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Abstract

For the purpose to reveal the metabolic pathway of GABOB the analyses were performed with the GABOB containing fluid perfused through the liver and the brain of rabbits, and the following results were obtained. Qualitative observations by paperchromatography on the fluid containing GABOB after perfusing the organs proved the presence of some amino acids. These were identified as glycine, glutamic acid and glutamine. The observation on the GABOBcontaining fluid perfused the organs showed a decrease in GABOB and an increase in these amino acids. Quantitative observation proved a considerable increase in glycive and a moderate increase in glutamic acid and glutamine with a marked decrease in the amount of GABOB injected. From these results it is believed that GABOB is decomposed into glycine and acetic acid probably passing the stage of γ-aminoacetoacetic acid in one way and into glutamic acid by the transamination of GABOB with α-ketoglutaric acid in the other.

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STUDIES ON γ-AMINO-β-HYDROXYBUTYRIC ACID

III. METABOLISM OF γ-AMINO-β-HYDROXYBUTYRIC ACID IN PERFUSION THROUGH THE LIVER AND BRAIN*

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In the previous experiment using the human and rabbits brains, the author[1] suggested that the oxygen consumption of brain is not so closely correlated with γ-amino-β-hydroxybutyric acid (GABOB), but there is a possibility that the decrease in GABOB contents in epileptic brain by the accelerated decomposition with its raised oxygen consumption may be correlated to the epileptic attack. This time the author observed the disintegration of GABOB in the livers and brains of rabbits by perfusing them with the diluted homologous blood containing GABOB. The purpose is to know whether GABOB is actually metabolized in these organs or not, as the metabolic pathway of GABOB is still obscure.

MATERIALS AND METHODS

Thirty-two adult white rabbits, both male and female, weighing about 2kg were used. These animals were divided into 2 groups, 16 animals including 8 controls in each. Just before the operation about 40 ml. of blood was taken from each animal from femoral artery inserting polyethylene tube 1 mm. in diameter. Blood was drawn from the tube by using syringe. The accumulated blood was defibrinated and used for the perfusion of the organs from the same animals. The livers were excised from the animals belonging to the first group by opening the abdomen without anesthesisa. These liver were put into the chamber of OHASHI's perfusion apparatus for liver, 37.5°C and the liver were perfused through the portal vein with the diluted blood by OHASHI's method[2]. The animals belonging to the second group were decapitated and the head containing brains were perfused through both arteria carotis interna with the same solution as that used for liver perfusion by applying INOUE's method[3]. For the perfusion of organs the blood from the same individual was used. The blood was diluted

* The outline of this paper was reported at the 33rd Meeting of the Japan Biochemical Society.

3-fold with Ringer's solution and 100 milligram per cent of GABOB was added, and the pH of this diluted blood was adjusted to 7.4 by adding N/10 NaOH. For perfusion 90 ml. of the diluted blood was used for each experiment, the inflow pressure was 10 to 15 mmHg and the amount 45 ml. per minute for the liver, and 20 ml. per minute for the brain. The perfusion was continued for 60 minutes in the liver and 30 minutes in the brain.

For the control the similar solution without GABOB was perfused under the same conditions and in the same manner.

Crude material GABOB was donated by ONO Pharmaceutical Co., Ltd. GABOB used for this experiment was obtained from this material by recrystallization from the saturated solution adding pure ethanol till this solution became muddy. This was left standing for 24 hours at 1° to 5°C and the supernatant was decanted. The precipitate was washed with ethanol twice and dried at room temperature.

