A Study on the Relation of Changes in Temperatures of the Medulla Oblongata and Cerebral Cortex to the Changes in Respiratory Action as well as Electrical Brain Waves in the Process of Death from Cold[1),2)]

by

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Introduction

A large number of studies on death from cold have been presented by many investigators (Reviewed by Yanagi, 1939). But in order to obtain more essential knowledge of its pathology, this subject is now being explored in our laboratory under the direction of Prof. H. Nakamura from the different point of view by newer methods, i: e. a study on the changes in function of the brain, using electroencephalograph, which has been demonstrated by Tanaka (1948) and on the rôle of the heart, which has been made clear electrocardiographically by Morita (1948) in the process of death by cold.

And thus their findings reached to the conclusion suggesting that the neural centers of brain play a greater part while the heart a less dominant rôle, during the cooling process of living bodies.

Furthermore in the paper presented here a study on the changes in function of the medulla oblongata, where the indispensable mechanism for the neural control of respiration is localized, will be discussed. In other words, these experiments were undertaken to investigate the changes in breathing and temperature of the medulla and to ascertain the relation of respiratory act to the changes in the electrical brain waves previously observed by Tanaka under similar conditions in body-cooling process.

Mtehods

The procedure applied in these experiments, which, for convenience of

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reference, will be described later briefly, is otherwise identical to that previously used by Tanaka and Morita, except for a set of thermocouples for medullary temperature and a recording apparatus for respiratory movement.

Rabbits unanaesthetized were used, weighing 2 to 3 kg. They were placed in the abdomen-up position with limbs firmly fixed on a small table.

a) Serial registration of cerebral and heart potentials

A different electrode, utilizing a tack 6mm in length ordinarily used, was tuck into the brain through the skull at area striata (right), reaching accurately to the cerebral cortex. Another different electrode for the heart, silver round plate about 1.5 cm in diameter, was sealed on to the skin at the heart apex while an indifferent electrode which was common to both the brain and the heart was attached at the area praecentralis (left). The electrodes were connected, through a 4-stage resistance-capacity coupled amplifier for the brain waves and a 3-stage amplifier for heart potentials, to a commercial oscillograph (Yokokawa Co.), activating H-type vibrators, or visual inspection and serial registration of potentials. (Connection diagram and others in detail may be consulted in the previous works by Tanaka and Morita.) Thus the potential fluctuations of cerebral cortex and heart muscles were recorded on the paper where, in my case, the respiratory movements were traced as well.

b) Recording respiration

Respiration was registered in the following way. A small curved needle cannula, about 3 cm length, was inserted into the right pleural cavity, connecting with a rubber tube to one arm of U-tube manometer. The latter contains a saturated $CuSO_4$ solution. The level of solution moves up and down in unison with the changes in intrapleural pressure. In another arm of the U-tube, two electrodes faced each other at moderate distance were placed in this solution, the upper one was sealed on the bottom surface of a small float which may follow the movement of level. These electrodes were connected with a vibrator (H-type) of the same oscillograph to allow the battery current (1.5 v) regulated by suitable resistance to circulate.

Now the changes in intrapleural pressure on expiration and inspiration cause an up- and down movement of level of $CuSO_4$ solution in U-tube i. e. of the upper electrode attached to the float. Consequently varying the distance between both electrodes, fluctuates the intensity of direct current in circuit involving the vibrator. The changes of intensity of direct current corresponded quite well to changes in repiration without a lag of time.

This apparatus employed for recording the respiration records the changes in rate accurately, but gives less reliable indication of tonic changes in respiration

since it was liable to be affected by an exsudate or blood coagula in cannula, jerks of body and other factors. Therefore, as far as the depth of the respiration is concerned, it was necessary to refer to the direct observations of the general appearance of the animal. The graphical tracings of respiration thus recorded are shown in the middle of the paper (see fig. 2) in which downstroke represents inspiration and upstroke represnts expiration. Time-markings are recorded on upper side of the strip. Heavy dots indicate 1/10 second and slight dots indicate 1/100 second. Photographs were taken, as a rule, at 10 minutes intervals and whenever necessary.

c) Body temperature measurement

Cortical and medullary temperatures were measured by two thermocouples specially made for this purpose and rectal temperature by an alcohol thermometer, 30 cm in length, inserted for a distance of 8 cm beyond the anal sphincter. The thermocouples were made of enamelled and with silk insulated copper and constantan wires, 1 mm in diameter (gauge no. 36), and in length of 3 m between the cold and the hot end, being long enough to reach a low temperature chamber. The hot junctions were protected with metal capsules, 1.0 to 1.5 cm length, utilizing hypodermic needles (1/2). These capsules were bent nearly at right angles at the point of 1.1 cm (in thermocouple for medulla) and 4 mm (in thermocouple for cerebrum) respectively from the tip of hot junctions. This was done for the convenience of fixing and keeping the position of hot juncton in the tissue constant. (In order to simplify, when necessary, the following abbreviated terms will be used thereafter, M-thermocouple for thermocouple for medulla, and C-thermocouple for that of cerebrum.)

