Nagoya J. med. Sci. 30: 429-452, 1968

EXPERIMENTAL STUDY ON THE ULTRASONIC ATTENUATION OF THE INJURED BRAIN

WITH SPECIAL EMPHASIS ON RELATIONSHIP BETWEEN CEREBRAL EDEMA AND CEREBRAL CIRCULATION

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ABSTRACT

In the field of ultrasonic diagnosis, its attenuation property in the brain has been also explored. Investigated are the ultrasonic attenuation of the brain following experimental head injury as well as cerebral circulation and water content of brain, the purpose of which are to analyse the essential mechanism of ultrasonic attenuation as its relationship to the brain edema. I studied the continuous changes of brain by means of epidural air shot, intracarotid oil injection and by extreme reduction of cerebral blood flow. Reduction of ultrasonic attenuation on brain is primarily due to the increased water content of brain tissue, whereas reduction of cerebral blood flow increases ultrasonic attenuation values to some extent.

INTRODUCTION

The diagnosis of brain edema, which often gives difficulty to the clinical neurosurgeons, still remains as a difficult problem and unsolved aspect in clinical medicine. The method currently practiced is to study brain edema using electron microscope, but this being fundamentally morphological, thus different approach for pursuing dynamic aspect of cerebral edema to be investigated.

A-scope method of ultrasonic diagnostic apparatus has been explored for diagnosis of intracranial organic disorder such as acute head injury or space taking lesions, and this method has been found to be a useful diagnostic aid. In another field of ultrasonic diagnosis, its attenuation property in the brain has been also explored. From this standpoint, there may be many advantages in following the physicochemical changes of brain tissue in time sequence. For clinical application of above method, however, still many fundamental problems remain to be studied.

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Received for publication September 11, 1967.

In this study, the author attempted to investigate the relationshipo between the ultrasonic attenuation of the brain versus experimental head injury, as well as cerebral circulation and water content of brain to analyze the essential mechanism of ultrasonic attenuation as to its relationship to the brain edema.

Medical application of ultrasound was first described by Dussik[1] in 1942, stating the possibility of an useful diagnostic procedure. In 1949, he reported on the demonstration of cerebral ventricles using the ultrasonic ray, *i.e.*, to differenciate cerebral parenchyma and cerebrospinal fluid by the difference of their transmission properties[2]. Thereafter, transmission method has been persued by Ballentine, Bolt, Hueter and Ludwig[3] (1950), and Güttner *et al.*[4] (1952).

On the other hand, Ludwig[5] (1950), French, Wild and Neal[6] and others studied the pulse reflection method of neoplastic masses by their acoustic property. They emphasized the advantage of ultrasonic pulse reflection method from the study on the echo-analysis of postmortem brain tumor.

Experiences and studies on ultrasonic diagnosis of intracranial diseases such as brain tumors, intracranial hematomata, etc. were reported by Kikuchi, Tanaka, Wagai and others[7] (1953), Leksell[8] (1961), de Vlieger[9] (1959), Lithander[10] (1960), Jeppsson[11] (1961), Gordon[12] (1959), Jefferson[13] (1962) and Iwata, Tomiyasu *et al.*[14] (1964).

They emphasized the midline-echo as an important diagnostic landmarks. Also attempt was made to pick abnormal echoes from brain tumors, intracranial hematomata, and another space occupying lesions.

On the other hand, the change of ultrasonic attenuation value was reported in the cases of epilepsy[15][16], brain edema[17][18][19][20], brain tumor[21] and cerebral circulatory disorders[22][23][24].

METHOD

Adult mongrel dogs, weighing 7 to 15 kg, were used for these experiments. After anesthesia was induced with sodium thiobarbiturate (30 mg per kg body weight) given intraperitoneally, the animals were endotracheally intubated and their respiration was assisted whenever necessary. Barbiturate was added later intravenously, if necessary, to keep sufficient anesthetic depth.

As the apparatus for ultrasonic attenuation measurement, models SSD-2 (Japan Radio Co.) and NJZ-109 A, step-attenuator for ultrasound (JRC) were used. The crystal transducers at the frequency of 5 MC/S, 10 mm in diameter were used for probes.

A small trephine opening of 12 mm in diameter was made on each temporal region. Then transducers were fixed on each temporal area so as to have the axis of each transducer on the same line, and the gaps between the dura and the transducer were filled with degassed water or glycerol solution. At

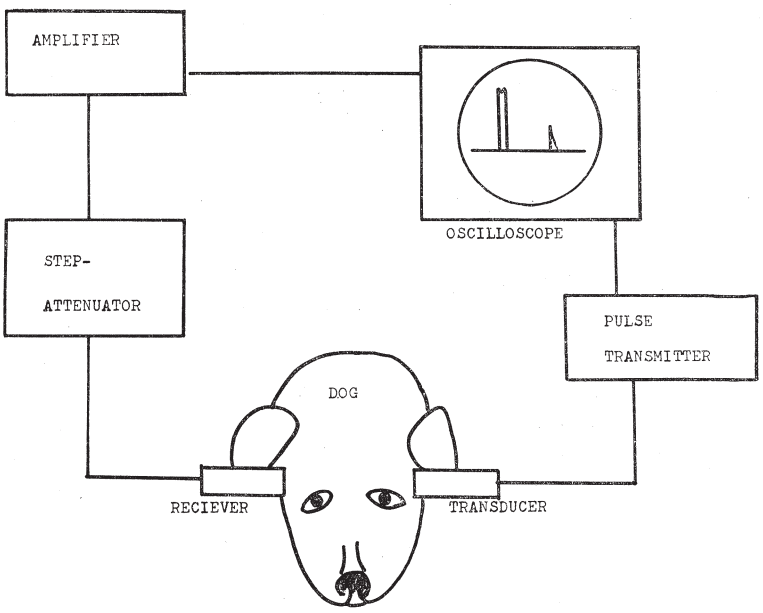

FIG. 1. Block diagram of apparatus for experiment.

times, the apparatus was calibrated and corrected with the test piece of this apparatus. The standard intensity of attenuation on the cathode ray tube screen was adjusted so that echo height was to be 10 mm with the step-attenuator. From this step-attenuator unit, attenuation value was calculated, the unit being decibel (dB) per cm of brain width (Fig. 1).

