XII. EXPERIMENTS ON THE METABOLISM OF GLYCINE

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THE metabolism of glycine has been a subject of controversy for a long time. As the simplest amino-acid, it was chosen as one of the first objects of investigation. But in the course of research it was seen that glycine behaved differently in many ways from the higher members of the series and that biochemical and physiological effects easily demonstrated with the higher aliphatic amino-acids proved to be absent or inconsistent with glycine.

The deamination of an amino-acid is considered to be one of its chief metabolic changes, and it was this mechanism on which early research was carried out. As long as the latter was confined to experiments involving the metabolism of the whole body, the results obtained by different investigators seemed to agree in general that glycine when fed *per os* [Salkowski, 1880; Nencki & Schultzen, 1872; Friedmann, 1908; Ringer & Lusk, 1910] or injected into the animal was almost quantitatively deaminated, the equivalent amount of NH_3 or urea being excreted by the animal. More recent experiments of Kiech & Luck [1931], however, demonstrated the existence of a lag between amino-N disappearance as estimated from the N content of the minced carcass, and urea formation following glycine administration. They conclude that "calculations of the extent of amino-acid metabolism from the amount of urea excreted during the first 8 hr. following amino-acid administration are likely to be seriously incorrect". Contradictions also arose when attempts were made to localize the deaminating effect on glycine by perfusion experiments with liver. Salaskin [1898] was the first to report urea formation during perfusion of liver with blood containing added glycine. Fiske & Karsner [1914], who repeated the experiment with improved technique, could not confirm these findings, whereas Jansen [1915] obtained small amounts of urea after perfusion. It is conceivable that the long duration of the experiments used by some of these workers (4-5 hr.) may finally have caused decomposition of the urea. The fate of the 2-C skeleton remaining after the deamination of glycine is also controversial. Feeding experiments such as carried out by Ringer & Lusk [1910] on phloridzinized dogs demonstrated an increased carbohydrate excretion following glycine administration which Pflüger [1910] could not confirm. More recently the same negative result was reported by Wilson & Lewis [1930], whereas Butts & Dunn [1935] observed no glucogenetic effect in one case, but report a small increase in carbohydrate content in another case.

Nor has agreement been reached hitherto either by means of the slice technique or by the use of tissue extracts. After Neubauer [1909] had discovered the principle of oxidative deamination in phenyl-substituted amino-acids. Patey & Holmes [1929] observed NH_3 production in chopped kidney tissue with added glycine. Krebs [1933] observed a slight deamination of glycine following its incubation with kidney and liver slices, but practically no effect was observed with kidney extracts, although a great number of other amino-acids were readily deaminated. Later Krebs [1935, 1], Bernheim & Bernheim [1935] and Kisch [1936],

confirmed the stability of glycine to deamination and oxidation, and Neber [1936] showed that of all the amino-acids tested glycine alone resisted deamination by intestinal mucous membrane. Reid [1936] obtained an increase in liver glycogen after injection with alanine, but a decrease following injection of glycine. Finally, similar results were experienced by Bach & Holmes [1937] when investigating the glucogenetic effect of amino-acids following their incubation with liver slices. Glycine proved to be the only amino-acid tested which caused no formation of carbohydrate, in fact a carbohydrate disappearance.

Thus it appears that glycine is probably metabolized by the body when fed to the whole organism, but the experimental conditions under which its deamination might consistently be demonstrated *in vitro* in different organs are not fully known. An attempt to elucidate such conditions and to clarify the mechanism of deamination seemed to be justified and represented the problem for this work.

METHODS

Animals used. Young rats, chiefly albinos, all males with an average wt. of 200 g. normally fed on ordinary stock laboratory diet, were used for all experiments with surviving tissue slices and chopped organs. Cats were used for the perfusion experiments. Tissue extracts were prepared from organs of pig.

Method of perfusion. Two cats were used for each experiment, and ether was used as anaesthetic. One cat served as blood donator; from it about 140 ml. blood were drawn to which about 80 ml. blood of the second cat were added later. The blood was defibrinated, filtered and made up to 400 ml. with bicarbonate-Ringer solution. In the meantime a cannula was inserted into the hepatic portal vein of the liver of the second cat. When the kidney was perfused the cannula was inserted into the renal artery. The gall bladder was ligated in the case of liver perfusion. The respective organs were then removed from the animals, the blood of the second animal was treated in the same way as that of the first and added to the blood-Ringer solution. The Dale & Schuster apparatus was used for the perfusion with a flask oxygenator. Care was taken that the oxygenation was efficient. The blood colour changed from dark red after it had passed the organ to bright red after oxygenation. The speed of perfusion was such that the whole volume of the perfusion fluid circulated in the system within 7 min. Samples of the organs were clipped off at the beginning and at the end of the experiment and the cut surface was seared with a hot iron. The temp. was kept at 37° and it was observed that the perfusion fluid was evenly distributed throughout the organ perfused. The perfusion fluid circulated through the organ for 20 min. in absence of substrate. After that time glycine solution was added. Samples were taken at suitable intervals, the volume of each being recorded in order to control the volume of the remaining fluid. The duration of the perfusion varied in the different experiments from 90 to 150 min. At the end of the experiment the remaining perfusion fluid was emptied from the apparatus. The samples were immediately deproteinized by means of trichloroacetic acid, and NH_3 - and NH_2 -N estimations were carried out at once. For the isolation of glycine, $\text{HgCl}_2 + \text{HCl}$ was used as deproteinizing agent; the procedure of isolation is described below.

Experiments on surviving tissue slices and extracts. Technique of slicing was the same as described previously [Bach & Holmes, 1937]. The wt. of tissue slices was 100 mg. wet wt. in all exp. Slices could be conveniently cut from kidney, liver and brain. It was more difficult in the case of spleen, but it was possible to obtain sufficiently thin slices from this organ after some practice. The spleen slices then showed satisfactory respiration. The blood attached to the spleen

slices was not removed. Portions of the thinner parts of the diaphragm were used. Testis material was used without further treatment. The mucous membrane of the small intestine was used after washing the latter with water, cutting it lengthwise and scraping it off the intestinal wall. For the experiments with frog muscle an extract was prepared in the usual way. Heart tissue was cut in slices with scissors as it was not possible to slice it in the ordinary way with the razor. The tissue was in all experiments suspended in 3 ml. phosphate buffer pH 7.2, this volume including any added substrates.

Kidney extract was made from acetone powder [Keilin & Hartree, 1936] by suspending it in buffer solution, shaking it for 10 min. at room temperature followed by centrifuging. 2 ml. supernatant fluid, corresponding to 100 mg. acetone powder, were used for the experiment, 1 ml. buffer or substrate being added.

