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Regulatory effects of blood constituents on the function and metabolism of the cat brain in perfusion ezperiments. Brain perfusion with artificial blood containing low molecular dextran and amino acids

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Abstract

As a link in a series of studies on the effects of blood constituents on the brain function by means of brain perfusion, we used four kinds of artificial blood; namely, the blood containing a low molecular dextran, one containing glutamic acid, one containing essential amino acid group and the one containing both essential amino acid group and glutamic acid. During the perfusion experiments we observed the effects of blood constituents on the function and metabolism of the perfused brain and obtained the following results. 1. When a low molecular dextran is used as the colloid osmotic pressure agent instead of hydrodextran, the amount of the blood flow in the brain is maintained roughly at a certain fixed level throughout the experiment, showing no gradual decreasing tendency. 2. When using the artificial blood supplemented with glutamic acid, EEG of the perfused brain shows an increase in the appearance rate of β32 and β33 bands, approaching closely to the pattern of EEG of unrestrained controls at arousal state. 3. In the case of the blood added with essential amino acids similar to the case using the blood with glutamic acid, EEG approaches towards the alert pattern of the controls. 4. When the perfusion is done with the artificial blood lacking in amino acids, about one hour after the start of the perfusion the amount of glutamic acid and its related compounds in the brain can no longer be maintained at normal level and the decrease, being so marked, brings about a marked decrease also in total amino acid content. 5. When the perfusion blood contains glutamic acid, essential amino acid group or both, the concentrations of amino acids of the brain glutamic acid group and the total amino acid can be maintained approximately at normal level for the duration of over one hour.

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REGULATORY EFFECTS OF BLOOD CONSTITUENTS ON THE FUNCTION AND METABOLISM OF THE CAT BRAIN IN PERFUSION EXPERIMENTS

—— BRAIN PERFUSION WITH ARTIFICIAL BLOOD CONTAINING LOW MOLECULAR DEXTRAN AND AMINO ACIDS ——

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The brain perfusion method in situ plays a unique role in the study of brain metabolism. One of the characteristic features of this method is that it enables us to study the effects of selected blood components on the physiological functions of the brain, eliminating the metabolic products of other organs from the circulating blood.

GEIGER *et al*[1]. used bovine serum albumin to keep the colloid osmotic pressure of artificial blood in their brain perfusion but they gave no detailed description of electroencephalogram (EEG) during the perfusion. IKEDA[2] used high molecular hydrodextran instead of bovine serum albumin but he found the functional level of the perfused brain to be low. GEIGER *et al*.[3,4] recognized that the most important factor that operates on the function of perfused brain is the constituents of the artificial blood.

Therefore, it is most desirable to raise the functional level of the perfused brain as close to normal physiological state as possible in such perfusion experiments. With this point in mind we performed the present perfusion experiments. As a link in the studies on the effects of artificial blood constituents on the function and metabolism of the perfused brain, we conducted the perfusion of cat brain using low molecular dextran (average molecular weight, 40,000) known to be a useful osmotic pressure maintaining agent in the field of surgery. The results[2] of this study were compared with those obtained by the use of high molecular dextran (average molecular weight, 75,000).

Further investigation was conducted to see the effects of the addition of amino acids to the artificial blood on the function and metabolism of the perfused cat brain. Namely, in this instance, the brain perfusion was carried out with the artificial blood containing glutamic acid, that with essential amino acids, and

that with both glutamic acid and essential amino acids to see physiological effects of amino acids contained in the blood on the brain function, especially on EEG, as well as their role on the concentration of intrinsic amino acids in the brain. The present report describes the advantages of the addition of amino acids to artificial blood in brain perfusion.

MATERIALS AND METHODS

For the brain perfusion, adult cats weighing 2.5—3.5 kg were used. The perfusion method was a slight modification of Geiger and Magnes' method. Namely, an open system in which the blood passing through the brain would not reenter into the brain (Fig. 1). For the preoperative anesthesia 0.7 ml/kg of Nembutal was injected intraperitoneally. In the brain perfusion with four different artificial blood, perfused brain was taken out within 60—90 minutes of perfusion.

In our previous experiment hydrodextran (average molecular weight, 75,000 ±25,000) was added to artificial blood in order to keep the colloid osmotic pressure, but this time a low molecular dextran (Daigo Nutrient Chemical Co. Product) (Table 1) was used. This artifical blood was supplemented with cytidine monophosphate-2 Na[5,6], bovine serum albumin, which constituted the Solution I, Solution I added with 10 mg/dl of glutamic acid was taken as Solution II, Solution I supplemented with 0.3 ml/dl of essential amino acid mixture

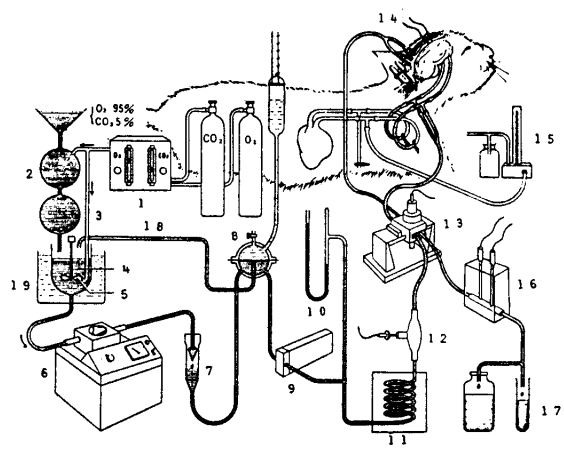

Fig. 1 Diagramatical Drawing of the Perfusion Apparatus
1: Gas Flow Meter, 2: Oxygenater, 3: Mixed Gas, 4: Deposit, 5: Stirrer, 6: Roller Type Pump, 7: Filter, 8: Pressure Regulator, 9: Transduser of Electromagnetic Flow Meter, 10: Pressure Meter 11: Heating Bath, 12: Thermister, 13: Oxymeter, 14: EEG Machine, 15: Pressure Meter, 16: pH Meter 17: Venous Blood Sample, 18: Feed Back, Arterial Sample 19: Cooling Bath

