Changes in Constituents in Arterial Blood Following Experimentally Induced Epileptic Convulsion in Dogs

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Many clinical and experimental studies on the blood following the convulsion in man2,6,7,27,38) and animals11,15,29,37) are already reported. It seems important to investigate the changes in the milieu interieur, as they have influences upon the function of various organs, especially of the brain. However, only few attempts have been made to study the progressive changes of the metabolism following convulsions.7,27,37)

In view of the present situation of the studies on the epileptic seizures, we attempt to investigate the changes in some constituents in the blood successively drawn from the artery. This enables us to have further knowledges on the metabolic changes and the regulation which may play a significant rôle in the recovery course following the nervous and humoral activation caused by electrical stimulation of the brain.

METHODS

Healthy dogs, varying in weight from 10 to 15 kg, were used for these experiments. The food was withheld for 24 hours before the experiment. The animal was placed in supine position and the blood samples were collected from femoral arteries under local anesthesia. For the electrical stimulation two round silver electrodes of 1.5 cm in diameter were applied to the skin about 1.5 cm superior to the eyes. The hair in these regions was previously cut to obtain the contact. The current used was 50 c/s AC of 50 and 100 V for five seconds. By such procedure the typical grand mal reactions as described by Feldman and other investigators11,20) could be obtained.

From the femoral arteries 20 to 25 ml of blood for various determinations was drawn at certain intervals into syringes whose inner surfaces were previously

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coated with paraffin oil. The blood was soon poured into flasks which contained sodium oxalate (1 mg per ml of blood) and paraffin oil, and were cooled under 7°C. A portion of cooled blood was immediately centrifuged without contact with air for the estimation of pH, phosphate and chloride in the plasma. Double oxalated blood was used for the measurement of the hematocrit value.

For control experiments in which no electrical stimulation was applied, the determination of the same constituents was carried out in five dogs under same experimental conditions to study the effect of successive blood sampling without convulsions.

The following constituents of blood samples were determined.

1. The relative volume of cells and plasma. This was determined by centrifugation of double oxalated blood for 30 minutes at 3,000 r.p.m. using a hematocrit of Hedin type.

2. pH. For the estimation of the hydrogen ion concentration in the plasma, quinhydrone method was used.

3. Chloride. The chloride concentration in the whole blood and the plasma was determined by Schales and Schales method. Chloride in cells was calculated as same as that of phosphorus mentioning below.

4. Phosphorus. Inorganic and acid-soluble organic phosphorus in whole blood and plasma were measured colorimetrically by the method of Fiske and Subbarrow. Attention was payed to prevent the decomposition of acid-soluble organic phosphorus. Thus, the concentrations of phosphorus in red cells were calculated according to the following formula:

\[ Cc = \left( Cb - Cp \frac{100 - H.C.}{100} \right) \frac{100}{H.C.} \]

where

- H.C. = hematocrit value
- Cc = concentration in red cells (mg/dl)
- Cb = concentration in whole blood (mg/dl)
- Cp = concentration in plasma (mg/dl)

5. Blood sugar. The sugar concentration in the arterial blood was determined by Hargedorn-Jensen's method.

RESULTS

1. Hematocrit value. In Table I, hematocrit value of convulsed and non-convulsed dogs are shown. In non-convulsed dogs variations in the hematocrit readings for about two hours remained 1. On the other hand, in convulsed dogs, a significant increase in the readings of cell volume (3-10, mean value 5) was observed at 3 to 10 minutes and returned to normal at about 35 minutes after the convulsion. This increase in the cell volume was also found in anesthetized, splanchnicotomized or adrenalectomized dogs following the convulsion.
dog into which 10 gamma/kg of adrenaline (Park Davis) was intravenously administered, the cell volume rose also remarkably, having sustained for 10 minutes, then fell gradually, and remained at a higher value than that in control period even at 60 minutes after the convulsion.

2. pH. The representatives among 14 convulsed dogs are demonstrated in Table I. A slight decrease in the plasma pH was found during 90 minutes by sampling of several specimens in control dogs. In convulsed dogs, however, pH of the plasma reached abruptly to the minimum value between 2 and 4 minutes and returned to normal at about 30 to 60 minutes after the convulsion. These decreases in the plasma pH were ranged between 0.12 to 0.18, and were significant since variations in pH during control period were ranged between 0.03 to 0.07. A marked fall of the plasma pH (from 7.55 to 7.34) was induced by the intravenous administration of adrenaline, but disappeared within 10 minutes following the injection.

