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## The Use of High-Intensity Ultrasound to Alter the Cellular Structure of the Anterior Pituitary

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*Introduction by Dr. Carstensen*

*The next paper concerns a topic of basic biological interest, namely, the possible use of high-intensity ultrasound to control hormonal functions of the body by means of its effect on the pituitary gland. The paper will be presented by Elizabeth Kelly.*

This preliminary report is concerned with the degenerative and regenerative processes occurring in the anterior pituitary gland as a result of ultrasonic irradiation. In order to study such processes by histological techniques, ultrasonic lesions of a variety of sizes and geometric configurations were produced in different positions in the anterior pituitary of cats and the resultant cellular changes were studied for sacrifice times from 1 hr to several months. The initial purpose of this research was the investigation of the possibility of using high-intensity ultrasound to produce selective effects, temporary and permanent, on the cellular composition of the anterior pituitary gland.

Figure 1 shows the stereotaxic apparatus used to support the cat in position in preparation for ultrasonic irradiation, the attached x-ray cassettes used in taking roentgenograms to identify landmarks on the sella turcica and the coupling pan which supports the transmitting liquid. A portion of the cat's skull is removed in order to maintain a precise focus and accurate control of the dosage for the sound beam within the brain. Degassed mammalian Ringer's solution is used as the liquid coupling agent between the sound transducer and the brain tissue. The sound source is lowered into the liquid in the coupling pan, and irradiation is accomplished through the intact dura mater. The sound is produced by a single X-cut quartz crystal and focused with a lens to produce a high-intensity beam at a frequency of 4 Mc/sec. The dosage of ultrasound applied to produce the cellular changes was uniformly high, but some variation of the level was utilized as one parameter for con-

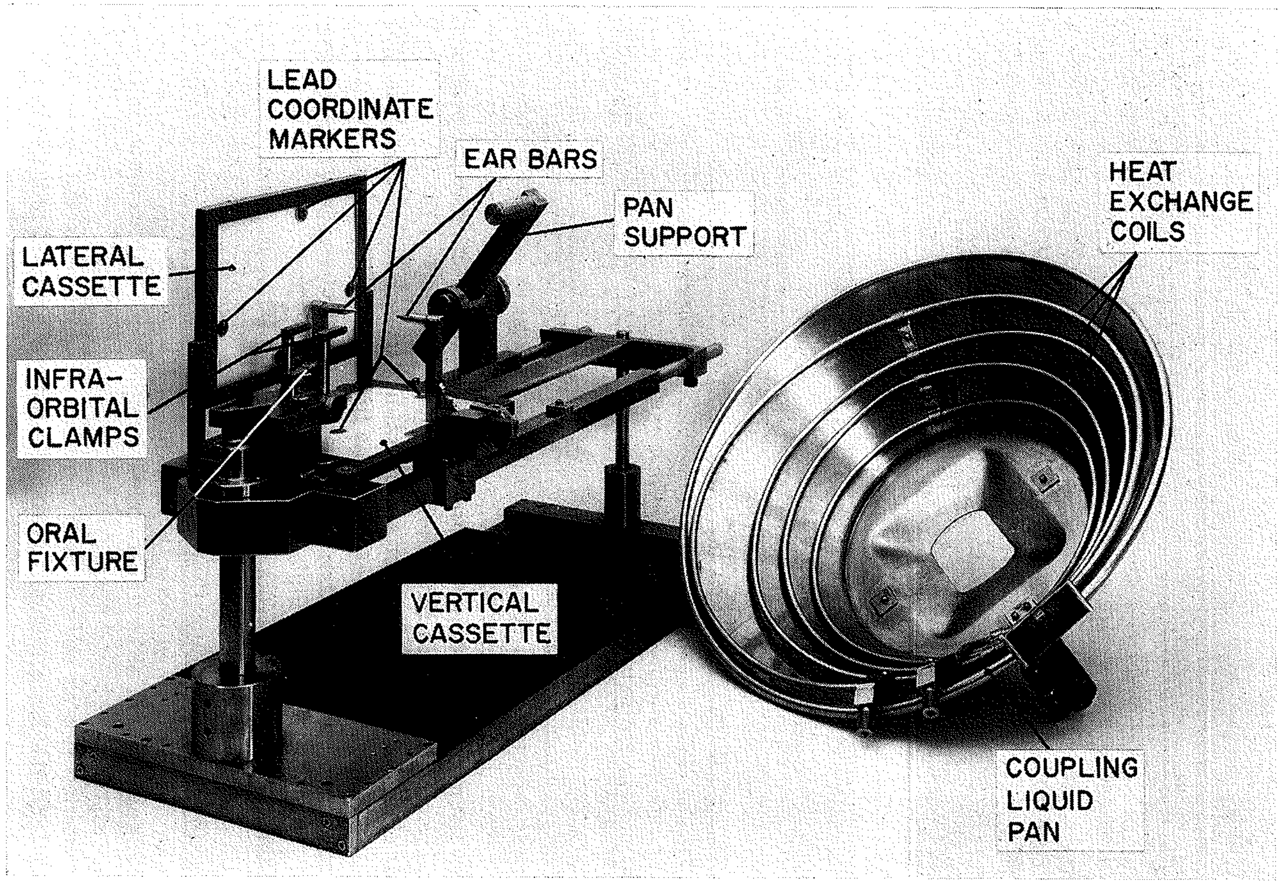



FIGURE 1. Stereotaxic apparatus and attachments.