Table 1. Constituents of the Artificial Bloods Used for Brain Perfusion

Solution number	Constituents			
I	Without Amino Acids			
	Bovine erythrocytes	40 %		
	Krebs-Ringer	60 %		
	1.3 % Sodium bicarbonate	10 ml/dl		
	Dextran (Average M. W. 40.000)	6 g/dl		
	Bovine serum albumin	0.5 g/dl		
	CMP-2Na	8 mg/dl		
	Glucose	100 mg/dl		
	Vitamin mixture (mg/dl)			
	A	100 i. u.		
	B_1	0.1		
	B_2	0.22		
	B_6	0.04		
	C	1.0		
	Nicotinamide	0.4		
	D-Pantothenol	0.1		
	B_{12}	4×10^{-5}		
	D	10 i. u.		
II	With Glutamic Acid			
	Solution I+Glutamic acid	10 mg/dl		
III	With Essential Amino Acids			
	Solution I+Amino acid solution (mg/dl)			
	Arg	2.71	Met	2.04
	Gly	1.80	Phe	2.88
	His	1.29	Thr	2.1
	Ile	1.98	Try	0.9
	Leu	3.0	Val	1.92
	Lys	5.76		
IV	With Glutamic Acid and Essential Amino Acids			
	Solution I+Glutamic acid and Amino acid solution (mg/dl)			
	Glu	10	Met	2.04
	Arg	2.71	Phe	2.88
	Gly	1.80	Thr	2.1
	His	1.29	Try	0.9
	Ile	1.98	Val	1.92
	Leu	3.0		
	Lys	5.76		

(Tanabe Product, SOH-AMIN) as Solution III, and Solution I added with glutamic acid and essential amino acids as Solution IV.

The composition of each artificial blood used is listed in Table 1. The amount of amino acids given in each group corresponded roughly to the quantity of amino acid in normal cat serum[7].

The level of brain function during the perfusion was determined on the basis of EEG, pupillary light reflex, conjunctival reflex, corneal reflex, reactions to stimulation, spontaneous movements. EEG was recorded from both the right and the left hemispheres with the thumb-tack poles fixed at the apex of the cranium one cm apart. Since the δ zone is apt to produce artifact, it was not included in the computation of the rate of wave appearance. In this experiment only the components of θ, α, β_1, β_2, and β_3 were recorded in the graph, and the standard deviation was placed in the ordinate. As the criterion for determining the EEG level of the perfused brain, the EEG frequency analyses were taken with seven unrestrained cats at attention, and the appearance rate of each band was estimated. As a result it was observed that the rate of α wave to be $15.1 \pm 2.03\%$; β_1 $19.2 \pm 2.52\%$; β_2 $12.9 \pm 2.42\%$; and β_3 $16.4 \pm 2.03\%$. This frequency is shown in a band formation in Figs. 4, 5, 6, 7.

Analytical methods of various blood and brain materials: Blood glucose content was determined by SOMOGYI's method in which deproteinized solution was analyzed by the glucose oxidase method of SAIFER and GERSTENFELD[8]. Lactic acid was measured by the method of BARKER and SUMMERSON[9]. Oxygen and carbon dioxide gases were measured by the micro-volumetric method of NATELSON[10]. The cerebral blood flow rate was measured by an electromagnetic flow meter, ME-2 type of Nihon Kōden apparatus. EEG recordings were taken by EEG apparatus and the frequency analyzer (Nihon Kōden Co.).

Isolation and quantitative analysis of brain free amino acids: Five percent TCA supernatant obtained from brain cortex was applied to Dowex 50×4 H^+ column and fractionated into neutral-acidic fraction and amino acid fraction. This amino acid fraction was put in Amberlite CG4B T2 acetate column (0.9×15 cm) and fractionated by the density gradient elution, where 4M acetic acid was added to 100 ml of 0.5 M acetic acid with the Unigrade of Mitamura Physical Laboratory as to increase the concentration of acetic acid linearly into neutral-basic amino acids, glutamic acid and aspartic acid (a modification of KURAHASI's method[11]). Glutamine contained in the neutral-basic amino acid fraction of Amberlite column was at first hydrolyzed against 2N HCl solution at 100°C for 2 hours, then it was taken as glutamic acid and again chromatographed on Amberlite column.

GABA was isolated by placing TCA supernatant in Dowex 50-×4 Na^+ column with sodium citrate buffer at pH 5.8 (BERL, LAJTHA and WAELSH[12]). The quantitative assay of total free amino acids and individual amino acid fractions was done by the ninhydrin method of ROSEN[13].

RESULTS

The cerebral blood flow in the perfused cat brain with a low molecular dextran: In the brain perfusisn with artificial blood containing a low molecular dextran (average molecular weight, 40,000) the cerebral blood flow increased steadily with the lapse of time without any decreasing tendency (Fig. 2). In a marked contrast, where the cat brain was perfused with an artificial blood containing high molecular dextran (average molecular weight, 75,000) the cerebral blood flow reached its peak in 20 minutes and thereafter it gradually decreased.

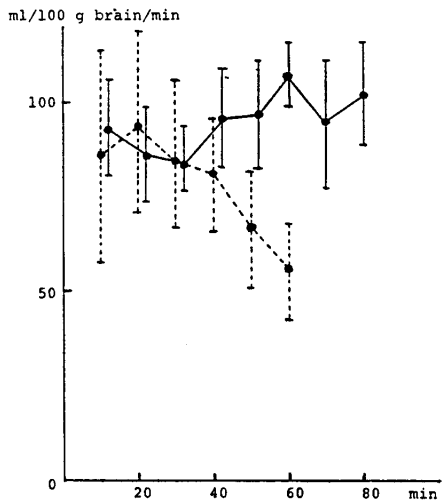

Fig. 2 Cerebral Blood Flow in Perfusion Experiments. Solid line: with low molecular dextran, broken line: with high molecular dextran[2].

