CONVERSION OF β-ALANINE IN VIVO AND IN ISOLATED LIVER TISSUE

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(Received May 16, 1958)

Until recently the paths of conversion of β-alanine in the animal body have been uncertain in many respects.

In previous reports [1, 2] it was shown that the following mechanism of decomposition of β-alanine is present in the kidney tissue of rats:

β-alanine
↓ pyruvic acid
α-alanine
↓ α-ketoglutaric acid
glutamic acid
↓
NH_3

In the present paper we give the results of experiments studying conversion of β-alanine in liver tissue of rats and also in vivo.

EXPERIMENTAL METHOD

Experiments with rat liver. The experiments were carried out with rat liver pulp (rats weighing 120-140 g). A sample of the pulp weighing 600 mg was placed in 3.4 ml of a phosphate-saline mixture free from calcium ions [4]. The total volume of the mixture of reagents was 4 ml and its pH 7.1. The experimental sample contained 40 μM of β-alanine. Incubation was carried out in an atmosphere of oxygen at 37°C for 160 minutes. Proteins were precipitated by heating the samples in a boiling water bath for 2 minutes, after which the reagent mixture was rapidly cooled in ice water and centrifuged. In the protein-free centrifugate thus obtained, the urea and ammonia were estimated. These estimations were made by Conway's method. The urea was estimated by the quantity of ammonia given off as a result of the action of urease. The amino acids were estimated by the paper chromatography method [7, 9].

Experiments on the intact animal. Experiments were performed on four young rats with marked vitamin B_6 deficiency [1] and on four healthy rats. The rats were specially selected to be of the same weight – 170 g each. Throughout the whole period of investigation both control and experimental rats were kept on a synthetic diet not containing vitamin B_6 [2]. Each experimental rat was given a daily intraperitoneal injection of 10 mg of isonicotinylhydrazide (INH) in physiological saline. Instead of INH, the control rats were injected with 100 μg of vitamin B_6. β-alanine was injected after determined intervals, also intraperitoneally and in a dose of 1 mg/g body weight of the animal. After receiving the injections of β-alanine, the rats were placed in a separate metabolic cage. The urine was collected according to the method of Levine and Smith [12] with the sole exception that in our experiments no liquid paraffin was poured into the urine-collecting funnel, and toluene was added to the receiver in place of NaF. The 48-hour sample of urine and the distilled water used for thorough

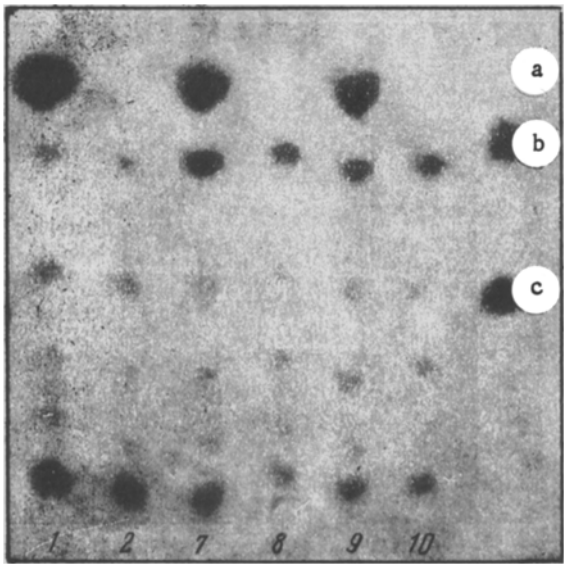

Fig. 1. Disappearance of β-alanine and excess formation of α-alanine during incubation of rat liver pulp in an atmosphere of oxygen. 1, 2 – Before incubation; 7, 8, 9, 10 – 160 minutes of incubation; a – β-alanine; b – α-alanine; c – glutamic acid.

washing of the cage and the funnel were combined, and the mixture was filtered through a Buchner funnel, evaporated down on a water bath and made up quantitatively to 50 ml in a measuring flask. The β-alanine in the solution was determined quantitatively by the chromatographic method [7, 9].

EXPERIMENTAL RESULTS

Experiments with rat liver tissue. During incubation of liver pulp from normal rats for 160 minutes in an atmosphere of oxygen, no excess ammonia could be determined in consequence of the added β-alanine. The chromatogram showed partial disappearance of β-alanine and some increase in α-alanine (Fig. 1), and it was clear that the quantity of β-alanine lost was greater than the α-alanine formed. It could be postulated that β-alanine is utilized in the liver tissue for the synthesis of urea [8, 11].