Paperchromatography was carried out on the diluted blood used for perfusion, before use and after the perfusion test. The diluted blood was deproteinized adding 2 volumes of pure ethanol and was filtrated through filter paper. 0.1 ml. of the deproteinized filtrate was spotted on filter paper, Toyo Roshi No. 50, 40×40, for two-dimensional paperchromatography. Each of two series of development was prepared for one sample using different solvent systems, i. e. one for butanol-acetic acid-water (4: 2: 1 v/v) and water saturated phenol, and the other for ethanol-acetic acid-water (4: 2: 1 v/v) and water saturated phenol (ammonia gas in the chamber). Qualitative determination of amino acids, which will be produced in the relation with the metabolism of GABOB, was made ninhydrine reaction by the routine method. For the quantitative estimation of amino acids, the one dimensional paperchromatography was carried out. For GABOB 0.1 ml. deproteinized filtrate of perfusion fluid was developed with butanol-acetic acid-water (4: 2: 1 v/v) on Toyo Roshi No. 50, 40×2, and for glycine, glutamic acid and glutamine the same amount of the sample was developed with water saturated phenol. For the performance of the quantitaive analysis the developed filter paper was thoroughly dried first and then 0.5 per cent ninhydrine solution was sprayed for the coloration, and colored spots corresponding GABOB, glycine, glutamic acid and glutamine were cut apart. Each sample was cut into slender strips and extracted with 0.2 ml. of 0.1 N NaOH for 24 hours left standing. Then it was neutralized with addition of 0.2 ml. of 0.1 N citric acid. The pH of each extract was adjusted to 5.0 by adding 1 ml. citrate buffer, and further extracted by shaking for 30 minutes for the complete extraction. To each of the extracts thus obtained, 2 ml. of 2 per cent ninhydrine solution and 0.2 ml. of tin chloride solution (prepared by dissolving 2 g $SnCl_2$ $2H_2O$ in 124 ml. citrate buffer) were added and colored by heating in the boiling

water bath for 20 minutes. Next, it was diluted with addition of 7 ml. of 50 per cent n-propanol. The absorption intensities were estimated by mean of the Beckman type spectrophotometer, Shimazu CO., at 410 mμ for GABOB and 570 mμ for glycine, glutamic acid and glutamine. For drawing the standard curves, pure GABOB, glycine, glutamic acid and glutamine were employed. With these the solutions of 0.1, 0.5, 1.0, 5.0, 10.0, 20.0, 30.0 and 40.0 milligram per cent were prepared in each sample.

RESULTS

The organs, both livers and brains, used for the observations showed not any changes such as swelling, color tone change or other changes during or after perfusion.

Qaulitative analysis:

In the two-dimensional paperchromatography, developed with butanol-acetic acid-water (4 : 2 : 1 : v/v) and water saturated phenol both of the perfused fluids from the liver and the brain showed some spots of amino acids. The enlargement of the color spot of each amino acid, i. e. *Rf*'s to be 0.32, 0.32 and 0.44 respectively in butanol-acetic acid-water (4 : 2 : 1 v/v) and *Rf*'s of 0.42, 0.23 and 0.63 in the water saturated phenol. The amount of these amino acid seemed to be increased in the cases of perfused fluids containing GABOB. In the paperchromatography of various amino acids developed with the same solvents, glycine, glutamic acid and glutamine gave the *Rf*'s of 0.32, 0.32 and 0.44 in butanal-acetic acid-water (4 : 2 : 1v/v) respectively and 0.41, 0.23 and 0.63 with the water saturated phenol. The results indicate that the *Rf*'s of amino acids showing an enlargement of color spots by addition of GABOB in the perfusion fluid correspond to those of glycine, glutamic acid and glutamine (Figs. 1, 2). Next, when each of the perfused media was mixed with pure glycine, glutamic acid and glutamine respectively and developed on two-dimensional paperchromatography with the same solvent systems, the spots of glycine, glutamic acid and glutamine were found to be completely superimosed on the spots of amino acids appeared in the perfused media and enlarged by addition of GABOB.

Results obtained by using the solvent of ethanol-acetic acid-water (4 : 2 : 1 v/v) and water saturated phenol (ammonia gas in the chamber) on both of the fluids perfused the liver and the brain showed also the spots of amino acids and they appeared enlarging in the case of the perfused fluids containing GABOB. The similar experiment as mentioned above on these cases proved that these spots correspond to those of glycine, glutamic acid and glutamine respectively, i. e. *Rf*'s of the amino acids in the fluids perfused liver and brain were 0.16, 0.18

Fig. 1. Diagrammatical demonstrations of ninhydrine reaction performed on the developed fluids before and after the perfusions through liver of rabbits.