Comparison of EEG of perfused brain (Fig. 3): As soon as the perfusion was started, EEG at first accompanied by spindle and spikes, showing the effect of nembutal, later exhibited predominantly fast activity. By 20—30 minutes after the start there appeared fast wave train with relatively low amplitude and this pattern resembled the pattern of arousal state at rest or the alert pattern of unrestrained cat. Such EEG was maintained for about one and half hours after the start of experiment, but thereafter slow wave components gradually ensued. In contrast to this, with the use of a high molecular dextran as in the previous experiment, spindle bursts persisted up to about 40 minutes of the experiment but later the amplitude gradually decreased and by 60 minutes EEG showed only a slow electrical activity.

Comparing this with the EEG in the present experiment using the low molecular dextran, the characteristic trait was that EEG showed fast activity more frequently and for about 60 minutes there could be seen practically no weakening of EEG with lapse of time.

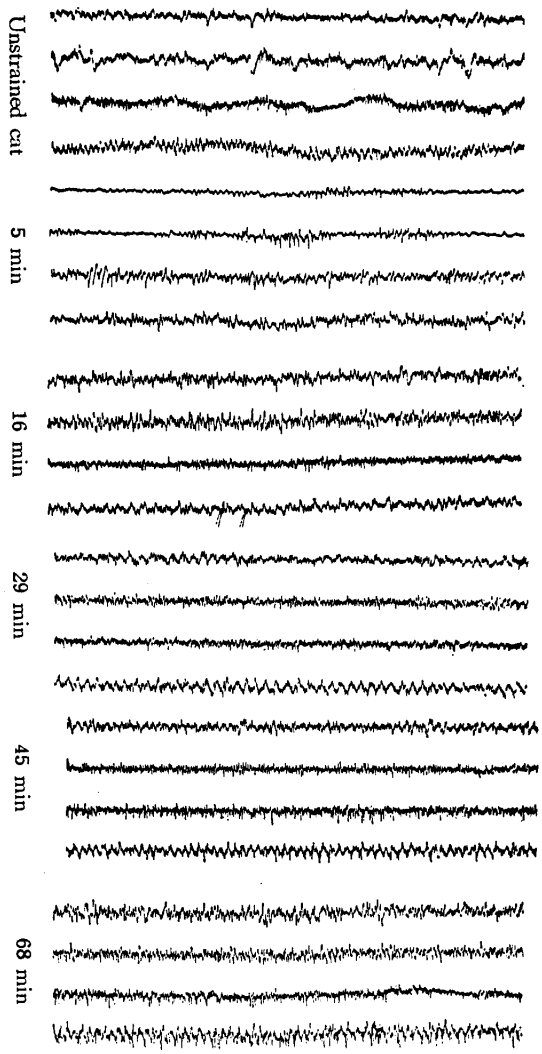

Fig. 3 EEG Pattern of the Perfused Cat Brain in the Course of Perfusion Time

Another striking feature was that the anesthetic effect of nembutal used as preoperative treatment disappeared very rapidly.

Frequency analysis of various EEG's during the brain perfusion with artificial blood containing a low molecular dextran (Figs. 4—7): After analysis of various EEG during the brain perfusion, the rates of appearance were assessed by taking the average of five successive epochs every five minutes from each of waves such as θ (4—8 cycles per sec), α (8—13 cps), β_1 (18—20 cps), β_2 (20—30 cps), and β_3 (30—60 cps). The average value of different experiments and standard deviation for each wave are calculated and listed in the figures. For the controls similar observations were carried out with the unrestrained cats at attention and their corresponding values are represented by dotted bands in Fig. 4—7.

In the brain perfusion with Solution I (Fig. 4) which contained no amino acids, by about 20—30 minutes when EEG was stabilized, the rate of appearance of α band was always higher than that of unrestrained cats at attention, and the rates of β_1, β_2, β_3 frequency bands were usually lower.

The perfusion blood containing glutamic acids (Fig. 5), in contrast to the Solution I, showed the rate of appearance of β_2 and β_3 bands to be practically the same as that of unrestrained controls. In the case of the perfusion with the blood containing essential amino acids (Fig. 6), the appearance rate of β_3 band was somewhat lower than that in the perfusion of the blood with glutamic acid

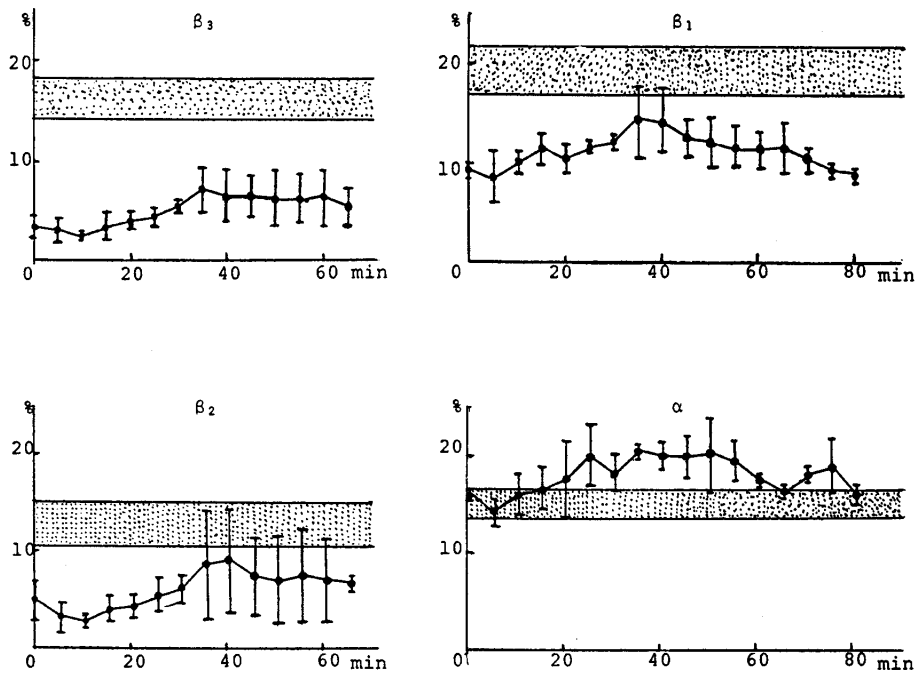

Fig. 4 Frequency Analysis of Various EEGs During the Brain Perfusion with Solution I. The dotted band indicates the control values obtained from unstrained cats at attention state.

but the other bands appeared at about the same rate.