The experiments which were carried out confirmed this hypothesis. As may be seen from Table 1 the quantity of surplus urea formed was almost equivalent to the quantity of β-alanine disappearing, and furthermore, as has already been mentioned, a slight surplus accumulation of α-alanine also took place.

Roberts and Bregeff [14] reported that in preparations of the liver and brain of mice a process of transamination of β-alanine and α-ketoglutaric acid takes place. It must be pointed out, however, that these workers used unpurified enzyme preparations in their work, and these may have contained proteinases, peptidases and transaminases. It is therefore impossible under these circumstances to exclude the possibility of the formation of different α-ketoacids under the influence of these enzymes from taking part in the process of transamination. For this reason the results described by these workers cannot, it seems to us, be regarded as sufficient proof that α-ketoglutaric acid is a primary acceptor of the amino-group of β-alanine.

Nor was the mechanism of decomposition of β-alanine in rat liver explained in our work. It is possible that in the liver of rats, by analogy with the renal tissue, pyruvate serves as the primary acceptor of the amino-group of β-alanine, and the α-alanine formed, directly or after transamination with ketoglutaric or oxalacetic acid, serves as a source of nitrogen used in the synthesis of urea [7]. This problem requires further study.

Experiments on the intact animal. It has previously been shown [7] that in kidney tissue of rats in vitro β-alanine takes part in a process of transamination with pyruvate. The findings described above suggest the possible conversion of β-alanine by transamination in liver tissue also. If in the intact animal in vivo, conversion of β-alanine is brought about by transamination, then in severe vitamin B_6 deficiency the elimination of β-alanine from the body in the urine in consequence of the pyridoxin deficiency must be greater than in control animals. After injection of pyridoxin into experimental rats their power of converting β-alanine must be restored.

TABLE 1

Conversion of β-alanine During Incubation of Liver Pulp of Healthy Rats (calculated per 600 mg of moist tissue)

Experiment	Lost β-alanine, μM	Surplus formation of N-urea, μM	Surplus formation of alanine μM
1	5.9	5.6	0.6
2	7.2	6.3	1.1
3	3.5	3.0	0.6

The urine for analysis after injection of β-alanine was collected over a period of 48 hours, since a specially conducted experiment showed that the urine which was collected on the third day after injection of β-alanine did not contain this amino acid. In the course of the first 8 days it is difficult to detect any difference in the quantity

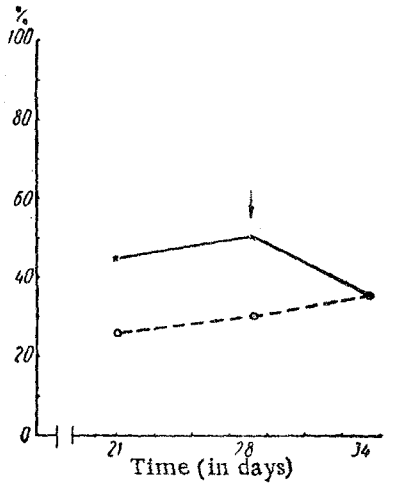

Fig. 2. The effect of INH on β-alanine metabolism in vivo in rats (mean results from Table 2).
— experimental; - - control; ↓ commencement of pyridoxin treatment. Along the ordinate is represented the quantity of β-alanine excreted in the urine as a percentage of the quantity of β-alanine injected

of β-alanine excreted in the urine of the experimental and control animals. On the 2nd day that the animals were kept on their synthetic diet (Table 2) the control rats excreted 23-29% of injected β-alanine in the urine and the experimental (vitamin B_6 deficient) rats – 40-47%, and on the 28th day the figures were 28-33% and 48-55% respectively. Thus vitamin B_6 deficiency increases the quantity of β-alanine excreted in the urine by approximately 20%. After injection of the experimental rats with pyridoxin this excretion of β-alanine falls and reaches the same values as in the control animals (Fig. 2).

Nevertheless the tissues of the experimental rats must evidently contain some quantity of vitamin B_6, since otherwise the animals would have died. A small part of the β-alanine injected into the experimental animals probably continues to take part in the processes of transamination. In addition β-alanine may be concerned in metabolic processes not dependent on pyridoxin enzymes: for example it may act as raw material for the synthesis of dipeptides such as carnosine and anserine [11, 12, 13], it may presumably be a source of formation of pyrimidine bases [10], and possibly it may undergo other conversions.