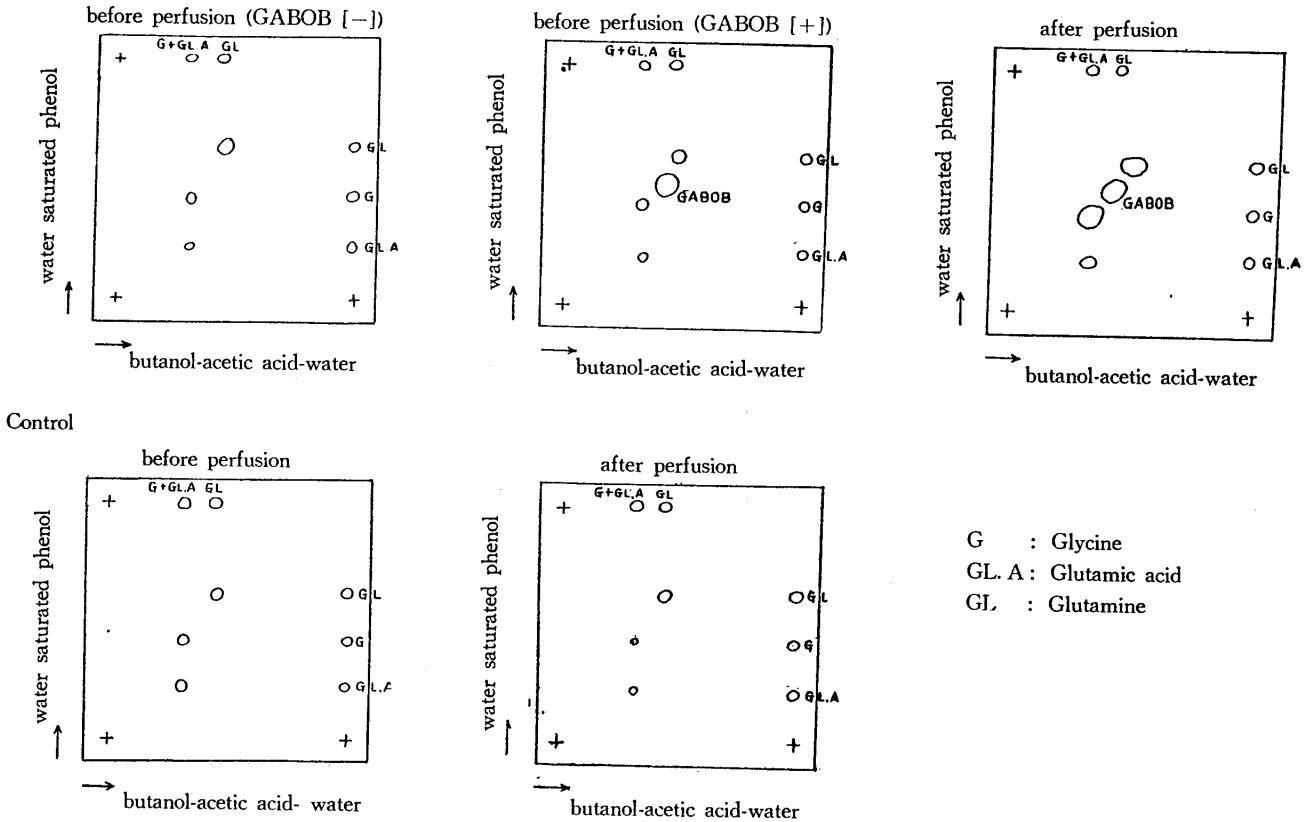
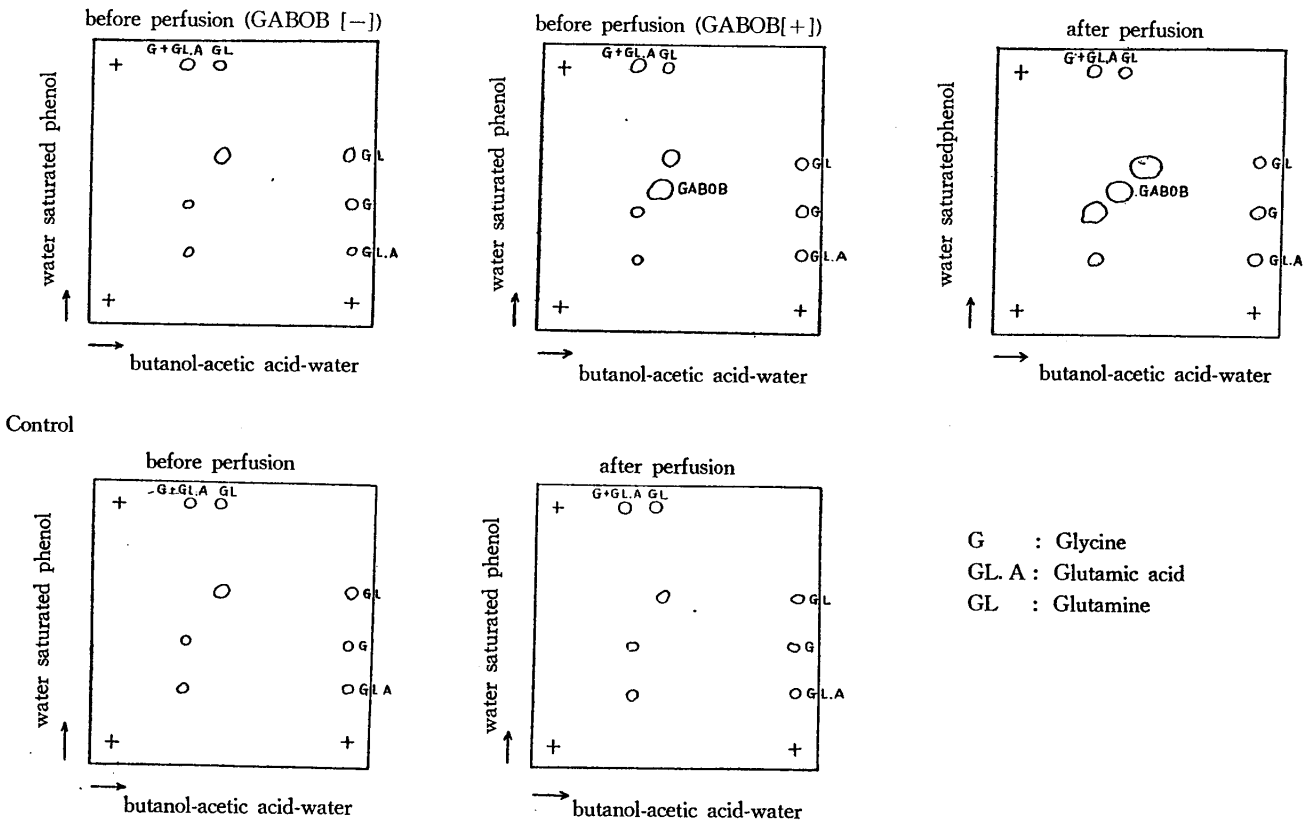