3. Inorganic and acid-soluble organic phosphorus. In preconvulsive period the concentration of inorganic phosphorus varied between 2.1 and 3.8 mg/dl in the whole blood, 2.3 and 4.5 mg/dl in the plasma, 1.3 and 3.2 mg/dl in red cells,
the mean values being 3.1, 3.6 and 2.5 mg/dl respectively. The concentration of acid-soluble phosphorus was negligible in plasma, and 11.1-28.6 mg/dl in whole blood, 23.1-50.0 mg/dl in cells, their mean values in six dogs being 16.2 mg/dl and 34.2 mg/dl respectively. These values were similar to those reported by others. In control dogs, the variations of the concentration of inorganic and acid-soluble phosphorus in the blood, plasma and cells remained within 20% of the initial values during successive determinations for 90 minutes (Fig. 1).

As shown in Fig. 2, a remarkable increase in the concentration of blood inorganic phosphorus (33.3-93.4% in whole blood, 33.3-70.0% in the plasma, 30.6-49.2% in the cells) was observed after the convulsion. Only in a case a decrease (38.0% decrease of the initial value), which was found after an initial rise, was obtained at 60 minutes following convulsions. In most cases, the maximum values were found at about 30 minutes after the convulsion, and returned to the initial value at about 2 hours following the electrical stimulation.

On the other hand, the changes in the concentration of acid-soluble phosphorus in cells remained within the range of variations during control period.

The changes of phosphorus compounds in the blood caused by the injection of adrenaline were rather complicated. A significant decrease in inorganic phosphate was found immediately after the administration. This decrease was more striking in the plasma (28.3%) than in the cells (18.6%), but the relation
in the decrease was reversed after 10 minutes following the convulsion, i.e. 48.6% decrease in the cells and 24.7% decrease in the plasma. Acid-soluble organic phosphorus in whole blood increased up to 30.9% within 10 minutes after the injection, though no significant change of its concentration in the cells was found.

4. Chloride ions. The concentration of chloride ions in blood of four control dogs were agreed with that reported by other authors, being 84.1–96.2 mequiv/l (mean, 89.6 mequiv/l) in blood, 109.3–120.6 mequiv (mean, 113.6 mequiv/l) in the plasma, 54.6–67.6 mequiv/l (mean, 57.4 mequiv/l) in the cell. The variations in both the plasma and the cell were within about 5% of the initial values during the control experiment for 90 minutes (Fig. 3).

Chloride in blood showed a significant increase following the convulsion. It showed the maximum increase at 2–3 minutes after the convulsion and went back to normal within 60 minutes after the stimulation. The rate of change of the chloride concentration in the cell was larger than that in the plasma, therefore the Donnan’s ratio of chloride showed a slight increase following the convulsions. The recovery period of the concentration of chloride in the cell to the preshock value was longer than that in the plasma.

5. Blood sugar. The sugar in control blood varied from 83 to 140 mg/dl with a mean of 111 mg/dl in six normal and two narcotized dogs. The fluctuation of the blood sugar level remained within 10% of the initial value in
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Fig. 3. Changes in concentration of chloride in plasma (above) and cells (below) induced by convulsions. Black and hollow circles: plasma chloride in convulsed and control dogs respectively. Black and hollow triangles: cell chloride in convulsed and control dogs respectively.

each dog, whether the animal was normal or narcotized, except one dog in which it attained as much as 15.6% of the initial value.

The results obtained from the non-narcotized convulsed and control dogs are illustrated in Fig. 4. The rise in the blood sugar reached to the maximum value (from 35 to 50% increase) within 2 minutes after the convulsion and then slowly declined. In a case, this rise was sustained at almost the same level over a period for 60 minutes. As seen in Fig. 5, the elevation of the sugar level in narcotized convulsed dogs was generally lower than that in non-narcotized dogs, although they were stimulated by the electric current of the same intensity. This difference can be explained partly by the fact that the grand mal reactions in narcotized dogs were less than those in non-narcotized dogs (see DISCUSSION). The maximum per cent increase in narcotized dogs was ranged between 29.0 and 33.3%.

