SELECTIVE ACTION OF ULTRASOUND ON NERVE TISSUE*

William J. Fry and John W. Barnard
Bioacoustics Laboratory
University of Illinois
Urbana, Illinois

Abstract

A description of the ultrasonic method of producing selective accurately localized lesions in central nervous systems is presented. The apparatus and technique are briefly described. Results of a study of ultrasonically produced lesions of the internal capsule of cats are discussed. The present state of our knowledge of the physical factors involved in the fundamental mechanism is outlined.

A focused beam of ultrasound can be used to produce selective, accurately localized lesions in central nervous systems which are quantitatively reproducible from one animal to another. Discrete lesions can be produced without destruction of blood vessels. A lesion in the depths of the brain can be effected without disturbance of the intervening tissue. Accurate localization is accomplished by focusing a fine beam of ultrasound in the region to be treated. Beam widths of two or three millimeters are readily obtained. The nature of the destruction depends upon the intensity and duration of the exposure. Dosage studies made on a series of animals are used as a guide in choosing the conditions of irradiation for structural and functional studies. Most of the work up to the present time has been accomplished at a sound frequency of 1 mc. The sound is generated by a vibrating X-cut quartz crystal excited in thickness mode. The sound is focused by a polystyrene lens placed in front of the crystal. Transmission of the sound from the irradiator to the tissue is accomplished through physiological saline. This liquid must be degassed to prevent the formation of bubbles which would interfere with the transmission of acoustic energy at the high levels used in these experiments. Sound intensities up to 1000 watts/cm^2 have been used in some of the studies. The bone overlying the region of the brain through which the sound is to enter

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the central nervous system must be removed in order to prevent overheating and to insure a well defined focused beam in the brain tissue. This implies that a sterile surgical procedure must be used throughout if the animal is to be kept for a period of time after irradiation for functional and behavior studies. For producing fine lesions we have used a four beam focussing irradiator of the type illustrated in Figure 1. Focussed beams from each of the four transducer heads are adjusted so that the focal regions are brought into coincidence. The animal is supported in a standard stereotaxic apparatus. The head is held rigidly in position in the machine. Localization of the focal spot in the region of the brain to be affected is determined as follows: Before the animal is placed in the apparatus a pointer attached to the irraditor and coinciding with the focal spot of the irradiator is positioned on the midline of the apparatus and is collinear with the line through the bars which support the skull of the animal at the ears. This point together with a plane determined by the stereotaxic machine is used as a reference system for mapping the position of structures in the brain by neuroanatomists. The coordinate values on the system which supports the four beam irradiator are read corresponding to this ear bar zero. One can then transform the coordinates from the system as used by the neuroanatomist to the coordinates of the system supporting the irradiator. The pointer is then removed from the irradiator and the animal is mounted in the stereotaxic machine. The necessary surgery is performed and irradiation can proceed. In practice, a flanged cap which is used to support the physiological saline, which acts as a transmitting medium for the sound, is tied to the skin of the animal. Figure 2 shows the animal mounted in the apparatus ready for irradiation with the irradiator supported over the head of the animal. Figure 3 shows a close-up view of the multibeam instrument positioned over the head of the animal. The cortex was exposed in this particular experiment. In general, it is not necessary to open the dura unless visual observation of the surface brain pattern is desired. Previous to preparing the animal, one determines the output of the transducer as a function of the driving voltage using an acoustic probe which has been previously calibrated on an absolute basis. The driving voltage corresponding to a desired sound level is then determined. For a specific irradiation one adjusts (1) the driving voltage across the crystal, (2) the pulse time and (3) the position of the coordinate system supporting the head to produce a lesion of the desired

shape and size in the central nervous system. One lays down a rectangular pattern of the shape desired with shots 0.5 mm between centers. Usually one minute intervals are allowed between adjacent shots. The pulse time for each shot has been in the range from 0.5 second to 10 seconds for most of the work. The second intensities used are in the range from 50 watts/cm^2 to 1000 watts/cm^2.

