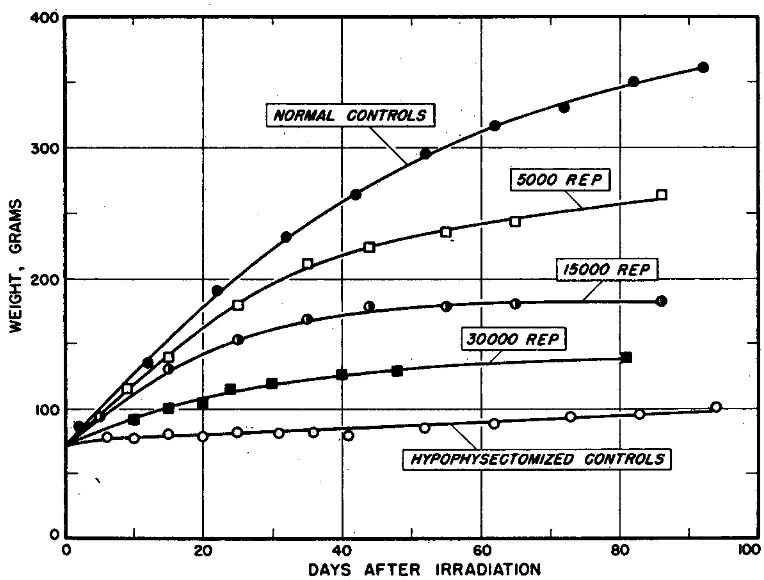

Fig. 8

Average gain in weight of groups of rats given various doses to the pituitary as compared to normal and to surgically hypophysectomized controls of the same age.

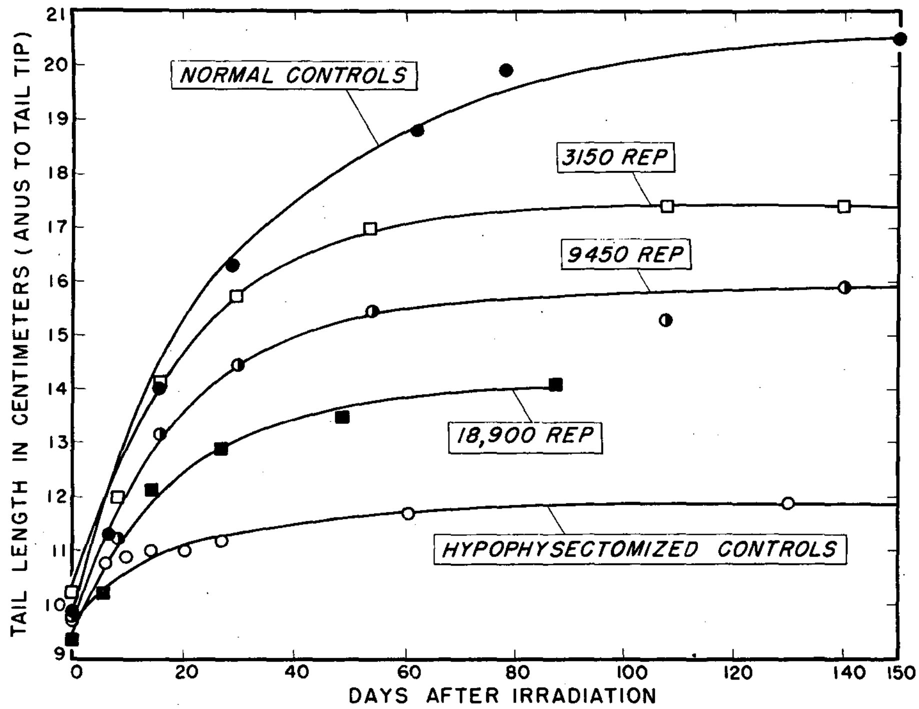

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Fig. 9

Average gain in tail length of groups of rats given various doses of irradiation to the pituitary as compared to normal and to surgically hypophysectomized controls of the same age. These are the same groups as illustrated in Fig. 8.

at intervals from 4 to 270 days after irradiation. From these autopsies it was found that only at very high doses (25,200 rep or more) did the irradiation cause cessation of pituitary function within a few days. At lower doses (12,600 rep or less) it was found that complete atrophy of the pituitary target organs occurred only after the lapse of several months. Fig. 12 shows the average weights of some of the endocrine organs and the pituitaries at various intervals after irradiation of the pituitary with 12,600 rep. Up to 40 days postirradiation there was evidence of only moderate damage to pituitary function, but after that time the weights of the pituitary and pituitary target organs rapidly declined. Examination of the figure indicates that there was an immediate effect on growth of the thyroid, whereas the testes and dependent organs showed no great difference in weight from the weight of the testes of normal rats of comparable age during the first 45 days. At a later time all of the target organs became atrophic, following atrophy of the pituitary itself. By 130 days there was almost complete atrophy of all pituitary target organs. The testes descended into the scrotum, enlarged and differentiated normally during the early period, subsequently atrophied and returned to the abdominal cavity. The speed with which atrophy occurred varied with dose. In one half of the animals, testes had atrophied and left the scrotum three months after irradiation of the pituitary with 12,600 rep. After 6,300 rep testes of only one half of the animals had withdrawn from the scrotum 10 months postirradiation. Thus the different doses resulted in similar final degrees of pituitary destruction, but required different postirradiation intervals to become manifest. The only outstanding difference in the effect of pituitary irradiation on the different pituitary functions was that body growth and growth of the thyroid were affected almost immediately at all doses, whereas the testes were the last of the target organs to atrophy.

Forty days after treatment the microscopic appearance of the pituitary target organs of animals given large doses of irradiation to the pituitary (18,900 and 25,200 rep) was that which is characteristic after surgical hypophysectomy. The thyroid epithelium became flattened and the colloid compact, the adrenal cortex became thin and a characteristic subglomerular lipid-free zone was present, and the testis showed atrophy of interstitial tissue as well as failure of spermatogenesis in the seminiferour tubules. For representatives organ weights, see Table II.

In animals given smaller doses (12,600 rep), the microscopic examination of the target organs revealed the same sequence of development of atrophy, paralleling the change in organ weights. In the early postirradiation period the histology of the thyroid, adrenal and testis was not different from that of normal controls. At later periods these organs became more atrophic. By 4 months or more the microscopic appearance of the target organs of the animals receiving the lower doses of irradiation approached that of the completely hypophysectomized rat.

At 6,300 rep to the pituitary and a postirradiation period of 9 months, the pituitary target organs had undergone almost complete atrophy. The thyroid epithelium was flattened, being only slightly higher than after surgical hypophysectomy. The adrenal cortex was depleted of lipid almost to the same degree as after hypophysectomy, showing the coarse lipid granules and subglomerular lipid-free area. The interstitial tissue of the testis was deficient and the tubules were atrophied to two cell layers with only a few desquamating primary spermatocytes.