The blood sugar in splanchnicotomized animals was elevated as high as that in normal animals following convulsions, but in a dog denervated more than a week before the experiment, it appeared to return to its initial value earlier than that in the intact animals. On the other hand, only a slight and transitory increase, which was within the range of variations in control value, was found
Fig. 4. Comparison of blood sugar level in convulsed dogs with that in non-convulsed dogs (expressed as per cent of the initial values). Black and hollow circles; convulsed and control dogs respectively. Triangle; non-convulsed dog which was adrenalectomized and splanchnicotomized 3 hours before the first determination of the blood sugar.

Fig. 5. Changes in blood sugar in narcotized dogs. Black circle; convulsed dogs. Hollow circle; non-convulsed dogs.

in four adrenalectomized dogs, whether they were splanchnicotomized or not. Moreover, in an adrenalectomized dog, about 25% decrease was induced by the convulsions. These findings show that the adrenals play a significant rôle
for the remarkable elevation of the blood sugar level following convulsions, and that the impulse through the splanchnic nerve may have only some summative or prolonging effect on the rise in the blood sugar by convulsions.

DISCUSSION

It has been indicated that the autonomic response to the electroshock is a non-specific reaction originated from diencephalo-hypophysis and hyperactivity of neuro-vegetative system, and is characterized by a 'syndrome sympathique médullosurrénal' as in the case of defense reaction or emotional excitement. On the other hand, the convulsion induces an anoxic state due to violent muscle exertion with transient apnea as in the case of hard exercise or severe hemorrhagic shock. Therefore, the general metabolic changes in convulsions can be compared with those obtained from the experiments on the emotional excitement, hard exercise and also traumatic hemorrhagic shock.

It has been reported that the rise in blood sugar by stimulation of hypothalamic nuclei and pique diabétique and by reflex was much reduced by denervation of the adrenals. The remained slight rise in the blood sugar is thought to be mainly due to the liver activated by impulses through the hepatic nerve. Same conclusion was presented from the experiment on hyperglycemia
induced by emotional excitement. However, it will be recalled that the splanchnicotomy is not a sufficient denervation of the adrenals in dogs. According to our results in convulsed dogs, little change in the rate of increase in the blood sugar level was found after the section of the splanchnic nerves as is reported in the case of asphyxia, while no significant rise was obtained in adrenalectomized dogs. These findings seem to support the view that the marked rise in blood sugar following convulsions may be mainly due to the direct stimulation of adrenals by anoxemia suggested by Malméjac, Chardon and Gross (1950).

As mentioned above, the degree of the rise in blood sugar following convulsions was less in narcotized dogs than in non-narcotized ones. This might be also due in part to the possibility that the anoxemia by weaker grand mal reaction in the narcotized dogs is caused to a lesser extent than in non-narcotized ones.

The rise in the blood sugar by convulsions was much more intense and prolonged than that induced by intravenous injection of adrenaline of 10 gamma/kg. It may be possible that 10 gamma/kg adrenaline is overdose for the induction of the maximum hyperglycemia in dogs, since it has been reported that the most effective dose is 4 gamma/kg/min in cats. Moreover, it must be kept in mind that the abrupt fall in the blood sugar following convulsions in adrenalectomized dogs can be due to waste of sugar by muscle exertion and also partially by activation of vago-insulin system.

The increase in the hematocrit readings and the decrease in the glomerular filtration rate following convulsions, which are explained to be related to the activation of splenic and renal nerves by the stimulation of sympathetic system, disappeared within 20-30 minutes after the stimulation. In the case of emotions and also of convulsions, an increase in the reabsorption of water in renal tubules due to the increased secretion of ADH was suggested, and it seems to be possible that the liberation of anterior pituitary hormones, especially ACTH, is accelerated directly (via diencephalon) and indirectly (by the increased secretion of adrenaline) and then the secretion of glyco corticoids is induced. Those may co-operate for the prolonged elevation of blood sugar level with the action of the hormone from the adrenal medulla.

Some authors reported that the decrease in plasma inorganic phosphate following the initial rise was observed within 45 minutes after hard exercise for short period in man and animals. According to their results, it takes more than two hours for the recovery of this decrease in the concentration of inorganic phosphate. In our results, only in a few cases of narcotized and weakly convulsed dogs, such a fall in plasma inorganic phosphate was noted at 10-20 minutes after the convulsions. And the extent of the rise in blood sugar was slight. However, the concentration of inorganic phosphate in the plasma following
typical convulsions increased and sustained for more than an hour in most cases. The same pattern of changes in inorganic phosphate was reported by Ward and Call in convulsed rats. This evidence shows that the typical convulsions are characterized by an abnormal metabolic state accompanied by an extraordinary oxygen debt as the severe hemorrhagic shock, and is explained to be due to the inhibition of phosphorylation. Moreover, this seems to support the view that the convulsions and the severe hemorrhagic shock have many common features in the metabolic state of organs.