The type of accurately localized lesion which has been produced within the depths of the mammalian brain by a focussed beam of ultrasound is shown in Figures 4 through 7. A series of cats were irradiated in the region of the internal capsule and thalamus in transverse planes from 9 to 11 mm in front of the ears. The internal capsule consists entirely of nerve fibers, including among others the nerve fibers from the motor cortex, located in the front end of the brain, which terminate in the neighborhood of the motor cells of the spinal cord. The internal capsule of these cats is illustrated at a plane of irradiation in Figure 4. A series of animals were irradiated for 4.00 sec at 210 watts/cm^2 with a rectangular array of shots, the array being 7.5 mm laterally by 2 mm longitudinally. The animals were observed after the irradiation for functional disturbances. Characteristic changes were produced in the placing reactions of the limbs, that is, changes in such things as the cortical reflex response to touching of the ankle to the edge of a table. Such changes are indicative of damage to the motor pathway. The cats were sacrificed at 1 day, 3 days, 12 days, and 30 days after irradiation to study the time course of the effects. They were perfused through the arterial system with formalin at the time of sacrifice and their brains removed. Degeneration of the pathway from the motor cortex to the spinal cord was studied by Marchi preparations. This staining technique shows the degenerating fibers as black dots on a yellow background (Figure 5). Tissue sections in the irradiated region were stained with thionine or hemotoxylin and eosin which are especially useful for observing changes in cell bodies and blood vessels. Figures 4, 6, and 7 illustrate this study. One day after irradiation there is no observable change in the tissue as observed in the microscope.* Sound exerts at this dosage no direct effect on blood vessels. The primary action of the sound is probably not a destructive effect upon the gross cell membrane but is a disturbance at a submicroscopic

* Further investigations have shown that histological changes may be observed within 10 min. after exposure to ultrasound.

level. At 3 days leucocytes begin to appear indicating reaction to tissue damage. Figure 6 shows the region of the lesion under low power magnification. In animals sacrificed at twelve days phagocytes are engaged in removing the debris in the damaged area. At 30 days this process is continuing and scar tissue formation has started (Figure 4). At this dosage the nerve cell bodies of the thalamus seem relatively immune to effects by the sound but the fibers of the internal capsule are irreversibly affected. This procedure makes feasible the severing of deep fiber bundles in the central nervous system in primates or man with the following considerable advantages over surgical techniques: 1. No disturbance to brain tissue except in the focal region. 2. No cutting of any brain tissue. 3. No disturbance to the blood vessels. 4. No opening of the dura.

Physical factors which experience changes during acoustic propagation and which must be considered in any study of the fundamental mechanism of the action of the sound on the tissue were investigated. The frog was used as the biological test object for these studies. Conclusions reached here may not necessarily apply unchanged to mammals. The region of the spinal cord containing the motor cells which control the hind legs was irradiated. The end-point used was paralysis of the hind legs.

Temperature changes which occur when ultrasound is propagated through tissue may produce biological effects in the following ways: A. The temperature of the tissue exceeds some value above which damage occurs. B. High temperatures exist at interfaces in the tissue which are not detectable by a small thermocouple. C. Temperature changes associated with cavitation are responsible. D. Heating which occurs at gas nuclei in the tissue is the cause. A number of independent arguments are presented against the possibility that the temperature of the tissue exceeds a critical value above which damage occurs. First, experiments on cooled frogs show that ultrasound produces effects on nerve tissue when the temperature level in the cord is less than 20^{o} C. Second, summation of the biological effects in nerve tissue produced by sound pulses separated by periods of time