Barcroft cups and manometers were used. In the aerobic experiments the cups were evacuated and filled with O_2 . In the anaerobic experiments the cups were 3 times evacuated and filled with N_2 passed over hot Cu filings. The exp. temp. was 38° .

The substrates after adjustment to pH 7.2 were in most cases added in dangling Keilin cups which were shaken off after equilibrium had been obtained. In the centre pot of the Barcroft cups was placed filter paper saturated with 0.3 ml. of 10 % KOH.

At the end of the experiment 2 ml. were drawn from the cups and made up to 10 ml. Aliquot portions of this fluid were used for NH_3 and urea estimations. Other portions used for estimations of total, amino-, amide- and purine-N, creatine and pyruvic acid, were deproteinized either with trichloroacetic acid (final concentration 6 %) or, in the later experiments with dialysed $Fe(OH)_3$ which gave more consistent results owing possibly to the avoidance of decomposition of the glycine condensation products.

Estimation of NH_3 and urea. To the portion reserved for the NH_3 estimation 3 drops 3 % acetic acid were added, and NH_3 estimated after distillation [Parnas & Heller, 1924], collection in 0.01 N HCl and titration with 0.01 N NaOH. The portion of the fluid reserved for the urea estimation was incubated for 30 min. with urease at 60° followed by NH_3 estimation.

Estimation of NH_2-N . The deproteinized fluid was centrifuged, carefully neutralized and made up to 10 ml. To a suitable amount about 10 drops of $Mg(OH)_2$ suspension were added, the mixture placed on the boiling water bath for 25 min., the fluid being then acidified with 2 drops of glacial acetic acid. The estimation of NH_2-N was made with the Van Slyke apparatus.

Estimation of amide-N. To an aliquot portion of the deproteinized fluid H_2SO_4 was added to a final concentration of 5 %. It was placed on a boiling water bath for 30 min. after which it was neutralized and the NH_3 estimated.

Estimation of total N. Total N was estimated in a Kjeldahl apparatus, conc. NH_3 -free H_2SO_4 with added Se and Cu salts being used for the incineration. The titration was carried out with 0.01 N HCl.

Estimation of purine-N. To 2 ml. deproteinized fluid 2 ml. 40 % $NaHSO_3$ and 2 ml. 10 % $CuSO_4$ were added. The reddish precipitate turned brown after 3 min. boiling. The fluid was centrifuged, the precipitate washed with 1 % acetic acid and centrifuged again followed by total N estimation on the precipitate [Krüger & Schmidt, 1905; Schmidt, 1933].

Estimation of creatine. A modification of Walpole's direct method [Walpole, 1911] of estimating creatine was used, diacetyl serving as reagent; it was prepared by distillation of dimethylglyoxime with H_2SO_4 [Walpole, 1911]. To 5 ml. experimental solution 0.5 ml. sat. Na_2CO_3 and 2 drops diacetyl were added.

The mixture was immediately placed in a boiling water bath for exactly 1 min. and then in cold water for 5 min. After a further period of 20 min. during which the samples were left standing at room temperature the red colour was measured as described below. Standard solutions with known amounts of creatine and controls with water were prepared in the same way. Since the control samples with water yielded a yellowish tint with the reagents alone, methods had to be developed to eliminate this interfering colour. Neither a colorimeter nor a tintometer served the purpose. In the later experiments the readings were carried out by means of a spectrophotometer. The light passing through the coloured solution of the water control was compared at different wave-lengths with that

Fig. 1.

Fig. 2.

Fig. 1. Photometer readings. ■—■ With 100 g. creatine solution.
○—○ With the water control.

Fig. 2. Standard curve for creatine estimation. For definition of unit see text.

transmitted by known amounts of creatine. It will be seen from Fig. 1 that the two curves are almost contiguous down to 630 mμ (absorption = b arbitr. units), below which they show a divergence reaching a maximum value at approximately 530 mμ (absorption = a arbitr. units). The final standardizing curve was plotted by estimating a and b both for the water control and for mixtures containing different known amounts of creatine (a' and b'). A straight line was obtained when values of creatine in arbitrary absorption units from the equation: $(a' - b')_{\text{creatine}}$ minus $(a - b)_{\text{control}}$ were plotted against μg. creatine (Fig. 2). The influence of glycine on the colour developed during the creatine estimations was found to be negligible. That of glycoeyamine was allowed for by adding this substance to the water control.

Estimation of pyruvic acid. The estimation of pyruvic acid was carried out on the deproteinized fluid according to the method of Clift & Cook [1932]. Special care was taken for reasons described that the excess NaHSO_3 was titrated with I_2 immediately in order to avoid possible decomposition of the product of condensation of glycine with pyruvate (see below).

Reagents. I have to thank Dr D. E. Green for a gift of cozymase solution and Mr E. M. Crook for that of coenzyme II. The other reagents were obtained from British Drug Houses, Ltd.

EXPERIMENTAL

In vitro experiments were first made on the deamination of glycine and *dl*-alanine under conditions in which many amino-acids besides alanine are readily deaminated. In all the cases in which $\text{NH}_3\text{-N}$ was estimated, urea-N was determined at the same time, the total of both being given as " $\text{NH}_3\text{-N}$ ". The results from Table I show clearly that while alanine readily gives rise to NH_3 production, no such formation of NH_3 takes place under the same experimental conditions with glycine.

Table I. NH_3 formation from glycine and *dl*-alanine in extracts of pig kidney

Experimental period: 2 hr.					
Kidney powder used for extract mg.	Amino-acid-N added mg.	mg. NH ₃ -N			Extra NH ₃ -N formation as % of added NH ₂ -N
		Without added substrate	With added amino-acid	Extra NH ₃ -N formation	
Glycine					
100	0.56	0.14	0.16	0.02	0.4
200	1.12	0.21	0.12	-0.09	-8
100	1.12	0.05	0.05	0	0
100	1.12	0.08	0	-0.08	-7
50	0.56	0.07	0.04	-0.03	-5
250	1.12	0.14	0.14	0	0
*	1.12	0.08	0.08	0	0
Alanine					
100	0.47	0.14	0.294	0.154	65†
200	0.94	0.21	0.39	0.18	38†
100	0.94	0.05	0.35	0.30	64†
100	0.94	0.08	0.35	0.27	57†
50	0.47	0.07	0.24	0.19	81†
250	0.94	0.14	0.42	0.28	60†
*	0.94	0.08	0.40	0.32	68†
Average for alanine					62

* 1 g. rat kidney was ground with sand and buffer and centrifuged, the supernatant fluid being taken for the experiment.

† % disappearance is calculated from the amount of added *d*-alanine (i.e. $\frac{1}{2}$ *dl*-alanine).