Fig. 2. Diagrammatical demonstrations of ninhydrine reaction performed on the developed fluids before and after the perfusions through brain of rabbits.



G : Glycine
GL. A : Glutamic acid
GL : Glutamine

Fig. 3. Diagrammatical demonstrations of ninhydrine reaction performed on the developed fluids before and after the perfusions through liver of rabbits.

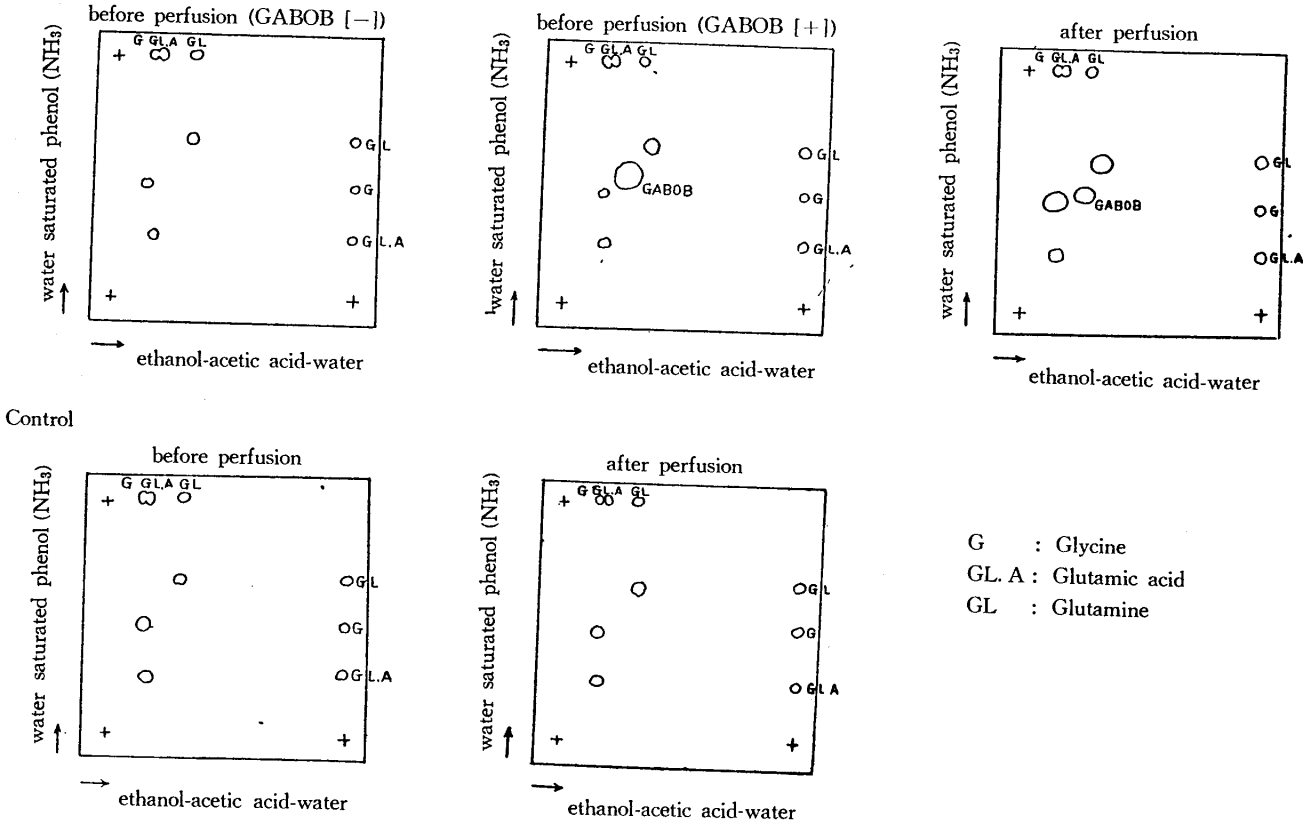
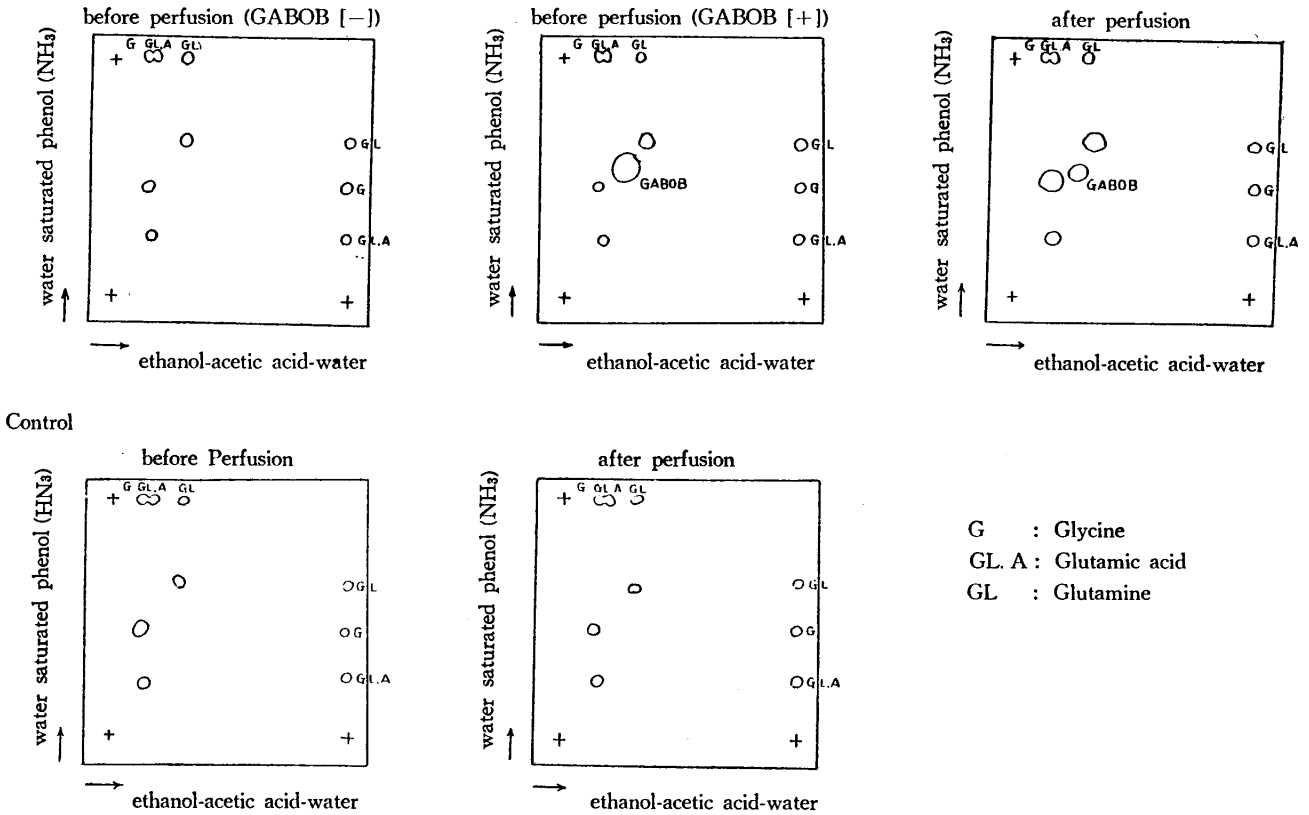