In the case where the artificial blood contained both glutamic acid and essential amino acids (Fig. 7), there could be seen no significant difference from the perfusion of the blood with essential amino acids and the rate of each frequency was approximately identical with each other.

In the perfusion with Solutions II, III and IV on one hand and Solution I on the other, there could be observed a distinct difference in the frequency of β_2 band.

However, what can be seen common in all the perfused cat brain is that the frequency of β_1 band is clearly lower than that of unrestrained controls.

The amount of glucose uptake in the perfused brain (Table 2): In the four groups of brain perfusion with the blood containing low molecular dextran the glucose consumption in every group in the presence or absence of amino acids was stabilized within 20—30 minutes of the perfusion and there was no significant difference in the amounts of glucose uptake amoug these four groups. On the other hand, in the brain perfusion with a high molecular dextran the glucose uptake was 0.37 μ mole/g brain/minute after 20 minutes of the perfu-

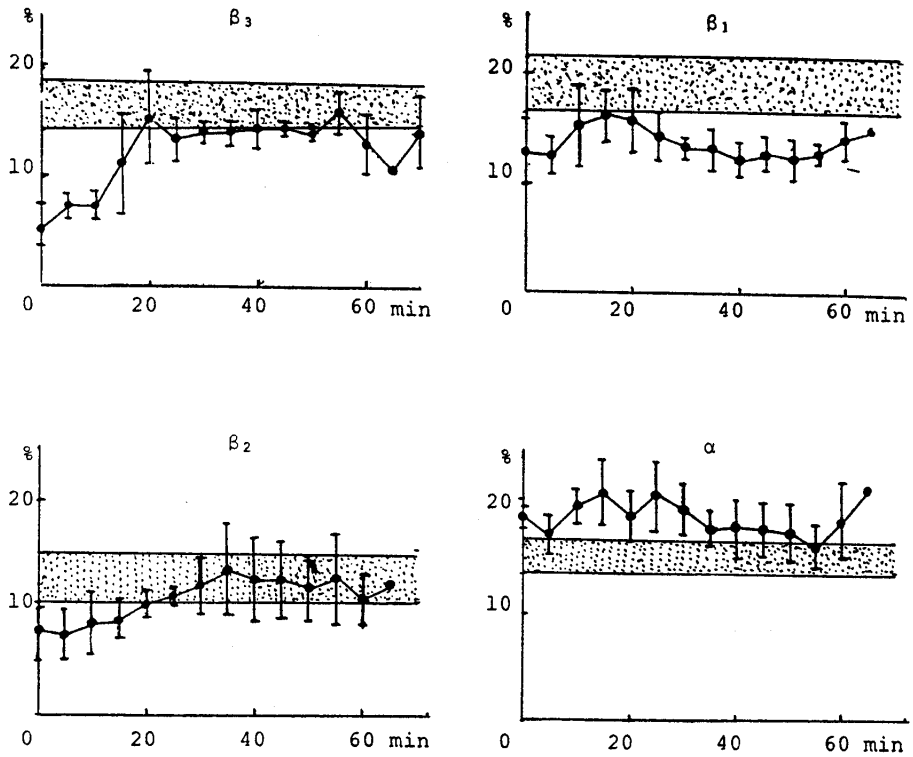

Fig. 5 Frequency Analysis of Various EEGs During the Brain Perfusion with Solution II. The dotted band indicates the control values obtained from unstrained cats at attention state.

Table 2. The Amount of Glucose Uptake in the Perfused Brain (μmole/g brain/min)

Perfusion Time \ Solution No.	I (4)	II (3)	III (8)	IV (6)	High molecular dextran[2]
10 min	−0.12±0.05				0.27±0.06
15		0.25±0.07	0.29±0.14	0.37±0.11	
20	−0.05±0.05		0.30±0.11	0.39±0.10	0.37±0.26
30	0.41±0.14	0.36±0.02	0.34±0.12	0.44±0.15	0.33±0.12
40	0.53±0.13	0.40±0.11	0.39±0.13	0.41±0.12	0.22±0.13
50	0.49±0.12	0.44±0.14	0.39±0.09	0.41±0.12	0.12±0.12
60	0.42±0.06	0.40±0.09	0.40±0.08	0.37±0.11	
70	0.49±0.12	0.37±0.05	0.37±0.09	0.41±0.11	

Solution number : See Table 1.
Number of experiments was shown in parenthesis.