The same difference in behaviour between the two amino-acids was observed regarding the O_2 consumption. Fig. 3 gives the course of the respiration of kidney extract in presence of glycine + *dl*-alanine. It will be seen that while with alanine O_2 consumption reached the theoretical value calculated on the basis of oxidative deamination of the *d*-isomeride, glycine does not increase the O_2 consumption of the tissue at all. It is also shown that an addition of glycine + alanine does not affect the respiration as compared with that of alanine alone in tissue extracts. This phenomenon is equally shown by comparison of columns 4 and 6 in Table II in which the results of further respiration experiments are recorded. A comparison of columns 3 and 5 reveals a slight inhibition of O_2 consumption in presence of glycine alone.

Table III shows the effect of a mixture of glycine and alanine when incubated with various tissues. The object of these experiments was to investigate whether a "Stickland effect" such as observed in bacteria [Stickland, 1934; Woods, 1936] would affect NH_3 production. Results from Table III show that this is not the case. There is very little or no difference between the formation of NH_3 , the disappearance of $\text{NH}_2\text{-N}$ and the O_2 consumption in presence of alanine alone and those in presence of alanine plus glycine.

Fig. 3. Respiration of pig kidney extract with added *dl*-alanine and glycine.Table II. *Respiration of kidney extract in presence of glycine and dl-alanine*

Duration of exp. (min.)	Amino-acid conc. (%)	μl. O ₂ consumption			
		Without added substrate	With alanine	With glycine	With glycine and alanine
60	0.1	25	169	15	174
60	0.2	87	322	23	—
60	0.2	—	322	10	322
90	0.1	31	185	21	192
90	0.2	93	387	28	—
90	0.2	—	358	19	355

Table III. *Experiments with various tissues in presence of a mixture of glycine and dl-alanine*

Duration of exp. min.	Tissue	Substrate	Amino-acid-N added mg.	With added substrate		
				Extra NH ₃ -N formed mg.	Extra O ₂ consumption μl.	% of NH ₃ -N disappeared mg.
60	Kidney extract	Glycine	0.56	-0.03	10	—
60		<i>dl</i> -Alanine	0.47	0.26	144	—
60		<i>dl</i> -Alanine + glycine	1.03	0.33	149	—
100	Kidney extract	Glycine	1.12	—	21	0
100		<i>dl</i> -Alanine	0.94	—	399	23
100		<i>dl</i> -Alanine + glycine	2.06	—	377	31
60	Kidney extract	Glycine	1.12	0.06	10	—
60		<i>dl</i> -Alanine	0.94	0.18	322	—
60		<i>dl</i> -Alanine + glycine	2.06	0.20	322	—
65	Kidney extract	Glycine	1.12	0	35	—
65	and spleen	<i>dl</i> -Alanine	0.47	0.13	102	—
65	slices	<i>dl</i> -Alanine + glycine	1.59	0.14	97	—

For further evidence determinations of NH₂-N were carried out, the results of which, shown in Table IV, seem to confirm the previous findings. While in the case of the alanine experiments an average of 82 % of the NH₂-N (calculated on

Table IV. *Disappearance of NH₂-N from glycine and dl-alanine in kidney extract*

Experimental period: 2 hr.

NH ₂ -N added mg.	mg. NH ₂ -N			Disappearance of added NH ₂ -N	Disappearance of NH ₂ -N in % of NH ₂ -N added
	Without substrate	With amino- acid added	Increase with amino-acid		
	Glycine				
1.12	0.47	1.50	1.03	0.09	8
1.12	0.47	1.82	1.35	-0.23	0
1.12	0.80	1.83	1.03	0.09	8
dl-Alanine					
0.94	0.26	0.69	0.43	0.51	108*
0.47	0.26	0.50	0.24	0.23	100*
0.94	0.80	1.24	0.44	0.50	100*
0.94	0.47	1.14	0.67	0.27	58*
0.94	0.47	1.20	0.73	0.21	45*
Average for alanine					82*

* Disappearance in % of added *d*-alanine-N (i.e. $\frac{1}{2}$ *dl*-alanine).

the basis of the *d*-isomer) disappeared, practically all the glycine NH₂-N was present in most of the cases at the end of the experimental period. Another phenomenon observed was a discrepancy between the average disappearance of alanine-N of 82 % and an NH₃-N formation of 62 % (according to Table I). (Both series of determinations were carried out in the same experiment in order to obtain comparable values.) This phenomenon has previously been observed by Krebs [1933] and also by Neber [1936].

Table V. *NH₃ formation and O₂ consumption in tissue slices of various organs in presence of glycine*

Tissue wet wt. 100 mg. Duration 2 hr. Amount of glycine added 1.12 mg. glycine-N, except in exp. * in which it was 0.96 mg. Glycine conc.: 0.2 % in all experiments.

Tissue	Extra NH ₃ -N with added glycine mg.	Glycine-N disappeared mg.	Extra O ₂ con- sumption with added glycine, μl. after 60 min.
Rat kidney	0.04	—	- 40
Rat heart	0.03	—	—
Rat heart	0	0.05	- 9
Rat spleen	0.05	—	- 16
Rat spleen	-0.03	—	- 12
Rat spleen	0.01	—	—
Rat intestine	0.01	—	—
Frog muscle	-0.04	—	0
Rat kidney	0.03	-0.10	- 12
Rat heart	0.07	0.11	—
Rat kidney*	—	0.16	—
Rat kidney* 3.7 mg. glutathione	—	0.09	—
Rat kidney pH 7.8	—	0.07	—
Rat kidney pH 6.8	—	0.09	—
Rat testis	—	0	—
Rat brain	—	0.15	—
Rat spleen	—	0.12	—
Rat kidney	—	—	- 61
Rat kidney	—	—	- 5
Pig kidney	—	—	- 5
Rat liver	—	—	14
Rat diaphragm	—	—	6

In the experiments recorded in Table V tissue extracts were replaced by tissue slices of various organs. None of the tissues used produced any appreciable amount of NH_3 or urea from glycine and none or little $\text{NH}_2\text{-N}$ disappeared. In fact the respiration experiments revealed a slight but distinct inhibition in presence of glycine in almost all cases.

From the results obtained so far it seemed obvious that the deamination of glycine cannot be demonstrated by incubating it *in vitro* with either tissue extract or tissue slices under conditions such that with other amino-acids NH_3 or urea is readily formed and O_2 is taken up. Therefore perfusion experiments with added glycine on the intact organ were carried out as recorded in Tables VI and VII. The technique has been described earlier. $\text{NH}_3\text{-}$ and $\text{NH}_2\text{-N}$ estimations

Table VI. *Perfusion of cat liver*

Quantities: mg. N/100 ml. perfusion fluid or per g. liver.