Cold junctions were placed in a Dewer flask which contained some ice water. Each of hot junctions was calibrated against a standard certified Hg-thermometer calibrated to 0.01°C, using two suspension type galvanometers. (Riken, period 5 sec, sensibility 1.0×10^{-7} volt ; Yokokawa, 4 sec, 4.6×10^{-7} v)

Each thermocouple was inserted into tissues after passing through for a distance of 6 cm subcutaneously, to eliminate an error which might be produced by cooling from outside along the thermocouple wire. The location of insertion were as follows : the area praecentralis (right) for C-thermocouple and a slight lateral point, usually left, to protuberantia occipitalis externa for M-thermocouple were respectively chosen and these locations were previously trephined by a tack or a small drill, which was tightly fitted for thermocouple's diameter. C-thermocouple reached the cerebral cortex (2 mm depth) accurately. C-thermocouple was inserted from above mentioned place to obex in sagital direction up to the depth as far as the thermocouple could reach. But the latter is bent, as noted previously, at the point of 1.1 mm from the hot junction and therefore did not reach the

substance of medulla, which was verified at autopsy in all the cases. This was for the reason that it was concieved to be desirable to avoid the effects by stimulation of vagal and other medullary nuclei overlying the reticular formation. Therefore the temperature measured here is that at the point 1.1 mm below the skull surface at protuberantia occipitalis exterma. But it may be permitted to consider that the temperature obtained here is a representative one of the reticular formation, basing on the assumption that both locations are very close to each other anatomically. Insertion of C-thermocouple was accompanied by no bleeding and no effect, but introduction of M-thermocouple, in two or three cases, resulted in small hemorrhage which however stopped at once ; in a very few cases, a gross hemorrhage, which was probably due to a variety of vena cerebelli superior mediana. In latter case, the further experiment was discontinued.

It seems here necessary to consider the effects of insertion of thermocouples to physiological functions. Refering to these effects is necessary in analysing the

Table

Exp. 23 (Rabbit, 2.3 kg. ♀ white) room temp. +18.0°C.

Time in min.	Body temperature in °C			E. E. G.
	medullary	rectal	cerebral	amplitude in μV (average)
control period	37.8	34.0	33.8	104 120
Exposure to cold environment (-40°C air)				
5	36.8	32.0	32.8	75
15	35.3	30.4	31.2	60
33	32.8	28.8	28.5	a series of continuous faint fluctuations of isometric line
45	30.9	27.0	27.0	〃 〃
63	28.7	25.5	25.2	〃 〃
93	24.8	21.4	21.0	〃 〃
105	23.2	19.6	19.4	〃 〃
120	21.0	17.3	17.0	reappearance of brain waves (18c/s, 20~100 μV, being superposed on slow waves (3 c/s)
128	18.6	16.2	14.4	disappeared
140	14.8	15.0	11.2	

Remarks :

* Respiratory rate in rabbit is about 40 per min. in normal condition, but

** These faster waves (so-called Berger rhythm), superposing on 2~3 c/s

data obtained in these experiments in order to compare with the results attained in the unoperated experiments previously made by Tanaka and Morita. These effects will be described and discussed in the following.

d) The effects of insertion of thermocouples

As shown in fig. 2, there were no appreciable changes in either brain waves or E.C.G. referable to the insertion of thermocouples. The insertion of C-thermocouple caused no effects. But by the insertion of M-thermocouple there were observed following changes. Slight change in respiration, an increase in some cases and a decrease in others were noted. These, however, were negligible since they returned to normal condition a few minutes later. These changes in rate were rather due probably to a mechanical stimulus caused by the enforced flexed position, which was temporarily necessary for convenience of insertion of M-thermocouple, than due to direct stimulation of ascending and descending pathways of respiratory

1.

	Respiration		E. C. G.	
cycles per sec.	rate per min.	depth & others	pulse rate in min.	other changes
14** 7~5	73*		238	
7~5	50	deep	244	
6~4	58	decreased its depth gradually	245	
	59		195	bradycardia
	64	regular, shallow (See text for details)	154	
	〃		135	
	28		90	ST-drop (slight)
	29		74	sinus arrhythmia
	32	agonal	25	ST-drop, diphasic T
	0	cessation of breathing	23	atrio-ventricular block, marked ST-drop, widening of QRS
			†	

it was usually almost doubled by insertion of intrapleural cannula.
waves, lasted ca. 5 sec. and occurred once in 10 sec..

Table

Exp. 16 (Rabbit, 2.9 kg. ♀ White) room temp. +11.5°C.

Time in min.	Body temperature in °C			E. E. G.
	medullary	rectal	cerebral	amplitude in μv (average)
control	38.0	35.0	34.3	80
Exposure to cold (-40°C air)				
5	36.6	—	31.8	107
10	36.0	30.2	31.0	80
40	30.5	27.0	26.6	60
60	27.8	24.6	24.9	faint fluctuations of isometric line
91	24.8	21.5	21.0	〃 〃
120	20.5	18.0	18.0	reappearance of brain waves (17～15 c/s, 50～40 μV), lasting 15 min.
135	19.2	16.5	15.4	disappeared
150	13.6	14.8	11.2	

Remarks :

* Superposing on 2 c/s waves, lasted about 2 sec. in intervals of 5～10 sec.