Fig. 4. Diagrammatical demonstrations of ninhydrine reaction performed on the developed fluids before and after the perfusions through brain of rabbits.



and 0.31 in the ethanol-acetic acid-water (4 : 2 : 1 v/v) showing the good coincidence with those glycine, glutamic acid and glutamine respectively. In the cases developed with the solvent of water saturated phenol (ammonia gas in the chamber), the *Rf*'s of the amimo acids found in the perfused media also showed good coincidence with those of glycine, glutamic acid and glutamine, 0.46, 0.25 and 0.65 respectively (Figs, 3, 4). The observations on the perfused solutions mixed with pure glycine, glutamic acid and glutamine showed the superimposed three amino acids on those amino acids found in the perfused media, identifying the amino acids found in perfused media to be glycine, glutamic and glutamine

Quantitative analysis:

The quantitative estimation on GABOB on the perfusion media proved that the added GABOB in the media is reduced by one third of original level after the perfusicn both through the liver and the brain, as shown in Table 1.

Table 1. Quantitative analysis of GABOB.

Analysis on the fluids perfused liver

Case No.	before perrfusion (γ)	after perfusion (γ)	decrease (γ)
1	34	22.5	11.5
2	26.5	21	5.5
3	32.5	12	20.5
4	32	25	7
5	27	20.5	6.5
6	24	21	13
7	33	17	16
8	27.5	15.5	12
average	30.8	19.3	11.5

Analysis on the fluids perfused brain

Case No.	before perfusion (γ)	after perfusion (γ)	decrease
1	29	18.5	10.5
2	27	19	8
3	28.5	24	4.5
4	31	24	7
5	29.5	17	12.5
6	29	20	9
7	30.5	26.5	4
8	30	22.5	7.5
avesage	29.3	21.4	7.9

Note: Values represent the contents of GABOB in 0.1 ml. of deproteinized filtrate of perfusion fluid.

As expected from the qualitative observation on the amino acids, the quantitative estimation on amino acids proved that the contents of glycine, glutamic acid and glutamine are increased in the GABOB containing medium after perfusion in comparison with the control, i.e. glycine showed a considerable increase though the increase in glutamic acid and glutamine were slight. The increasing rates of these amino acid were almost the same both in the fluids perfused liver and those perfused brain (Tables 2, 3, 4, 5).

Table 2. Quantitative analysis of glycine, glutamic acid and glutamine. Analysis on the fluid perfused liver.

	Glycine			Glutamic acid			Glutamine		
Case No.	before perfusion (GABOB [−]) (γ)	after perfusion (GABOB [+]) (γ)	increase (γ)	before perfusion (GABOB [−]) (γ)	after perfusion (GABOB [+]) (γ)	increase (γ)	before perfusion (GABOB [−]) (γ)	after perfusion (GABOB [+]) (γ)	increase (γ)
1	0.3	1.4	1.1	0.2	0.5	0.3	1.7	5.7	4.0
2	0.7	1.5	0.8	0.4	1.55	1.15	5.2	6.1	0.9
3	0.9	2.8	1.9	0.3	0.4	0.1	3.3	5.0	1.7
4	0.3	1.8	1.5	0.2	0.35	0.15	2.3	3.7	1.4
5	0.4	0.6	0.2	0.4	0.7	0.3	4.5	5.7	1.2
6	0.3	0.65	0.35	0.45	0.6	0.15	4.3	5.3	1.0
7	0.6	1.7	1.1	0.5	0.6	0.1	3.6	5.4	1.8
8	0.7	1.55	0.85	0.45	1.1	0.65	3.8	5.1	1.3
average	0.53	1.5	0.97	0.38	0.73	0.35	3.6	5.3	1.7

Note: Values represent the content of amino acids in 0.1 ml. of depoteinized filtrate of perfusion fluid.