Time course of perfusion I min.	NH ₃ - and urea-N						Amount of glycine- N added (mg.)
	In perfusion fluid			In perfused liver			
	NH ₃ - plus urea-N			NH ₃ - plus urea-N			
	NH ₃ -N	Urea-N	urea-N	NH ₃ -N	Urea-N	urea-N	
	Without added glycine						
0	0.4	4.6	5.0	0.18	0	0.18	0
20	0.4	4.1	4.5	0.18	0	0.18	0
	With added glycine						
25	0.28	5.57	5.85	—	—	—	55
80	0.63	8.17	8.80	—	—	—	55
110	1.12	9.28	10.40	0.12	0.35	0.47	55
Final result perfusion I	+0.72	+5.18	+5.90	-0.06	+0.35	+0.29	55
Final result perfusion II	+3.93	+1.33	+5.36	0	-0.38	-0.38	96.5
Final result perfusion III	-0.14	+3.68	+3.54	-0.03	-0.18	-0.21	33.6

Table VII. *Perfusion of cat kidney*

Quantities: mg. N/100 ml. perfusion fluid or per g. kidney.

Duration of perfusion min.	NH ₃ and urea-N ₂				NH ₂ -N in perfusion fluid
	In perfusion fluid			In perfused kidney	
	NH ₃ -plus urea-N			NH ₃ -plus urea-N	
	NH ₃ -N	Urea-N	urea-N	urea-N	
	Without added glycine				
0	0.28	7.00	7.28	0.71	10.5
15	0.28	—	—	—	7.35
	With added glycine				
75	0.70	—	—	—	—
105	0.28	5.60	5.88	0.77	43.7
Final result	0	-1.40	-1.40	+0.06	36.35
	Amount of glycine-N added				34.3

were performed both on the perfusion fluid and the organs used. Glycine was isolated from the former as the β -naphthalene-sulphonic compound.

In Table VI the course of a liver perfusion (I) has been briefly outlined whereas from liver perfusions (II) and (III) only the final balance is given for

simplicity's sake. Table VII shows the course of kidney perfusion and also includes $\text{NH}_2\text{-N}$ determinations.

From the final results of Tables VI and VII it is evident that the NH_3 and urea formation observed during the perfusion is insignificant, representing only 5–10 % of added glycine-N. The results of the perfusion without added substrate revealed no significant changes during the initial period. The changes in the N content of the perfused organs were negligible. The $\text{NH}_2\text{-N}$ content in the final perfusion fluid corresponded approximately to the amount of glycine-N added at the beginning of the experiment (34.3 % added as glycine-N and 36.3 % found as $\text{NH}_2\text{-N}$ at the end of the experimental period). The method of $\text{NH}_2\text{-N}$ determination was checked by adding a known amount of glycine to a quantity of perfusion fluid, the $\text{NH}_2\text{-N}$ of which had been estimated while in circulation. The estimated $\text{NH}_2\text{-N}$ values were found to agree satisfactorily with the calculated amounts, the average experimental error being 6 %. By the isolation of glycine from the perfusion fluid it was ascertained that the $\text{NH}_2\text{-N}$ found at the end of the experiment was in fact glycine-N. This was done by adding to 250 ml. of the fluid, 100 ml. 2 N HCl and 100 ml. 5 % HgCl_2 , filtering the precipitate, removing the Hg by H_2S , neutralizing and finally concentrating *in vacuo* to c. 20 ml. A small precipitate filtered off was disregarded. To the clear filtrate an ethereal solution of 3 g. β -naphthalenesulphochloride was added and the mixture was shaken for 6 hr. during which 24 ml. of N NaOH were gradually added. The mixture was again filtered and acidified with 2 N HCl, the precipitate filtered and recrystallized from water. The yield varied in the different experiments from 38 to 62 % of the amount expected in accordance with the $\text{NH}_2\text{-N}$ determinations. The substance was β -naphthalenesulphoglycine. (Found: C, 54.08; H, 4.39; N, 5.53; S, 11.9. Calc. for $\text{C}_{12}\text{H}_{11}\text{O}_4\text{NS}$: C, 54.3; H, 4.16; N, 5.28; S, 12.07.) No other NH_2 compound was found to be present in the perfusion fluid.

Further experiments (not recorded) were made to investigate the effect of fixatives on the deamination of glycine. In liver slices no NH_3 formation or disappearance of $\text{NH}_2\text{-N}$ occurred, however, with glycine in presence of fixatives. Ornithine was added as fixative for NH_3 and NaHSO_3 for glyoxylic acid, possibly formed. The presence of glucose also had no effect when added with glycine to kidney slices.

Further, the possibility of a collaboration of more than one organ in the metabolism of glycine was considered; since as mentioned earlier glycine administered *per os* appears to be readily attacked. The example of the formation of uric acid by collaboration of liver and kidney, as shown by Schuler & Reindel [1933], inspired these investigations. Mixtures of kidney and liver tissues and of kidney and spleen tissues were chosen.

In the experiments in which glycine was added to such mixtures (Table VIII) it will be seen, in the case of kidney slices plus spleen slices, that while no NH_3 or urea was formed, $\text{NH}_2\text{-N}$ disappeared in some cases to an extent of approximately 70–95 % of the $\text{NH}_2\text{-N}$ added. No disappearance of $\text{NH}_2\text{-N}$ however was observed in the case of the mixture of kidney and liver slices, irrespective of the addition of substrates such as alloxan or ketoglutaric acid. (Alloxan is known to catalyse the oxidation of amino-acids *in vitro* [Strecker, 1862] and α -ketoglutaric acid has been reported to serve as an "acceptor" for amino groups [Braunstein & Kritzman, 1937, 1].) Kidney slices and kidney extract produced equal effects when mixed with the spleen slices.

Estimations of total N were carried out on the deproteinized filtrates of the experimental fluid. An example is given in Table VIII. No change of total N was observed, hence the $\text{NH}_2\text{-N}$ disappearing was not used for synthesis of proteins.

Table VIII. *N* changes and O_2 consumption in mixtures of tissue slices in presence of glycine

Duration of exp.: 2 hr. Amount of glycine-N added: 1.12 mg. except in exp. † where 0.96 mg. was used. Rats were used for all tissue slices. Concentration of kidney extract: 50 mg. dried powder from pig kidney.