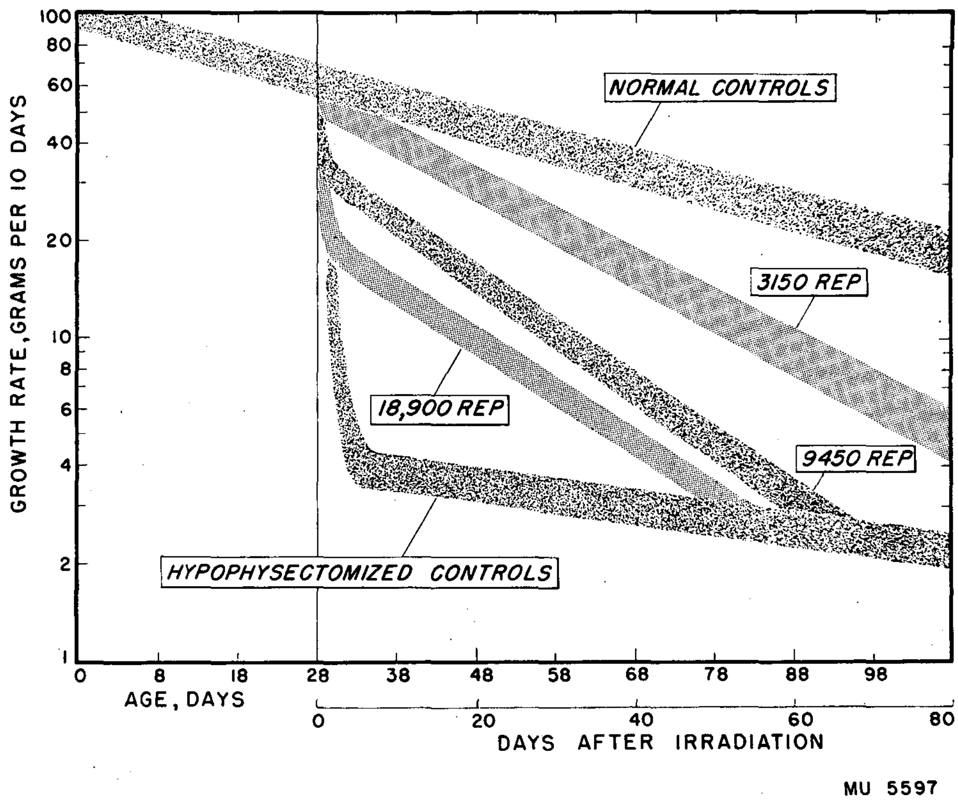

Fig. 10

Average growth rates for rats reconstructed from weight data. Because of the great natural fluctuation in animal weights, this chart is very inaccurate. Nevertheless it shows a striking difference in growth rates of normal and of surgically hypophysectomized rats, also the drop in growth rate immediately after irradiation with increasing doses. The initial drop in growth rate increases with dose. At a later time the growth rate decreases faster than normal, indicating progressive decrease in function of the pituitary.

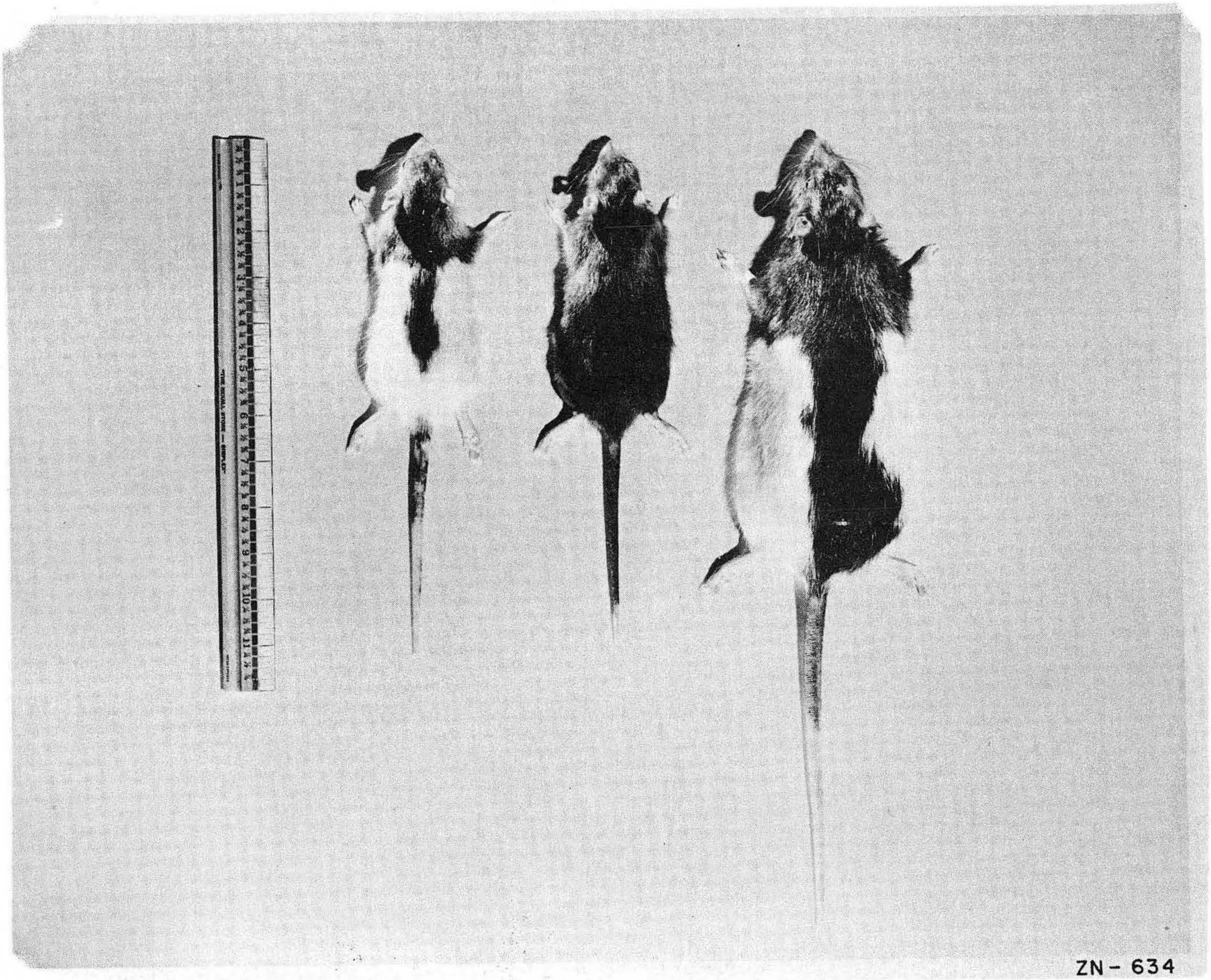

Fig. 11

A rat (middle) 5 months after irradiation of the pituitary with 30,000 rep, flanked by normal (right) and surgically hypophysectomized (left) controls of the same age. The irradiated rat was a typical hypophysectomized rat with a small well formed body with soft infantile fur and without obesity.