trolling the effect of the irradiation on the cellular structure. Details of the irradiation dosages will not be discussed in this presentation except to indicate that for the average ultrasound dosage applied, the particle velocity amplitude was approximately 600 cm/sec. This corresponds to approximately 90 atm pressure amplitude[1] for the focused field. The lesions were produced by a series of closely spaced ultrasonic exposures (0.2 to 0.3 mm separation). The irradiation time for a single exposure was also used as a variable to control the effects on the cellular structure but was, in general, close to 0.5 sec.

The cytological studies were made on the hypophyses of cats sacrificed at times varying from 1 hr to 3 months after ultrasonic irradiation. The two groups of pituitary cells considered in this paper fall under the general classification of chromophiles, namely, the acidophils (alpha cells) and basophils (beta and delta cells). These two groups are of considerable importance and are concerned with the function of various hormones such as ACTH, TSH, corticotrophin, and somatotrophin (1). Figure 2 shows a Gomori-stained transverse section of the anterior pituitary of a normal cat – the cytoplasm of the acidophils appears red, that of the basophils blue. Consideration of Figure 2 appears to indicate a greater concentration of acidophils in the lateral areas with the basophils more restricted to the central area. Such an arrangement would agree with the observations of Dawson regarding the location of the different types of cells of the anterior pituitary gland of the cat (2).

Figure 3 shows a gallocyanin-stained tissue section of the anterior pituitary of a normal cat – the nuclei of the various cells are stained blue with only a very light staining of the cytoplasm. The DNA and RNA of the nucleus are affected by this stain, as well as the RNA of the cytoplasm. The normal tissue of this figure can be compared to that of Figure 4 which shows a gallocyanin-stained transverse section of the pituitary of a cat sacrificed 3 days after irradiation. The tissue at the base of this figure illustrates a so-called thermal lesion (the thermal lesion extends dorsal to the base on the left side), that is, a lesion produced primarily by a transfer of heat from the bone surrounding the pituitary. (The ultrasonic absorption coefficient of bone is much greater than that of soft tissue.) The most striking effect of the thermal lesion is the almost complete absence of cells. Such thermal lesions can be prevented by placing the focus of the sound beam more dorsally in the structure, but it is of interest to study such lesions and compare them with ultrasound lesions that are not complicated by direct heat transfer. For example, in the tissue section illustrated in this figure, the "ultrasound" lesion is apparent dorsal to the thermal lesion at the base of the gland, but, in contrast to the thermal lesion, it exhibits only slight changes with the gallocyanin stain. In this regard, it is of interest to compare Figure 5, which shows a magnified view of the normal or unirradiated area of the gland shown in Figure 4, with Figures 6 and 7 which show, at the same magnification, the ultrasound lesion regions and the thermal lesion region of the same gland. In the unirradiated area

[1] Expressed in terms of intensity for a plane wave, this pressure amplitude would correspond to an intensity of 2700 w/cm $^{2}$.

FIGURE 1. Stereotaxic apparatus and attachments.

(Fig. 5) the nuclei of the cells are quite distinct, with some indication of the surrounding cytoplasm. In the ultrasound lesion area (Fig. 6) the nuclei are a little swollen and the cytoplasm shows vacuolization and there is some depletion in the number of cells. However, in the thermal lesion area (Fig. 7) it is evident that the effect of the heat has been quite drastic, with an almost total depletion of nuclei. Since all of these sections were stained with gallocyanin, which reacts with nucleic acid, it would appear reasonable to conclude that in the case of the thermal lesion the nucleic acids were affected by the thermal energy. The Feulgen test, which is a specific for DNA, yielded a negative result indicating that essentially no DNA remained in the thermal lesion area.

Figure 8 shows a tissue section of the pituitary gland of the same cat just described, but the stain applied is Gomori. As indicated previously, after application of this stain, the acidophils appear red, and the basophils blue. The nuclei of the cells are also stained. If one now examines the thermal lesion area, which appeared almost devoid of cells when stained with gallocyanin, it is evident that there is a considerable cell population. In addition, in the upper area of the gland the extent of the ultrasound lesion is more clearly defined, although in the gallocyanin stain it was only just detectable. Figure 9 illustrates a magnified view of the normal or unirradiated region of this Gomori-stained tissue section, while Figures 10 and 11 respectively show at the same magnification the ultrasound lesion and thermal lesion regions. It is quite evident that in the ultrasound lesion area, the secretion granules are greatly depleted and those that are still present are not as deeply stained as previously. Many of these cells are undergoing degeneration, but the nuclei are present in the scattered acidophils. By contrast, for the thermal lesion, the secretion granules are now deeply stained but no nuclei are present. The blood vessels still contain blood elements.

Summarizing the data for this cat, it can be concluded that with a sacrifice time of 3 *days* and for the specific ultrasound dosage applied, it is possible to distinguish two types of lesions. In the "thermal" lesion, the nuclei are destroyed, while the cytoplasm appears intact. In the ultrasound lesion, the nuclei are relatively unaffected, while a large percentage of the secretion granules are depleted.