long compared to the interval required for the tissue to return to its initial temperature proves that paralysis is not dependent upon achieving any particular temperature level. Third, on the basis of the experimentally determined linear relation between reciprocal of minimum paralysis time and acoustic pressure amplitude it appears that the absorption of a constant amount of acoustic energy by the tissue is not required to produce the effect. Consider now the second possible manner in which temperature might be involved, i.e., through heating at interfaces. A relatively simple calculation proves that such an hypothesis is untenable. For example, assume that all the sound that is absorbed is absorbed at interfaces and consider a volume of material consisting of cells of a diameter of 10 microns. If we subject such a material to a sound intensity as high as a 100 watts/cm^2 choosing values for the physical parameters of the material those characteristic of nerve tissue we obtain for the maximum temperature rise at the interfaces a value of about 0.001^{o} C. Another possible manner in which temperature might produce biological effects is, as indicated above, in association with cavitation defined here to include both the process of cavity formation and collapse in the tissue and the growth and collapse of a gas nucleus in the tissue under alternate tension and compression forces. It has been shown that the biological effects of ultrasound on the tissue of the central nervous system (frog spinal cord) are the same whether the exposure is made under a hydrostatic pressure of one atmosphere or under a pressure sufficiently high to insure that no tension forces exist in the biological material during acoustic propagation. This eliminates possible temperature changes associated with cavitation as important in the mechanism since in the absence of tension forces, cavitation does not take place. Another possible way in which temperature might enter is through high temperatures, produced during acoustic propagation, at or near the surfaces of small gas nuclei which might be present in the tissue. This could occur, of course, in the absence of tension forces. A mechanism based on the existence of such temperature changes is unlikely for these reasons. Gas nuclei of diameters equal to or greater than about 0.5 micron would be detected microscopically. However, there is no evidence for the existence of such nuclei. Assume then that if gas nuclei are present they are less than 0.5 micron in diameter. Since the maximum temperature rise occurs at the surface of

the bubble and this temperature level increases with increasing bubble radius we compute the temperature change at the surface of a gas nucleus with a diameter equal to 0.5 micron from a formula derived by M. D. Rosenberg. The difference between the temperature at the surface of such a bubble and the average temperature of the medium for a sound intensity of 100 watts/cm^2 at a frequency of 1 megacycle is about 0.3° C. This indicates that the effect under consideration cannot be important in the mechanism of the effect of ultrasound on the nerve tissue. Secondly, no evidence of the growth of gas nuclei is seen under the microscope in nerve tissue irradiated under a hydrostatic pressure of one atmosphere and fixed within two minutes after exposure.

The second physical factor which received consideration as a possible participant in the mechanism of the action of the sound on the tissue was cavitation which we defined in the discussion of the temperature factor to include both the process of cavity formation and collapse and the expansion and contraction of a gas nucleus. Since it is possible to produce by ultrasound the effects described in the central nervous system (frog spinal cord) when the animal is under a hydrostatic pressure sufficiently high to prevent cavitation then this process cannot enter into the mechanism.

The factor now receiving consideration with respect to a possible fundamental role in the mechanism is viscous force action between submicroscopical structural components of the protoplasm and the imbedding media. It is expected that studies as a function of frequency and in standing wave fields will indicate the possible significance of this mechanism.