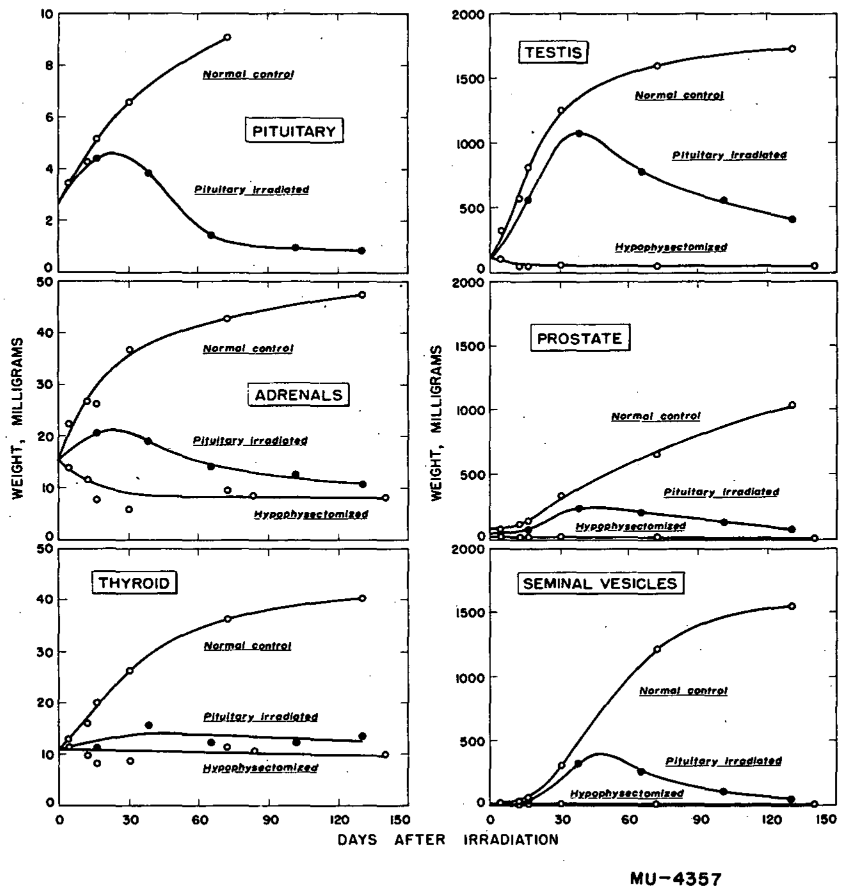

Fig. 12

Average weights of the pituitaries and some of the endocrine organs at various intervals after irradiation of the pituitary with 12,600 rep. Each point represents the average of 9 rats.

Table II

Representative Organ Weights of Rats Given High Doses of Irradiation to the Pituitary, Normal Rats, and Surgically Hypophysectomized Rats, 75 Days after Radiation.

Treatment	Body Weight g	Tail Length cm	Adrenals mg	Thyroid mg	Testes mg	Seminal Vesicles mg	Prostrate mg
18,900 rep	77	11.0	9	8	122	7	25
25,200 rep	75	11.6	6	6	136	6	28
Surgical Hypophysectomy	93	11.6	7	7	107	5	26
Normal	303	19.1	35	23	3181	833	624

Circulating red cell volumes were determined two months after irradiation of the pituitary with 12,600 rep in order to demonstrate whether the anemia which characteristically follows surgical hypophysectomy could be shown to develop after irradiation of the pituitary. The red cell volumes were determined by the Fe^{59} labeled cell dilution method in six irradiated rats, in five normal rats and in five surgically hypophysectomized controls. The hematological data are presented in Table III. It can be seen from the table that at this period after irradiation there was a small but definite anemia as judged by the hemoglobin, hematocrit, and circulating red cell volume. These determinations were done at a time when pituitary atrophy was only partial.

No gross injury to the cranial nerves in the path of the beam was seen and no functional aberrations were noted. The rats given the small total body irradiation (fast neutron background)equal to the highest background irradiation received by the experimental groups showed no significant changes in growth or in endrocrine organs even after 9 months.

The normal controls of this series survived 2 years or more. Half of the 100 hypophysectomized rats used as controls in this series died within 25 days after operation. Half of the animals given 25,200 rep also succumbed within 25 days; after 18,900 rep half survived after 45 days. After 12,600 rep most of the animals survived longer than 200 days but the rate of death was greater than in the normal control.

To determine whether the results obtained by pituitary irradiation could be explained by unintentional injury to surrounding tissue, several groups of rats were subjected to irradiation of the area surrounding the pituitary (a beam of the same size being directed above, anterior or posterior to the pituitary). It was found that doses of 12,600 and 18,900 rep to the surrounding area resulted in rapid death of all animals whereas doses of 3,150 rep did not cause death and resulted in no significant changes in pituitary weight, growth rate or state of the pituitary target organs.

Pituitary Histology

Pituitaries of 78 experimental and 44 control rats were examined histologically. The glands were fixed in Zenker-formol, sectioned at 4 micra after nitrocellulose embedding, and stained with a modified Mallory-Azan stain (Koneff, 1938).[23]

The effect of ionizing irradiation was studied on day 4, 12, 16, 25-30, 163-200 and 279-286 following irradiation with a 6,300 rep dose; on day 4, 12, 16, 25-30 and 72 after a 12,600 rep dose, and on day 25-50 following irradiation with massive doses of 18,900 and 25,200 rep.

Definite postirradiation effects could be seen even grossly at autopsy. Glands removed soon after irradiation (12-16 days) were already slightly smaller than their controls and were hyperemic. Pituitaries of rats sacrificed after a longer posttreatment interval, or those from animals subjected to the highest doses of irradiation, were considerably reduced in size, flattened, often pale, and without clear outline of individual lobes. The majority of rats subjected to 18,900 or 25,200 rep doses (5 of 8) showed considerable distention of the

Table III

Hematological Data on Normal Rats, Rats Given 12,600 rep to the Pituitary, and Surgically Hypophysectomized Controls. Irradiated or Operated Two Months Previously.

Treatment	Body Weight g	Hemoglobin g/100 ml	Hematocrit %	Red Blood Cell Volume/100 gm. Body Weight ml
Normal	291	14.2	48.4	2.18
	289	14.0	45.5	2.44
	334	13.8	47.0	2.57
	271	13.6	46.9	2.26
	321	11.2	42.5	2.02
	301	13.4	46.1	2.29
Irradiated	146	12.6	43.3	2.04
	140	10.6	37.0	1.66
	136	11.8	40.4	1.99
	135	13.6	43.5	2.12
	170	13.2	40.6	1.89
	120	12.4	38.6	1.90
	141	12.4	40.6	1.93
Hypophysectomized	110	10.0	33.9	1.58
	101	11.8	36.4	1.58
	86	10.2	31.5	1.36
	74	10.2	31.0	1.41
	76	10.6	31.7	1.42
	68	10.4	33.0	1.58
	86	10.5	32.9	1.49