Attention is drawn to the gradual increase in the quantity of β-alanine excreted by the control rats (Table 2, Fig. 2). A parallel fall is observed in the weight of both the experimental (25-33%) and control animals (18-23%). This is due to the lack of balance of the synthetic diet in respect to a number of factors (besides vitamin B_6) directly or indirectly concerned in the metabolism of β-alanine.

In conclusion we must compare our findings with the experimental results of Pihl and Fritzson [13]. These authors showed that in the course of 5 hours, more than 90% of the labeled carbon of the carboxyl group of β-alanine injected into rats was excreted in the form of CO_2. In other words in 5 hours more than 90% of the injected β-alanine has undergone decomposition. In the control rats in our own experiments, in two days about 25% of the

TABLE 2

The Effect of INH on the Metabolism of β-alanine in Vivo in Rats

Date		2/5/58 start of expts.	2/24/58 after 19 days			3/3/58 after 26 days			3/9/58* after 32 days		
Rat no.		rat wt. (in g)	β-alanine injected A (in mg)	β-alanine excreted B (in mg)	$\frac{B}{A}100$	A (in mg)	B (in mg)	$\frac{B}{A}100$	A (in mg)	B (in mg)	$\frac{B}{A}100$
Experimental	1	170	122	58.0	47.5	122	67.4	55.2	127	52.2	41.1
	2	170**	0	none			none		0	none	
	3	170	122	49.4	40.3	120	57.2	47.8	115	35.1	30.4
	4	170	136	62.1	45.6	132	65.7	49.8	1***	–	–
Control	5	170	141	40.7	28.9	135	44.5	32.9	138	53.2	38.5
	6	170**	0	none		0	none		0	none	
	7	170	143	36.2	25.2	134	37.7	28.2	130	42.1	32.4
	8	170	133	31.4	23.5	–	–	–	130	48.5	37.2

* After 4 days of treatment of the experimental animals with pyridoxin (150 μg per day)

** A blind test was done as a control of the absence of β-alanine from the urine of rats not injected with β-alanine.

*** Rat died.

injected β-alanine was excreted in the urine in an unchanged form, and consequently the decomposition of this amino acid did not in any case exceed 75%, i. e. was considerably less than in the experiments of Pihl and Fritzson. The difference between the results obtained is due to the different quantity of β-alanine injected in the experiments: with Pihl and Fritzson this amounted to 1-1.4 mg per rat, and in our experiments it was about 100 times greater, i. e. it amounted to 120-140 mg.* Summing up, it can be stated that after the parenteral injection of rats with 120-140 mg of β-alanine, the quantity of this aminoacid excreted unchanged in the urine of vitamin B_6 deficient animals is greater than in controls, which gives additional evidence of the participation of pyridoxin enzymes in the metabolism of β-alanine.

In isolated liver tissue the added β-alanine acts as a source for the formation of extra urea and also of α-alanine.

SUMMARY

β-alanine was incubated with chopped liver tissue of rats in oxygen for 160 minutes at 37°C. It was established that a certain part of added β-alanine (about 12%) disappears, causing an excessive production of urea and of a small quantity of β-alanine.

In parenteral administration of β-alanine an increased urinary excretion of this amino acid is observed in B_6-deficient rats in which the process of β-alanine transformation is disturbed.

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* Experiments in which rats were injected with 200 mg of β-alanine and the urea nitrogen of the urine was subsequently analyzed as $N-NH_3$ and $N-NH_2$ were also carried out by A. N. Parshin, T. A. Goriukhina and E. A. Gromyko [5]. However, their published results cannot be used for comparison for the following reasons: these workers injected rats with 200 mg of β-alanine or 31.5 mg $N-NH_2$, and they found in the urine an excess of nitrogen which was considerably greater than this figure, composed of three fractions ($N-urea + N-NH_3 + N-NH_2$) totalling from 46.1 to 117.7 (!) (instead of 31.5 mg). Furthermore these workers estimated the β-alanine excreted by the value of the $N-NH_2$, determined in the urine by a nonspecific "copper" method, which was increased five times.

** See English translation.

*** In Russian.