The Formation of Glycine and Serine

THE INFLUENCE OF THE ADMINISTRATION OF GLYCINE, DL-SERINE AND OTHER COMPOUNDS ON LEVELS OF TISSUE GLYCINE AND SERINE

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(Received 8 March 1957)

The rates of conversion of glycine into serine and serine into glycine have been determined by administration of isotopically labelled glycine and serine (Arnstein & Neuberger, 1953; Elwyn & Sprinson, 1954; Arnstein & Keglević, 1956; Arnstein & Stanković, 1956). The present investigation, which is concerned with the net synthesis of glycine from serine, and of serine from glycine, was carried out with unlabelled glycine and serine and the methods were similar to those used in a previous investigation of tissue-glycine levels (Simkin & White, 1957). Greenberg & Sassenrath (1955) have made a more extensive but less detailed investigation of the effect of administration of several amino acids including glycine and serine.

METHODS

Animals, diet and method of dosage were described by Simkin & White (1957). The dose level of amino acids and of sodium benzoate was usually about 400 μ moles/100 g. body wt. In the rat the volume injected was generally 1.0 ml., except with DL-serine where its low solubility made it necessary to use 1.5–2.0 ml. If several compounds were administered to the same animal they were, if possible, dissolved in the same solution, but where this was not possible the total volume injected did not exceed 2.0 ml. Control rats were injected with an equal volume of water. Rabbits were usually given 10 ml. (20 ml. for DL-serine). Pyruvic acid was neutralized with NaHCO_3 before dilution to the required volume.

Collection and deproteinization of tissues

Blood. This has been described previously (Simkin & White, 1957).

Other tissues. The method of Simkin & White (1957) was used when only glycine was determined, but this was modified, as suggested by Schurr, Thompson, Henderson & Elvehjem (1950), when filtrates were prepared for the estimation of serine by microbiological assay, in the following way. The frozen sample (3–4 g.) was heated with 10 ml. of water in a boiling-water bath for 3 min. The cooled sample was comminuted with a further 10 ml. of water and the requisite amount of tungstate mixture. Tungstic acid filtrates were stored under toluene in a refrigerator overnight and brought to pH 6.8–7.0 before assay.

Determination of serine

Serine was estimated by microbiological assay, with *Leuconostoc mesenteroides* P60 and a slight modification of the method of Henderson & Snell (1948). Sodium benzoate (final concn. 0.1 mM) did not interfere with the growth of the organism (cf. Nurmikko & Virtanen, 1951). Krueger (1950) used a similar method of assay and reported that β -hydroxybutyric and pyruvic acids had no effect upon the growth rate of this organism.

Plan of experiments

This was, in general, as described by Simkin & White (1957). The mean control value obtained from all experiments was used for rats. Microbiologically available serine has been termed 'free L-serine'.

RESULTS

The results obtained for the levels of glycine and serine in rat liver and blood and in rabbit blood are presented in Tables 1–3. Table 1 contains values obtained in rat liver after administration of glycine, Table 2 those after administration of serine and its precursors, and Table 3 those for levels of glycine and serine in rat and rabbit blood.

Administration of glycine

The intraperitoneal injection of glycine caused a rapid rise in the concentration of free glycine in rat liver (Table 1). The concentration of free L-serine in liver was similarly affected. The ratio of the concentration of serine to that of glycine remained approximately constant. The control value of this ratio was 0.45, and the mean value after administration of glycine was 0.46 ($\pm$ s.d. 0.14, 8 values).

Table 3 shows the results of experiments on the effect of injection of glycine into the rat and rabbit. The blood glycine level increased about 12-fold at 0.5 hr. after injection. The concentration of free glycine in rat liver and in rat and rabbit blood increased also after simultaneous injection of equimolecular quantities of glycine and benzoate (Tables 2 and 3), but the increase was smaller than that observed when a similar dose of glycine was administered alone (Table 1). [A similar result was obtained by de Vries & Alexander (1948), who

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studied the free glycine concentration of human plasma.] Although the rise in glycine concentration observed was smaller than when glycine alone was administered, the concentration of free serine in rat liver still increased (Table 2).