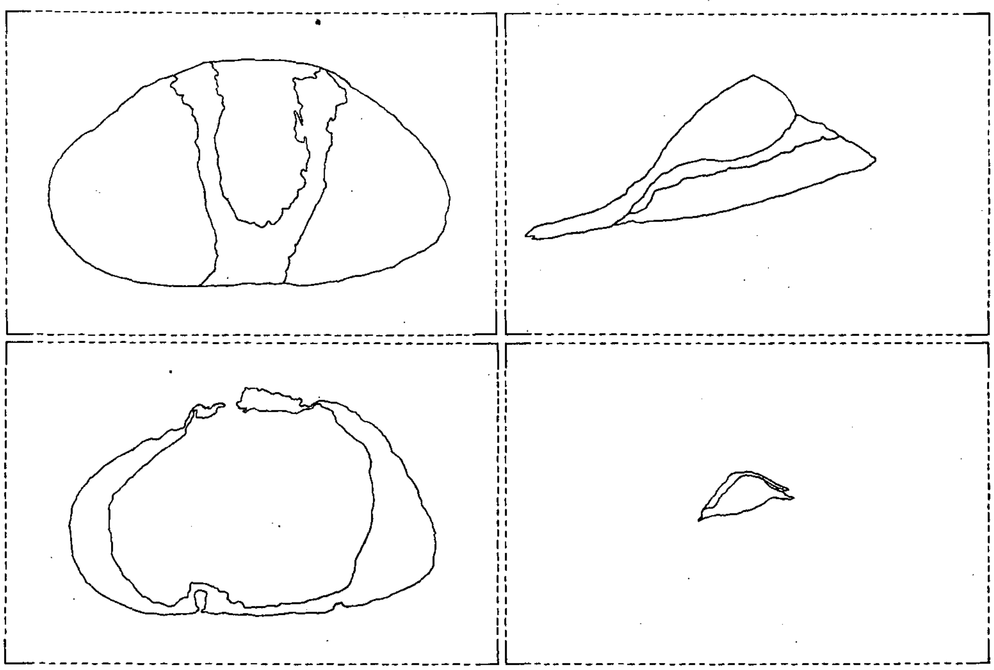

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Fig. 13

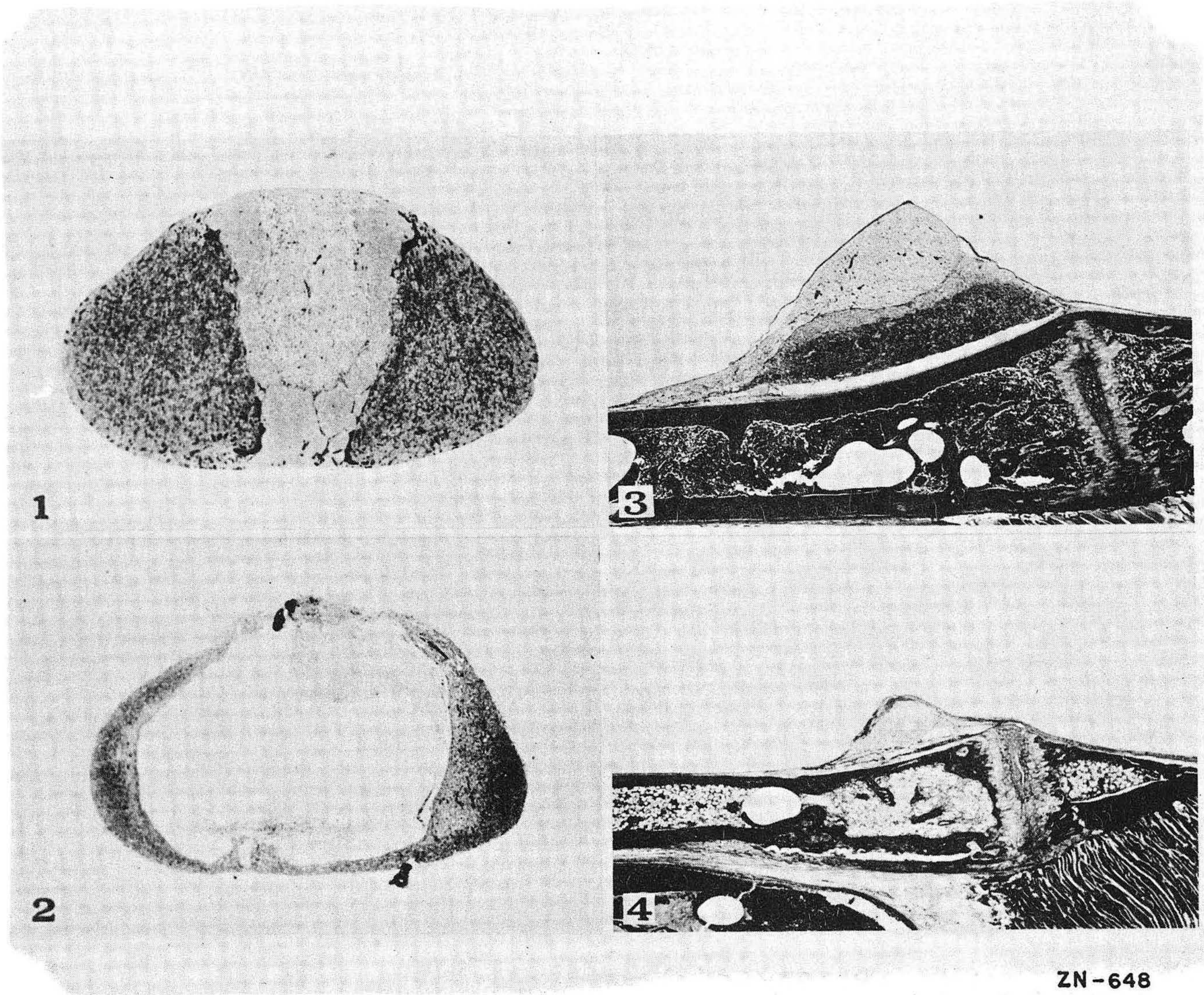

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Fig. 13

Horizontal and sagittal sections of pituitaries at magnifications x 23 and x 18 respectively; Mallory-Azan stain.

1

Pituitary of normal 53-day-old rat.

2

Pituitary of an experimental rat 25 days after irradiation with 18,900 rep. Note distention of cleft and reduction of the anterior lobe tissue.

3

Sagittal section through the pituitary of a 259-day-old, normal rat in its normal position. The stalk and all parts of the hypophysis are clearly shown.

4

Sagittal section through the pituitary of an experimental rat 256 days of age, 227 days after irradiation with 18,900 rep. The parenchyma of the pituitary has been replaced by scar tissue.

cleft, which was filled with colorless or blood-tinged fluid (Fig. 13b). It was also noted that after prolonged post irradiation periods, there was a firm adherence of covering membranes to the pituitary tissue.

The effect on pituitary weight of different doses of ionizing irradiation is shown in Table IV.

Table IV

Weights of Irradiated Pituitaries at Different Postirradiation Intervals

Days after irradiation	4	12	16	25 to 39	49	72	163	200	279 to 286
Normal controls (mg)	3.5	4.3	5.2	6.5	.	9.1	10.6	11.2	12.8
6,300 rep	3.4	4.2	3.6	4.0			1.1	1.0	1.4*
12,600 rep	3.3	3.4	3.5	3.0		2.8			
18,900 to 25,200 rep				2.1	1.1				

*Actual weight of the pituitaries was probably less than the figures of the table show because some fragments of the covering membranes could not be removed.

The effects of irradiation with 6,300 rep will be described in some detail as the effects of this dose on the pituitary were studied at intervals throughout a considerable span of time.

Morphological changes in the pituitaries 4 days after irradiation were slight though detectable. Some pyknosis in chromophils, increased numbers of mitoses, abnormal nitoses and degranulation in acidophils were seen.

By day 12 the effects were more pronounced. Mitoses were found in all lobes of the hypophysis. Degranulation of chromophils, especially of acidophils, was obvious. Vacuolated basophils and degenerating acidophils were seen throughout the anterior lobe.