For the determination of cerebral blood flow (CBF), Aizawa's modification[25] of Kety and Schmidt's method[26] was used in this study. A polyethylene tube of 0.5 mm in inner diameter was inserted into the sinus confluens through the external jugular vein. After canulation was completed, 2 mg per kg body weight of heparin solution was administered intravenously for prevention of blood coagulation. The arterial blood sample was withdrawn from the femoral artery and the venous blood from the sinus confluens. The gas mixture used was 15% N_2O gas and 85% air as described by Kety and Schmidt. The O_2 and CO_2 content of blood was determined by the manometric technique of Van Slyke and Neill. The cerebral vascular resistance (CVR), cerebral oxygen derivery (CDO_2), cerebral oxygen consumption ($CMRO_2$) and cerebral oxygen intake retio ($CERO_2$) were culculate as follows;

$$CBF = \frac{100 \times V_{12}}{\int_0^{12} (A - V)dt} \text{ (ml/100 g of brain/min)}$$

$$CVR = \frac{MABP}{CBF} \text{ (mmHg/100 g of brain/min)}$$

$$CDO_2 = \frac{CBF \times \text{arterial } O_2}{100} \text{ (ml/100 g of brain/min)}$$

$$CMRO_2 = \frac{CBF \times (A-V)O_2}{100} \text{ (ml/100 g of brain/min)}$$

$$CERO_2 = \frac{CMRO_2}{CDO_2} \text{ (\%)}$$

MABP (mean arterial blood pressure obteined directly from the femoral artery by means of a mercury manometer ...Hg)
$(A-V)O_2$ (cerebral arteriovenous oxygen difference...vol.%)

The animals were sacrified by means of an overdose of intravenous thiobarbiturate and bleeding from main vascular trunks. The calvarium was removed quickly, and brain stick samples were taken from the both temporal regions. Water content of the brain was measured by Kimoto and Takai's modification[27)28)] of evaporation method[29)]. As soon as the samples of brain pieces were obtained, the gray matter and the white matter were weighed separately with the chemical balance, [P_0]. Negative pressure desiccator was used to evaluate the brain water and oversaturated sodium chloride was placed under the cup. Then the desiccator was immersed in water bath for 72 hours at 30°C; brain samples were weighed [P_1]. Materials were dried for 24 hours at temperature of 110°C; the dried pieces of brain were weighed [P_2].

Under the saturated vapour pressure at temperature of 30°C

$$\text{Free Water (F.W.)} = \frac{P_0 - P_1}{P_0} \times 100 \quad (\%)$$

$$\text{Bound Water (B.W.)} = \frac{P_1 - P_2}{P_0} \times 100 \quad (\%)$$

Total Water (T.W.) = Free Water + Bound Water

In this experiment of the experimental brain injury, cysternal pressure was also measured for the observation of change in the cerebrospinal fluid pressure.

RESULTS

A) Normal values on canine brains

The values on ultrasonic attenuation are 6.00±0.585 dB/cm, on the average.

The values of cerebral circulation are shown in Table 1. The mean values on 10 dogs are CBF 51.01 ml/min, MABP 119.0 mmHg, CVR 2.403 mmHg, CDO_2 6.922 ml, $CMRO_2$ 2.999 ml and $CERO_2$ 45.23%.

In gray matter, the values for the water content of brain were, free water 71.35%, bound water 5.78% and total water 77.14%; and in white matter, free

TABLE 1. The Values of Cerebral Circulation on Normal dogs

	CBF	MABP	CVR	CDO_2	$CMRO_2$	$CERO_2$
No. 1	56.7	144	2.55	7.80	3.17	40.8
2	42.5	106	2.50	7.10	3.50	49.0
3	44.0	110	2.50	5.09	2.33	47.4
4	54.0	104	1.93	6.07	2.57	40.7
5	51.5	128	2.46	7.53	3.97	53.0
6	60.0	120	2.00	7.37	3.34	45.5
7	40.5	120	2.96	7.05	2.57	37.0
8	45.3	142	3.12	6.17	2.86	46.2
9	58.6	112	1.89	9.48	2.93	31.0
10	50.7	104	2.02	6.60	2.75	41.7
mean	51.01	119	2.403	6.922	2.999	43.23
S.D.	±7.03	±15.2	±0.425	±1.31	±0.497	±6.5

TABLE 2. The Values of Cerebral Water Content on Normal Dogs

	Gray Matter			White Matter		
	F.W.	B.W.	T.W.	F.W.	B.W.	T.W.
No. 1	70.1	5.8	75.9	61.4	6.5	67.9
2	72.3	5.4	77.7	63.6	6.6	70.2
3	70.0	5.9	75.9	61.4	6.4	67.8
4	71.1	6.3	77.4	60.0	6.5	66.5
5	71.0	6.5	77.5	60.1	6.5	66.6
6	71.0	5.5	76.5	60.0	6.5	66.5
7	73.2	5.7	78.9	63.8	5.3	69.1
8	72.1	5.2	77.3	63.0	6.3	69.3
mean	71.35	5.79	77.14	61.66	6.33	67.99
S.D.	±1.127	±0.442	±0.758	±1.62	±0.423	±1.379

water 61.66%, bound water 6.33% and total water 67.99% (Table 2).

The cerebrospinal fluid pressure was 91.5 mm H_2O.

B) Contrast study

The animal was secured on the operating table. A burr hole was made in the region of the parietal lobe, into which air shot was made. The change in the cerebral ultrasonic attenuation was continuously measured (Fig. 2). A decrease of attenuation values was seen in 3–4 hours after the head trauma, and thereafter increase was noted (namely transmission force decrease). 24 hours after the induced trauma the absorption curves were not uniform from case to case probably because of difference in the anesthetic level, so that in this experiment values were studied within 4 hours after head trauma.

C) The effects of extradural air shot (Fig. 6)

After the intraperitoneal barbiturate induction and endotracheal intubation, a small trephine opening of 7 mm in diameter was made on the left parietal

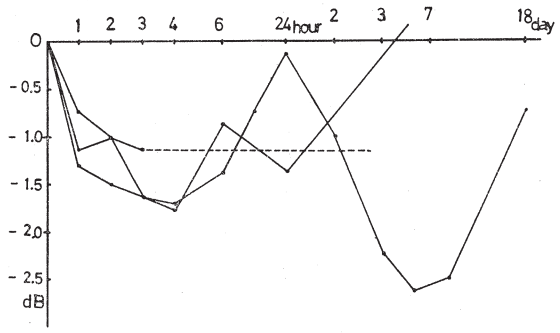

FIG. 2. Ultrasonic attenuation value after brain injury.

region. Brain injury was induced by 18,700 erg of air shot by air gunn through the burr hole.