Administration of serine and serine precursors

The concentration of free serine in the liver was raised to a considerable extent by administration of DL-serine, acetate, pyruvate and DL- β -hydroxybutyrate (Table 2). There was not a marked rise in the level of glycine. The ratios of the concentrations of serine to those of glycine were well above the

control value (0.45) and ranged from a minimum of 0.75 after the administration of pyruvate to 1.40 after administration of DL-serine.

The concentration of serine, after administration of DL-serine + benzoate, was raised to approximately the same extent as in the absence of benzoate, and the administration of serine failed to prevent the decrease in glycine concentration normally found in the presence of benzoate (Simkin & White, 1957); the ratio of serine to glycine concentration was about 2.8.

After administration of glycine and benzoate the concentrations of both free L-serine and glycine in

Table 1. *Effect of intraperitoneal injection of glycine on the concentration of free glycine and serine in rat liver*

Each determination was carried out on the pooled livers from two rats. Mean control values for undosed animals were: free glycine, 268 ± 42 s.d. (33 expts.); free (microbiologically available) L-serine, 120 ± 9 s.d. (7 expts.). Column headings in hr. refer to time of death; doses were injected at 0 hr.

Expt.	Dose (μ moles/100 g. body wt.)	Free glycine (μ moles/100 g. of fresh tissue)					Free L-serine (μ moles/100 g. of fresh tissue)			
		0.5 hr.	1.5 hr.	2.0 hr.	3.0 hr.	5.0 hr.	0.5 hr.	1.5 hr.	3.0 hr.	5.0 hr.
A	360	980	—	530	—	—	—	—	—	—
B	190	655	—	355	—	—	—	—	—	—
C	380	835	535	—	525	420	—	—	—	—
D	460	1250	770	—	405	255	365	335	170	105
E	390	950	515	—	345	—	455	275	265	—
F	430	1160	—	—	—	—	410	—	—	—

Table 2. *Effect of intraperitoneal injection of serine and serine precursors on the concentration of free glycine and serine in rat liver*

Each determination was carried out on the pooled livers from two rats. Mean control values for undosed animals: free glycine, 268 ± 42 s.d. (33 expts.); free (microbiologically available) L-serine, 120 ± 9 (7 expts.). Column headings in hr. refer to time of death; doses were injected at 0 hr.

Expt.	Compound admin- istered	Dose (μ moles/100 g. body wt.)		Free glycine (μ moles/100 g. of fresh tissue)					Free L-serine (μ moles/100 g. of fresh tissue)				
		Serine or precursor	Benzoate	0.25 hr.	0.5 hr.	1.5 hr.	3.0 hr.	5.0 hr.	0.25 hr.	0.5 hr.	1.5 hr.	3.0 hr.	5.0 hr.
G	DL-Serine	380	—	—	260	300*	—	—	—	—	—	—	—
H		410	—	300†	245‡	—	—	—	—	—	—	—	—
I		1000§	—	—	310	280	—	—	—	—	—	—	—
J		400	—	—	320	315	305	265	—	—	—	—	—
K		440	—	—	300	270	255	—	—	415	295	260	—
L	DL-Serine +	420	420	—	140	185	115	105	—	—	—	—	—
M	benzoate	410	380	—	145	115	90	—	—	430	295	250	—
N	Acetate	400	—	180	—	—	—	—	195	—	—	—	—
O		1000	—	—	240	—	—	—	—	265	—	—	—
P	Pyruvate	400	—	215	—	—	—	—	165	—	—	—	—
Q		1000	—	—	210	—	—	—	—	190	—	—	—
R	DL- β -Hydroxy-	400	—	260	—	—	—	—	230	—	—	—	—
S	butyrate	1000	—	—	270	—	—	—	—	260	—	—	—
T	Glycine +	350	350	—	730	405	200	240	—	—	—	—	—
U	benzoate	400	370	—	605	260	110	—	—	375	255	190	—
V	Benzoate	—	460	—	90	125	100	105	—	90	125	95	150
W		—	470	—	—	115¶	170	90	—	—	120¶	165	75

* 2 hr.

† 0.35 hr.

‡ 0.75 hr.

§ Owing to the sparing solubility of DL-serine, 600 μ moles/100 g. of this dose were given by stomach tube.

|| 0.9 hr.