By day 16 the glands were hyperemic. Brilliance of staining was lost. Acidophils were decreased in number. They were degranulated and were degenerating. Some basophils were markedly degranulated; others showed cytoplasmic vacuolation resembling that found in "castration" and "thyroidectomy" cells or showed fragmentation of the cytoplasm and pyknosis of the nuclei. Atypical mitoses were found in all lobes and apparently were responsible for the appearance of cells with varying degrees of nuclear and cytoplasmic hypertrophy or atypical nuclear shape*. It was also noted that by this

*Attention should be drawn to the excellent demonstration of Wm. Bloom and R. E. Zirkle (Proc. Am. Assoc. of Anatomists, Anatomical Record, 1953) of the disordered mitosis induced by irradiation in vitro of the chromosomes of the newt resulting in daughter cells with abnormal nuclei.

time there was a definite lack of uniformity of cell and nuclear size as well as staining reaction of individual cells of anterior and intermediate lobes. Pools of homogenous material, stained blue by Mallory, were found among the parenchyma cells in several glands.

After 25 to 30 days all the alterations noted earlier were still present, but were more marked. Young scar tissue had begun to appear in the parenchyma, evidently at the site of destruction of the parenchyma.

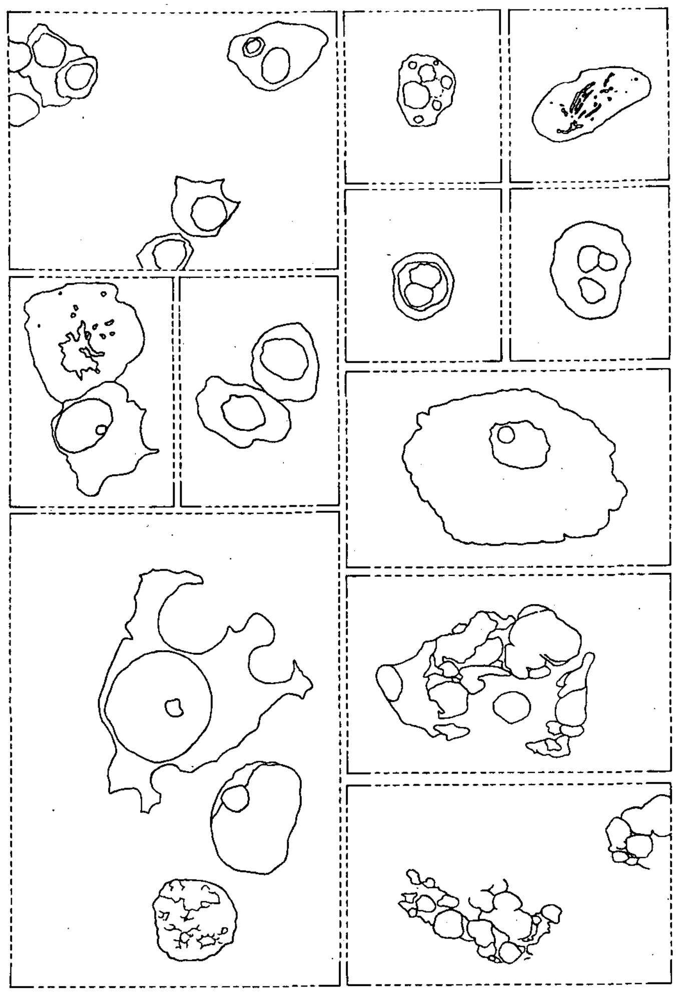
It is of interest to note that the damaging effect of irradiation on the parenchymal elements was similar in all three lobes of the pituitary. Abnormal mitoses, multinuclear cells, giant cells, "foam cells", hypertrophy and abnormalities of the nuclei were common in all parts of the glands. Cells of the various lobes, thus changed, could not at times be distinguished morphologically. Alterations found in different cells of the pituitary parenchyma are shown in Fig. 14.

After 163 to 286 days the average pituitary weights were reduced to 1.2 mg. In 18 rats the weights ranged from 0.8 mg. to 1.7 mg. The covering membrane was thickened by scar formation and fibers were continuous with the scar tissue within the parenchyma (Fig. 16d). The lack of distinct subdivision of the glands into individual lobes, noted at autopsy, was due in part to thickening of the covering membrane, and in part to localized irregular invasion of tissue from one lobe by that from another. The anterior lobes were often markedly hyperemic. Histologically, small localized hemorrhages were gound in the glands, especially in the anterior lobe.

All structural changes and degenerative processes, previously described for individual cells, were also found at this postirradiation period. The continued presence of abnormal mitotic divisions may be interpreted either as due to the initial injury of resting cells, which was not evidenced until the infrequent mitoses occurred, or may be interpreted to mean that the injurious effect of the radiation was carried down through succeeding generations of daughter cells during the 6 to 9 months postirradiation.

Although degenerative changes in the three different parts of the pituitary were similar and frequently led to the formation of indistinguishable cells, the three lobes still maintained sufficient characteristics so that they could usually be recognized. The anterior lobe, in addition to the hemorrhages and scar tissue previously mentioned, consisted almost entirely of nongranular cells. Some of them, in contrast to typical chromophobes, varied considerably in size, were sharply outlined and often showed a negative image of the Golgi apparatus. Among these atypical chromophobes occasional abnormal acidophils and basophils were distinguishable. In a small region, the so-called gonadotropic area in the anterior aspect near the pars intermedia, clumps of easily recognizable basophils were present. The majority of these cells, however, were signet ring forms or had darkly stained peripherally-located granular material. The intermediate lobe had lost its usual uniformity of cellular and nuclear size and staining reaction, but could be recognized by occasional groups of typical cells. In the posterior lobe the localized hemorrhages and degenerative changes in the cells constituted the usual alterations.

The effects on the pituitary of a dose of 12,600 rep will not be described separately as there were only slight quantitative and no qualitative differences from the effects of 6,300 rep doses.



MU 5599

Fig. 14

Photographs taken with oil immersion objective at magnification x 1000; Mallory-Azan stain.