Immediately after the air shot pulse became slower, respiration ceased, and bilateral mydriasis, extention of extremities, and weaker protective reflexes observed. Then, tachycardia followed and spontaneous respiration ceases for 4-5 minutes and is maintained by artificial respiration. Later pupillary and corneal reflexes reappeared and pulse returned to almost normal rate. Occasionally some of the animals abnormal respiration and irregular pulsation after air shot died within several minutes; on autopsy, diffuse bleeding at the base of brain was revealed, especially at the anterior pontine portion.

The change of the ultrasonic attenuation: 15 minutes after, comparing with normal values, the absorption of ultrasound became reduced; namely 30 minutes after −0.55 dB, in 1 hour −0.66 dB, in 2 hours −0.93 dB. The maximum absorption was noted in 3 to 4 hours, being −1.05 and −1.08 dB (Fig. 3).

The changes in the cerebral circulation: 1 hour after, the average values on 10 dogs are CBF 32.73 ml, MABP 103.2 mmHg, CVR 3.205 mmHg, CDO_2 4.447 ml, $CMRO_2$ 2.445 ml and $CERO_2$ 54.61%. CBF decrease was 36%, MABP a decrease of 13.3%, CVR an increase of 34%, CDO_2 a decrease of 36%, $CMRO_2$

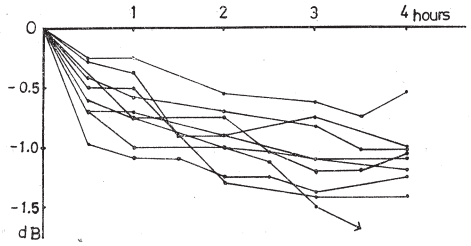

FIG. 3. The changes of ultrasonic attenuation after extradural air shot.

a decrease of 19% and $CERO_2$ an increase of 33%. 4 hours after, the average values on 7 dogs are CBF 33.78 ml, MABP 127.0 mmHg, CVR 4.24 mmHg, CDO_2 4.69 ml, $CMRO_2$ 2.28 ml and $CERO_2$ 44.51%. CBF decreased by 34%, MABP increased by 6.7%, CVR increased by 76%, CDO_2 decreased by 32%, $CMRO_2$ decreased by 24%, and $CERO_2$ increased by 3% (Table 3).

The changes in water content: the values after 1 hour, in gray matter were as follows: F.W. 73.96%, B.W. 6.08% and T.W. 80.04%, and in white matter F.W. 63.78%, B.W. 6.72% and T.W. 70.50%. In gray matter, F.W. showed an increase of 3.7%, B.W. an increase of 0.5% and T.W. an increase of 3.8%, and in white matter, F.W. an increase of 3.4%, B.W. an increase of 6.1% and T.W. an increase of 3.7%. The values after 4 hours, in gray matter F.W. 75.72%, B.W. 5.64% and T.W. 81.36%, and in white matter, F.W. 64.84%, B.W. 6.68% and T.W. 71.52%. In gray matter, F.W. an increase of 6.1%, B.W. a decrease of 0.3% and T.W. an increase of 5.5%, and in white matter, F.W. an increase of 5.2%, B.W. an increase of 5.5% and T.W. an increase of 5.2% (Table 4).

TABLE 3. The Values of Cerebral Circulation after Epidural Air Shot

	CBF	MABP	CVR	CDO_2	$CMRO_2$	$CERO_2$
Mean Value (after 1 hour)	32.73	103.2	3.205	4.447	2.445	54.61
S.D.	±4.89	±15.9	±0.464	±0.758	±0.250	±7.18
Normal rate (%)	−36	−13.3	+34	−36	−19	+33
Mean Value (after 4 hours)	33.78	127.0	4.240	4.690	2.280	44.51
S.D.	±12.5	±13.5	±1.885	±1.55	±0.93	±18.8
Normal rate (%)	−34	+6.7	+76	−32	−24	+3

TABLE 4. The Values of Cerebral Water Content after Epidural Air Shot

	Gray Matter			White Matter		
	F.W.	B.W.	T.W.	F.W.	B.W.	T.W.
Mean Value (after 1 hour)	73.96	6.08	80.04	63.78	6.72	70.50
S.D.	±1.81	±0.72	±0.93	±2.30	±0.71	±2.62
Normal rate (%)	+3.7	+0.5	+3.8	+3.4	+6.1	+3.7
Mean Value (after 4 hours)	75.72	5.64	81.36	64.84	6.68	71.52
S.D.	±0.82	±0.64	±0.30	±2.12	±0.84	±1.52
Normal rate (%)	+6.1	−0.3	+5.5	+5.2	+5.5	+5.2

In this experiment, cerebrospinal fluid pressure by cysternal puncture is measured. And this increased to 142.1 mmH_2O (+55%) in one hour and to 143.7 mmH_2O (+57%) in 4 hours following head injury.

Cut surfaces of these brains revealed localized petechial hemorrhage of the contused area of about 10 mm in diameter.

After the recovery from the immediate shock status, hypoxic condition of the brain tissue lasted for at least 3 days as stated in previous reports[30)31)32)]. It was revealed that both CBF and $CMRO_2$ decreased within 1 hour and 4 hours following brain injury, while CVR increased. Water content of brain also shows actual increase in these stages at these periods. The ultrasonic attenuation values decreased, starting in 15 minutes and continued gradually to decrease till 3 hours following injury when it showed maximum change, and then the value remained the same for next 2 hours. Comparing the values obtained one hour and four hours after the trauma, it could be concluded that the water content increased, cerebrospinal fluid pressure was elevated, CBF showed some changes, and ultrasonic attenuation value decreased in general. From these observations, it is believed that there is close correlation between brain ebema and following properties such as ultrasonic attenuation value, water content, CBF, and cerebrospinal fluid pressure[20)].

D) The changes after the carotid salad oil injection (Fig. 7)

Edstrom's method[33)] was used to make experimental brain edema by carotid injection of vegetable oil to avoid respiratory arrest, extreme cardiac failure, or decerebrated response of acute stage which were seen in the experiment with air gun shot.