¶ 1 hr.

Table 3. *Effect of intraperitoneal injection of serine, glycine and benzoate on the concentration of free glycine and serine in blood*

Each determination for rats was carried out on the pooled blood of two animals. Mean control values for undosed animals were: free glycine 20 ± 4 s.d. (29 expts.); free (microbiologically available) L-serine 22 ± 8 (4 expts.). For rabbits, blood samples were withdrawn at the stated times and the control value (0 hr.) for each animal is given. Column headings in hr. refer to time of death of rats, or time of taking sample from rabbits; doses were injected at 0 hr. Lettered experiments refer to rats and numbered experiments to rabbits.

Expt.	Compound administered	Dose (μ moles/100 g. body wt.)		Free glycine (μ moles/100 g.)					
		Glycine or serine	Benzoate	0 hr.	0.25 hr.	0.5 hr.	1.5 hr.	3 hr.	5 hr.
C	Glycine	380	—	—	—	260	106	52	44
T	Glycine + benzoate	350	350	—	—	114	52	18	23
J	DL-Serine	400	—	—	—	19	18	17	17
L	DL-Serine + benzoate	420	420	—	—	10	12	9	11
1	Glycine	430	—	63	720	860	820	530	310
2	Glycine + benzoate	390	390	57	210	250	187	120	69
3	DL-Serine	300	—	167	165*	157	151	162	143
4	DL-Serine + benzoate	290	290	107	84*	74	47	27	16
Free L-serine (μ moles/100 g.)									
V	Benzoate	—	460	—	—	20	16	17	16
W	Benzoate	—	470	—	—	10†	—	16	12
5	Benzoate	—	400	33	—	36‡	29	30	—

* 0.3 hr.

† 1 hr.

‡ 0.75 hr.

the liver were increased initially above the control value in almost the same proportion, but subsequently the serine level decreased more slowly than the glycine level (Table 2). The ratio of serine to glycine concentration rose from 0.62 to 1.9. Administration of DL-serine did not result in any significant change in the concentration of free glycine in rat or rabbit blood (Table 3).

Administration of benzoate

The concentrations of free glycine and L-serine in rat liver after administration of benzoate are given in Table 2. There is no evidence for a large change in concentration of free serine, but since the variation (s.d. ± 32) appears to be greater than that in the controls (s.d. ± 9) no conclusion can be drawn as to the possibility of a small variation. The concentration of free glycine in the same experiments decreased, as is usual after benzoate administration (Simkin & White, 1957). The change in the concentration of free serine in the blood of a rabbit after the administration of benzoate was small (Table 3). Injection of serine with benzoate did not prevent the decrease in free glycine of the blood, which is characteristic of benzoate administration.

DISCUSSION

Formation of serine from glycine

The change in tissue levels of serine after administration of glycine show that there is a rapid net synthesis of serine from glycine, which indicates that the rate of formation of serine from glycine varies

markedly with the tissue level of glycine. It had already been shown (e.g. by Arnstein & Neuberger, 1953) that glycine may provide both the hydroxymethyl group of serine and the remaining part, but investigations with labelled glycine did not exclude the possibility that the rate of the reaction was limited by a reaction step following zero-order kinetics. It is clear that this is not so, and that glycine from dietary sources can act as an effective precursor of serine.

This fact is of considerable interest in connexion with the glycogenic action of glycine. Although Hess (1950) found that glycogen was rapidly formed from administered DL-serine, there is a time lag of several hours before glycogen is formed from administered glycine (see Arnstein, 1954), and it has been suggested (Arnstein, 1954) that a possible explanation of this lag is the limited net rate of conversion of glycine into serine. The results obtained from the present investigation do not support this explanation.

Formation of glycine from serine

It is known that glycine is readily formed from serine, since administration of isotopically labelled serine results in the production of glycine with a specific activity as high as 63% of that of the administered serine (Elwyn & Sprinson, 1954). We have found, however, that the tissue levels of glycine after administration of serine showed little or no increase above normal values, which indicates that the rate of conversion of serine into glycine varies little with the tissue level of serine. An

increase in the tissue level of serine resulting from administration of nitrogen-free precursors also fails to change the tissue level of glycine, showing that the lack of effect of administered serine is not caused by permeability barriers. Moreover, when serine is formed from administered glycine, and the level of glycine is reduced by benzoate, the serine level decreases more slowly than that of glycine.