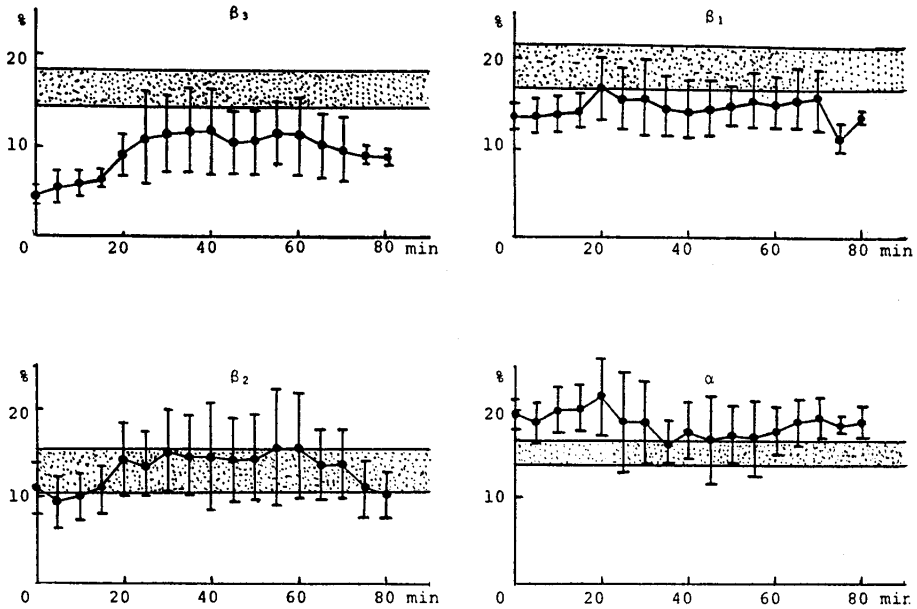

Fig. 6 Frequency Analysis of Various EEGs During the Brain Perfusion with Solution III. The dotted band indicates the control values obtained from unstrained cats at attention state.

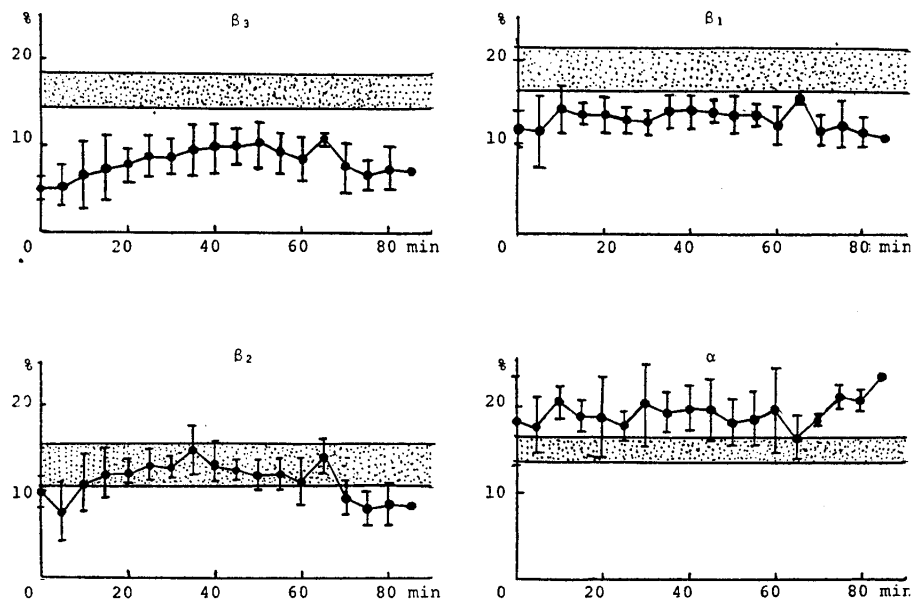

Fig. 7 Frequency Analysis of Various EEGs During the Brain Perfusion with Solution IV. The dotted band indicates the control values obtained from unstrained cats at attention state.

sion[2], and with this as a peak it gradually decreased, and by 50 minutes of perfusion it fell down to 0.12 μ mole/g brain/minute. In the brain perfusion with a low molecular dextran the amount of glucose uptake in average was 0.43 —0.48 μ mole/g brain/minute, showing no gradual decreasing tendency as in the case with a high molecular dextran.

The amount of glucose uptake during the first 20 minutes of perfusion showed negative value in some cases. This was due to the rise of glucose level in the systemic blood in the course of surgical operation. This point has been suggested by ALLWEIS and MAGNES[14].

Time-lapse changes of lactic acid liberation (Table 3): In the brain perfusion with high molecular dextran the amount of lactic acid liberated into the veins of the brain rapidly increased with lapse of time; namely, by the 10-minute perfusion, it amounted to 0.22 μ mole/g brain/min, by 50 minnutes, 0.72 μ mole/g brain/min and by 50-minute perfusion the liberated amount increased over 3-fold.

Table. 3. The Amount of Lactic Acid Liberation of the Perfused Brain into the Cerebral Veins (μ mole/g brain/min)

Solution No. / Perfusion Time	I (4)	II (3)	III (8)	IV (6)	High molecular dextran[2]
10 min	0.16±0.09				0.22±0.08
15		0.39±0.22	0.39±0.06	0.34±0.07	
20	0.20±0.04		0.30±0.08	0.34±0.08	0.34±0.29
30	0.21±0.10	0.34±0.21	0.29±0.13	0.31±0.11	0.49±0.26
40	0.18±0.12	0.34±0.09	0.30±0.14	0.30±0.08	0.49±0.27
50	0.29±0.12	0.28±0.16	0.30±0.12	0.22±0.08	0.72±0.65
60	0.18±0.16	0.31±0.10	0.27±0.19	0.22±0.11	
70	0.20±0.05	0.29±0.12	0.32±0.16	0.21±0.12	

Solution number: See Table 1
Number of experiments is shown in parenthesis.

On the other hand, in the perfusion with a low molecular dextran, with exception at 50 minutes, the amount of lactic acid liberated was always low, being less than 0.21 μ mole/g brain/min, showing not any increasing tendency with lapse of time as observable in the perfusion with high molecular dextran.