Dogs were anesthetized with intraperitoneal barbiturate and intubated through the trachea. The left common carotid artery was exposed through the median incision of the neck. Dogs were secured in prone position and then ultrasonic attenuation of brain was measured to obtain basic values. 0.1 ml of salad oil per kg body weight was injected into the left common carotid artery without stopping the blood flow. Subsequently, ultrasonic attenuation values on these animals' brains were measured for four hours with an interval of half an hour, and in the mean time, blood pressure and cerebrospinal fluid pressure were measured (Fig. 4, Table 5, 6).

Water content of the brain was measured at the end of the experiment, usually four hours after the carotid oil injection and in seven dogs, one hour after the injection.

The intracarotid injection of salad oil on dogs resulted in some respiratory changes, namely irregularity and decrease in respiratory rate and tidal volume, but no remarkable change was seen in their pupils or other vital signs. Five dogs showed a severe depression of respiration and died within two hours in spite of administration of cardiotonics and respiratory stimulants. Values

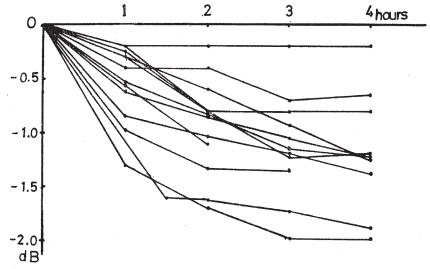

FIG. 4. The changes of ultrasonic attenuation after carotid injection of salad oil.

TABLE 5. The Values of Cerebral Circulation after Intracarotid Oil Injection

	CBF	MABP	CVR	CDO_2	$CMRO_2$	$CERO_2$
Mean Value (after 1 hour)	32.53	102	3.27	4.53	2.18	47.90
S.D.	±3.70	±16.1	±0.631	±0.843	±0.534	±6.73
Normal rate (%)	−36	−11	+36	−34	−27	+11
Mean Value (after 4 hours)	27.28	94	3.51	3.39	1.70	51.12
S.D.	±5.13	±19.4	±0.877	±0.864	±0.417	±9.91
Normal rate (%)	−46	−21	+46	−50	−43	+18

TABLE 6. The Values of Cerebral Water Content after Intracarotid Oil Injection

	Gray Matter			White Matter		
	F.W.	B.W.	T.W.	F.W.	B.W.	T.W.
Mean Value (after 1 hour)	73.82	5.12	78.93	63.10	5.18	68.28
S.D.	±1.50	±0.48	±1.38	±0.77	±0.57	±1.06
Normal rate (%)	+3.5	−11.5	+2.3	+2.3	−18	+0.3
Mean Value (after 4 hours)	76.42	5.19	81.61	66.91	5.06	71.97
S.D.	±0.92	±0.32	±0.96	±2.16	±0.38	±2.18
Normal rate (%)	+7.1	−10.4	+5.5	+8.6	−20	+5.2

obtained on these dogs were excluded in evaluating experimental results.

The changes in ultrasonic attenuation value on brain revealed a mean value of −0.76 dB, −0.94 dB, −1.12 dB and −1.28 dB one, two, three and four hours after the injection respectively. In other words, ultrasonic attenuation

value decreased in time sequence.

CBF 32.53 ml (36% decrease of normal value), MABP 102.6 mmHg (10.6% decrease), CVR 3.27 mmHg (35% increase), CDO_2 and $CMRO_2$ 4.53 ml (48% decrease) and 2.18 ml (44% decrease) were actually measured. These values showed the tendency in time sequence, as CBF 29.28 ml (46.5% decrease), CDO_2 3.39 ml (51% decrease) and $CMRO_2$ 1.70 ml (43.3% decrease) were measured.

Total water content of the brain revealed 2.3% increase in gray matter and 0.3% increase in white matter one hour after injection and 5.5% increase in the gray matter and 5.2% increase in the white matter four hours after the injection. Bound water decreased by 11.5% in gray matter and 18% in white matter one hour after, and 10.4% in gray matter and 20% in white matter 4 hours following the injection.

Cerebrospinal fluid pressure rised to 140 mmH_2O (arise of 43%) in mean value and to 175 mmH_2O (arise of 73%), one and four hours following injection respectively[34].

Macroscopically, the cerebral hemisphere showed marked swelling on the injected side and the cut surfaces of the brain showed swelling with petechial hemorrhages as well.

Therefore, brain swelling by carotid injection of salad oil resulted in the decrease of ultrasonic attenuation value, CBF, CDO_2 and $CMRO_2$ in time sequence and in an increase of the water content of brain in both gray and white matters. Cerebrospinal fluid pressure also showed remarkable increase. These changes are diagrammed on Fig. 7, and the influence of CBF change on the ultrasonic attenuation required the following experiment.

E) Ligation of both common carotid and vertebral arteries

Attempt was made to investigate the relationship of extremely decreased CBF and the ultrasonic attenuation.

Animals were anesthetized with intraperitoneal barbiturate, bilateral common carotid and vertebral arteries were exposed. These arteries were taped and trepanation openings were made over the temporal calvarium, and the ultrasonic attenuation was measured before and after the occulsion of these four vessels (Fig. 5).

Even though these four vessels were occluded, there were no gross changes seen on the condition of anesthetized dogs. Under these conditions, ultrasonic attenuation values of brain were measured continuously, and the CBF as well as the water content of the brain were measured at the end of the experiment, namely one hour to two hours after the ligation.

Generally, ultrasonic attenuation values increase immediately after the simultaneous ligation of these four vessels in the neck and then there were gradual increase of the values. Among these five dogs, the ultrasonic attenua-

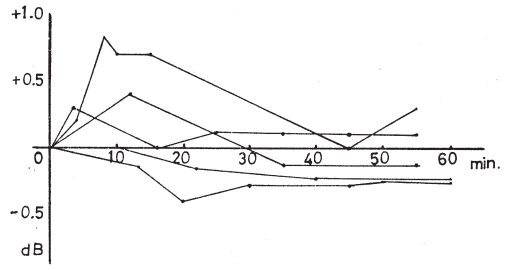

FIG. 5. Changes of ultrasonic attenuation after ligation of both common carotid and vertebral arteries.