Mixture of tissue used	Extra NH_3 -N with added glycine mg.	mg. NH_2 -N				Extra O_2 consumption with added alanine, μ l. after 60 min.	% of glycine-N disappeared
		Without glycine	With glycine	Difference	Disappeared		
Kidney slices† + Liver slices	—	0.20	1.07	0.87	0.09	—	9
Kidney slices† + Liver slices + Alloxan <i>M</i> /100	-0.02	0.17	1.14	0.97	0	- 2	0
Kidney slices + Liver slices + Ketoglutaric-acid <i>M</i> /40	-0.01	0.17	1.22	1.05	0.07	-38	7
Kidney slices + Spleen slices	0.04	0.38	0.63	0.25	0.87	49	78
Kidney slices + Spleen slices	0.02	—	—	—	—	—	—
Kidney slices + Spleen slices	0.03	0.34	0.61	0.27	0.84	—	75
Kidney extract + Spleen slices	0.10	—	—	—	—	-12	—
Kidney extract + Spleen slices*	0	0.73	0.80	0.07	1.05	29	94
Kidney extract + Spleen slices	0.01	0.48	0.81	0.33	0.79	—	70
mg. Total N							
		Without glycine	With glycine	Difference	Disapp.		
Estimation of total N in exp. *		0.06	1.20	1.14	0		

The disappearance of NH_2 -N in presence of mixed kidney and spleen tissue, not occurring in presence of either of the tissues alone, may be interpreted either as an enzymic effect or an effect of non-enzymic substances present in the tissue mixture. In order to answer this question, further experiments with *Kochsaft* of various tissues were carried out. The results are shown in Table IX. Kidney *Kochsaft* added to kidney slices caused a disappearance of approximately 25 % of the NH_2 -N. Spleen *Kochsaft* alone had about the same effect, which was hardly increased by addition of kidney *Kochsaft*, but which appeared to be a little intensified in presence of kidney slices. Heart *Kochsaft* caused no effect. A comparison of the results of Table VIII with those of Table IX shows that the effect of *Kochsaft* alone or of spleen *Kochsaft* plus kidney slices on the disappearance of NH_2 -N was considerably smaller than that of kidney slices plus spleen slices. O_2 consumption was slightly inhibited with added glycine, as in previous experiments, and extra NH_3 -N formed in presence of glycine was insignificant.

The partial disappearance of NH_2 -N under the experimental conditions described, which was not accompanied by a corresponding NH_3 or urea formation led to an investigation of other products of decomposition of glycine. The possibility of the formation of amides and creatine was considered next.

Table IX. *Experiments with kidney slices and kidney Kochsaft in presence of glycine*

Tissue	Substrate other than glycine	mg. $\text{NH}_4\text{-N}$					Glycine-N added mg.	Other observations
		Without glycine	With glycine	Difference	Disapp. of $\text{NH}_4\text{-N}$	Disapp. of $\text{NH}_4\text{-N}$ in % of added $\text{NH}_4\text{-N}$		
Kidney slices	Kidney Kochsaft	0.22	1.10	0.88	0.27	23	1.15	Extra O_2 with glycine = -46
Kidney slices	Kidney Kochsaft	0.19	1.02	0.83	0.32	28	1.15	Extra O_2 with glycine = -13
Kidney slices	Kidney Kochsaft	0.14	0.96	0.82	0.33	29	1.15	Extra NH_3 0.15 mg.
Kidney slices	Kidney Kochsaft*	0.32	1.18	0.86	0.29	25	1.15	Extra NH_3 0.09 mg.
Kidney slices	Heart Kochsaft	0.16	1.27	1.11	0	0.06	1.17	—
Kidney slices	—	0.01	1.23	1.22	0	0	1.12	—
Kidney slices	Kidney Kochsaft	0.16	1.00	0.84	0.28	25	1.12	Creatine test (Tintometer reading) 60% increase with glycine
Kidney slices	Spleen Kochsaft	0.27	1.04	0.77	0.35	31	1.12	—
Kidney Kochsaft	Spleen Kochsaft	0.28	1.14	0.86	0.26	23	1.12	—
—	Spleen Kochsaft	0.14	1.02	0.88	0.24	21	1.12	—

Note. The Kochsaft was prepared by boiling 1 g. rat kidney for 7 min. on the water bath and centrifuging. 0.3 ml. of the supernatant fluid was used for all exp. except for exp. * where 0.6 ml. was used.

The formation of amide-N from glutamic acid has been recorded by Krebs [1935, 2]. No appreciable amount of amide-N however was found such as to justify the suggestion that the glycine-N disappeared had been retained as amide-N.

The formation of creatine from glycine has often been claimed, since numerous investigators have proved that patients suffering from the muscular dystrophies excrete less creatine, and since Brand *et al.* [1929; 1931] had found in these cases that following the injection of glycine creatinuria was increased. The same effect was observed after the administration of glycoxyamine [Bodansky, 1936]. This has been isolated from the urine of dystrophic patients following administration of glycine [Weber, 1935]. It is generally assumed that glycoxyamine acts as a precursor to creatine, the former being methylated by glycine to form the latter. Evidence for the conversion of glycoxyamine into creatine in the body has been given by early workers [Jaffe, 1906; Thompson, 1917]. The methylation was assumed to take place in muscles and in kidney [Bodansky, 1936], although Shibuya [1937] failed to show such an effect in *in vitro* experiments with liver slices, and obtained doubtful results when perfusing liver of rabbits. Finally Davenport *et al.* [1938] supplied further evidence for this assumption.

Glycoxyamine was added to mixtures of kidney and liver slices, in order to see whether it was able to cause $\text{NH}_4\text{-N}$ disappearance as did Kochsaft. It will be seen from Table X that in a mixture of kidney and liver slices with added glycine, 46 % of the $\text{NH}_4\text{-N}$ disappeared in presence of glycoxyamine, whereas no appreciable disappearance of $\text{NH}_4\text{-N}$ occurred when glycine alone was added. It will also be observed that the individual tissues, i.e. liver alone and kidney alone, had no appreciable deaminating effect in presence of glycoxyamine. Thus glycoxyamine showed an effect similar to that of kidney or spleen Kochsaft. There is of course no evidence that glycoxyamine is one of the active constituents of spleen Kochsaft or kidney Kochsaft, although glycoxyamine has been isolated from the urine. The results of the following experiments on creatine formation, however, offer further support for the action of glycoxyamine.

In one of the experiments of Table IX creatine was determined approximately, and it was found to be present in a larger amount in presence of glycine than in its absence. More experiments of this type were made using chopped rat

Table X. *Disappearance of glycine-N in tissue slices in presence of glycoyamine*

Amount of glycine added: 1.12 mg. glycine-N, final concentration 0.2%. Amount of glycoyamine added: 1.5 mg., final concentration 0.05%.