The present investigation shows that there is no evidence for an increase in the level of free glycine in liver after administration of serine or its precursors (see, however, Christensen, Streicher & Elbinger, 1948; Greenberg & Sassenrath, 1955). The mean of 19 values after administration of DL-serine, acetate, pyruvate or DL- β -hydroxybutyrate was 264 ± 37 s.d.; the mean of 33 control values was 268 ± 42 . The fact that the value of the standard deviation of the mean free glycine level of liver after administration of serine or its precursors is very close to that of the control confirms that this calculation of the mean value of the determinations at different time intervals after dosage is permissible; calculation of such an average value would tend to obscure a temporary rise in the free glycine level of liver, but such a rise would lead to a large standard deviation. The mean of 12 values for free serine in liver was 270 ± 81 as compared with a control value of 120 ± 9 (7 expts.). The standard deviation of 81 corresponds to real differences in the effects of administering serine or its precursors, as well as to temporary changes in the level of free L-serine. The evidence shows that an increase in the tissue level of serine has much less effect on the conversion of serine into glycine than an increase in the tissue level of glycine has on the formation of serine. This may be expressed formally by saying that the kinetics followed by the serine $\rightarrow$ glycine reaction approach zero- rather than first-order. This is supported by the inability of DL-serine to restore the growth rate of glycine-depleted rats (White, 1941).

Availability of serine and glycine

The results of Arnstein & Neuberger (1953) show that the gross rate of formation of glycine from serine and other precursors is independent of the supply of glycine in the diet. As shown in the previous section, this rate is only slightly increased by the administration of serine. The present investigation shows that there is a net formation of serine from glycine after administration of glycine, which indicates that the gross rate of formation of serine increases with increasing glycine level.

These findings seem to suggest that in the absence of dietary sources of glycine the net production of glycine from serine will increase with the requirements of the organism for glycine. As the requirement for glycine increases, the tissue level of glycine will decrease. An extreme instance of this

is the marked decrease in free glycine of the liver after administration of benzoate (Table 2). The rate at which glycine is converted into serine will therefore decrease, so that if the serine level stays constant the net production of glycine would be greater than before. The present results suggest, however, that the reduction in the glycine level after administration of benzoate does not affect the rate of formation of serine. It is known that the α -C atom of glycine is a source of the β -C atom of serine, presumably via formaldehyde or a derivative at the same level of oxidation (Elwyn & Sprinson, 1954). When there is a plentiful supply of glycine, up to 60% of the β -C atoms of serine is derived from glycine, but at normal glycine levels glycine acts as precursor of the β -C atom of serine to a negligible extent (Arnstein & Neuberger, 1953). Under these conditions, therefore, glycine is of little importance as a source of 'formaldehyde', and a lowering of the glycine level would not appreciably diminish the availability of 'formaldehyde'. The fact that serine levels are maintained even when the glycine level is depressed by benzoate suggests that the rate of formation of serine is limited by the concentration of 'formaldehyde' rather than of glycine.

SUMMARY

1. The influence of the administration of benzoate, glycine, DL-serine, acetate, pyruvate or DL- β -hydroxybutyrate on the concentrations of free glycine and free L-serine in the liver and blood of the rat and the blood of the rabbit has been investigated. Administration of glycine resulted in a rise in the concentration of both glycine and serine. Administration of serine or its non-nitrogenous precursors resulted in a rise in the concentration of serine but not of glycine. Administration of benzoate alone resulted in a fall in the concentration of glycine but not of serine.

2. The significance of the results is discussed with reference to the kinetics of the interconversion of glycine and serine.

We wish to thank Dr F. W. Norris for providing a culture of *Leuconostoc mesenteroides*. We are indebted to Dr W. V. Thorpe and Dr H. G. Bray for their interest and discussion of this work and to Mr P. B. Wood, Mrs B. G. Taylor and Miss D. A. Clarke for technical assistance.