Table

Exp. 9 (Rabblt, 2.9 kg. ♀ white) room temp. +19.0°C

Time in min.	Body temperature in °C			E. E. G.
	medullary	rectal	cerebral	amplitude in μV (average)
control	38.7	36.0	36.3	75
Exposure to cold (-40°C)				
38	33.8	29.1	28.1	15
78	27.2	23.3	21.4	serious faint fluctuations of isometric line
125	19.6	17.8	14.2	40
129	19.2	17.6	13.2	disappeared completely
140	16.0	16.0	10.0	

Remarks :

* Never appeared "Berger rhythm".

** Reappearance of brain waves.

2.

	Respiration		E. C. G.	
cycles per sec.	rate per min.	depth & others	pulse rate in min	other changes
13*	101		278	
7~4	112		227	
6~3	113		〃	bradycardia
5~3	52		138	
	54		129	
	61		117	sinus arrhythmia
	67	em-barrassed	76	ST-drop
	0	cessation of breathing	20	complete atrio-ventricular block, inversed T
			†	

3.

	Respiration		E. C. G.	
cycles per sec.	rate per min.	depth & others	pulse rate in min.	other changes
7~3*	82		250	
〃	64		216	
	35		—	
6~4**	10	agonal	50	marked lengthening of QRS
	0	cessation of breathing	17	atrio-ventricular block
			†	

Table

Exp. 1 (Rabbit, 2.5 kg. ♂ white) room temp. +15.0°C.

Time in min.	Body temperature in °C			E. E. G.
	medullary	rectal	cerebral	amplitude in μV (average)
control	38.0	35.8	36.5	180
Exposere to cold (-40°C air)				
10	36.6	32.4	32.8	80
43	33.0	29.0	29.5	40
60	30.6	26.8	26.6	Any fluctuation was no more observed.
90	26.8	23.1	22.6	Some fluctuations with abnormal great amplitude (200~100 μV) and slow frequency (5~3/sec.) occurred occasionally.
115	25.0	19.2	19.0	abolished
137	20.4	15.8	14.4	
150	15.8	14.5	9.3	

Remarks :

* The "Berger rhythm" in 11 c/s, 90 μ V. occurred only once during

** 135′, ventricular automatism, 31 in rate.

Table

Exp. 3 (Rabbit, 2.6 ♀ white) room temperature +14.0°C.

Time in min.	Body temperature in °C			E. E. G.
	medullary	rectal	cerebral	amplitude in μV (average)
control	37.2	35.0	34.2	80
Exposure to cold (-40°C air)				
30	32.9	28.8	28.4	40
90	25.8	21.8	22.5	only irregular fluctuations of zero line
135	21.3	17.3	18.6	reappearance of 6~3 c/s waves, 100 ~70 μV, lasting 15 min.
150	19.0	15.2	17.0	disappeared entirely
180	15.4	11.3	13.1	
190	13.8	10.9	11.2	

Remarks :

* Faster waves (14 c/s, 80 μV), lasting 3~6 sec, appeared occasionally (at 10~20

4.

	Respiration		E. C. G.	
cycles per sec.	rate per min.	depth & others	pulse rate in min.	other changes
5∼2*	134		276	
6∼5	130		273	
6∼3	58		105	bradycardia
	51		119	
	48		97	sinus arrhythmia
	41	em-barrassed	71	
	0	cessation of breathing	**	
			†	

control observation.

5.

	Respiration		E. C. G.	
cycles per sec.	rate per min.	depth & others	pulse rate in min.	other changes
6∼3*	106		263	
6∼3	78		187	bradycardia
	60		〃	
	〃		65	widening of QRS
	21		102	sinus arrhythmia
	0	cessation of breathing	—	
			†	

sec. intervals), overlapping on 2∼3 c/s waves.

nerve impulses. Moreover, although there the nucleus fastigii and other cerebellar nuclei were located which participate in the extrapyramidal system, in no cases was there a sign of stimulation of this system. But there must be noted an increase of blood sugar caused by M-thermocouple insertion which did not appear in unoperated experiments. According to the blood examination which has been made by Uozumi in same material, it was detected that blood sugar increased up to a level of 0.14 mg percent following the insertion of M-thermocouple while the normal values showed about 0.09 mg percent. This increase in blood sugar may have been, as was stated in the previous work by Shinozaki (1926), due to the injury of the uvula of the cerebellum, since this injury was identified likewise in these experiments by autopsy.

Now a question arises, what effect this increase in blood sugar produces on the process which will be taking place in the following body-cooling experiments. With regards to the point, I should like to think as follows : the body-cooling process was not almost influenced by this increase of blood sugar because there were no essential differences between the data obtained in these experiments and in the previous findings presented by Tanaka and Morita but only a slight shortening of survival duration of the animal. This shortening of survival time may be presumed as due to physical factors such as the intrapleural cannula and the more abundant chances of cooling from outside along "two" thermocouples. (Only C-thermocouple was employed in the experiments by Tanaka.) This conception may be supported by the fact that blood sugar increase in similar degree was detected also in unoperated cases even by exposure to cold (Uozumi). Therefore this increase in blood sugar in present experiments ought to have been produced sooner or later, even if without a puncture of M-thermocouple, namely it was only produced previously artificially instead of naturally by cold.