Acknowledgment

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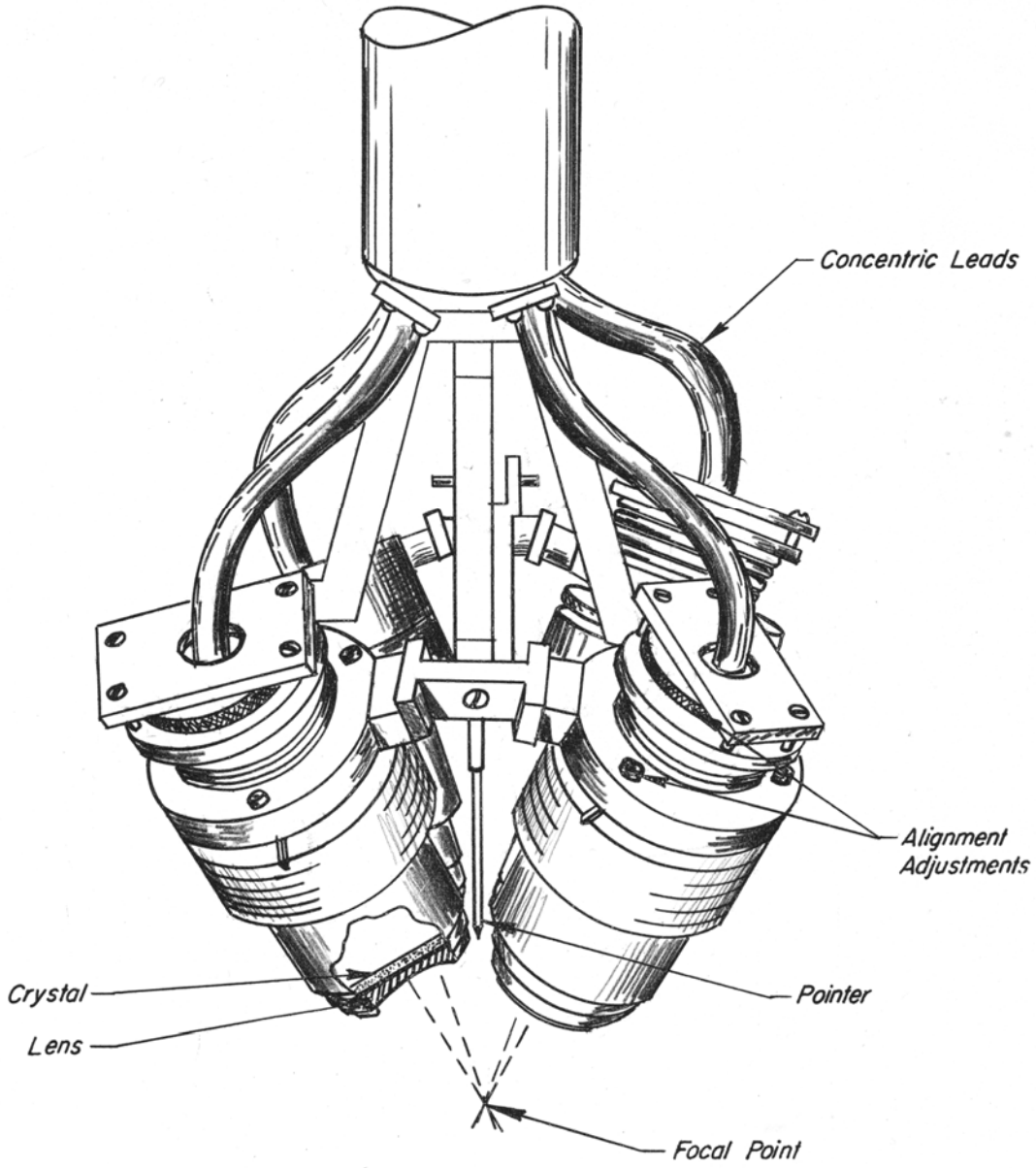

Figure 1. Four-Beam Ultrasonic Focussing Irradiator

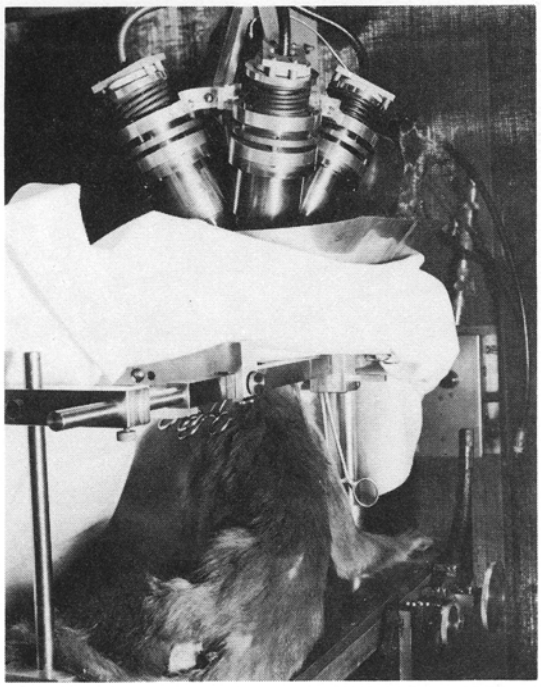

Figure 2. Apparatus setup for irradiation of a region of a monkey's brain.

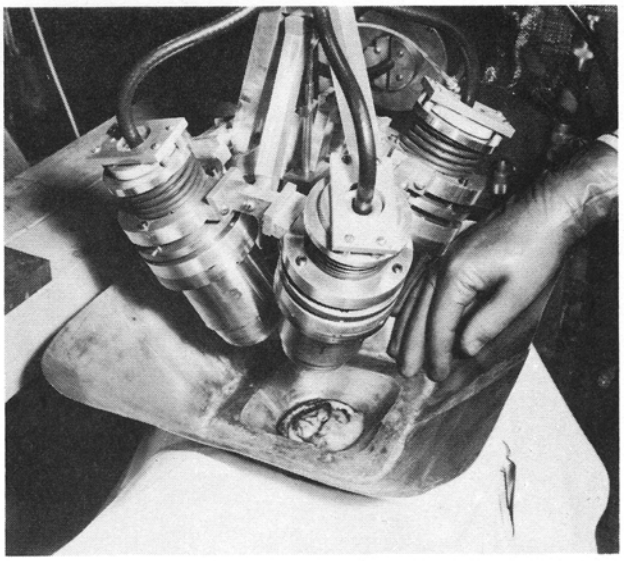

Figure 3. Closeup view of four-beam irradiator positioned over the brain of a monkey.