1

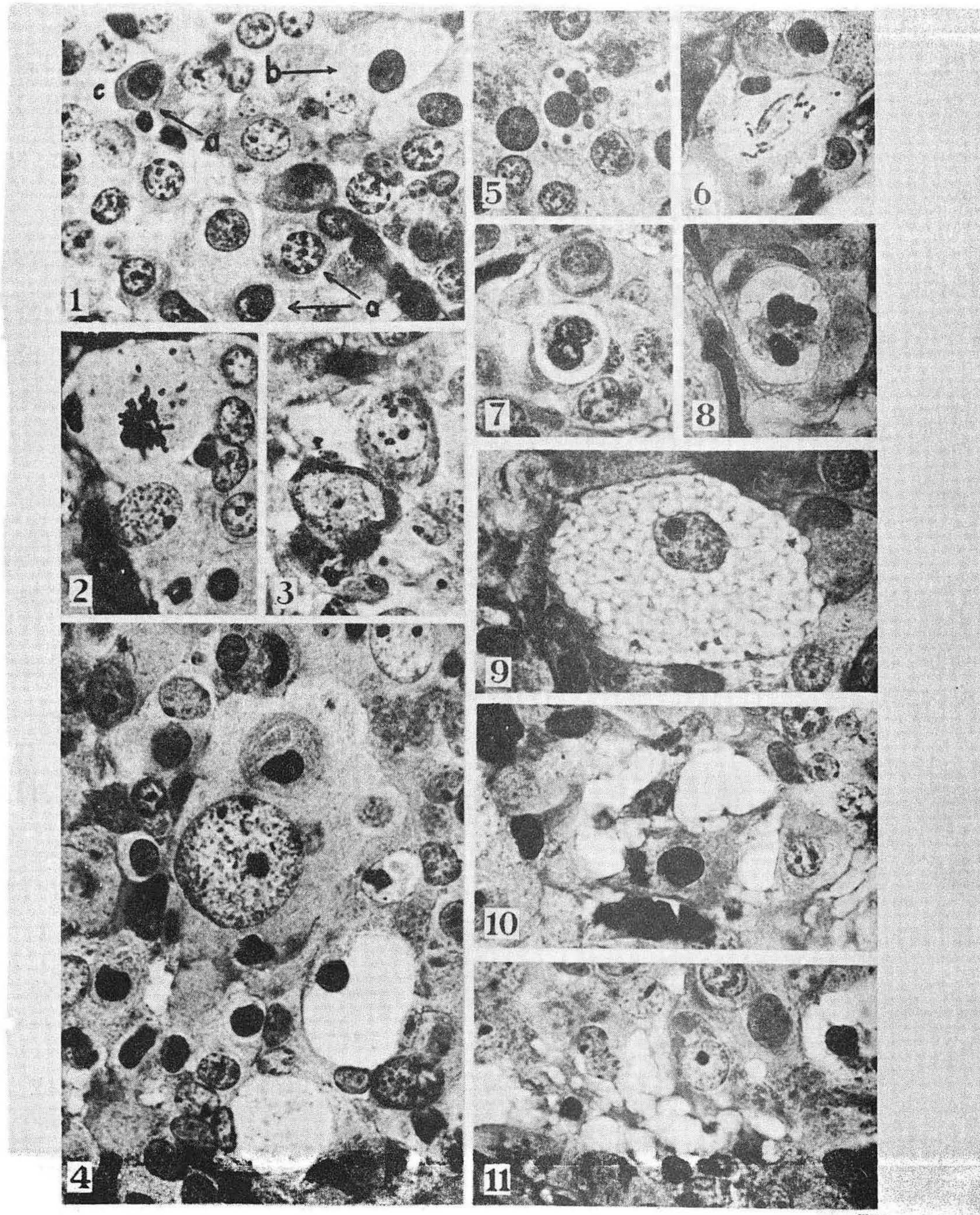
Anterior pituitary of a normal rat 72 days old, showing normal appearance of a acidophils; b basophils; and c chromophobes. The remaining figures (2 to 11) show the changes found in different hypophyseal cells 12 to 286 days following irradiation.

2 and 6

Anterior pituitary cells in abnormal mitotic division. Note scattered position and abnormal shape of individual chromosomes.

5, 7 and 8

These figures show abnormal number of nuclei in single cells. Fig. 5 (intermediate lobe) shows a cell containing (within the 4 micra section) 8 nuclei of different sizes. Figs. 7 and 8 show anterior lobe cells containing 2 and 3 nuclei.



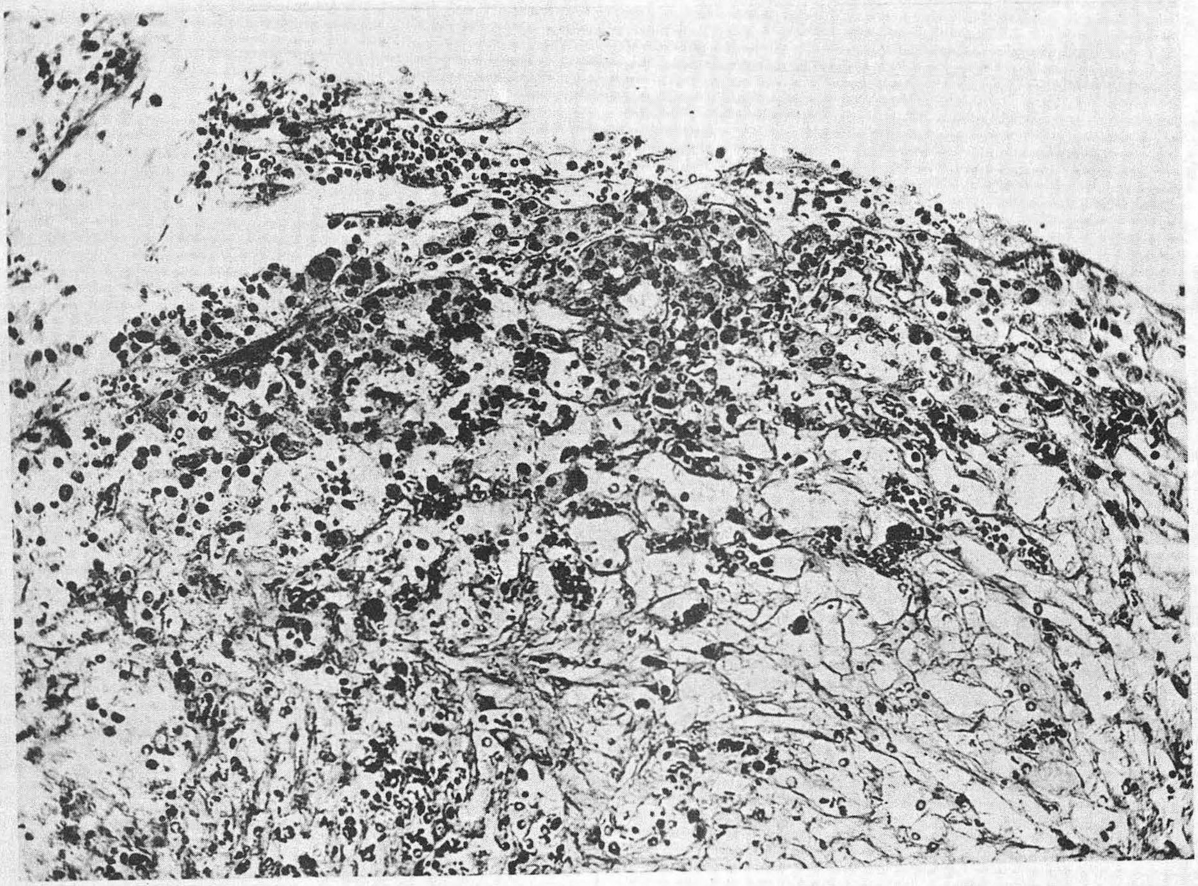
ZN - 644

Fig. 14

3
Two anterior lobe acidophils which show general nuclear and cytoplasmic size increase, each containing large Golgi apparatus.

2, 4 and 9
These figures show hypertrophied anterior lobe cells (basophil, acidophils and chromophobes) from irradiated pituitaries. Note that cytoplasm, nucleus and nucleolus all show hypertrophy. Also note that in some cases, hypertrophied cytoplasm has surrounded neighboring smaller cells.

4, 7, 8, 9, 10 and 11
These figures show different stages of cytoplasmic degeneration. Fig. 9 shows shrinkage of the cytoplasm. Fig. 8 shows early cytoplasmic degeneration. Fig. 4 shows late degenerative change. Fig. 9 shows multiple uniform-sized vacuoles ("foam cell"). Figs. 10 and 11 show late degenerative changes in basophils.