Figure 12 (gallocyanin stain) demonstrates a series of lesions, with normal pituitary tissue between the affected areas.[2] A greater dose of ultrasound was used than that applied to produce the lesions demonstrated in the previous figures, and the cat was sacrificed after a 7-day interval. If the lesions in this figure are compared with that of the previous gallocyanin-stained lesions, it is immediately evident that the destruction in the ultrasound lesion is much greater than in the previously illustrated ultrasound lesions. Further, there is no sharp demarcation between the thermal lesion and the ultrasound lesion. Figure 13 represents a Gomori stain of the same region shown in Figure 12, and here again it is evident that the destruction is extreme. One does not

[2] The torn area is a result of a mechanical break of the tissue due to poor fixation.

FIGURE 2

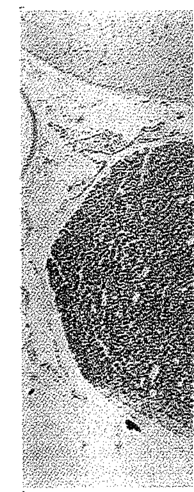


FIGURE 3

FIGURE 4

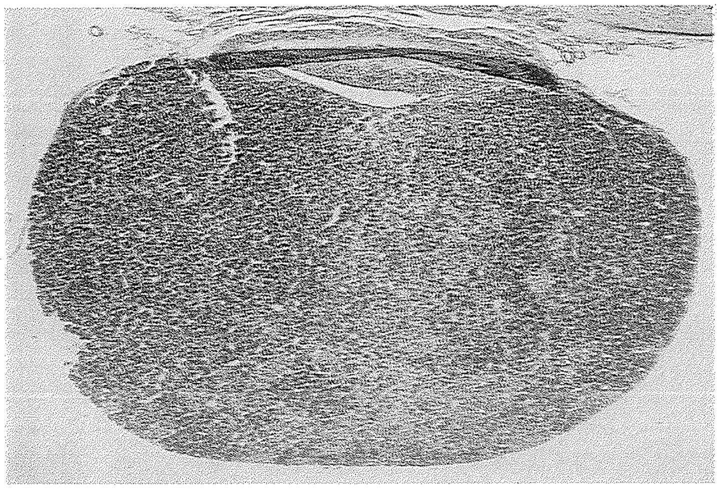


FIGURE 2

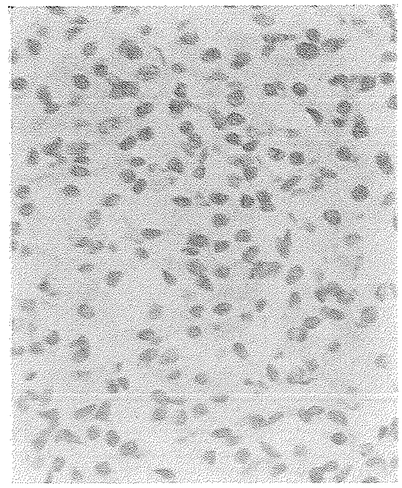


FIGURE 5

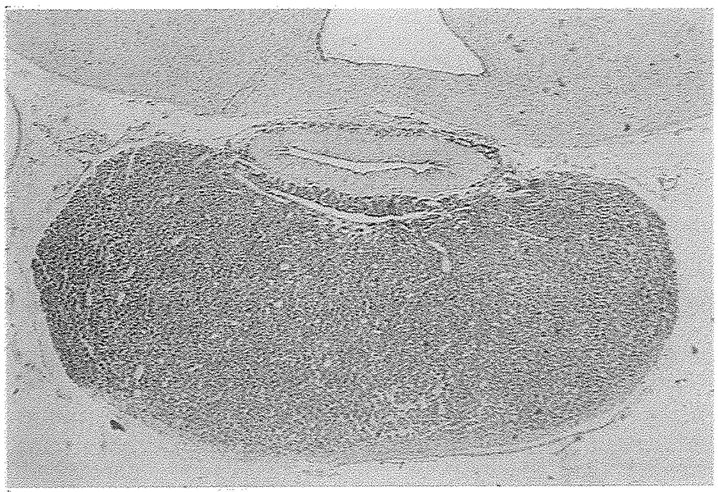


FIGURE 3

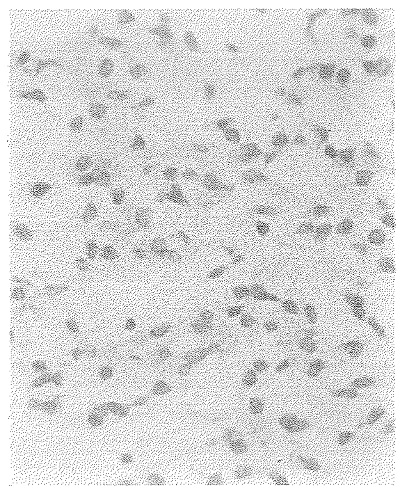


FIGURE 6

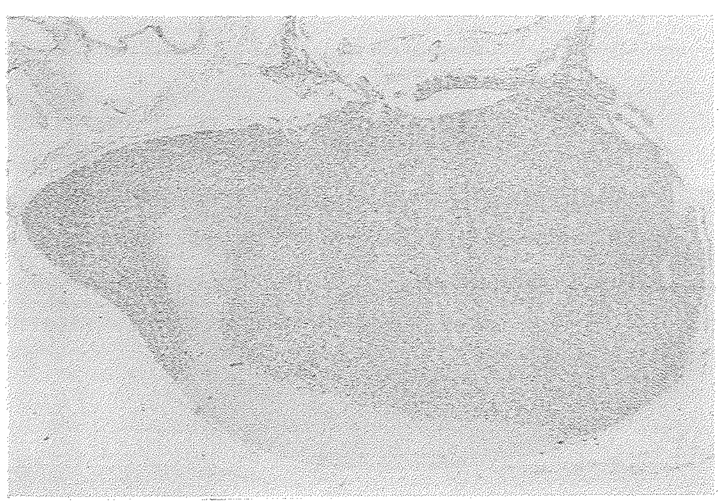


FIGURE 4

FIGURE 7

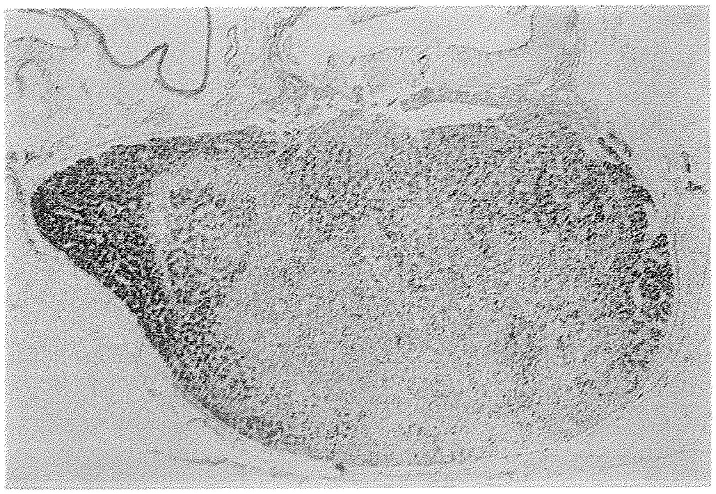


Figure 8

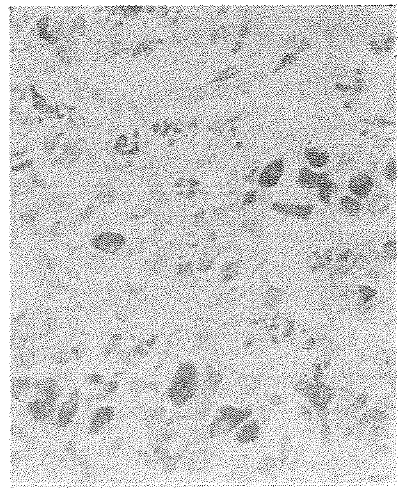


Figure 11


Figure 14


Figure 9

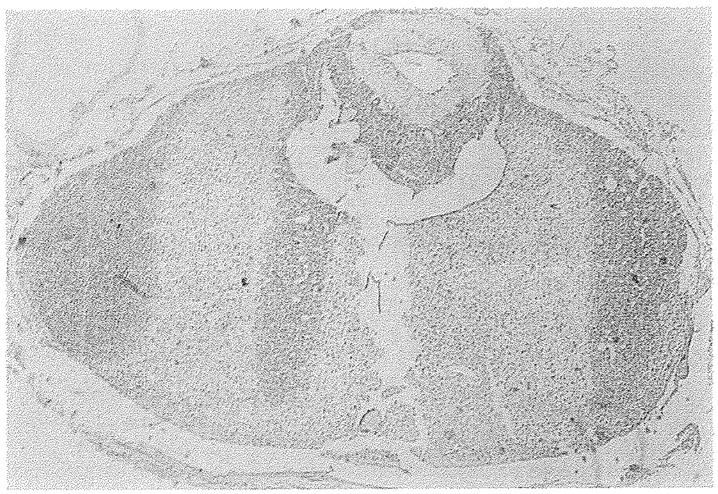


Figure 12

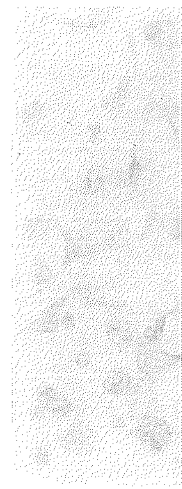
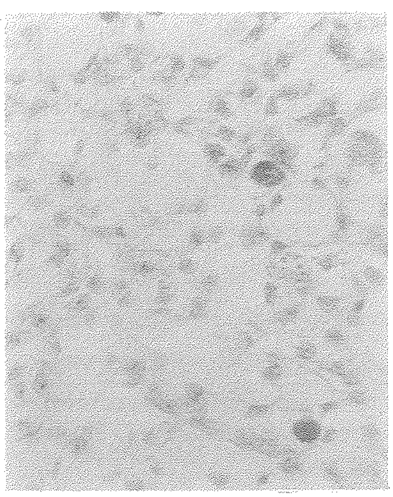