Results and some comments

The data obtained in these experiments are summarized in tabs. 1-5 and only one representative case (exp. 23) is illustrated in fig. 1 and shown in fig. 2 in abbreviated forms by trimming for sake of space economy.

a) Body temperatures

In normal condition, the medullary temperature was found to be the highest, showing 37.2°C to 38.7°C (the average 37.7°C), while the cerebral and rectal temperature about 3 to 4°C lower than the former. The cerebral temperature revealed the lowest value 33.8 to 36.5°C averaging 35.2°C. As shown in fig. 1, after the exposure to cold, body tmperatures declined gradually at an uniform rate, figuring approximately linear curves, up to 20°C or less (medullary) at which the

abolishment of respiratory movement occurred. Throughout the observation period, in all experiments, the medullary temperature was 3 to 4°C much higher than the corresponding temperatures in other places and in most cases, the rectal temperature situated in the middle and the cerebral temperature was slightly (about 1°C) below the rectal temperature. The death of the animal (the complete cessation of heart activity) occurred at a temperature of 16°C or less (rectal).

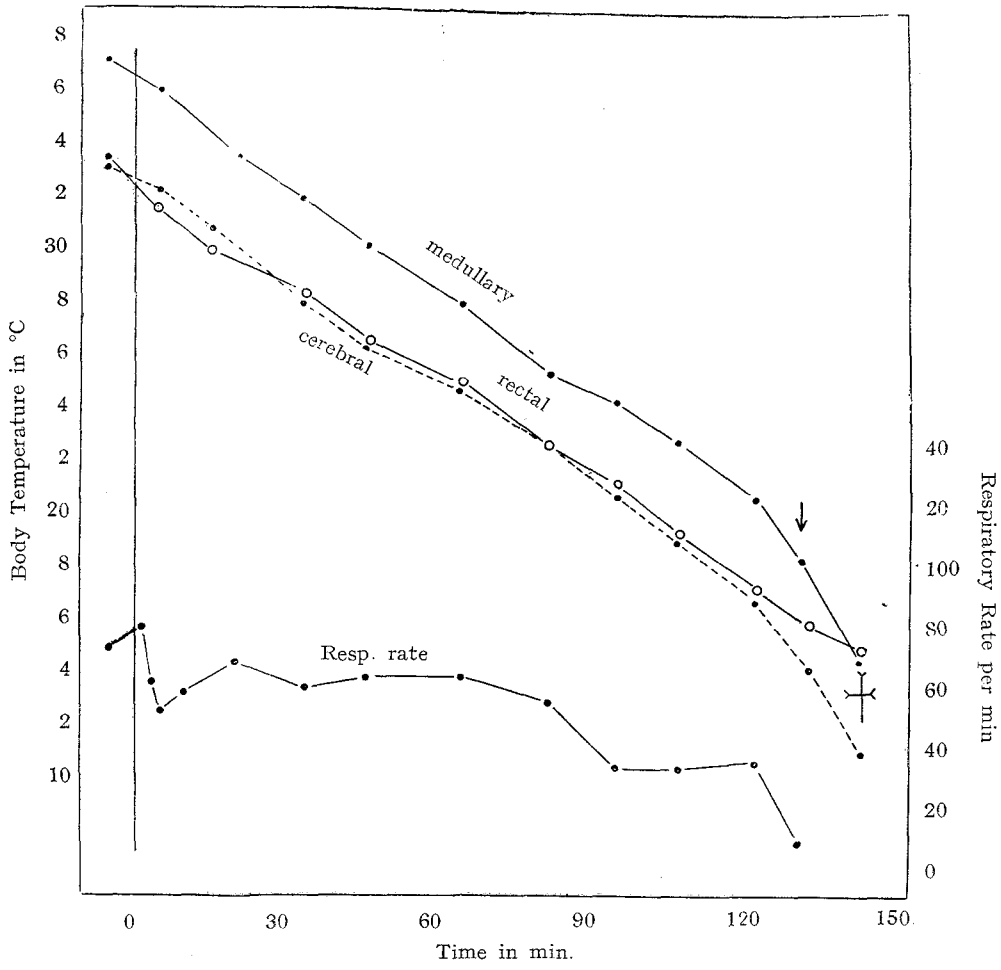
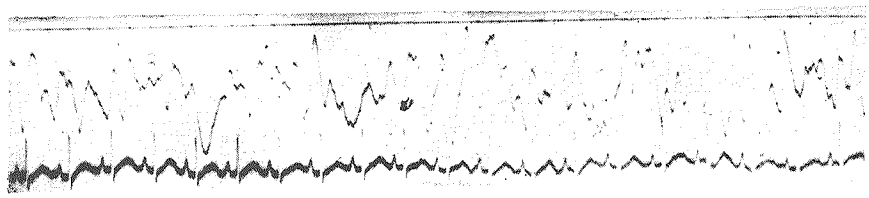


Fig. 1. (exp. 23) Changes in body temperature and respiratory rate after exposing the rabbit to cold environment (-40°C).
Medullary : medullary temperature, Rectal : rectal temperature, Cerebral : cerabral temperature. ↓ : cessation of respiration.