The metabolic acidosis is caused by experimentally induced convulsions in rats. In our experiments the same state was found in convulsed dogs by determining pH and also carbon dioxide content in blood. Changes in the acid-base balance is one of the factors which may have influences upon the functions of organs, especially of the central nervous system, since the excitability of neurones was changed by a shift in pH and be counted up one of the reasons for the therapeutic effect of convulsions.

Plasma pH and plasma carbon dioxide content, though the latter was determined in only four cases, changed in parallel. They showed the minimum values at 2–3 minutes after the convulsion and gradually returned to normal from 30 to 60 minutes after the electric shock. Although the hyperpnea which helps the driving carbon dioxide out of the blood begins to appear immediately after the termination of the convulsions, it is not sufficient to avoid the metabolic acidosis caused by an anoxia. The concentration of inorganic phosphate in the plasma did not change in the same way as pH in the plasma. This seems to indicate that the concentration of lactic acid in blood may mainly be related to couple with the acid-base balance.

As described above, such an increase in the concentration of chloride in blood of dogs was found following convulsions, as reported in the case of hard exercise in man and animals. It is worth mentioning that the rate of increase in chloride in the cell was much larger than that in the plasma, in other words, there was an increase in Donnan's ratio which indicates chloride in the plasma moves into the cell as if there is some mechanism to maintain the concentration of plasma chloride constant. One of the factors concerning this mechanism may be metabolic acidosis mentioned before, since the increase in Donnan's ratio run parallel with the decrease in plasma pH. In connection with this, it will be remembered that the same pattern of chloride shift is aroused during the course of general adaptation syndrome. With reference to the view described by Penn, such an increase in plasma chloride is accounted for the shift of water from the extracellular space to the intracellular space as the result of the rise in the intracellular osmotic pressure of muscles by contraction of muscles during grand mal reactions.

Rapoport and Guest reported that the concentration of cell acid-soluble
organic phosphorus depends upon the factors which have influences on the enzyme system of glycolysis in red cells, that is, blood pH, the concentration of plasma inorganic phosphate and of chloride in the cell. Thus the concentration of cell acid-soluble organic phosphorus increases with the rise in the concentration of plasma inorganic phosphorus, while the former decreases with the diminution of blood pH and with the increase of cell chloride concentration. In our convulsed dogs, little change in the concentration of cell acid-soluble organic phosphorus was found. This can be explained by supposing as the result of the balanced state of these three factors.

**SUMMARY**

1. Hematocrit value, pH, chloride, sugar, inorganic phosphate and acid-soluble organic phosphorus in arterial blood of dogs were successively determined before and after the electrically induced convulsions and the following results were obtained.

2. The hematocrit value increased abruptly within 2–3 minutes and sustained the maximum value for more than 10 minutes, then returned towards normal at 30 minutes after the convulsion.

3. pH in the plasma decreased, attaining the minimum value at about 2–3 minutes and returned to normal by 60 minutes after the convulsion.

4. The concentration of chloride in the blood increased soon after the electroshock, and showing an increase in the Donnan’s ratio, then returned to the initial value within 30–60 minutes following the electrical stimulation.

5. A marked rise in the blood sugar was found immediately after the convulsion and then slowly decreased, but it remained at a level higher than the initial one even 60 minutes after convulsions. This rise was little affected by the bilateral splanchnicotomy, but almost abolished after adrenalectomy.

6. The concentration of the plasma inorganic phosphate increased remarkably after the convulsion, showing the maximum value at about 30 minutes, then gradually diminished, but did not return to normal even at 60 minutes after the convulsion. On the other hand, cell inorganic phosphate showed a slight increase. No significant change in the concentration of acid-soluble organic phosphorus in cells was found.

7. The changes in the constituents of arterial blood by electric convulsions were discussed with regard to the results obtained from the experiments on the emotional excitement, hard exercise and hemorrhagic shock in man and animals.

The outline of this work was reported in the 28th General Meeting of Japanese Physiological Association (1951).

**References**