Tissue	Substrate added other than glycine	mg. NH ₂ -N			Disappearance of NH ₂ -N	Disappearance of NH ₂ -N as % of added NH ₂ -N
		Without glycine	With glycine	Difference		
Kidney	Glycoyamine	0.1	1.12	1.02	0.1	9
Liver	Glycoyamine	0.1	1.03	0.93	0.19	17
Kidney + liver	Glycoyamine	0.2	0.81	0.61	0.51	46
Kidney + liver	—	0.2	1.07	0.87	0.09*	9
Kidney + spleen	Glycoyamine	0.35	1.12	0.77	0.35	31

* The amount of glycine-N added in this exp. was 0.96 mg.

Table XI. *Chopped heart of rat in presence of glycine and glycoyamine*

Concentration of glycoyamine: 0.1%. Amount of glycine added: 1.12 mg. glycine-N

Substrate added	NH ₂ -N mg.	Amide-N mg.	NH ₂ -N mg.	NH ₂ -N disapp. mg.	Creatine μ g.	O ₂ consumption
None	0.08	—	—	—	800	—
Glycine	0.26	—	—	—	740	154
Glycoyamine	0.19	—	—	—	770	135
Glycine + glycoyamine	0.22	—	—	—	1000	161
None	0.08	0.02	0.08	—	570	93
Glycine	0.15	0.03	0.09	0.11	570	84
Glycine + glycoyamine	0.19	0.08	0.92	0.28	710	98

heart and adding glycoyamine and glycine. The more reliable results of this series, as the technique for creatine estimation had to be improved in the course of this experimental work, are shown in Table XI. It will be seen that no extra creatine is formed with either glycine alone or glycoyamine alone, but small creatine rises were observed where glycine and glycoyamine had simultaneously been added. Furthermore, the disappearance of NH₂-N was observed to be greatest when both substrates had been added. It will be seen that in these experiments small but definite NH₃ formation has taken place. Again a small increase in O₂ consumption was observed with added glycine + glycoyamine.

These results may point to a possible *in vitro* methylation of glycoyamine by means of glycine, but the difficulties of estimating accurately small amounts of creatine prevent any definite conclusion in this respect.

Amongst substances possibly responsible for the disappearance of NH₂-N coenzymes had to be considered. The coenzyme responsible for the deamination of the unnatural amino-acids recently discovered by Warburg & Christian [1938] and Straub [1938] exists in sufficient quantities in most of the tissues and tissue extracts used. Hence an effect by adding it could not be expected.

Table XII shows experiments with cozymase and coenzyme II added to glycine under anaerobic conditions. Coenzyme II showed no effect. Cozymase usually caused a disappearance of NH₂-N of 40–65%, but sometimes much less.

Further experiments were made with pyruvic acid. Table XIII shows the results in kidney extract. They seem to be much more consistent than the experiments in Table XII and reveal that in presence of pyruvate the further

Table XII. *Effects of cozymase and coenzyme II on the disappearance of glycine-N in tissue slices under anaerobic conditions*

Duration of exp.: 2 hr. Amount of glycine added: 1.12 mg. glycine-N.

Tissue	Addition	With added glycine	
		NH ₂ -N disappearance mg.	NH ₂ -N disappearance as % of added NH ₂ -N
Kidney slices	Cozymase	0.74	66
Kidney slices	None	0	0
Kidney slices	Cozymase	0.48	43
Kidney slices	Cozymase	0.71	62.5
Kidney slices	Cozymase	0.12	10
Liver slices	Cozymase	0	0
Kidney slices	Cozymase	0.08	7
Kidney slices	Coenzyme II	0.14	12.5
Kidney slices	Coenzyme II	0.01	0
Liver slices	None	0	0

Table XIII. *Effect of pyruvate on the disappearance of glycine-N in kidney extract under anaerobic conditions*

Duration of exp.: 2 hr. Amount of glycine added: 1.12 mg. glycine-N (1.02 mg. in exp. *). Concentration of pyruvate: 1%.

Tissue extract	Substrates added	With added glycine	
		NH ₂ -N disappearance mg.	NH ₂ -N disappearance as % of added NH ₂ -N
Kidney extract	{ Cozymase + pyruvate	0.47	42
	{ Cozymase + pyruvate	0.39	35
	{ Pyruvate	0.41	36.5
	{ Pyruvate	0.40	35
	{ Pyruvate	0.61	40.5*
Boiled kidney extract	{ Pyruvate	0.21	21*
	{ Pyruvate	0.24	21
Water control (no tissue extract)	{ Pyruvate	0 (aerobic)	0 (aerobic)
	{ Pyruvate	0.20	18
	{ Pyruvate	0.16	15.5*

addition of cozymase is unnecessary for the disappearance of NH₂-N. 35–42 % glycine-N disappeared in presence of pyruvate and in presence or absence of cozymase. Boiled extract had a small effect compared with unboiled extract. Under aerobic conditions no disappearance of NH₂-N occurred when tissue extract was replaced by water, whereas, under anaerobic conditions, a small disappearance was observed. This suggested some catalytic effect of the tissue.

In some examples of large disappearance of NH₂-N, estimations of NH₃ and urea-N, amide-N, total N and purine-N were performed simultaneously. There was no appreciable change observed in any of the N fractions tested, except in NH₂-N (Table XIV). This meant that the disappearance of NH₂-N could not be accounted for by the formation of any of the above N compounds, and suggested that the glycine had not lost its N but had condensed with another substrate, using in this way its NH₂ group to form a nitrogenous condensation product.

In estimating the simultaneous disappearance of pyruvic acid with the disappearance of NH₂-N the decomposition of a possible condensation product by the

Table XIV. *Simultaneous determination of nitrogenous substances in exp. with added glycine and pyruvate under anaerobic conditions*

	mg. N with added glycine		
	Initial	Final	Change
NH ₃ + urea-N	0.08	0.08	0
	0.08	0.11	0.03
NH ₂ -N	1.29	0.54	-0.75
	1.38	0.99	-0.39
Amide-N*	0.10	0.10	0
Purine-N	0.38	0.38	0
Total N	1.96	1.90	-0.06
Pyruvate*	1.77	1.68	-0.09
	2.90	1.95	-0.95
	3.84	2.77	-1.07

* In these exps. kidney slices were used.

addition of NaHSO₃, used for the estimation of pyruvic acid, presented a difficulty. When the estimation was performed quickly, however, a disappearance of pyruvic acid was observed with added glycine (Table XIV).

It will be recalled that in almost every experiment recorded the O₂ consumption of tissue slices and extracts was slightly inhibited in presence of glycine. As a result of the experiments described with added *Kochsaff*, glycocyamine, pyruvate etc. a theory of condensation of certain factors with glycine has been advanced. Now, most of the substances mentioned are known to increase O₂ consumption when added to tissue in absence of glycine. It seems feasible, therefore, that if a condensation of glycine with such substances occurs, they may be prevented from increasing the O₂ consumption. From Fig. 4 it will be seen

Fig. 4. The effect of tissue diffusate on the inhibition of tissue respiration by glycine.

that the inhibition by glycine is intensified in presence of added tissue diffusate. The latter was prepared by shaking 100 mg. kidney slices in 3 ml. phosphate buffer solution for 2 hr. 1 ml. of this solution was added to another portion of kidney slices suspended in 2 ml. buffer solution.