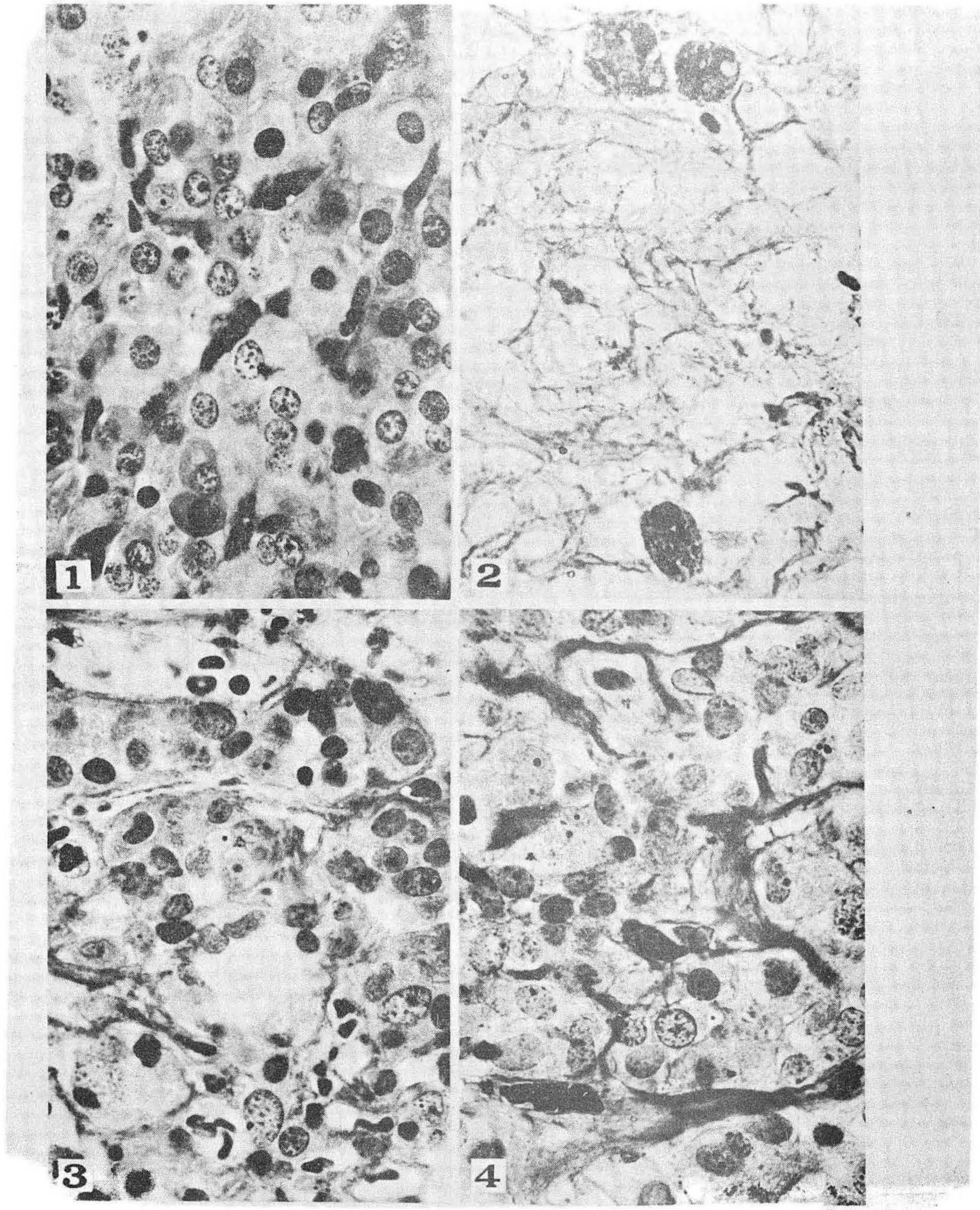


ZN - 647

Fig. 15

Photograph taken with 8 mm objective, magnification x 200; Mallory-Azan stain.

Anterior pituitary of a rat on the 49th day following irradiation with 25,200 rep. Anterior pituitary parenchyma has been replaced by scar tissue except a few scattered small nests of cells (upper part of the photograph). In such regions some chromophobes, acidophils and basophils could be recognized. (For details, see Figs. 16 and 17.)



ZN-646

Fig. 16

Photographs taken with oil immersion objective at magnification x 800; Mallory-Azan stain.

1

Section of anterior pituitary of a normal 72-day-old rat. Note general appearance and distribution of cells.

2

Section of anterior pituitary of a rat on the 49th day following irradiation with 25,200 rep (same pituitary as shown in Fig. 15). Note complete absence of parenchymal cells; presence of young scar tissue, three macrophages.

3

Same pituitary as Fig. 2. This figure shows remnant of surviving anterior lobe parenchyma. Note abnormality of size and shape of the chromophobes. Also note degenerating cells. No recognizable acidophils or basophils have been found in this particular field.

4

Anterior pituitary of a rat 286 days following irradiation with 6,300 rep. Note that only chromophobes are present. Also note heavy scar tissue.

Changes in pituitaries of rats subjected to massive doses, 18,900 and 25,200 rep, were studied only between 25 and 50 days following the irradiation. At 25 days, owing to the great distention of the vestigial cleft, the intermediate lobe was usually stretched and often fragmented. Scarring was more pronounced a month after irradiation than it was after the lower doses, and the characteristics of the scar tissue suggested that it had appeared earlier. There were small hemorrhages in the anterior lobe in close proximity to the scarred areas. Surviving anterior pituitary tissue consisted mostly of abnormal nongranular cells with occasional chromophils recognizable by a few characteristically stained granules. Intermediate and posterior lobes showed profound degenerative changes accompanied by the appearance of abnormal cells and giant cells.

Two months (50 days) after irradiation with 25,200 rep all that remained of the pituitary was a small, pale, transparent fragment of tissue in which the individual lobes were not distinguishable. Histological examination showed obliteration of the general pattern of subdivision into lobes. This was due to the extensive degeneration of the parenchyma and heavy scar formation which was in continuity with the surrounding membranes. Small numbers of surviving parenchyma cells still recognizable occurred in nests measuring at most 170 x 580 and 200 micra. Even these cells were atypical and usually nongranular (Fig. 15).

Alterations in all lobes of such pituitaries are shown in Figs. 13, 15, 16, and 17.

In summary, the injurious effects of deuterons on the pituitary were detectable within 4 days after treatment with 6,300 rep. Changes were more definite on the 12th day and well advanced by day 16. Thereafter the damage to all parts of the pituitary appeared to be progressive throughout the remaining period of observation (up to the 286th day). The earliest and most extensive damage was observed in the acidophils. Basophils were less affected by the deuterons and after 286 days could still be found in nests at the anterior aspect of the pituitary in close proximity to the intermediate lobe. They showed varying abnormalities in their structure, but were possibly capable of function.

Massive doses of deuterons (18,900 and 25,200 rep) produced the same qualitative damage to the individual cells as the lower doses. However, the effects were shown more rapidly and were more extreme.

It should be noted that although mitoses were present 6 to 9 months after irradiation, they were abnormal in appearance and abnormal cells resulted. There was no evidence within the period of these observations of recovery of the parenchymal cells.

Discussion

Only by giving extremely high doses of irradiation (18,900 rep or more) was it possible to bring about complete and immediate destruction of the pituitary of the rat, as judged by growth stasis comparable to that after hypophysectomy and by inhibition of differentiation of the testis, the most sensitive of the target organs to small amounts of functional pituitary tissue. At lower doses (12,600 rep or less) the pituitary continued to increase in size for some time and then proceeded to undergo atrophy. The greater the dose the more rapid the atrophy.