Actual and theoretical amounts of oxygen consumption in the perfused brain (Table 4): On the assumption that the remainder obtained by subtracting the amount of lactic acid liberated into cerebral venous blood from the amount of glucose consumed by the brain will always undergo aerobic glycolysis, the values of theoretical oxygen consumption were computed from

Table 4. The Amount of Oxygen Consumption in the Perfused Brain (μ mole/g brain/min)

Perfusion time \ Sulution No.	I (4)	II (3)	III (8)	IV (6)	High molecular dextran[2]
10 min	1.83±0.36				2.19±0.83
15		1.29±0.58	2.10±0.61	1.70±1.07	
20	1.56±0.61		2.05±0.49	1.83±0.78	2.28±0.87
30	2.05±0.04	1.56±0.32	2.19±0.33	1.65±0.53	2.14±0.71
40	2.37±0.62	1.61±0.18	2.10±0.48	1.61±0.44	2.01±0.71
50	2.54±0.53	1.65±0.50	2.14±0.44	1.56±0.50	1.92±0.65
60	2.46±0.64	1.65±0.18	2.23±0.55	1.56±0.61	1.65±0.65
70	2.63±0.68	1.79±0.41	2.14±0.42	1.52±0.73	

Solution number: See Table 1.
Number of experiments is shown in parenthesis.

individual average values, and these were compared with corresponding values in the perfusion with a high molecular dextran (Fig. 8).

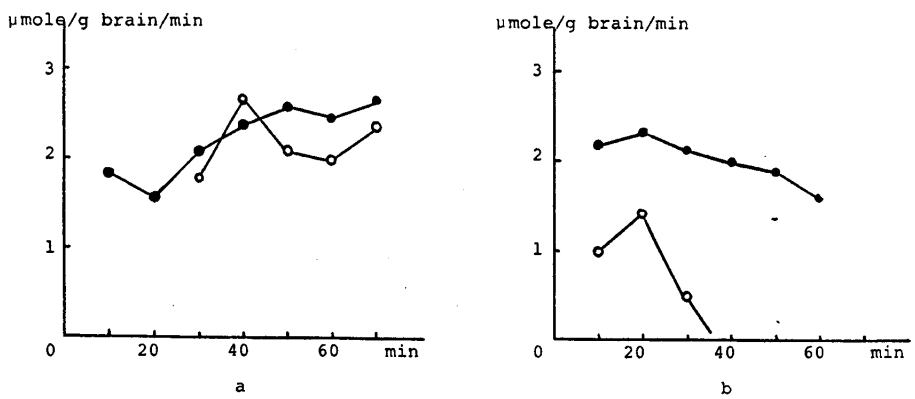

Fig. 8 Comparison of Actual and Theoretical Amounts of Oxygen Consumption in the Perfused Cat Brain with the Use of High Molecular Dextran and in Those with Low Molecular Dextran

a: Low molecular dextran (Solution I), b: High molecular dextran[2], solid line with filled circles: Actual amount of oxygen consumption, solid line with open circles: Theoretical amount of oxygen consumption.

In the brain perfusion with hydrodextran actual amounts of oxygen consumed by the brain were far greater than theoretical values[2]. Whereas in the brain perfusion with a low molecular dextran the actual values of oxygen consumption by the brain were close to theoretical values[15], and such a mutual relationship between the amount of oxygen consumption, that of glucose uptake and the amount of lactic acid liberated, hardly changed after 20—30 minutes of the perfusion.

Our finding that the theoretical oxygen consumption of the brain perfused with artificial blood containing high molecular hydrodextran was far greater than the actual oxygen consumption coincided with the findings of available reports, but in the perfusion with a low molecular dextran we obtained more physiological respiration.

Table 5. Amino Acids Concentration in the Perfused Cat Brain (μmole/g brain)

Solution No. / Free Amino Acid in Brain	I (2)	II (5)	III (14)	IV (10)
Glutamic Acid	4.56±0.26	7.24±2.08	6.20±1.21	7.19±1.38
Aspartic Acid	0.62±0.03	1.59±0.54	1.67±0.53	1.24±0.40
Glutamine	1.74±0.50	2.86±0.86	3.71±1.61	5.17±1.23
GABA	1.89±0.94	2.38±0.45	1.78±0.67	1.48±0.79
Total Amino Acids	19.8 ±4.60	32.1 ±6.85	36.8 ±5.19	34.5 ±9.68

Solution number: See Table 1.
Number of experiments is shown in parenthesis.

Concentration of amino acids in the perfused brain (Table 5)[16]*:*

i) In the brain perfusion with the basic artificial blood not containing any free amino acids, the amount of amino acids of the brain decreased markedly after 60—90 minuites' perfusion, showing a decreasing tendency of every amino acid in the glutamic acids and its related amino acids, and the amount of total amino acids is decreased to one half the normal level[7]. Among them glutamic acid and aspartic acid were markedly decreased while the decrease of GABA was slight.

ii) In the perfusion with the artificial blood containing glutamic acid in the amount equivalent to the sum of concentration of glutamic acid and glutamine in normal cat serum (Solution II), it is possible to maintain the total amino acid level at normal.

iii) When the brain perfusion is done with Solution III in which essential amino acids are added to the artificial blood in the amount equivalent to the essential amino acid concentrations of serum of normal cat, total amino acids, glutamic acid as well as aspartic acid all can be maintained at normal level during the perfusion.

iv) In the perfusion with the artificial blood containing free amino acids in the concentration approximately the same to the concentration of amino acids in the serum of normal cat whose blood contains both glutamic acid and essential amino acids, the concentration of free amino acids of perfused brain can be kept close to normal level.

Table 6. Physiological Traits of the Perfused Cat Brain (Solution Number: See Table 1.)

