all the flasks, 15 mg of desoxycorticosterone was added. Desoxycorticosterone in 150-mg quantity was dissolved in 33.4 ml propylene glycol and 16.6 ml distilled water. Autoclaving readily dissolved the steroid and sterilized it. Five ml was added to each flask.

The adrenal glands of cats, dogs, rats, guinea pigs, and chickens were removed under sterile technique while the animals were under ether anesthesia. Half a human adrenal from a case of Cushing syndrome removed by operation was also tested. Immediately following the removal, with the least lapse of time and manipulation, the glands were sliced with a sharp razor blade into 3 or 4 longitudinal slices, and placed in the flasks containing the medium and ingredients. To prevent bacterial contamination, 10,000 or 50,000 units of penicillin G were added. The flasks were incubated at 37° C, and every 48 hr the medium was replaced by freshly made broth and ingredients, on three occasions.

In another series of experiments, slices of the kidney, liver, ovary, testis, cardiac muscle, striped muscle, spleen, lung, brain, thyroid, bone marrow, and pancreas of the cat, dog, and rat, as well as human placenta and human prostate, were incubated in broth containing 15 mg desoxycorticosterone, 100 mg ascorbic acid, 25 mg thiamine, 5 mg pyridoxine, 5 mg riboflavin, 5 mg nicotinic acid, and 5 units of insulin. The broth in the flasks with the glandular tissue was kept sterile by the addition of penicillin G. The medium was changed every 48 hr on three occasions by substituting freshly made medium.

Fresh adrenals, kidneys, testis, and human placenta were separately tested for the presence of cortisone. The glandular tissue was ground up with glass sand in a mortar and extracted with either 20% trichloracetic acid or *N* HCl, or both, for 25 hr at 37° C. It was then extracted with ether, *N*/10, NaOH and then *N* HCl. This neutral extract was then tested for the presence of cortisone by paper chromatography.

Each sample of broth obtained from the culture flasks every 48 hr was tested for the presence of cortisone. Samples of the adrenal gland were removed at 24-hr intervals throughout the period of incubation and preserved in 10% formalin for histological studies. The broth was first adjusted to pH 1 with *N* HCl. The proteins were then precipitated with 20% trichloracetic acid. The supernatant and the precipitate were then extracted separately with ether, and then the extracts were combined, washed with *N*/10 NaOH and then *N* HCl, and finally evaporated to about 5 ml. The extracts were then tested individually for the presence of cortisone by the propylene-toluene chromatography method at the end of 72 hr, by spraying with 5% potassium iodide and 0.3% iodine solution.

The following results were obtained in these experiments:

1. Extracts of adrenals, liver, kidneys, testis, and human placenta were negative for cortisone. Table 1 shows the result of incubating various tissues with desoxycorticosterone, vitamins, and insulin.

2. Incubation of adrenal in media containing desoxycorticosterone, insulin, ascorbic acid, thiamine, pyridoxine, riboflavin, and nicotinic acid gave the most constant and potent paper chromatography test. Most of the positives were obtained in the second sample of broth. It was almost always negative in the first 48 hr of incubation. Positive results were also obtained in the third sample of broth.

3. Desoxycorticosterone plus adrenal gland and insulin gave the second highest positive; vitamin B complex was next, followed by ascorbic acid, although sometimes simple incubation of adrenals with desoxycorticosterone also yielded positive results.

4. When desoxycorticosterone and adrenal gland were incubated with glutathione, or with glutathione, insulin, ascorbic acid, and vitamin B complex, the results were consistently negative.

5. Positive chromatograms were obtained in a third of the experiments where the liver or testis was incubated with desoxycorticosterone, insulin, ascorbic acid and thiamine, riboflavin, pyridoxine, and nicotinic acid. The kidney and, to a lesser extent, the ovary also gave positive chromatograms.

6. All the other tissues tested gave negative results.

7. The adrenals of the cat and man (one case tested) gave the highest positives, followed by those of the dog, rat, and guinea pig, in the order cited, whereas the adrenal of the chicken was always negative.

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On the Detection of Intracranial Pathology by Ultrasound[1]

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This report deals with the initial progress of a long-range program on the application of ultrasonic techniques to medical problems (*1*). An immediate goal is the detection and localization of intracranial tumors and cerebral anatomic abnormalities.

There are two basic methods of using ultrasound for diagnosis. One uses echoes reflected from interfaces within an object, the other utilizes selective transmission through an object. In either method the useful range of vibration frequency appears to be of the order of a

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few megacycles per second, and ultrasound in this range is conveniently generated and detected by piezoelectric crystals. A comparative study of these two approaches has led us to investigate first the transmission method.