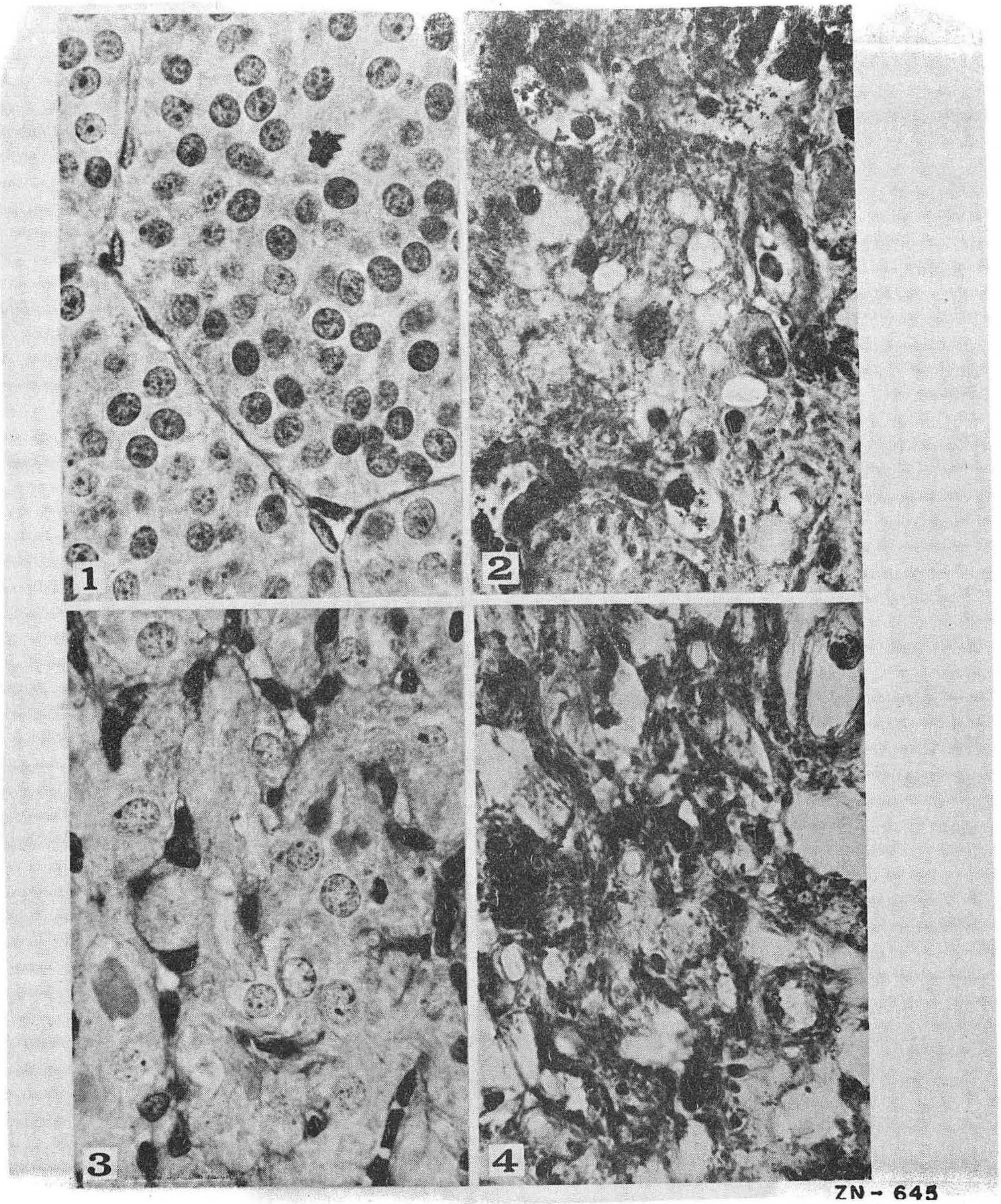


Fig. 17

Photographs taken with oil immersion objective at magnification x 800; Mallory-Azan stain.

1

Field selected to show typical appearance of the intermediate lobe of a normal 72-day-old rat.

2

Intermediate lobe of a rat on the 49th day following irradiation with 25,200 rep. Note that the normal pattern of tissue is lost; remaining cells show varying degrees of degeneration; scar formation.

3

Field selected to show posterior lobe of a normal 72-day-old rat.

4

Posterior lobe of a rat on the 49th day following irradiation with 25,200 rep. Note complete replacement of parenchyma by scar tissue; a few macrophages present.

It was hoped at the onset of this work that a differential radiation sensitivity of the various cell types of the anterior pituitary might be demonstrated. However, at the doses employed, all cell types and all lobes were markedly injured. There was some evidence that the pituitary basophils were slightly less sensitive to irradiation than acidophils, as a few survived doses at which practically no acidophils could be identified. The only outstanding difference in the effect of pituitary irradiation on the different pituitary functions was that there was some effect on body growth and thyroid morphology manifested within a few days, whereas the testis was the last target organ to atrophy at any given dose. This might be interpreted as a differential effect on pituitary function; however, it is more probable that the irradiated pituitary gives the same end result as incomplete hypophysectomy, although the entire pituitary was irradiated. Smith[20] showed that only minute fragments of the anterior pituitary need remain for stimulating effects to be shown by the testes and adrenal. The thyroid and body growth were far less sensitive to minute fragments. Pituitary weight and histological examination have shown us that at lower doses marked destructive changes in the pituitary become manifest only after long periods. During the earlier postirradiation period the degranulated anterior lobe cells were still producing hormone in amounts that were adequate to maintain the more sensitive target organs. Later, when the destructive changes in the pituitary were more advanced, even the more sensitive target organs ceased to respond. Although it has been reported by Ghilarducci[10] (1922) and Lawrence[6] (1937) that regeneration of the pituitary occurs after small doses of irradiation, no evidences of regeneration have been observed at the doses and time intervals employed in these experiments.

The early reports suggesting stimulation of pituitary function after irradiation appear to have employed much smaller doses than the smallest used in this series. It is possible that these workers who reported stimulation of body growth after pituitary irradiation (Rahm[7], 1922, and Epifanio[8], 1932) could have been observing the development of obesity resulting from irradiation damage to the hypothalamus, since the criteria of increased growth was increased weight gain. There was no tendency to develop obesity in any of the rats of this series.

It must be remembered that the conclusions here are all based on doses of irradiation which resulted in eventual serious damage to the pituitary. Further work must be done at lower doses and longer postirradiation periods.

Conclusions

(1) The use of deuteron particles for alteration or destruction of a localized volume of tissue deep within the body has been demonstrated by irradiation of the pituitary of the rat employing doses of from 3,150 to 25,200 rep.

(2) It has been shown that the degree of destruction observed following irradiation of the pituitary is a function of time as well as dose.

(3) Only by giving high doses of irradiation (18,900 rep or more) was it possible to bring about rapid destruction of the pituitary. With lower doses (6,300 rep) the destruction was not complete for many months after irradiation (9 months).

(4) There was no evidence of stimulation of any function of the anterior pituitary with the doses and periods of observation employed in this experiment.

(5) There was no evidence that the pituitary was able to recover after being damaged by this type of irradiation at dose levels employed.

(6) The different cells of the anterior pituitary were found histologically to be almost equally sensitive to irradiation.

(7) Body growth and thyroid growth were impaired soon after irradiation at each dose level, but degeneration of the testes occurred much later.

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