	Solution No. / Perfusion time (min)	I			II			III									IV					
		1	2	3	1	2	3	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6
Conjunctival reflex	30	+++		+	++	++		+++	+	++	+++	+++	+++	−	+	+++	+++	++	−	−	+	
	40	+++		+	++++	++		+++	++	+++	+++	+++	+++	−	+	+++	+++	++	+	−	−	+
	50			+	+++	+++	+	+++	+++	+++	+++	+++	+++	−	+	+++	+++	++	+	−	−	−
	60	++		+	+++	+++		+++	+++	+++	+++	+++	+++	−	+	+++	+++	++	−		−	−
	70	++		+	+++			+++		++	+++	+++	+++		+	+++	+++	++			−	−
	80	++		+	+++					++	+++				+	+++	+++					
corneal reflex	30	+++		+	++	++		+++	+	++	+++	+++	+++	−	++	+++	++	+	−	−	−	−
	40	+++		+	+++	++		+++	++	+++	+++	+++	+++	−	++	+++	++	+	−	−	−	−
	50			+	+++	+++	+	+++	+++	+++	+	+++	+++	+	++	+++	++	+	−	−	−	−
	60	++		+	+++	+++	++	+++	+++	+++	+	+++	+++	+	++	+++	++	+	−	−	−	−
	70	++		+	+++		++	+++		++	+	+++	+++		++	+++	++	−			−	−
	80	++		+	+++					++	+				++	+++	++					
touch sensation	30	+++			+++	+++		+++	+	++	+++	+++	+++	−	++	+++	+++		+	−	+	−
	40	+++			+++	+++	++	+++	+	++	+++	+++	+++	−	++	+++	+++	++	+	−	+	−
	50				++	+++	++	+++	+++	++	+++	+++	+++	+	+	+++	+++	++	+	−	−	−
	60	++			++	+++	++	+++	+++	++	+++	+++	+++	+	+	+++	+++	++	−		−	−
	70	++			++		++	+++		+	+++	+++	+++		+	+++	+++	−			−	−
	80	++			++						+++	+++	+++			+++						
spontaneous movement	30	+++			+			+		+	++	+++	+	−	−	+++	−	−	−	−	+	−
	40	+++			−			+	−	+	++	+++	+	−	−	+++	+	−	−	−	+++	−
	50	+++			−		+++	+	−	−	++	+++	+	+	−	+++	−	−	−	−	++	−
	60	+++			−			+	−		+	+	+	+	−	+++	−	−			+	−
	70	+++					++				+	−	+		−	+++					−	−
	80										+	−	+		−	+++						
pupillary light reflex	30	+++			+						+++		+++		+				+			+
	40	++			+						+++		+++		+				+			
	50	++			+					+	+++		++		+				++			
	60	+			+						+++		++		+							
	70	+			+						+++		++		+							
	80	+									+++		++		+							
reaction of EEG to stimulation	30	++	+++		+			+			+++		++	+++	+		+	+++	+	+	+++	
	40	++	+++	+	+			+	+		++		++	+++	+	+++	+	++++	+		+++	
	50	++	+++	+	+	+		+		+	++		++	+++	++	+++	+	++++	+		+++	
	60	+	+++	+	+	+		+		+	++		++	++	++	++	+	++++	+	−	+++	
	70	−		+	+	+				+	++		++		++	+	+	+++	+		+++	
	80	−			+	+					++		++		++	+					+++	

Other physiological traits (Table 6) : Table 6 illustrates the pupillary light reflex, corneal reflex, conjunctival reflex, the responses to touch and pain and spontaneous movements during the perfusion. The record was taken also of the reaction on EEG to the sound stimuli during the perfusion.

DISCUSSION

The brain perfusion in which a known constituent of artificial fluid is made to perfuse through the brain is the most suitable method for studying the regulation of the brain metabolism by changes in the blood components.

Ever since GEIGER *et al.*[3,4] started the brain perfusion experiments with cat, it has been demonstrated that the important factor that controls the function of a perfused brain is the constituents of the artificial blood perfused. GEIGER *et al.*[1] used bovine serum albumin (Cohn's Fraction V) as the agent to maintain colloid osmotic pressure of artificial blood, but because of its cost and instability of bovine serum albumin, we used high molecular dextran[2] and then high molecular hydrodextran[2] but the results with these agents were not so satisfactory. Therefore, we tried with a low molecular dextran instead of high molecular one in the perfusion experiments[17]. As a result we have recognized a marked elevation of the function of perfused cat brains.

It has been found that the use of a low molecular dextran as the colloid osmotic pressure agent in the perfusion eliminates the tendency of a gradual decrease in the cerebral blood flow and it enables us to keep the blood flow at a certain fixed level throughout the experiments (Fig. 2).

GELIN[18] states that the use of a high molecular dextran in the brain perfusion induces a relatively early erythrocyte aggregation and the stoppage of blood circulation to the peripheral circulatory system, and bring about such irreversible changes as intravascular sludging and further thrombus formation. THORSEN and HINT[19] have recognized that a low molecular dextran with molecular weight of less than 60,000 prevents erythrocyte aggregation. BERNSTEIN[20] has observed that the administration of a low molecular dextran can overcome aggregation induced by the intravenous injection of diatrizoate sodium in dog. Further, the use of a low molecular dextran is known to improve the circulation in the peripheral system (SUNADA *et al.*[21] SHIRAHIGE *et al*[22].). It seems that one of the reasons for the persistent maintenance of the cerebral blood flow by the low molecular dextran during the brain perfusion is the prevention of sludging of peripheral blood vessels of the brain.

A characteristic difference of a low molecular dextran in every case from the use of a high molecular dextran lies in its power to efface completely the effect of nembutal used as preoperative treatment within about 20 minutes of

the perfusion as observed from EEG pattern. It is obvious that the low molecular dextran has a stronger power to dispose of anesthetic drug from the tissue than a high molecular dextran.

The course traced by EEG of the perfused brain using a high molecular dextran seems to be affected by anesthetic drugs, i. e. up to about 40 minutes of the perfusion there appears spindle burst and just before this, burst disappears itself, and the electrical activity is gradually weakened, then becomes flattened out[2]. It is known that high molecular dextran induces aggregation of erythrocytes in the capillary vessels[23] and decreases the blood flow in the cerebral cortex[24], finally culminating in the metabolic disturbance of tissues by the fall in the oxygen consumption[25]. The rapid weakening of EEG as observable in the brain perfusion with a high molecular dextran and the accompanying decrease in the cerebral oxygen consumption, the decline in the glucose consumption as well as a sharp increase in the lactic acid liberation seem to be due to the cerebral vascular insufficiency.