Figure 15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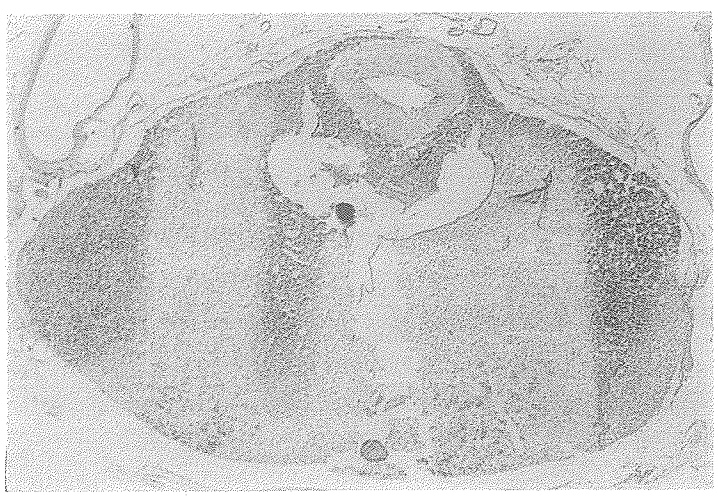


Figure 13

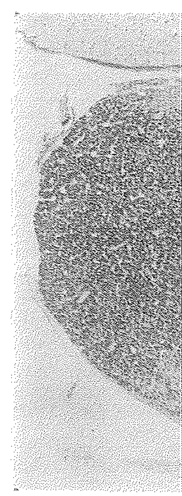
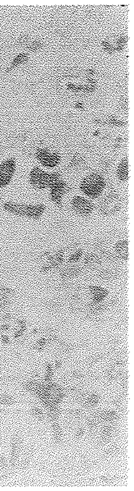
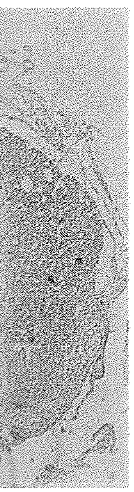
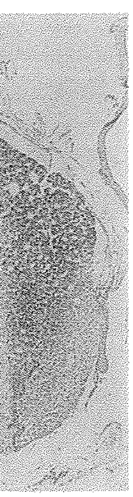