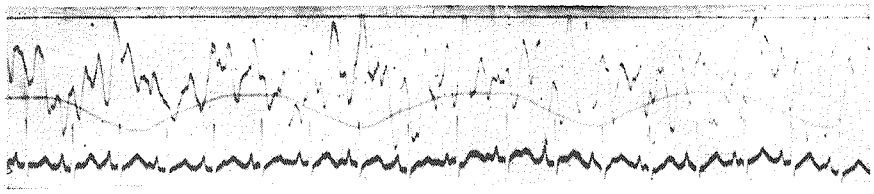
b) Potential fluctuations in the cerebral cortex

The spontaneous cortical potential fluctuations of the rabbit which were taken at area striata, as seen in fig. 2, consist of roughly two kinds of waves, i. e. relatively faster frequency waves (8 to 15 cycles per sec.), so-called "Berger rhythm" or "alpha waves", and slower frequency waves (2 to 7 cycles per sec.). But it must be here noted that the so-called "alpha waves" in rabbits are generally more complicated and less regular form often superposed on slow frequency waves of 2 to 3 cycles per sec.

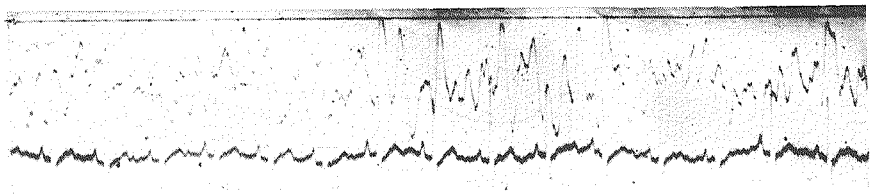
By means of this leading method employed in these experiments, the average amplitude of cerebral fluctuations is in general considerably great, ranging individually from 80 to 180 microvolts.



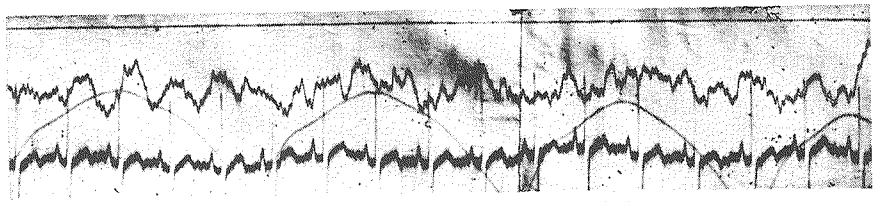
a.



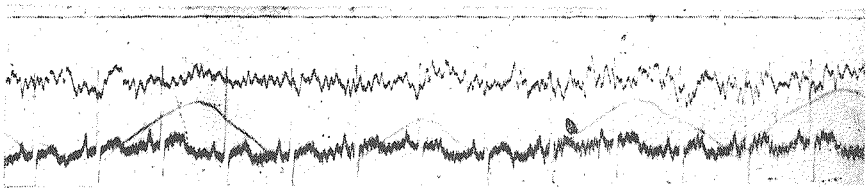
b.



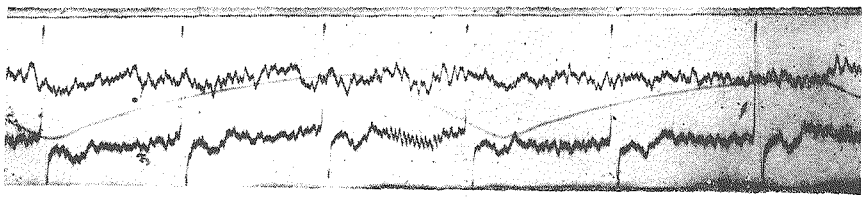
c. M. 37.8°C R. 34.0°C 33.8°C.



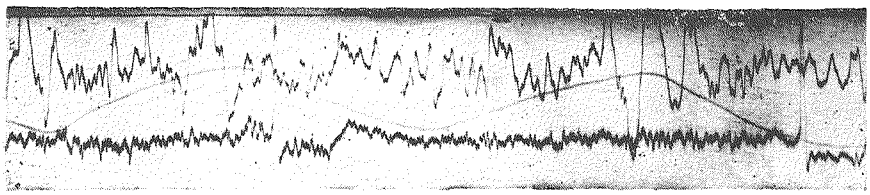
d. (5′) M. 36.8°C R. 32.0°C 32.8°C.



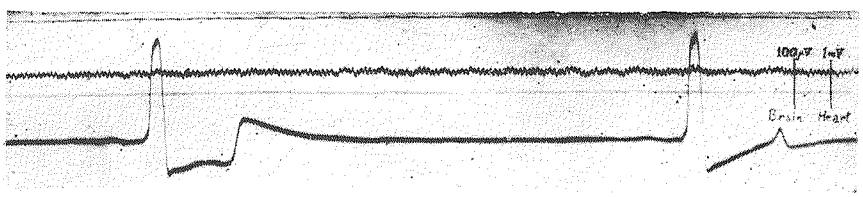
e. (33′) M. 32.8°C R. 28.8°C 28.5°C.



f. (93′) M. 24.8°C R. 21.4°C 21.0°C.



g. (120′) M. 21.0°C R. 17.3°C 17.0°C.



h. (128′) M. 18.6°C R. 16.2°C 14.4°C.