Table 3. Quantitative analysis of glycine, glutamic acid and glutamine. Analysis on the fluid perfused liver (Control).

	Glycine			Glutamic acid			Glutamine		
Case No.	before perfusion (GABOB [−]) (γ)	after perfusion (GABOB [−]) (γ)	increase (γ)	before perfusion (GABOB [−]) (γ)	after perfusion (GABOB [−]) (γ)	increase (γ)	before perfusion (GABOB [−]) (γ)	after perfusin (GABOB [−]) (γ)	increase (γ)
1	0.7	0.8	0.1	0.7	1.1	0.4	4.8	5	0.2
2	0.35	0.45	0.1	0.3	0.5	0.2	4.8	5.2	0.4
3	0.5	0.55	0.05	0.45	0.5	0.05	2.2	2.8	0.6
4	0.35	0.5	0.15	0.2	0.45	0.25	2.3	5.2	2.9
5	0.9	1.1	0.2	0.3	0.9	0.6	2.6	3.8	1.2
6	0.4	0.7	0.3	0.25	0.4	0.15	3.4	4.5	1.1
7	0.75	0.9	0.15	0.2	0.3	0.1	2.1	4.7	2.9
8	0.4	0.7	0.3	0.3	0.5	0.2	3.6	4.2	0.6
average	0.54	0.71	0.17	0.34	0.62	0.28	3.2	4.4	1.2

Note: Value represent the content of amino acids in 0.1 ml. of deproteinized filtrate of perfusion fluid.

Table 4. Quantitaive analysis of glycine, glutamic acid and glutamine. Analysis on the fluid perfusd brain.

Glycine

Case No.	before perfusion (GABOB [−]) (γ)	after perfusion (GABOB [+]) (γ)	increase (γ)
1	0.8	2.2	1.4
2	0.4	0.8	0.4
3	0.4	0.9	0.5
4	0.3	1.5	1.2
5	0.35	1.1	0.75
6	0.5	1.2	0.7
7	0.3	0.9	0.6
8	0.7	1.5	0.8
average	0.47	1.26	0.79

Glutamic acid

Case No.	before perfusion (GABOB [−]) (γ)	after perfusion (GABOB [+]) (γ)	increase (γ)
1	0.2	0.5	0.3
2	0.2	0.3	0.1
3	0.3	0.45	0.15
4	0.4	0.7	0.3
5	0.45	1.2	0.75
6	0.5	1.1	0.6
7	0.6	0.9	0.3
8	0.35	0.75	0.4
average	0.38	0.74	0.36

Glutamine

Case No.	before perfusion (GABOB [−]) (γ)	after perfusion (GABOB [+]) (γ)	increase (γ)
1	2.2	5.0	2.8
2	2.7	5.5	2.8
3	2.5	3.8	1.3
4	3.4	6.0	2.6
5	6.0	7.0	1.0
6	4.7	5.9	1.2
7	4.5	5.6	1.1
8	3.6	4.8	1.2
average	3.7	5.45	1.75

Note: Value represent the content of amino acids in 0.1 ml. of deproteinized filtrate of perfusion fluid

Table 5. Quantitative analysis of glycine, glutamic acid and glutamine. Analysis on the fluid perfused brain (Control).

Glycine

Case No.	before perfusion (GABOB [−]) (γ)	after perfusion (GABOB [−]) (γ)	increase (γ)
1	0.7	0.8	0.1
2	0.35	0.45	0.1
3	0.5	0.55	0.05
4	0.35	0.5	0.15
5	0.8	1.1	0.3
6	0.4	0.7	0.3
7	0.3	0.7	0.4
8	0.5	0.8	0.3
average	0.49	0.7	0.21

Glutamic acid

Case No.	before perfusion (GABOB [−]) (γ)	after perfusion (GABOB [−]) (γ)	increase (γ)
1	0.7	1.3	0.6
2	0.3	0.6	0.3
3	0.2	0.5	0.3
4	0.55	0.6	0.05
5	0.2	0.45	0.25
6	0.35	0.55	0.2
7	0.4	0.6	0.2
8	0.25	0.45	0.2
average	0.37	0.63	0.26

Glutamine

Case No.	before perfusion (GABOB [−]) (γ)	after perfusion (GABOB [−]) (γ)	increase (γ)
1	3.8	5.0	1.2
2	4.8	5.2	0.4
3	2.2	5.2	3.0
4	4.2	5.3	1.1
5	2.4	3.8	1.4
6	3.5	4.6	1.1
7	3.8	5.1	1.3
8	4.2	5.3	1.1
average	3.7	4.9	1.2

Note: Value represent the content of amino acids in 0.1 ml. of deproteinized filtrate of perfusion fluid.