DISCUSSION

The possibility of amino-transfer

When it was demonstrated in the first part of this work that glycine in absence of any further substrate is not metabolized in tissue extracts, tissue slices or in perfusion experiments with liver and kidney, because no NH_3 or urea was formed and no $\text{NH}_2\text{-N}$ disappeared, it must be remembered that similar observations have been made before with other amino-acids, which led to different conclusions. The first observations of Needham [1930], who found an increase in succinic, fumaric and malic acids, but no change in either $\text{NH}_2\text{-N}$, $\text{NH}_3\text{-N}$, or amide-N, when glutamic and aspartic acids were incubated with minced muscle, were followed more recently by the experiments of Braunstein & Kritzmann [1937, 1, 2] and on the amino-transfer of amino-acids [Knoop & Oesterlin, 1925]. The former workers observed the formation of glutamic acid from glycine in presence of α -ketoglutaric acid. No change in the $\text{NH}_2\text{-N}$ content can be expected under these conditions, i.e. the observations made by these workers are similar to those in the "negative" cases of this experimental work. But it may be argued that had amino-transfer taken place in those cases the result would have been the formation of another amino-acid from a suitable keto compound present, and deamination of this newly formed amino-acid would probably have been the necessary consequence. Thus, kidney tissue containing strongly deaminating enzymes would have given rise to NH_3 . This was generally not observed in these "negative" cases, although an NH_3 formation of 8–10 % of the $\text{NH}_2\text{-N}$ added, and a corresponding $\text{NH}_2\text{-N}$ disappearance of about the same order, occurred in isolated cases. If these small changes in $\text{NH}_2\text{-N}$ can be considered as significant, the possibility remains that apart from the mechanism suggested by the later experiments, such a deamination either of glycine direct or of an amino-acid formed by the amino-transfer of glycine may take place to a small extent, explaining the findings of other workers who reported NH_3 formation from glycine of about the same order. These small amounts of NH_3 have in accordance with these other workers been observed under aerobic conditions only.

The astonishing stability of glycine towards organs, whether extracted, sliced or perfused, gives the impression that a more complicated mechanism than generally attributed to the process of deamination of amino-acids may exist, involving simultaneous contributions by various factors. It seems difficult, therefore, to localize the metabolism of glycine in a single organ without the concept of a contribution by compounds originating in other organs and introduced by the blood stream. This is probably why it was possible to show deamination of glycine administered *per os* or by injection, thus involving the whole metabolic system of the body. Attempts to show *in vitro* effects on glycine incubated with single organs were either frustrated or led to inconsistent results possibly depending on the accidental presence of preformed compounds necessary for the metabolic reaction. The latter could only consistently be observed when metabolites or enzymes of one organ were introduced into the enzymic system of another, thus setting up the necessary system for a metabolic attack on glycine.

The enzymic nature of the reaction

The experiments described may give some indication as to whether or not enzymes participate in the reactions observed. For the sake of the following

considerations, it may be permissible to divide the effects of the different biological materials on the disappearance of glycine-N into three groups:

- group— causing a disappearance of 0–10 %,
- group+ causing a disappearance of 10–30 %,
- group++ causing a disappearance of 30–80 %.

The following table summarizes the results of the experiments with different tissues:

1. Kidney slices and kidney extract	—
2. Liver slices and other slices alone	—
3. Kidney + liver slices	—
4. Spleen <i>Kochsaft</i> alone	+
5. Kidney slices + kidney <i>Kochsaft</i>	+
6. Kidney <i>Kochsaft</i> + spleen <i>Kochsaft</i>	+
7. Kidney slices or kidney extract + spleen slices	++
8. Kidney slices + spleen <i>Kochsaft</i>	+

Thus under the experimental conditions described neither kidney nor any other organ alone, nor kidney + liver, is capable without further addition of compounds to cause disappearance of glycine-N (see 1, 2 and 3). Kidney + spleen slices causes a strong effect (see 7). The reaction between glycine and compounds present in spleen *Kochsaft* can take place to a small extent in absence of enzymes (see 4 and 6). The reaction responsible for the disappearance of glycine-N is catalyzed by enzymes present in kidney slices + spleen slices (compare 7 with 4, 5, 6 and 8).

Since in the experiments described the only metabolic change of glycine observed consisted in a disappearance of $\text{NH}_2\text{-N}$, a condensation of glycine with a non-enzymic component of the tissue was suggested. Two possible enzymic actions of the tissue may be therefore considered: either a catalytic effect on the mechanism of condensation, or an enzymic action, perhaps involving cozymase, which may lead to the production of tissue components with which the glycine condenses.

Results of the anaerobic experiments, with cozymase (Table XII) again indicate enzymic action of the tissue, since no effect was observed with kidney slices alone, whereas a definite disappearance of $\text{NH}_2\text{-N}$ occurred in most experiments with kidney slices and added cozymase. Cozymase may therefore react either with the enzyme which catalyses the condensation, or cozymase may be concerned with the enzymic formation of suitable substrates for the condensation. The first possibility can be excluded by the results of the experiments with pyruvate (Table XIII), as cozymase + pyruvic acid and pyruvic acid alone exert identical effects. For the second question the nature of the non-enzymic components of the condensation has to be taken into consideration.

The nature of the non-enzymic components of the glycine condensation

(a) *Keto compounds.* These compounds play an essential part in the metabolism of kidney and liver, and condensation of amino-acids with aldehydes and keto bodies is well known to take place purely chemically [Schiff, 1901; Sørensen, 1908]. More recently a similar simple condensation has been described by Herbst [1936] between amino-acids and pyruvic acid. Akasi [1937] reports the isolation of octopine, i.e. a reduced Schiff's base, from muscle of Octopoda: this has recently been synthesized by Knoop & Martius [1938] by condensation of pyruvic acid with arginine.