Fig. 2. (exp. 23)

1) Timemarking is on top side in each strip. Heavy dots indicate 1/10 second, slight dots indicate 1/100 sec.
2) Calibration curve for 100 microvolts (E.E.G.) and that for 1 mV (E.C.G.) are shown at right side of the bottom strip.
3) Sinnsoidal curve in the middle in each strip shows respiratory tracing, in which downstroke represents inspiration while upstroke represents expiration.

a : control period, b : introduced a intrapleural cannula, c : inserted thermo-couples, d : 5 minutes after exposure of the animal to cold (-40°C), e : 33 minutes (a continuous series of irregular and faint fluctuations of isometric line.) f : 93 minutes later, g : 120 minutes (reappearance of brain waves), h : 128 minutes (complete cessation of brain waves and respiration). For other details see text.

C : cortical temperature, M : medullary temperature, R : rectal temperature.

Immediately after the subjecting to cold, the cortical potentials suppressed temporarily, and 2 or 3 minutes later there was observed a tendency to revert to normal state. But as the body temperature continued to decrease, the depression of the cortical potentials became progressively pronounced, being substituted by less faster frequecy waves (5 to 7 cycles per sec.), and never appeared the "alpha waves". And then after the appearance of continued 3 to 5 cycles per sec. waves (so-called "delta waves"), the electrical brain waves, showed no regularity of rhythm, as may be seen in strip "e" of fig. 2, became only a continuous series of irregular and faint fluctuations of isometric line, losing the normal characters of the brain waves, in 30 or 90 minutes in all cases from the onset of cooling (28.5 to 21.4°C cerebral).

This lack of normal character of brain waves was regarded by Tanaka in his previous work as indicative of depression of cortical functions and the time from which the rabbit dashed into the paralytical stage. Following this stage, the brain waves remained the same, the irregular fluctuations of zero-line, being occasionally interspersed by small flings which corresponded to the peaks of respiratory tracing, until the reappearance of the brainwaves-like fluctuations occurred before the cessation of respiratory act. (See the strip "g" in fig. 2.) This reappeared brain waves characterized by great amplitude of 20 to 100 microvolts, the frequency of 15 to 18 cycles per sec., and the irregularity of form in various degree. In some instances (exps. 9, 1 and 3), at this stage there were observed slower frequency waves (3 to 6 cycles per sec., 40 to 200 microvolts) instead of faster waves. The typical beautiful spindle-like "alpha waves" which were previously detected by Tanaka at this stage, were not observed in these experiments. Following the reappearance of the brain waves which lasted 5 or 20 minutes, the cortical fluctuations became only a straight line and disappeared completely (11.2 to 9.3°C cerebral).

c) Changes in E. C. G.

The electrocardiographical changes will be described here simply because they have been already reported in detail and accurately in Morita's work.

As the body temperature declined, the heart rate slowed gradually, without showing other marked changes in heart activity. The essential changes, such as the failure of the heart muscle or the disturbance of atrio-ventricular conduction, as indicated by the deformation of spikes or the lengthening of PQ-intervals, began to appear only after one hour or more from the start of observations, but in slight degree. It must be here noted that the changes in E.C.G. are surprisingly inconspicuous, but a slowing of the rate, even by the time at which the cortical fluctuations revealed themselves as some faint and irregular fluctuations of zero-line, losing the normal character of brain waves.

At the time of terminal reappearance of brain waves, although there were observed signs of a moderate or fairly marked failure in heart activity, such as sinus arhythmia, a drop of ST, a considerable lengthening of conduction period of impulse, but the heart could have beaten still vividly. Following the abolishment of the brain waves and respiratory movements, however, the heart acted less vividly and weakened extremely (artio-ventricular block, negative P, diphasic or inversed T-waves) and then ceased the beating completely. The cessation of beating of the heart occurred at least 10 minutes after the preceding abolishment of respiration and at a temperature of 16°C or less (rectal).

d) Respiration

The rabbit in this posture, fixed abdomen-up, performs the costo-abdominal respiration, amounting about 40 in rate. By the introduction of the intrapleural cannula, the respiration was usually doubled or more in rate and became somewhat shallow. The insertion of the M-thermocouple caused, as stated in the preceding paragraph, no marked changes in rate and depth, even if it caused any change, it was slight and restored to the former state a few munites later. The following observations for respiration are compared with the respiration at the time of insertion of the thermoccouples.

Immediately after the subjecting of the animal to cold, the respiration revealed temporarily a labile appearance, probably due to a reflexogenic stimulus by cold, lasting several minutes and in most cases, showed a slight increase in rate. But the respiration turned presently into a steady, regular, slow and very deep respiration which lasted for a considerably long time. As the body temperature continued to fall, however, the respiration became gradually shallow, decreasing its rate, but remained still regular.