The rationale for attempting to employ such a technique for the detection of intracranial lesions is based on the following facts: When a beam of ultrasound is sent through a portion of tissue, the amount of energy that arrives at a receiver is influenced by absorption along the path, by refraction and scattering, and by reflections at any intervening interfaces. These acoustic variables in turn are determined by such physical properties of tissue as density, elasticity, homogeneity, and viscosity.

The influence of these different properties of living matter on high-frequency sound waves is not generally the same as on other forms of physical energy, such as the electromagnetic radiation of x-rays. The attenuation of ultrasound as it traverses the fluid-filled ventricles is less than that through cerebral tissue. Utilizing this property, Dussik *et al.* (*2*) have obtained ultrasonic ventriculograms on a large number of patients. The purpose of our work is to refine and extend this method, and to study the basic physical and physiological problems involved.

At present x-ray evidence of distortion of the ventricular system is of great value in the diagnosis of neurological disorders. To obtain this information, however, the ventricular fluid must be replaced by air. A method that would yield substantially the same information, but without the necessity for air injection, would constitute a distinct improvement over present techniques.

It has also been shown that ultrasound may produce tissue damage. The skin, for example, will be injured by a minimum intensity of 5 w/cm² peak, if continuous irradiation is applied to a given area for 10 min at a frequency of 800 kc (*3*). Pain, which seems to come from deep within the tissues and is presumed to originate at the periosteum, will occur when an intensity of 2 w/cm² peak is applied for 40 sec at this same frequency (*4*). Below 1.8 w/cm² no pain is caused, irrespective of the duration of irradiation. The Dussiks were able to record a received signal from a beam of transcranially transmitted ultrasound the intensity of which at the transmitter was about 1 w/cm². They report no evidence of brain damage from the use of this intensity on more than 200 patients. On a theoretical basis this seems likely, because ultrasound at a frequency of 800 kc is attenuated about 30 db as it travels through scalp, subcutaneous tissue, and skull; the intensity that reaches the first cortical layer of the brain is so low that, according to all available evidence, it would be incapable of damaging living tissue, regardless of the duration of application.

Nevertheless, we have been unable to find experimental evidence as to the maximum intensity and duration of application that can be applied to a given area of nervous tissue without producing interruption of function (*5*). We are in the process of investigating this problem and to date have irradiated transdermally 2 dogs, a cat, and

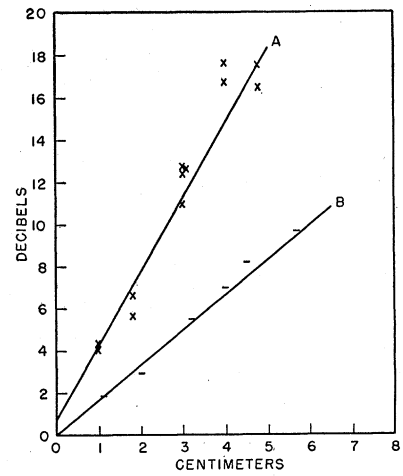

FIG. 1. Attenuation measurements in fresh hog-brain: *A* through whole brain including cortical convolutions; *B*, through blocks of white matter.

2 human subjects. The 2 dogs were exposed to ultrasound at a frequency of 2.4 Mc. In one, an intensity of 3 w/cm² was applied for 11.5 min; in the other, an intensity of 1.5 w/cm² was applied for 15 min. In neither animal was histological evidence of brain damage found.

The cat was exposed to a frequency of 800 kc at an intensity of 15 w/cm² for 5 min. Electroencephalographic recordings taken during the period of radiation showed no pathological changes. The animal suffered superficial scalp necrosis but showed no evidence of neurological deficit.

The 2 human subjects were also irradiated by placing the 800-kc transmitter in contact with the scalp and using electroencephalographic control. Peak intensities of the order of 5 w/cm² (2 w/cm² average over the irradiated surface) for periods up to 9 sec were sufficient to cause moderate scalp pain but did not alter the pattern of the EEG.

Since the success of the transmission method depends basically on whether there is sufficient difference in the attenuation of ultrasound as it passes through cerebral tissue with and without ventricle, measurements of attenuation in tissue, including brain, have been made with frequencies of 1.25 Mc and 2.5 Mc (*6*). Fig. 1 shows the attenuation of fresh hog brain as a function of sample thickness. Curve *A* represents measurements on whole brain, curve *B* measurements on blocks of brain tissue containing white matter only. Average attenuation in *A* is 4 db/cm, and in *B* it is 1.7 db/cm. Measurements on human brain are in agreement with these findings. Ultrasound at these frequencies is attenuated about 50 db/cm by the bones of the skull.

The interposition of the ventricular system in the path of an ultrasonic "beam" that is scanning the brain increases the energy received by amounts up to 20 db (a factor of 10 in voltage). This differential is more than sufficient to indicate the position of the ventricles.