REFERENCES

- Arnstein, H. R. V. (1954). *Advanc. Protein Chem.* **9**, 80.
- Arnstein, H. R. V. & Keglević, D. (1956). *Biochem. J.* **62**, 199.
- Arnstein, H. R. V. & Neuberger, A. (1953). *Biochem. J.* **55**, 271.
- Arnstein, H. R. V. & Stanković, V. (1956). *Biochem. J.* **62**, 190.

- Christensen, H. N., Streicher, J. A. & Elbinger, R. L. (1948). *J. biol. Chem.* **172**, 515.
- de Vries, A. & Alexander, B. (1948). *J. clin. Invest.* **27**, 665.
- Elwyn, D. & Sprinson, D. B. (1954). *J. biol. Chem.* **207**, 459.
- Greenberg, D. M. & Sassenrath, E. N. (1955). *Cancer Res.* **15**, 620.
- Henderson, L. M. & Snell, E. E. (1948). *J. biol. Chem.* **172**, 15.
- Hess, W. C. (1950). *J. Amer. chem. Soc.* **72**, 1407.
- Krueger, R. (1950). *Helv. chim. acta*, **33**, 2157.
- Nurmikko, V. & Virtanen, A. I. (1951). *Acta chem. scand.* **5**, 97.
- Schurr, P. E., Thompson, H. T., Henderson, L. M. & Elvehjem, C. A. (1950). *J. biol. Chem.* **182**, 29.
- Simkin, J. L. & White, K. (1957). *Biochem. J.* **65**, 574.
- White, A. (1941). *Yale J. biol. Med.* **13**, 759.

Determination of Manganese in Tissue extracts by means of Formaldoxime

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(Received 20 March 1957)

Sideris (1937) described a method for the estimation of Mn^{2+} ions in ashed tissue which was based on the spot-test reaction of manganese with formaldoxime ($CH_2=N\cdot OH$). Gum ghatti was used to stabilize the colour. The present modification of this method was developed for the study of the uptake of Mn^{2+} ions by tissues from dilute solutions (10^{-3} to $10^{-5}M$). The estimation is made directly on trichloroacetic acid or perchloric acid extracts of the tissue without prior ashing. The technique is simple, involving the addition of a single solution, and the colour developed is extremely stable without addition of any protective colloid. Ferric iron which also reacts with formaldoxime may be removed by extraction as ferric thiocyanate. Between 0.01 and 0.25 μ mole (0.55–13.7 μ g.) of manganese can be estimated in this way. Yellow tissue constituents absorb at the same wavelength as the formaldoxime-manganese complex and this may reduce the accuracy of the determination at low levels of Mn^{2+} ions. The method is therefore not suitable for the determination of endogenous tissue manganese without prior ashing, but it is very convenient for the determination of manganese at the levels often used in enzyme experiments.

METHOD

Solutions. Manganous chloride (A.R., 0.1M) in glass-distilled water was diluted to give a working standard of 0.0005M.

A solution of formaldoxime hydrochloride (British Drug Houses Ltd.), 0.5 g. in 10 ml. of glass-distilled water, was made; 0.1 ml. of this solution was diluted with 1 ml. of 10N-NaOH immediately before use.

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Procedure

Tissue samples (liver homogenates or mitochondrial suspensions) were deproteinized with trichloroacetic acid (final concn. 0.8N) or $HClO_4$ (final concn. 0.5N). The clear supernatant obtained after centrifuging constitutes the tissue extracts which were analysed. Up to 3.0 ml. of the tissue extract was placed in a test tube. With the smaller samples the volume was adjusted to 3.0 ml. with water. To ensure rapid mixing 1 ml. of formaldoxime was squirted from a 1 ml. all-glass hypodermic syringe into the test solution; mixing was completed at once by shaking the tube. Less accurate results were obtained when the mixing was not rapid. After 10 min. the optical density was measured in 1 cm. cells in the Unicam spectrophotometer SP. 600 at 450 $m\mu$ (see Fig. 1). The solutions were read against a manganese-free solution treated in the same way. The optical density of the blank solution increases slightly for the first 2 hr. and then steadily fades. However, in the test

Fig. 1. Absorption spectra of manganese and nickel complexes with formaldoxime. Solutions (3 ml.) containing 0.25 μ mole of Mn^{2+} or Ni^{2+} were treated with 1 ml. of formaldoxime solution.