At the time in which the brain waves were only a continuous series of irregular and faint fluctuations of zero-line, the respiratory act was still firm and regular, (32.8 to 25.8°C medullary) although the rate was already slowed by about half and the depth was fairly decreased as compared with the initial period. This fact, the author believes, offers the evidence that the central respiratory mechanism in medulla is maintained still fairly intact even by the time at which the cerebrum has already lost its normal activity. However with the further fall in body temperature, the respiration became gradually more shallow, although the decrease in rate was not so much, and finally proceeded to terminal stage.

At this agonal terminal stage, which, with the exception of exps. 1 and 3, corresponded to the time of reappearance of brain waves, the animal was extremely exhausted, showing the embarrassment of breathing and the respiratory action became so weak and faint as to be hardly traceable to be recorded.

Finally the animal ceased to breath entirely, being preceded by gasping

movements accompanied by opening the mouth and slight movements of the nose (20.4°C or less medullary). Whereas the cortical fluctuations were abolished almost simultaneously, except exps. 1 and 3, with the cessation of breathing and never appeared thereafter, the heart continued still to beat for a period of 10 minutes or more.

Discussions

It is in general believed by many physiologists that the central controlling mechanism of breathing is localized in the reticular formation extending along the floor of the fourth ventricule. But as to its more definite extent, their observations were often conflicting. In the recent works, however, by Henderson and Craigie (1936), Gessel, Bricker and Magee (1936), and Pitts et al (1939) who have postulated a functional subdivision of the respiratory center into the inspiratory and expiratory portion, all their findings agree quite well in showing that the respiratory center may be limitted above at the level of the upper quarter of hypoglossal nucleus and below by the pyramidal deccussation. According to their investigations, it may be known that the respiratory center resides in fairly wide area in the reticular formation extending above and below the obex.

Therefore in my experiments, as mentioned in the preceding paragraph, it was assumed that the temperature of the obex represents that of the respratory center.

Respiration in normal condition is performed automatically by centrogenic impulse built up in the reticular formation. The automaty of respiration is controlled by the chemical changes (such as O_2 lack and CO_2 excess i. e. the change of pH) in blood which passes through the respiratory center or, according to Heymans and his collaborators (1930, 1930a, b), by reflexogenic impluse arising from the carotid sinus which acts as a chemoceptor to the changes of CO_2 and O_2 content or pH of blood. In addition, respiratory act is regulated by stimuli from the sensory endings of the vagi in the lungs which tend to limit both inspiration and expiration in ordinary breathing (so-called Hering-Breuer Reflex). Moreover the respiration is readily influenced by many other sensory stimuli. And thus the respiratory mechanism is considerably complicated in itself and liable to be influenced by external and internal stimuli. For example, this neural central mechanism is sensitive to the temperature of the blood passing through the respiratory center. Cold blood evokes slow respiration while hot blood causes accelerated respiration. The influence of cold applied directly to the medulla was demonstrated by Fredelicq (1883), Dittler (1912) and Nicholson (1936), resulting, in their investigations, in depression or even complete arrest of breathing.

These facts suggest that the central respiratory mechanism is associated with the temperature of the medullary tissue. In general all the physiological

activities are dependent upon the fundamental processes of tissue oxidation which are inherently a series of chemical reactions to which is applicable the van't Hoff law stating that the speed of a chemical reaction is doubled or more for each increase of 10°C in temperature. It seems therefore reasonable to consider that the physiological function of tissue reduces generally with the fall of its temperature.

Immediately after subjecting the animal to cold, in present experments, the respiration showed temporarily a labile appearance, in most cases a slight increase in rate. This is, as noted above, due presumably to a reflexogenic stimulus caused by cold, and should be included in the same category of "vital reaction" which was called previously by Tanaka and Morita in meaning that living bodies react promptly by a slight increase of heart rate and body temperature and a suppression of cerebral potential fluctuations upon cold stimulus.

However several minutes after exposure to cold, the respiration turned into a steady, slow, regular and deep breathing. This may be interpreted as meaning that at this stage the animal is endeavoring extraordinarily to take a great amount of oxygen by deep respiration, in response to the demand of the internal organs where the respiratory metabolism is now taking place even more or to the fullest extent, in order to balance the increased heat out-go resulting from external cold and to maintain the normal body temperature. In spite of the continuous extraordinary increase in heat production the body temperature regulating mechanism is not equal to the task confronting it in such an extreme low environmental temperature and breaks down and thus the body temperature begins to decline.

With the fall of body temperature, consequently the fall of the temperature of cerebral blood flow, accelerated by cooling from the skull surface, and the decrease in the amount of blood supply to the cerebral cortex, as presumably judged by slowing of heart beat, the frequencies and amplitudes of cortical potential fluctuations decrease or diminish, indicating the progressive reduction of cerebral activities. And then eventually electrical brain waves became only a continuous series of irregular and faint fluctuations of isometric line, losing the normal character of brain waves. This lack of normal character of brain waves was, as stated in previous paragraph, interpreted by Tanaka to mean the almost complete depression of normal functions of cerebrum and the beginning of the paralytical stage. This conception may be supported by the symptomatic appearance of the animal revealing at this stage, such as an occasional dull movement of body, no reaction to light, sound, and pricking, or moderate dilation of pupils. This epoch occurred at a body temperature of 28.5 to 21.4°C (cortical), but, in previous experiments of Tanaka, at 30.6 to 26.4°C. The difference of temperature in both instances is to attribute to my experimental unskilfulness and to more abundant chances of cooling from outside along the "two" thermocouples. (In Tanaka's experiments only one thermocouple for cerebrum was used.)