Pyruvate was shown in fact to cause disappearance of $\text{NH}_2\text{-N}$, and here again, the failure to detect a production of NH_3 and other nitrogenous products of decomposition strongly suggested the formation of a product of condensation of glycine with pyruvate. This suggestion was supported by a disappearance of pyruvic acid simultaneously with the $\text{NH}_2\text{-N}$ as observed in the cases recorded. The lability of such a condensation product probably prevented the establishment of a clear stoichiometric comparison between the disappearance of $\text{NH}_2\text{-N}$ and pyruvate. Finally, the fact that the presence of cozymase was required for the reaction with kidney slices alone, but not when pyruvate was added, suggested a link with dehydrogenase systems known to be present in kidney, by means of which keto bodies may be formed. Thus pyruvic acid possibly produced by the lactic-pyruvate dehydrogenase system represents an example of such a reaction. Other keto-compound systems, such as hydroxybutyric-oxaloacetic or more likely the malic-oxaloacetic dehydrogenase, may be taken into consideration, the last being reported to be present in high concentration in kidney [Green, 1936]. Adler *et al.* [1938] consider that the fixation of NH_3 by keto-acids in animal cells takes place with the help of aspartic dehydrogenase, the activity of which is dependent on cozymase. Although no evidence of a direct link between such dehydrogenase systems and the disappearance of $\text{NH}_2\text{-N}$ has been produced in this work, it may be pictured that the equilibrium of such a system may be shifted towards the formation of keto compounds in presence of glycine as a fixative for such keto compounds. This problem will be a matter of further investigation.

(b) *Glycocyamine*. The underlying idea of these experiments was the general methylating capability of glycine as shown by a number of workers [Hoppe-Seyler, 1930; Challenger, 1936] and the special methylating action of glycine on glycocyamine to form creatine (*v. supra*). The mechanism of the methylation of glycocyamine by glycine may be pictured to take place as follows:

The condensation of the deaminated glycine with glycocyamine is presented above in a scheme slightly different from that set up by other workers. Glyoxylic acid was considered to be an easier condensing material than glycollic acid which has been postulated by other workers, to act as the methylating agent following the deamination of glycine. The former view is supported by findings of Milhorat & Toscani [1936] who obtained a greater rise in creatine, when glycine was administered to patients with muscular dystrophy than when glycollic acid was used. The scheme postulates as the first step a deamination of glycine. According to Table XI a small NH_3 production was observed when glycine or glycocyamine was added to heart tissue. Thus this was the only tissue tested in which glycine was shown to produce NH_3 on incubation. Furthermore, the O_2 consumption was definitely increased with glycocyamine + glycine whereas

it was decreased with either glycine or glycoeyamine alone. Although the changes mentioned are small, they may support the evidence for the formation of creatine *in vitro*.

General metabolic action of glycine

It was the object of these experiments to demonstrate the principle of condensation of glycine in place of its simple deamination. Many more possible condensations are conceivable, a number of which are known to take place, such as the formation of hippuric acid by condensation with benzoic acid, the condensation to carbamidoacetic acid [Hill, 1932], by condensation with alloxan, and possibly others. Owing to its instability the product of condensation with keto bodies cannot be regarded as an end-product in the metabolic path. The compound may either undergo tautomerization as suggested by Braunstein & Kritzmann [1937, 1, 2] and Knoop & Martius [1938] followed by splitting with the formation of a new amino-acid, thus completing amino-transfer. Or it may be reduced similarly to octopine and thus be stabilized. In this way glycine may act as fixative for keto compounds producing an equilibrium effect in systems responsible for the formation of such compounds.

The latter phenomenon may lead to a basis of the well-known "specific dynamic effect" of glycine by picturing its role as an acceptor of aldehydes and keto products thus catalysing the reaction within the enzymic systems concerned. By its condensing capacity it may act as a general reagent for the removal of compounds the metabolism of which would otherwise come to a standstill.

SUMMARY

1. Glycine, unlike alanine and other amino-acids, is little or not at all deaminated when incubated with slices of kidney, liver, spleen, diaphragm and brain of rat, or with extracts of kidney, testis and other organs. The NH_3 and urea formation and the disappearance of $\text{NH}_2\text{-N}$ does not exceed 8% of that corresponding to the amount of glycine added and is not increased by fixatives such as NaHSO_3 and ornithine. 65–100% of added *dl*-alanine (calculated on the basis of the unnatural isomeride) was deaminated under the same conditions. The deamination of alanine was not increased by the addition of glycine.

No NH_3 and urea formation was observed during the perfusion of liver and kidney of the cat with glycine. At the end of the experiment all the glycine was recovered as $\text{NH}_2\text{-N}$. Glycine was isolated from the perfusion fluid as the β -naphthalenesulpho-derivative.

2. When glycine was incubated with a mixture of kidney and spleen slices or kidney extract + spleen slices, a disappearance of 70–80% of the $\text{NH}_2\text{-N}$ was observed, without a corresponding formation of NH_3 or urea. The total N content of the deproteinized fluid was unchanged before and after the experiment. On the other hand a mixture of kidney and liver slices with or without added activators, for example alloxan, caused no such effect. From experiments using kidney slices with added *Kochsaft* of kidney or spleen a condensation of glycine with heat stable compounds under the influence of kidney and spleen enzymes is suggested.

3. O_2 consumption of tissue slices and extracts is slightly inhibited by glycine. This phenomenon is believed to be caused by its condensation with oxidizable compounds, thus protecting them from oxidation. Under the same

experimental conditions the oxidation of *dl*-alanine corresponds to the theoretical amount required for oxidative deamination of the unnatural isomeride.

4. Glycocyanine was shown to cause a disappearance of $\text{NH}_2\text{-N}$ similar to that produced by kidney and spleen *Kochsaft* when added to glycine in presence of mixtures of tissue slices.

5. A small creatine and NH_3 formation was demonstrated *in vitro* from glycine when incubated with heart tissue in presence of glycocyanine. A scheme is suggested for the condensation of glycine with glycocyanine to form creatine.

6. 40–60% of the $\text{NH}_2\text{-N}$ disappeared when glycine was incubated with kidney slices or kidney extract in presence of cozymase or pyruvate under anaerobic conditions. The effect, was more consistent with pyruvate, which partly disappeared simultaneously, and was not increased with further addition of cozymase. The role of the latter is believed to be concerned with the formation of keto compounds. No change was observed in these experiments in the content of $\text{NH}_3\text{-}$, amide-, purine- and total N before and after the experiment. Pyruvate, when incubated with glycine in presence of boiled kidney extract under anaerobic conditions, and even in absence of tissue, induced a small disappearance of $\text{NH}_2\text{-N}$, but none in the absence of tissue under aerobic conditions.

7. The possible metabolic pathways of glycine are discussed with special reference to amino-transfer. A general tendency of glycine to condense with glycocyanine, keto-bodies and other compounds is suggested. The influence of cozymase, possibly introduced into the system by *Kochsaft*, may point to a link between dehydrogenase systems concerned with the formation of keto-compounds (pyruvic acid, oxaloacetic acid etc.), and the above-described condensation of glycine with such compounds. Glycine may thus shift the equilibrium in such systems in the direction of increased oxidative production of keto-acids, a possibility that would explain its well-known specific dynamic effect.

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