Figure 16







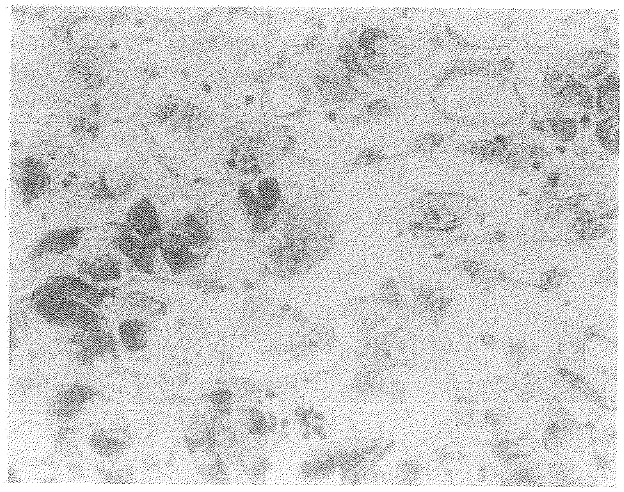


Figure 14

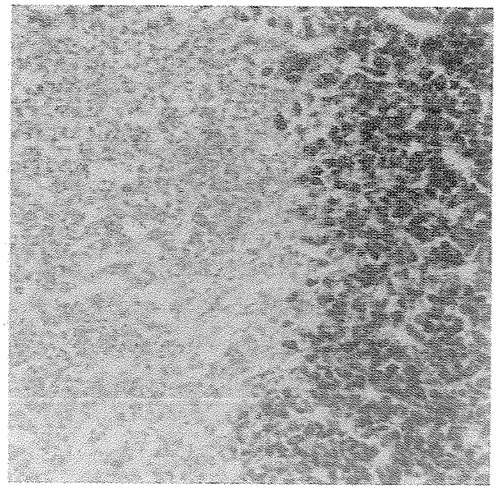


Figure 17

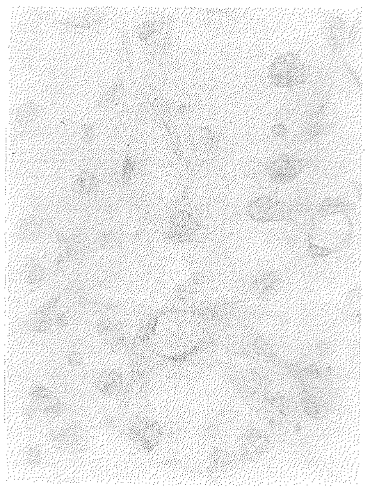


Figure 15

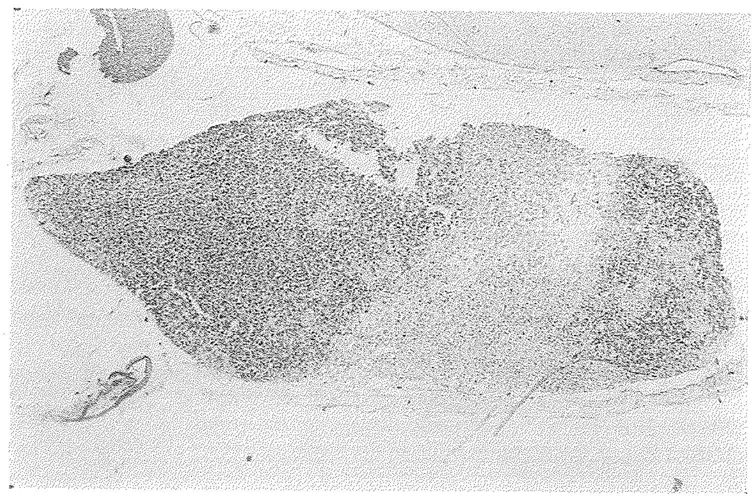


Figure 18

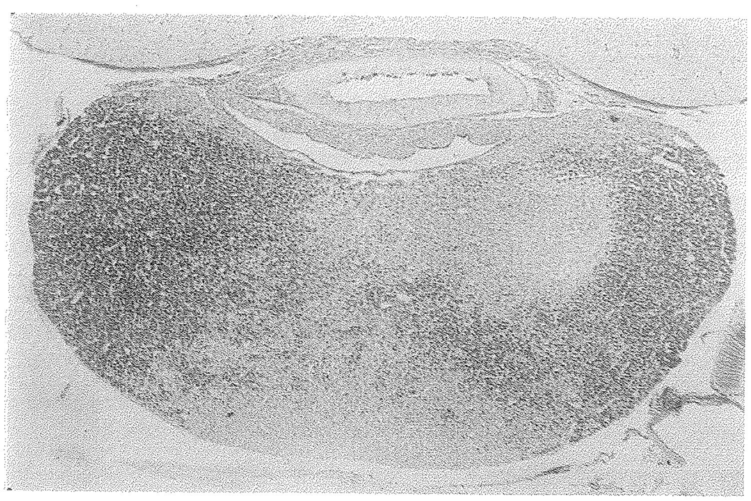


Figure 16

Figure 19

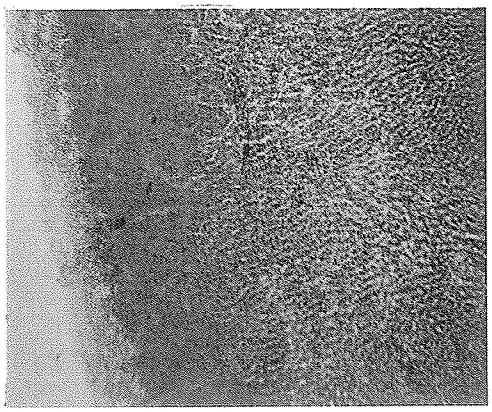


FIGURE 20

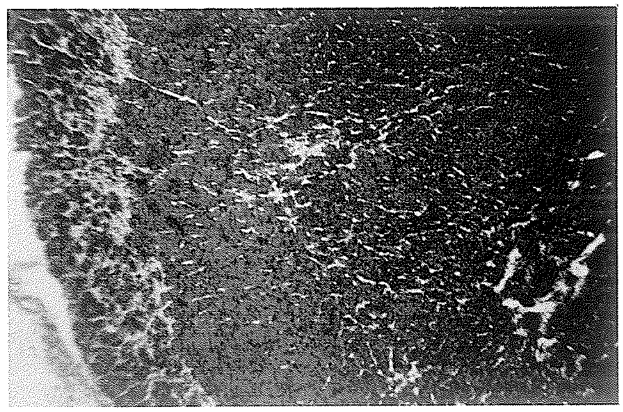


FIGURE 21

FIGURE 2. Transverse tissue section of anterior pituitary of normal cat – Gomori stain.

FIGURE 3. Transverse tissue section of anterior pituitary of normal cat – gallocyanin stain.

FIGURE 4. Transverse tissue section of anterior pituitary of cat sacrificed 3 days after ultrasonic irradiation of a portion of the gland – gallocyanin stain.

FIGURE 5. Magnified view of unirradiated region of anterior pituitary section shown in Figure 4 – gallocyanin stain.

FIGURE 6. Magnified view of ultrasonic lesion region of anterior pituitary section shown in Figure 4 – gallocyanin stain.

FIGURE 7. Magnified view of thermal lesion region of anterior pituitary section shown in Figure 4 – gallocyanin stain.

FIGURE 8. Transverse tissue section of anterior pituitary of same cat illustrated in Figure 4 – Gomori stain.

FIGURE 9. Magnified view of unirradiated region of anterior pituitary section shown in Figure 8 – Gomori stain.

FIGURE 10. Magnified view of ultrasound lesion region of anterior pituitary section shown in Figure 8 – Gomori stain.

FIGURE 11. Magnified view of thermal lesion region of anterior pituitary section shown in Figure 8 – Gomori stain.

FIGURE 12. Transverse tissue section of anterior pituitary of cat sacrificed 7 days after ultrasonic irradiation of separate regions of the gland – gallocyanin stain.