At this stage, the medullary temperature remains still at a higher level by 3 to 5°C than the corresponding cortical temperature. This may be due reasonably to the advantageous anatomical position of medulla against cooling, which is protected by thick occipital muscle layer and situated more close to the heart. This less decreasing of medullary temperature and blood supply, indicating a favorable condition for building-up process of respiratory impulse in the reticular formation, may account for a fairly intact respiration even by the time at which the cerebrum has lost its normal activity.

Following at this stage, however, with the further fall of body temperature, the decrease became gradually noticeable in respiratory depth, although not so much in rate, indicating the depression of whole respiratory system.

The animal was prostrated and showed no more reaction to any stimuli, such as light, sounds, pricking, and showed a slight movement of thorax accompanied occasionally by a faint nod-like movement of head in inspiration, and then approached to the terminal stage. Complete cessation of breathing occurred at 20.4°C or less (medullary), preceded by several gasping respiration and occasionally by a insignficant faint nose movement which was seen for a short period following the former. The "biological zero" (Bělehrádek, 1935) of medullary function obtained here is substantially identical with that attained in a classical work by Winternitz (1894) and in recent study by Niitsu (1935), both of which, however, were observed by rectal temperature.

The abolishment of electrical brain waves were observed almost simultaneously with the respiratory cessation or, in exps. 1 and 3, 20 or 30 minutes before. Here I should like to think that the latter two cases are probably rather the exceptions, being supported by the gross and accessory observations in other similar experiments in our laboratory which were however carried out under the different object.

Therefore it appears that the electrical brain waves (derived by means of such leading method as employed in these experiments) and respiration cease almost at the same time.

Although the genesis of reappeared brain waves at the terminal stage is not accountable at present, but it seems to the author that the experiments of Dittler (Literature 1912), Dittler and Garten (1912) on the diaphragmatic and phrenic nerve action currents in case of the local cooling of medulla and cervical cord suggest a hint to solve this problem. They stated in their works that by local cooling of cervical cord corresponding to the root of phrenic nerve, the freqeuncies of action currents of phrenic nerve and diaphragm decreased to approximately 1/10 of normal values, to 15 per second. This value of frequencies is identical roughly with that of brain waves obtained at this agonal stage in present experiments. At this stage, the animal is extremely prostrated and showed only a faint and weak respiratory

effort accompanied by a nod-like movement of the head. The latter which is a movement of a group of occipital muscles, never participating in normal respiration, may now offer an opportunity to make its action currents break through to electroencephalogram, in harmonizing with inspiratory movement. This conception is of course only a presumption, affirmance of which remains on the future study.

At this terminal stage, the heart activity showed marked failure, although there had been observed no striking changes so far, but a slowing of rate, and proceeded to the entire arrest of beating in 10 minutes or more after the cessation of breathing.

And thus the death of the animal occured. The immediate cause of the death is naturally the paralysis of cerebro-medullary function, especially of respiration. However, it should be considered that the paralysis was caused by the fall of temperature of the central nervous system, by a gradual decrease of blood supply to it, and consequently by a reduction of respiratory metabolism in the neural centers.

Summary and conclusions

1) The whole process from the onset of subjecting the rabbit, fixed abdomen up, to cold environment (−40°C) to death which occurred in about 2 or 3 hours, has been observed, recording the electroencephalo- and electrocardio-grams, and registering respiratory tracing as well.

2) Meanwhile the body temperature has been measured (the cerebral and medullary temperature by copper-constantan thermocouples and rectal temperature by an alcohol thermometer).

3) Following the exposure to cold, the body temperature declines gradually linear with an uniform rate up to 20°C or less where the cessation of breathing occurs.

4) Throughout the whole observation period, the medullary temperature remains higher by 3 to 4°C than the other corresponding body temperatures while the cortical temperature shows, in most instances, the lowest value.

5) Respiration shows more or less a slight increase in rate immediately after the exposure, but, in several minutes, turns into a steady, slow, regular and deep breathing. And with the progressive fall of body temperature, it becomes gradually shallow and proceeds finally to agonal stage, showing a nod-like movement of head, gasping breathing and faint movement of nose, and then ceases entirely. (20.4°C or less medullary)

6) The cerebral function is affected in an earlier stage of the experiments (in 30 minutes or more after cooling, at 28.5 to 21.4°C cortical) and then the abolishment of cortical fluctuations occurs, preceded by the reappearance of the brain-waves-like fluctuations, the genesis of which is discussed but remains for future

study.

7) The cessation of electrical brain waves and that of respiration occurred almost at the same time, with the exception of two cases.

8) It is concluded that the cause of death may be attributable to the paralysis of cerebro-medullary function sepecially of central respiratory mechanism, caused by the fall of medullary temperature and consequently by a reduction of respiratory metabolism in the neural controlling center of breathing.

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* sign indicates a paper which is written in japanese and which title has been provisionally translated into english by the author.
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