TABLE 7. The Values of Cerebral Circulation and Water Content after Ligation of Both Common Carotid and Vertebral Arteries

	CBF	MABP	CVR	CDO_2	$CMRO_2$	$CERO_2$	F.W.	B.W.	T.W.
No. 1	22.1	115	5.01	3.32	1.71	51.7	71.2 61.5	5.9 6.5	77.1 68.0
2	26.9	125	4.28	4.75	3.20	67.6	71.5 61.7	5.8 6.3	77.3 68.0
3	26.9	110	4.65	2.63	1.87	70.9	76.1 63.9	5.3 6.6	81.4 80.7
4	37.9	131	3.46	3.12	2.62	84.8	76.0 69.5	5.7 7.5	81.7 77.0
5	31.9	65	2.05	1.86	0.67	43.6	73.9 65.5	6.0 6.6	79.9 72.1

tion values again increased in one case and this particular case revealed no increase of water content in both gray and white matters of brain tissue (Table 7).

When all of four vessels were occluded, actual measurement of CBF revealed to be 50% of its normal value and the ultrasonic attenuation showed decrease only among the group of increased water content of the brain. In this experiment, in general, when the water content of brain did not increase, no decrease of the ultrasonic attenuation was seen. Therefore, the following can be concluded: increase of water content of brain causes increase of ultrasonic attenuation, and the water content of brain plays a decisive role upon the CBF influence on the ultrasonic attenuation of brain[24)35)].

DISCUSSION

The primary purpose of this study is to make continuous observation on the development of cerebral edema and successive measurement of the cerebral

edema by means of ultrasonic attenuation. It has been impossible to evaluate the degree of cerebral edema by means of morphological or chemical methods while the subject is alive, but only these physical devices give clue to the edematous condition of brain tissue when necessary.

In clinical practice of neurosurgery, it is important to know the degree of swelling of the brain tissue in time sequence, especially when treating the acute stage of brain injury or the intracranial space-taking lesion.

It is reasonable not to dare to state that the ultrasonic attenuation itself could demonstrate the status of cerebral edsma, nevertheless withholding above possibility, mechanism of the ultrasonic attenuation change on the cerebral edema is investigated as one can easily understand the posibility of close correlation between the ultrasonic attenuation change and change of physico-chemical condition of cerebral tissue, that is, the cerebral edema.

The factors which have influence upon the ultrasonic attenuation of brain tissue could be accounted in various aspects. They are, namely: 1) Scattering of ultrasonic ray in distance, 2) Fractional reflection by structual change of the brain, 3) Reflection of the ultrasonic ray at the boundary surface of the media, and 4) Absorption of ultrasound by viscosity of the medium, and etc[36]. Especially in the alive medium such as living brain tissue, many other factors, such as cerebral circulation, the temperature of the brain tissue or pathological changes of the brain should be added to the factors which influence on the ultrasonic attenuation of the brain[37]. IN other words, in the living body, not only the physical factors but also the biological factors should be considerd as the cause of ultrasonic attenuation change and this fact makes this study very complicated.

At present, there are two methods to measure the ultrasonic attenuation change, they are 1) pulse transmission method and 2) pulse reflection method[38) 39)]. The latter method is carried out by determining the penetrating intensity of reflected ultrasound after being projected on either scalp or cranial calvarium.

The former method, on the contrary, is carried out by determination of penetrating intensity of ultrasonic ray projected from the skull, the scalp, or the dura mater over brain of one side to the opposite side.

In both methods, much effort is exercised to minimize the measuring error by improving the direction of the ultrasonic ray as well as the position and fixation of the apparatus over the head[40]. In experimental animal study, we use a different type of the transducer which frequency ranging from 10, 5, 2.25 and 1 MC/S for various purposes.

Sound impedance (I) can be shown as the result of velocity of sound (C) times the density of substance (ρ), that is

$$I=C\rho \qquad (\mathrm{g/cm/sec})$$

For example, the sound impedance of brain is, calculated by Wagai[41],

0.151×10^6 and water $0\ 143 \times 10^6$. Therefore, the impedance of edematous brain is expected to be less as its density ρ should be smaller than the normal brain tissue dencity because of increased water content. Other measures are also taken to minimize the extracerebral influence upon the determination of the brain ultrasonic attenuation, first, by taking the temporal muscles out of the skull and then the temporal bone, and utilizing transducer of 5 MC/S in frequency.

A classical and reliable method of determining cerebral circulation was established by Kety and Schmidt in 1945, using nitrous oxide gas, is well known[26]. Since Kety and Schmidt's publications, many other methods have been described, namely N_2O method with application of Fick's principle, Kr^{85}-inhalation method, continuous determination of cerebral circulation by Kr^{79}-inhalation, Evans Blue pigment method, Indigo-carmine-green method, dilution method using radioactive materials labelled with P^{32} or RISA, method derived from cardiac out put, method using electromagnetic flow meter, and further local clearance method using Kr^{85} or Xe^{133} administered by internal carotid artery injection, and other methods[42].

Methodologically, however, there still remain some problems in these methods; with exception of N_2O method and Kr^{85}, P^{32} labelled material application. Here, N_2O method is believed, by the author, to be the most reliable one in the studies of cerebral metabolism. It is necessary to sample the venous blood circulated the brain tissue for this method; and in experimental animals, the vein from which the venous sample is withdrawn is somewhat different according to the animals.

This poses some problems because of a possibility of the venous blood from the brain with other blood; while in human the venous blood from the internal jugular vein represents purely cerebral circulated venous blood[25].

Aizawa and Takizuka, from anatomical cerebral vascular study, came to the conclusion that it is impossible to sample the unmixed venous blood of the dog which circulated the brain only. They stated that the sinus confluens of *Torcula Herophillii* or the transverse venous sinus are the most suitable veins of the dog for sampling the venous blood for the above mentioned study[25]. In this study, the venous blood was sampled through the small polyethylene catheter which diameter was enough not to cause circulatory disturbance of these venous blood flow as a whole.

In this study, cerebral blood flow was measured to be 51.0 ml/min and this mean value appeared more than Page's value of 40.0 ml/min[43] and Morita's report of 46.7 ml/min[30], while Aizawa reported it to be 53.3 ml/min[25] and Saito 51.5 ml/min[44]. These differences by the authors seem to be caused by the condition of the animal at experiment, but the author believe the differences are caused by the difference in method used for measurement by different authors. These differences mentioned before are not significant in this kind

of experiment. According to Aizawa and Takizuka, while the dog is in anesthetized state, the cerebral blood flow was determined to be 49.31 ml/min when the depth of anesthesia was that of autonomic movement, and 60.69 ml/min at the depth of absent corneal reflex with positive response to painful stimuli. At the deeper phase of anesthesia, increased cerebral blood flow of 80.05 ml/min was recorded when corneal reflex disappeared and animal'spupils dilated.