FIGURE 13. Transverse tissue section of anterior pituitary of same cat illustrated in Figure 12 – Gomori stain.

FIGURE 14. Magnified view of thermal lesion region of anterior pituitary section shown in Figure 13 – Gomori stain.

FIGURE 15. Magnified view of ultrasound lesion region of anterior pituitary section shown in Figure 13 – Gomori stain.

FIGURE 16. Transverse tissue section of anterior pituitary of cat sacrificed 21 days after ultrasonic irradiation of a portion of the gland – Gomori stain.

FIGURE 17. Magnified view of border between ultrasonically irradiated region and normal region of anterior pituitary section shown in Figure 16 – Gomori stain.

FIGURE 18. Transverse tissue section of anterior pituitary of cat sacrificed 3 months after ultrasonic irradiation of a portion of the gland – Gomori stain.

FIGURE 19. Magnified view of ultrasound lesion region of anterior pituitary section shown in Figure 18 – Gomori stain.

FIGURE 20. Tissue section of adrenal gland of normal cat – sudan III stain.

FIGURE 21. Tissue section of adrenal gland of cat sacrificed 3 months after ultrasonic irradiation of a portion of the anterior pituitary – sudan III stain.

see intact secretion granules in the ultrasound lesion as was evident in Figure 8. At the base of the slide, however, some secretion cells are evident. Figure 14 is a magnified view of the base region. The secretion cells are quite prominent but they do not exhibit nuclei. Also evident are the blood cells and the fibrous reticulum. Figure 15 shows a magnified view of the ultrasound lesion. The majority of the gland cells are gone, and a high macrophagic activity is underway. In some regions, free debris, fragments of cytoplasm and cell nuclei are stained.

The ultrasound lesions demonstrated in the upper areas of Figures 12 through 15 are due to the ultrasonic energy per se and are not complicated by extraneous heating, for example, heat transfer from bone. However, these lesions differ considerably from the ultrasound lesions demonstrated in Figures 4 through 11. It is evident, therefore, that depending on the dosage, ultrasound may produce a variety of effects on the cellular structure of the anterior pituitary gland, ranging from partial depopulation to complete destruction.

The sacrifice times (interval between irradiation and sacrifice) for the two experimental cats discussed above were comparatively short (3 and 7 days). Figure 16 demonstrates a high-intensity ultrasound lesion in which the sacrifice time was considerably longer, namely, 21 days. The predominantly blue stain of the lesion region indicates a general absence of acidophil cells since a Gomori stain was applied (compare with Fig. 2). A region of particular interest is the upper right-hand area, since in a normal cat the acidophil population would predominate in this area (2). Figure 17 is a magnified view of part of this area which includes the boundary between the unirradiated and the lesion zones. The absence of acidophil cells within the lesion area is quite striking. Preliminary evidence indicates that the basophils are regenerated cells. The same type of result, namely, the predominance of basophils in the region of the lesion was observed in cats sacrificed 30 days and 3 months after irradiation. For example, Figure 18 shows a Gomori-stained transverse tissue section of a pituitary of a cat sacrificed 3 months after irradiation. Again, the lesion region is stained predominantly blue, and a magnified view of this region (Fig. 19) indicates almost a complete absence of acidophil cells. If ultrasonic energy can be applied to selectively depress the acidophil population, the results would be of tremendous medical significance since the acidophils are associated with the production of growth hormone.

In addition to the histological studies of the pituitary glands of the irradiated cats, such studies were made on the adrenals, thyroids, and ovaries. A number of physiological tests were made on the animals during the intervals following irradiation. These latter included a study of the effects of the pituitary lesions on: (1) estrus cycle — as indicated by the vaginal smear test, (2) thyroid metabolism — as indicated by the cholesterol levels in the blood, and (3) sugar metabolism — as indicated by blood sugar levels and insulin tolerance levels. All of these studies are still in the preliminary stages so the results will not be discussed in detail at this time. However, it is of some interest to indicate here the results of some of these tests on the cat whose pituitary sections show a predominance of basophil cells in the lesion region 3 months after irradiation. The plasma cholesterol levels and the volume of urine output were normal. The histological studies of the thyroid and ovaries did not indicate any gross changes, except for an abnormal flatness of the epithelial cells of the thyroid and an indication of abnormally enlarged follicles in the ovary. The histological changes in the adrenal gland, however, were more drastic. Figure 20 shows a tissue section of normal adrenal gland stained for fat distribution with Sudan III. The various layers of the adrenal can be clearly distinguished, namely, the capsule on the outer edge, the glomerulosa zone, and the outer and inner fasciculata zones. The red stain represents fat deposits. Figure 21 illustrates a tissue section of the adrenal of the cat sacrificed 3 months after irradiation. It is quite apparent that considerable change has taken place in the amount and distribution of fat. The same layers are evident, the capsule, the glomerulosa, and the fasciculata (the blue area in the center is a portion of the medulla), but the most striking change is the tremendous increase in fat in the fasciculata zone. The fat is