In the apparatus used for these measurements, a pencil-

shaped ultrasonic beam is generated by a barium titanate transducer, which is driven by a stable c-w transmitter, modulated with 1,000 cps. This transducer has a rectangular aperture of 1 cm² and is mounted in a water tank opposite a receiver crystal (quartz) of variable aperture. The test object is supported between the 2 probes. The received signal is amplified, demodulated, and fed into a sound level recorder.

In one of our experiments a formalin-fixed brain was scanned by the ultrasonic beam. The line of scan was marked by small lead pellets, the ventricular system injected with diodrast, and a lateral radiograph obtained. Fig. 2 shows the degree of correlation achieved between

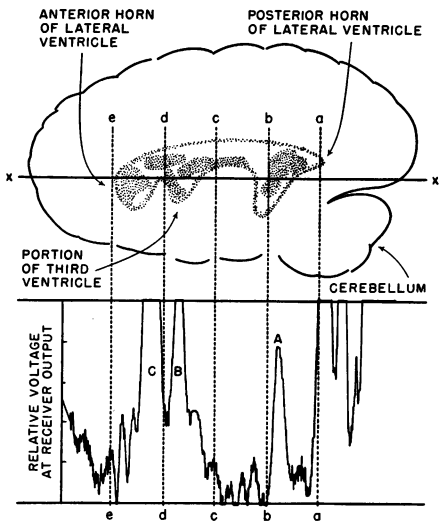

FIG. 2. Correlation between x-ray ventriculogram and utlrasonogram.

the two methods. The upper half of the illustration is a drawing of the brain specimen, with shading of those areas of the ventricular system most capable of being filled by diodrast. The line x-x is the line of scan, and the lower half of the figure is a reproduction of the sound level recording. At first the received signal is high in consequence of the decreased path through the tips of the frontal lobes. As they begin to increase in size, signal strength diminishes until the anterior horns of the ventricles are reached at e. The two peaks C and B on the ultrasonogram correspond to the shaded areas to the left and right of point d. The strong signal at C indicates the position of the largest portion of the anterior horns of the ventricles. Decreased signal strength appears at c and b as the sound beam passes out of the ventricles. At A another strong signal appears as the posterior portion of the lateral ventricles is partially traversed. To the right of a the signal strength again increases because of the decreasing path through the occipital lobes. In transcranial scanning this is compensated for by a greater energy loss resulting from oblique incidence of the rays as they strike the frontal and occipital regions of the skull.

Fig. 3 is a record taken on a living human subject in

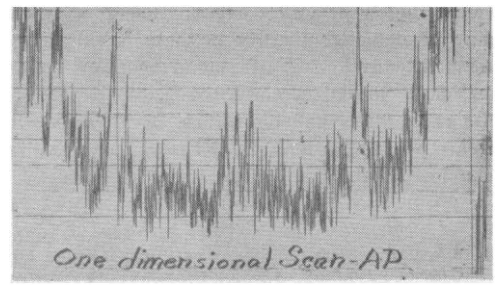

FIG. 3. Recording of live subject made in the unmodified tank, showing masking effect of reverberation.

an anterior-posterior direction. Symmetrical peaks that are believed to be significant appear on either half of the record. There is, however, a considerable amount of background "noise" from reverberant sound, and the attenuation caused by the skull is sufficient in some cases to bring the received signal down to levels below the reverberant intensity. Therefore, the tank had to be subdivided into 2 compartments sonically insulated from each other by the head. The new arrangement shown in Fig. 4 prevents leakage of reverberation from the transmitter compartment to the receiver compartment, both of which are lined with absorbent material to damp the undesired waves.

A simple recording device has been added to this apparatus in order to produce a contrast recording of a two-dimensional scan of the brain. We have been able to portray crudely the position of the ventricular systems of 3 normal subjects using this apparatus. No pain or other untoward effect has been produced. The intensity employed was less than 1 w/cm² at a frequency of 2.5 Mc.

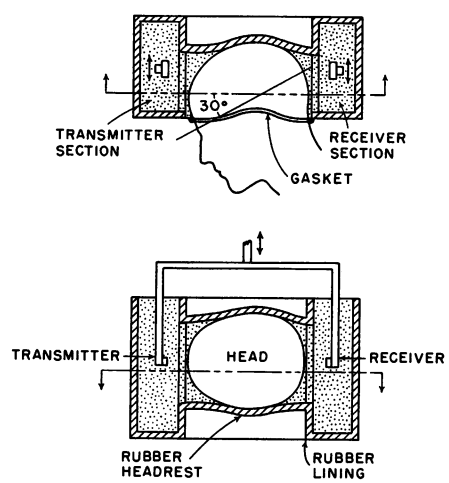

FIG. 4. Schematic diagram of tank for scanning brain of live subjects.