The first attempt to study the water content of the brain was made by Halliburton[45] in 1894 who measured it by weighing the fresh and the dried brain tissues. He demonstrated that the cerebral gray matter contained more water than the white matter. Later, Kotani[46] differentiated the water of the brain into two, namely the free water and the so-called bound water, and he reported that in case of "brain swelling" the bound water increased in the brain.

Reports by Stewart-Wallace[47], Windle *et al.*[48], Stern[49], Bakay[50] and Adachi[51] are all dealing with content of the brain water.

In Japan, Ambo[52] made "experimental brain swelling" on rabbits and measured the water content of brain by means of Hatchek's cobalt chloride method according to Goltner's theory[46].

So-called bound water is defined in various ways, but most authors have a common definition that it plays a significant role in the vital function and it does not become frozen below the temperature of 0°C and belongs to the hydrophile colloidal system. From this concept, there are various methods to measure it, namely, the method to measure the water unfrozen at the temperature of minus 20°C, namely the freezing point method, or the electromagnetic method to measure the changes of resistence on electric field. But it is rather difficult to measure it when the sample is of small amount because this requires rather a large amount of the specimen. For this reason, the cobalt method has been adopted for this study by many authors; however, this method has a few technical difficulties, for instance, in identifying the coloring time, preventing the infiltration cobalt chloride into the sample, and others[53].

The vapor pressure method takes a long time in measuring because the bound water is hard to evaporate. In this method, another difficulty is that the vapor pressure is difficult to keep constant since sulfuric acid has to be diluted during the measurement. Kimoto and Tomita[27] improved this method by applying the saturated sodium chloride solution instead. They report that the measuring error is small if the measuring condition such as the temperature is kept constant[28][54][55][56].

The author believes that the bound water of the brain plays an important role upon the factors which influence the penetrating property of ultrasonic ray in the brain as this can be considered as one of the high frequency electric current[57]. As the result of this experiment, the actually measured data of

the brain is as follows; in the gray matter of cerebral cortex, total water 77.14%, free water 71.35%, and bound water 5.79%; while in white matter, total water 67.99%, free water 61.66% and bound water 6.33%.

Maruta[58] classified head injuries into 3 groups according to their ultrasonic attenuation. 1) Ultrasonic attenuation value shows its maximum elevation immediately after head injury followed by gradual descent to normal in several days, 2) the maximum ultrasonic attenuation value comes 4-5 days after the injury, it shows the curve of reversed V-shape, and this group is usually accompanied with lateral shift of the midline echo, and 3) immediate maximum elevation of ultrasonic attenuation takes much longer time to be normalized than the first group.

He believes that the above classification corresponds to 1) simple type, 2) so-called brain edema type, and 3) cerebral contusion type of Araki's classification[59].

In experimental head injury study, Oka *et al.*[60] always observed a decreased ultrasonic attenuation value on the injured brain and this experimentally induced lesion was confirmed by macroscopical observation through the craniotomy opening. The brain tissue was swallen and was bulging out, and histological observation revealed extravascular fluid collection and scattering of granular layer of the cerebral cortex, etc. Usually the ultrasonic attenuation value on experimental head injury by the 'knocking' method shows immediate decrease of the value which is followed by a constant level in 30 minutes to one hour. In this experiment, macroscopic change of brain eedema was observed.

In this study, the ultrasonic attenuation value begins to decline in about 30 minutes following extradural air gun shot, and continues to diminish for 3 hours and reach at its minimum value in 3-4 hours. The brain shows macroscopic brain edema suggesting an increased water content of the brain.

On the change of the water content of the brain, according to Nakayama's report[61], 6 hours following epidural knock of brain, an increase was observed as follows: free water 2.9%, bound water 3.1% and total water 2.9% and 48 hours following the experimental injury, the maximum increase of free water, decrease of bound water, and increase of total water were noted.

In his report on the experiment with air gun shot onto the brain, Morita[53] states that the free water increases to 3% and total water 1.9%, while the bound water decreases to 8%. He reports an increase of the brain water content following ligation of bilateral jugular veins.

Gurdjian and Webster[62] state that the cerebral vascular system shows a tendency to dilate as the early stage response to head injury.

Evans[63] has developed vascular stasis theory. In their studies on experimental head injury with dogs, the cerebral blood flow and oxygen consumption of the brain decreased immediately following the brain injury and cerebral vascular resistence increased at the same time. These conditions remain the

same for several days, and later, increase of oxygen intake compensates the lowered oxygen consumption as the functional defense mechanism of brain. Lindquist *et al.*[64] reported that oxygen consumption of brain decreases by 24% and cerebral blood flow decreases by 17% within one hour following brain contusion of dogs.

According to Moriyasu[65] the change of cerebral circulation is closely correlated to the severity of brain injury, and above correlation is more than that with nephro-hepatic circulation. In severe cases, cerebral blood flow decreases by 30% togather with the increase of the vascular resistence and the elevation of the cerebrospinal fluid pressure.

In this study of experimental head injury, diminished cerebral blood flow and elevated cerebral vascular resistence were observed.