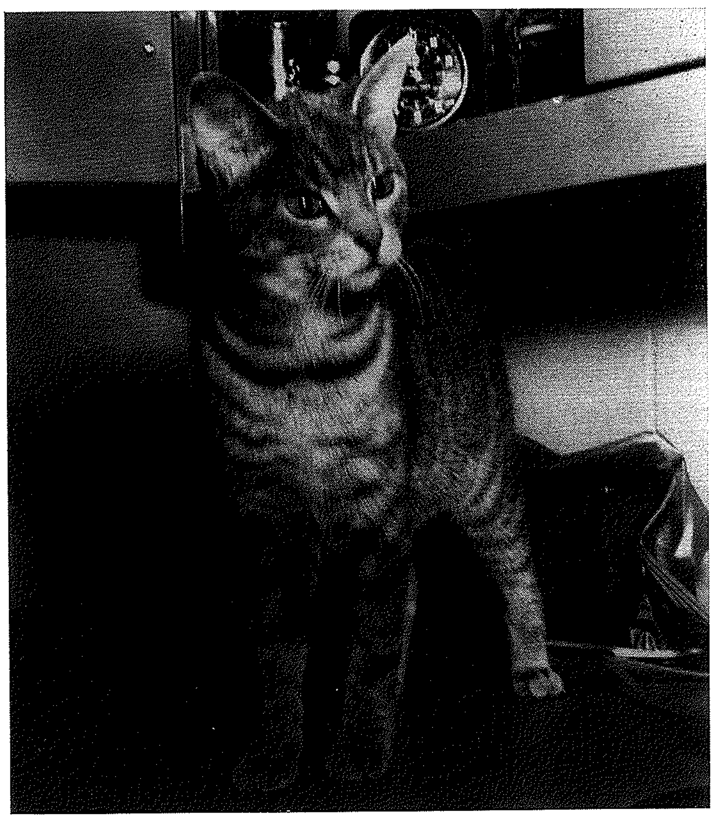


FIGURE 22. Photograph of cat which exhibited abnormal fat distribution within the fasciculata zone of the adrenal gland (see Fig. 21) following ultrasonic irradiation of a portion of the anterior pituitary. Picture taken on day of sacrifice, 3 months following the irradiation.

now heavily distributed over both areas of this zone and is also present in the glomerulosa. There is also an increase of connective tissue.

It is of interest to note that, in general, the cats appeared undisturbed by the pituitary irradiation after their initial recovery from the operative procedure. For example, Figure 22 is a picture of the cat that exhibited the abnormal fat distribution in the adrenal glands. This picture was taken on the day of sacrifice, and the cat was alert and healthy.

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## DISCUSSION

Dr. Carstensen: The information on regeneration of basophil cells would appear to be of great significance. Can you distinguish the regenerated area quite clearly?

Dr. Kelly: In the long-term cats, such as those sacrificed after 1- and 3-month intervals, the regeneration appears quite distinct. Connective tissue growth and basophil regeneration can be seen in the lesion area. In the case of cats sacrificed after shorter time intervals, such as 10 and 14 days, mitotic activity is evident in the lesion area.

Dr. Gordon: Ultrasound has been used previously to irradiate the anterior pituitary of carcinoma patients. I am a radiologist and, therefore, not intimately concerned with the results of such studies, but it is my general impression that this method has had some clinical success.

Dr. Kelly: Evidently the studies to which you are referring are concerned with complete destruction of the pituitary gland by ultrasound, that is, a hypophysectomy. It is relatively simple to perform ultrasonic hypophysectomies on experimental animals. The approach in this study, however, is to use the ultrasound to produce selective effects on the cellular components rather than to accomplish simple destruction.

Dr. Weissler: It is interesting that the pathology of the thermal lesion is different than that of the ultrasound lesion. Do you know what the relative temperatures were in the thermal lesion area and in the ultrasound lesion area?

Dr. Kelly: We did not make any temperature measurements. However, in previous studies at the Biophysical Research Laboratory concerned with the use of ultrasound for investigations of the central nervous system, temperature measurements were made in the irradiation area. Perhaps Professor Fry would care to comment on the result of those studies.

Dr. W. J. Fry: In the brain, for temperature rises of 18°C in white matter and 10°C in gray matter, we did not produce thermal lesions, although the usual ultrasonic selective lesion effects were produced. I would guess, therefore, that the temperature rise in the thermal lesion areas of the pituitary were certainly more than 20°C.

DR. CURTIS. Are these pituitary lesions the result of both heat and ultrasound, that is, are they combined lesions?

DR. KELLY: The lesions in the base, which we have called thermal lesions, are certainly dependent on the combined effects of direct heat transfer from the bone and ultrasound. It is convenient to distinguish them as thermal lesions since the lesions in the upper area are not complicated by direct heating, although heating is also involved. The essential point is that the effects on the cellular population for the two types of lesions are quite different. Of course, the effects are dependent also on the dose of ultrasound. With sufficient intensity, complete destruction can take place in the ultrasound lesion area.

DR. KIKUCHI: You could have avoided the problem of heat transfer from the bone by controlling the doses so that there was time for the heat to dissipate.

DR. KELLY: We knew that the heating of the bone could be avoided, but we did not make any attempts in that direction because we found it of interest to compare the histological results for the so-called thermal lesions and ultrasound lesions.