In the case of the brain perfusion with a low molecular dextran, although it is easy to maintain the level of EEG, after 60 minutes' perfusion it cannot be denied, though only gradually, that the brain function is weakened This suggests that the blood components that we used lack some substance associated with maintenance of the brain function. However, the addition of glutamic acid or essential amino acids or both to the artificial blood containing a low molecular dextran markedly improved the EEG levels of perfused brain.

Glutamic acid, when administered directly into the cerebral cortex microelectrophoretically, induces excitation of neurons. [26,27,28].

It is recognized that, when a certain amount of glutamic acid is injected into the carotid artery during the perfusion, EEG exhibits markedly low amplitude fast waves for a short time and subsequently high voltage slow waves or flat waves which persist for a long time[29]. Even when essential amino acid group is added to the artificial blood, EEG shows an increase of fast wave components[30] and it approaches the alert pattern of unrestrained cat. The amount of each essential amino acid employed in this experiment does not differ appreciably from the concentration of amino acid in the serum of normal cat, and hence it would hardly have any pharmacological action directly on the brain. It would seem reasonable to consider that the transformation of EEG to fast waves cannot at once be interpreted to be due to the direct action of these essential amino acids but rather due to the presence of the essential amino acid group in the serum, which, acting favorably on the maintenance of the stability of the brain metabolism, results in the heightening of brain metabolic activity so that it is the secondary appearance of a still higher brain function.

During the brain perfusion with a low molecular dextran there can be

observed no rapidly-rising tendency in the liberation of lactic acid with lapse of time as in the case of perfusion with Solution I (containing a low molecular dextran plus 8 mg/dl sodium cytidine monophosphate, CMP-2Na), the brain metabolism and the oxidative glycolysis are enhanced and these accelerative effects[5,6,32] prevent the accumulation of lactic acid in the brain.

For glucose taken up by the brain to be completely oxidized, theoretically one mole glucose requires 6 moles of oxygen. However, in the brain perfusion experiment a considerable amount of lactic acid is liberated in the venous blood. Six times μ mole of glucose consumed minus three times μ mole of lactic acid liberated from the brain into the venous blood represents the theoretical amount of oxygen in moles. However, the amount of oxygen actually consumed is slightly higher than these values. This tendency can be recognized more distinctly in the experiment with a high molecular dextran, but the experiments with a low molecular dextran have shown values far closer to the values of theoretical oxygen consumption. This seems to suggest that, viewed from the aspect of gylcolytic process, the brain perfused with artificial blood containing a low molecular dextran maintains far more physiological metabolic conditions than in the brain perfused with a high molecular dextran.

Concentration of amino acids in the brain: In the case of the absence of amino acids in perfusion blood as compared with the case of the presence of amino acids, the amounts of glutamic acids and its related amino acids in the brain decrease far more markedly. In this instance, there can be seen no decrease in the glucose uptake of the brain and along with the lowering of brain function the rate of conversion of glucose carbons to brain amino acid carbons is decreased[17], and for this reason it seems that the brain can no longer maintain the normal level of glutamic acid.

In the case where glutamic acid is present in the perfusion blood, the decrease in the amino acids of the brain is restored fairly well. In this instance, since the rate of direct conversion of the blood glutamic acid to the brain glutamic acid is less than one per cent[31], when the brain function is maintained at a high level, there ensues a high rate[30] of the conversion of glucose to the brain amino acid so that the decrease in the amino acid seems to be preventable to a certain extent.

In the presence of essential amino acids in the blood being perfused, despite a low rate of conversion of amino acids in the blood directly to the amino acids in the brain the amino acid level is maintained because the increased rate of the production of amino acids in the brain from the glucose metabolites.

In the tissue cultures of various species of mammalians including cancer cells, the importance of amino acids in the culture medium is well recognized[33].

Likewise from the nutritional viewpoint, it is necessary to give individual amino acids simultaneously lest the imbalance of amino acids causes the disturbance of health[34].

The interesting point in the relation between the physiological traits and EEG is that those that exhibit active physiological traits generally give alert pattern of low amplitude in the EEG and those of fast wave pattern in the EEG do not necessarily show active physiological traits.

SUMMARY

As a link in a series of studies on the effects of blood constituents on the brain function by means of brain perfusion, we used four kinds of artificial blood; namely, the blood containing a low molecular dextran, one containing glutamic acid, one containing essential amino acid group and the one containing both essential amino acid group and glutamic acid.

During the perfusion experiments we observed the effects of blood constituents on the function and metabolism of the perfused brain and obtained the following results. 1. When a low molecular dextran is used as the colloid osmotic pressure agent instead of hydrodextran, the amount of the blood flow in the brain is maintained roughly at a certain fixed level throughout the experiment, showing no gradual decreasing tendency. 2. When using the artificial blood supplemented with glutamic acid, EEG of the perfused brain shows an increase in the appearance rate of β_2 and β_3 bands, approaching closely to the pattern of EEG of unrestrained controls at arousal state. 3. In the case of the blood added with essential amino acids similar to the case using the blood with glutamic acid, EEG approaches towards the alert pattern of the controls. 4. When the perfusion is done with the artificial blood lacking in amino acids, about one hour after the start of the perfusion the amount of glutamic acid and its related compounds in the brain can no longer be maintained at normal level and the decrease, being so marked, brings about a marked decrease also in total amino acid content. 5. When the perfusion blood contains glutamic acid, essential amino acid group or both, the concentrations of amino acids of the brain glutamic acid group and the total amino acid can be maintained approximately at normal level for the duration of over one hour.

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