In the experimental study of cerebral embolism by carotid injection of sesame oil or salad oil, Edstrom *et al.*[33] and Blinderman *et al.*[66] observed development of remarkable swelling of the brain, and Sano and Hatanaka[67]

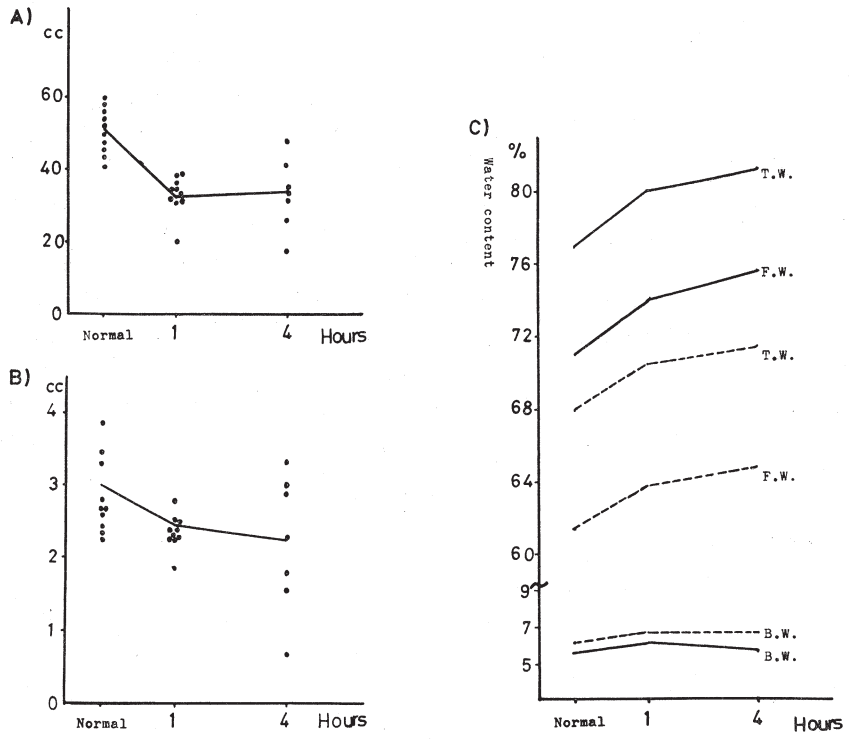

FIG. 6. Changes of cerebral blood flow (A), cerebral oxygen consumption (B), and water content (C) of brain after epidural air shot. Solid lines show gray matter and dotted lines white matter. T.W.-total water, F.W.-free water and B.W.-bound water.

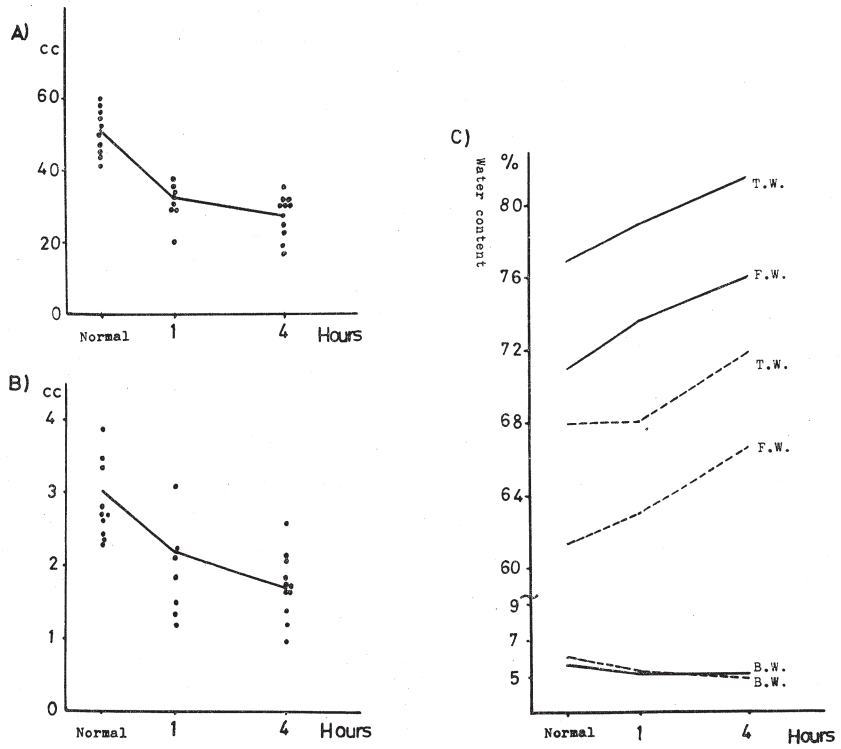

FIG. 7. Changes of cerebral blood flow (A), cerebral oxygen consumption (B), and water content (C) after carotid injection of salad oil.

found that this swelling of brain was caused by broken blood-brain-barrier. In author's experimental study with salad oil injection, cerebral blood flow decreased gradually and the water content of the brain increased, and further macroscopical brain swelling was recognized.

Meyer *et al.*[68] observed decreased cerebral blood flow and reduced oxygen tension of the brain tissue as the result of cerebral embolism caused by intracarotid injection of powder of pumice. They stated that these changes would act towards vasodilatation and would show the reduction of CO_2 tension as a result of formation of collateral circulation, and the elevation of $P\ CO_2$ indicate the developement of compensatory cerebral blood circulation.

Dogs rarely die in short period of time following ligation of bilateral common carotid and vertebral arteries in the neck. We ligated these four blood vessels in the neck of dogs in order to decrease the cerebral blood circulation in its extreme form and to determine the changes of ultrasonic attenuation of the brain. We observed, as the result of ligation of those vessels, that there is an immediate increase of cerebral blood crculation for

short period of time, then a gradual decrease.

Teraura[69] reported that there was no significant change of $P\,O_2$ in the cerebrum, cerebellum, medulla oblongata, and pons cerebri following ligation of bilateral common carotid and vertebral arteries of dogs. He believes this condition is compensated by elevation of blood pressure and well-developed collateral circulation in dogs.

Aizawa and Takizuka[25] reported that the dog brain has more vascular anastomoses than the human brain. There are many anastomoses between external and internal carotid arteries, between extracranial and intracranial arteries and many anastomotic vessels including muscular branches, internal mammary arteries, etc.

In this study, cerebral blood flow remained 1/2-1/3 of the normal value one hour after ligation, and the mean arterial blood pressure was fairly elevated. The ultrasonic attenuation value vary according to individual experimental animal to some extent, but from the data on determination of water content of brain, one can conclude that water content remains normal when ultrasonic attenuation value is normal or increased. The water content increases as the ultrasonic attenuation value decreases in spite of the fact that the cerebral blood flow decreases in above condition. Many studies have been carried out upon the essential nature of ultrasonic attenuation of the brain.

Hueter and Bolt[70] studied ventricular geometry by ultrasonic technique making use of the penetrating ability of ultrasound which should be different between the brain tissue itself and the cerebrospinal fluid.