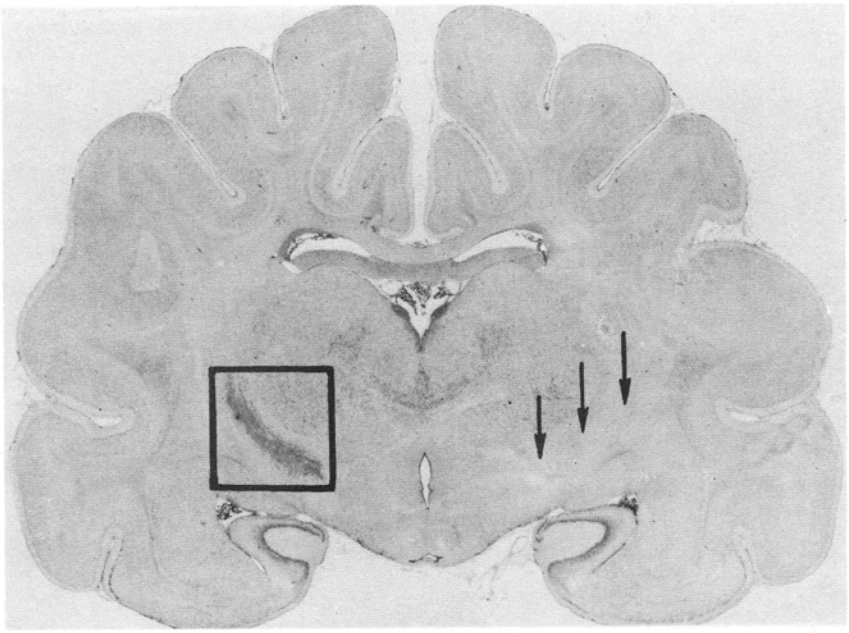

Figure 4. A transverse section of the brain of a cat showing the position of the internal capsule (arrows) in the plane of irradiation. The right side shows the normal unirradiated structure. The outlined area on the left shows the position of the irradiated zone and contains the lesion in the capsule as it appears 30 days after irradiation. The sound entered the brain at the top.

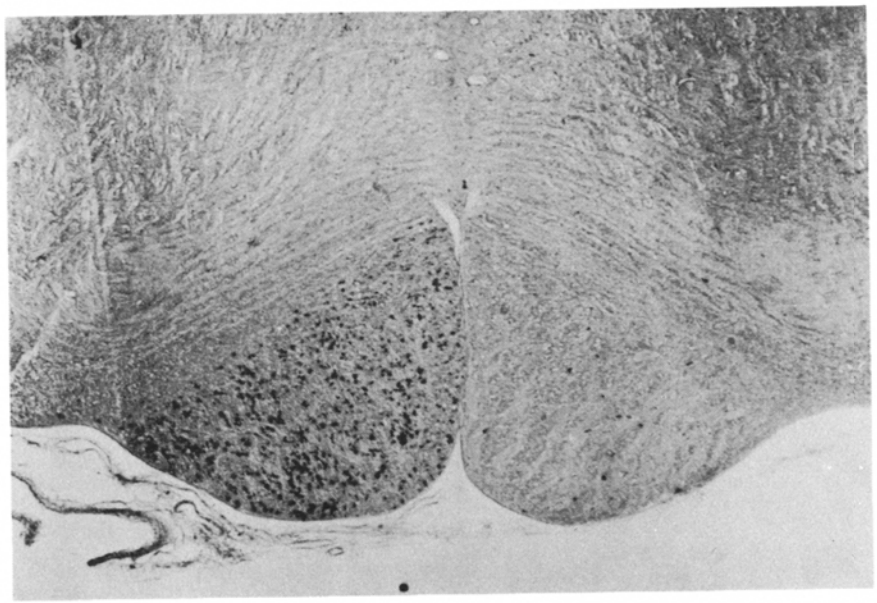

Figure 5. Degenerating fibers, indicated by the black dots, in a section of the brain between the irradiated region and the spinal cord. These fibers were irreversibly affected by irradiation of the internal capsule with ultrasound.