These investigations have demonstrated that an ultrasonic transmission method can yield ventriculograms without air injection by utilizing a level of intensity below any known threshold of pain or damage. The possibility also exists that types of brain tumors which exhibit

a sufficient degree of either calcification or cyst formation may be detected directly if there is sufficient difference in attenuation of ultrasound by these tumors in comparison to normal cerebral tissue. To establish the ultimate capabilities and limitations of ultrasonic methods, we are pursuing a number of fundamental studies on the behavior of ultrasound in biological tissues.

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A Slicer for Sampling Liquids[1]

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A mechanical slicer has been developed for sampling various levels of liquids contained in plastic tubes. This technique obtains small, accurately determined samples, with a minimum of stirring, and with high volumetric recovery.

Fig. 1 presents two views of such a slicer designed for ½-in. diameter plastic tubes of 8.2-cc capacity used in a quantity ultracentrifuge. The plastic tube *T*, filled with liquid to be sampled, is held by clamps *C* in plastic plates *P* and *Q* just above and below the desired level of division. A very thin (.007-in.) knife blade *B* made from razor blade stock[3] is carried in a rigid frame *F*, which slides in ways *W* machined in the plates. This frame is moved by a lever *L*. The sharpened edge of the blade cuts the tube wall, and the flat body of the blade serves as a sealing partition between the upper and lower levels of liquid. The plates are held together by two compressed springs *CS* adjusted to prevent leakage of the liquid above the blade. The upper liquid sample is removed with a hypodermic syringe or pipette. The clamps are loosened, the top section of the plastic tube is removed, and the blade withdrawn. A screw drive *D*, carrying an indicator *I*, which reads against a fixed scale *S*, raises the remaining portion of the tube to the level of the next slice, and the entire operation is repeated.

In order to prevent loss of liquid because of capillary action if surfaces become wet, a nonwetting grease is spread over the knife blade and other parts near the tube. We have used a mixture of 3 parts vaseline to 1 part mineral oil for this purpose, although probably a silicone grease is preferable. Since such greases are almost insoluble and chemically inert, this procedure will seldom affect subsequent analyses of the samples. Grease cups

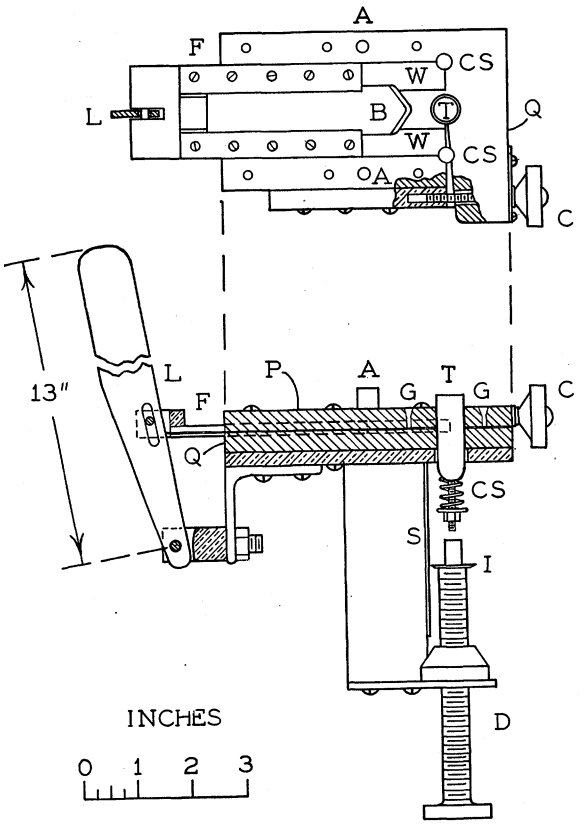

FIG. 1.

G, tapered to fit a hypodermic syringe, permit adding grease at any time.

For stability, the device is held in a heavy vise or bolted to a workbench. None of the dimensions is critical save that the plates must bear against the blade to insure proper slicing. In our instruments, each of the springs exerts a force of about 15 pounds. Our plates are made from ⅜-in. Plexiglas, with tapered pins *A* to insure alignment.

Using this technique to sample groups of three 8.2-cc tubes of ultracentrifuged serum, we recover about 92% of the liquid when dividing each tube into 10 samples. Part of the volume lost remains on the walls of the plastic tube and part in the 10 hypodermic syringes used. These residues are increased by the high viscosities in the lower regions of each tube, where we find 30g% of protein. Sampling similar tubes filled with water and using only one syringe, we recover 99% or more of the volume.

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[2] We wish to express our appreciation to R. H. Turner and his group for the suggestion of this device and for encouragement during its development.

[3] We are grateful to Howard Gambrill, Jr., of the Gillette Razor Blade Company, Boston, Mass., for this stock.