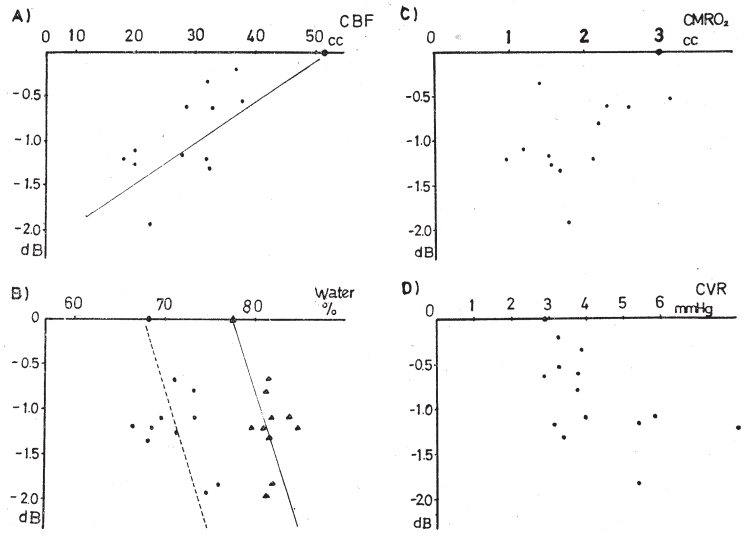

FIG. 8. Relationship between ultrasonic attenuation value and CBF (A), water content (B), $CMRO_2$ (C) and CVR (D).

Wild and Ried[71] observed a larger reflection wave when the muscle bundle of beaf steak was placed at right angle to the direction of projected sound. Ishikawa[37] reported that nerve fibers of the formalin fixed brain showed maximum ultrasonic attenuation value when it was placed in the right angle to the direction of projected ultrasonic ray.

Fry[72] stated that the living tissue can be considered as the particles suspended in the solution as viscous force has lineal relationship to absorption coefficient and frequency of ultrasound.

Carstensen[73] believed that the ultrasonic absorption in the various solution does not lineally correlate to the frequency, but to the concentration of protein. According to him, acoustic properties of the blood is mainly due to its protein content. If the brain tissue is dehydrated by formalin-fixation, specific gravity increases and ultrasonic attenuation value increases. Contrarily, when the brain is put in distilled water and absorbs the distilled water in, the opposite observation are made[74]. Therefore a conclusion is made that the change of water content of brain is the major factor of ultrasonic attenuation changes of the brain. Oka *et al.* reported on the removed brain as follows: ultrasonic attenuation index of the brain 0.61, the brain of the knocked head 0.41, and the brain after intravenous administration of distilled water 0.44, with frequency of 1 MC/S. In this experiment the values found are as follows; the ultrasonic attenuation index of the brain 6.00, that of the air gun shot brain 4.92 and the brain with infarction 4.72, being measured with frequency of 5 MC/S.

Maruta *et al.*[23] studied, after the ligation of the external carotid arteries and their branches, the relationship of common carotid flow and the cerebral ultrasonic attenuation using a electro-magnetic flow meter by means of acetylcholine, respectively. They observed fairly high coefficient of correlation of 0.891 over the scalp and of 0.970 over the cranium; and they concluded that when cerebral blood flow increases, ultrasonic attenuation decreases, and *vice versa*.

CONCLUSION

In conclusion, the brain edema evidenced by the ultrasonic attenuation is a reliable one as there is close relationship between the severity of the brain edema and the change of ultrasonic attenuation of the brain. In other words, the brain edema can be continuously followed from its developmental to the final stage by the determining ultrasonic attenuation value. The mechanism of ultrasonic attenuation change on brain edema is due primarily to the change of the water content of the brain and a detailed relationship is shown on Fig. 8. In case of brain edema, the increase of water content of the brain is more pronounced in its gray matter and thus ultrasonic attenuation decreases, because the ultrasonic ray easily penetrates through the brain tissue when it gets edematous. Blood flow of the brain decreases in its edematous condition

and, from this experiment, this fact seems to be secondary in influencing the change of ultrasonic attenuation of the brain.

From the data described above, it is conclude that measuring of ultrasonic attenuation can be useful and, in the future, a promising procedure in understanding and observing the development of the brain edema.

SUMMARY

Continuous determination of ultrasonic attenuation of the brain, fractional weighing of water content of the brain, and measurement of cerebral blood circulation were made in the experimentally induced head injury and brain edema. Followings can be summarized.

1) Ultrasonic attenuation value was measured by means of the pulse transmission method with two transducers of 5 MC/S, cerebral blood circulation was determined with nitrous oxide method and water content of brain tissue was determined with evaporation method in dogs. Brain injuries were introduced by epidural air gun shot and by oil injection into common carotid artery.

2) Ultrasonic attenuation values of the brain in epidural air shot experiment showed, comparing with normal values, −0.66 dB/cm after one hour, −1.08 dB/cm after 4 hours; and those of oil injected brain were −0.76 dB/cm at one hour, and −1.28 dB/cm at 4 hours, respectively.

3) Cerebral blood circulation values are as follows; in epidural air shot experiment, CBF, CDO_2 and $CMRO_2$ decrease by 36%, 36% and 19% 1 hour after, by 34%, 32% and 24% 4 hours after air shot. In oil injection experiment, CBF, CDO_2 and $CMRO_2$ shows a decrease of 36%, 48% and 44% in 1 hour, a decrease of 46.5%, 51% and 43.3% 4 hours after oil injection.

4) Total water content of brain tissues reveals an increase of 3.8% in gray matter and of 3.7% in white matter in 1 hour, and increase of 5.5% in gray matter and of 5.2% in white matter in 4 hours following air shot experiment. In oil injection experiment, total water content shows 2.3% increase in gray matter and 0.3% increase in white matter in 1 hour, and 5.5% increase in gray matter and 5.2% increase in white matter in 4 hours, and the bound water decreases in the gray and white matters in 1 and 4 hours following oil injection.

5) After the ligation of common carotid and vertebral arteries, ultrasonic attenuation gradually increases and CBF shows 50% decrease of its normal value.

The reduction of ultrasonic attenuation by the brain is primarily due to increased water content of the brain tissue, and the reduction of cerebral blood flow increases ultrasonic attenuation values to some extent.

AKNOWLEDGEMENTS

I wish to express my sincere appreciation to Professor Dr. Yoshio Hashimoto for valuable guidance, to Lect. Dr. Kinjiro Iwata for kind advice and to Dr. Takayuki Shibata for active assistance.

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