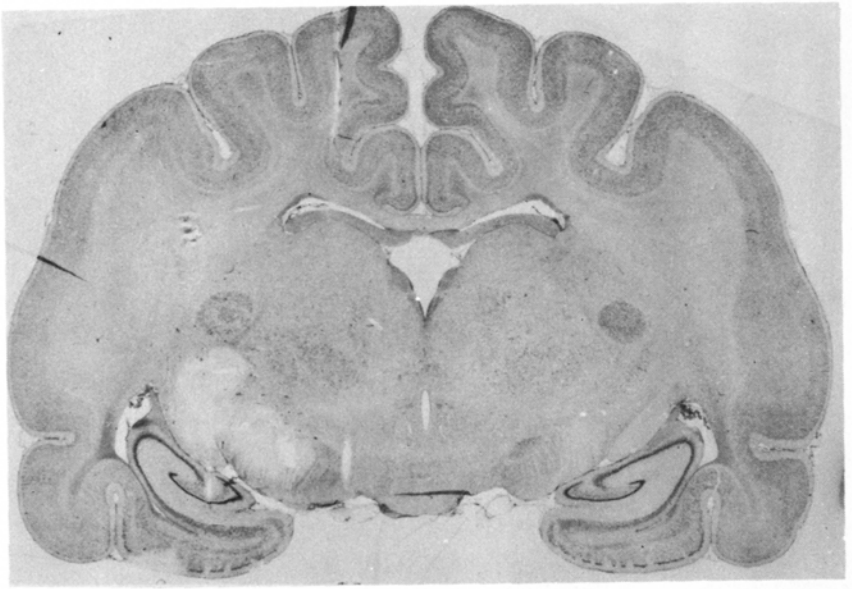

Figure 6. A transverse section of the brain showing the appearance of the internal capsule (left side) in the region of irradiation 3 days after subjection to the ultrasound.

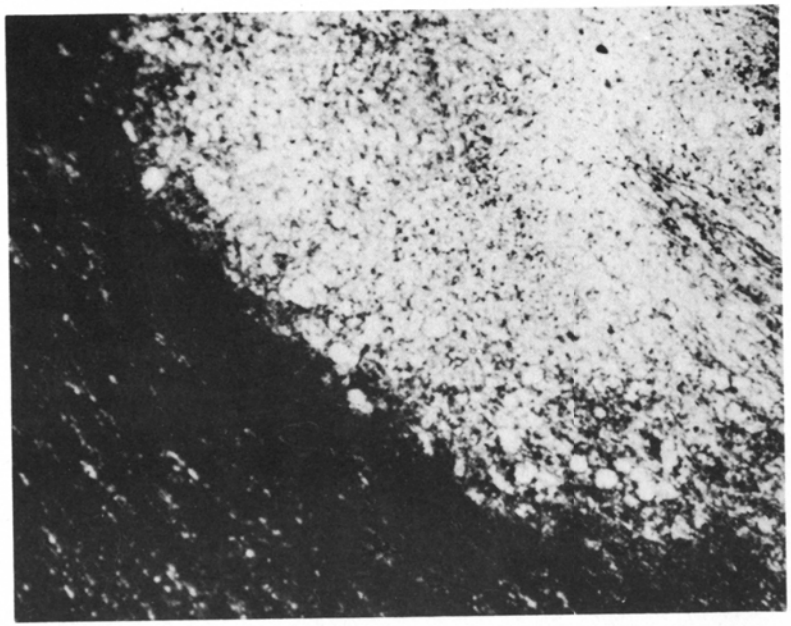

Figure 7. A transverse section of the internal capsule 12 days after irradiation (medium power magnification). The boundary between the normal and affected tissue is clearly visible. (Normal tissue on left side.)