DISCUSSION

As suggested by SUGIURA *et al.*[4], GABOB seems to be converted to γ-aminoacetoacetic acid by oxidation first and then further oxidized decomposing into glycine and acetic acid. Another way for catabolism may be the formation of glutamic acid and malic acid by the transamination with α-ketoglutaric acid. OKUMURA *et al.*[5,6], and KONDO[7] observed the increased excretion of glycine and β-alanine in urine after the intravenous injection of γ-aminobutyric acid into rabbits. From these observations it was supposed that γ-aminobutyrie acid would be oxidized to GABOB producing glycine by the way just mentioned and on the other hand, β-alanine through γ-amino-α-hydroxybutyric acid.

In the perfusion test of liver and brain with the fluid containing GABOB, it has been revealed that a quantity of glycine, some glutamic acid and glutamine are produced by consuming GABOB. This indicates that GABOB will be metabolized to glycine probably passing the stage of γ-aminoacetoacetic acid, and supports the view proposed by SUGIURA *et al.*[4], OKUMURA *et al.*[5,6], and KONDO[7]. Thus the possible metabolic pathway of GABOB is as follows:

$$\underset{\text{GABOB}}{NH_2\text{-}CH_2\text{-}\underset{\displaystyle OH}{\underset{|}{CH}}\text{-}CH_2\text{-}COOH} \rightarrow \underset{(\gamma\text{-aminoacetoacetic acid})}{(NH_2\text{-}CH_2\text{-}CO\text{-}CH_2\text{-}COOH)} \rightarrow \underset{\text{Glycine}}{NH_2\text{-}CH_2\text{-}COOH}$$

However, there is a possibility that GABOB is decomposed by another metabolic pathway, though it may not be the main way. The increase in glutamic acid and glutamine in the perfused fluid containing GABOB seems to suggest the existence of a metabolic pathway where GABOB is converted to glutamic acid by transamination with α-ketoglutaric acid as suggested by SUGIURA. The glutamine will be formed from glutamic acd.

CONCLUSION

For the purpose to reveal the metabolic pathway of GABOB the analyses were performed with the GABOB containing fluid perfused through the liver and the brain of rabbits, and the following results were obtained.

Qualitative observations by paperchromatography on the fluid containing GABOB after perfusing the organs proved the presence of some amino acids. These were identified as glycine, glutamic acid and glutamine. The observation on the GABOB containing fluid perfused the organs showed a decrease in GABOB and an increase in these amino acids.

Quantitative observation proved a considerable increase in glycive and a moderate increase in glutamic acid and glutamine with a marked decrease in the amount of GABOB injected.

From these results it is believed that GABOB is decomposed into glycine and acetic acid probably passing the stage of γ-aminoacetoacetic acid in one way and into glutamic acid by the transamination of GABOB with α-ketoglutaric acid in the other.

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REFERENCES

1. YOSHIKAWA, T.: To be published.
2. OHASHI, K.: Studies on the apparatus to be used for irrigating hepatomized liver. J. Gastroenterology, **8**, 683, 1933 (in Japanese)
3. INOUE, K.: A method to keep a decapitated rabbit cerebrum alive by irrigating fluid. Brain and Nerve, **2**, 215, 1951 (in Japanese)
4. SUGIURA, M. and S. SENO: Metabolic patterns of γ-aminobutyric acid in brain. Saishin-Igaku, **12**, 2386, 1957 (in Japanese)
5. OKUMURA, N. and T. KONDO: Metabolism of γ-aminobutyric acid (I). Excretion of glycine and β-alanine in the urine after the administration of γ-aminobutyric acid. J. Yonago Medical Association. **7**, 52, 1956 (in Japanese)
6. OKUMURA, N., S. SUMIDA and T. KONDO: Metabolism of γ-aminobutyric acid. J. Jap. Biochem. Soc. Vol. **28**, Special No. 95, 1959 (in Japanese)
7. KONDO, T.: Amino acid metabolism in brain (I). Study on the excretion of γ-aminobutyric acid in urine after injection. J. Jap. Biochem. Soc. **30**, 444, 1959 (in Japanese)