THE NON-ENZYMATIC TRANSAMINATION AND DECARBOXYLATION OF AMINO ACIDS IN PRESENCE OF CELLULOSE

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Summary

The general characteristics of the transamination reaction between α-amino acids and α-keto acids occurring by the action of heat in presence of cellulose have been studied. Although the reaction is maximum at acidic pH, it proceeds even at alkaline pH. Monocarboxylic as well as dicarboxylic amino acids readily take part in the reaction. A mechanism has been proposed for the formation of decarboxylation products from glutamic and aspartic acids when they are heated in presence of keto acids. Cobalt and manganous ions were found to inhibit the reaction.

The mechanism of acid-catalysed model transamination reaction has been studied extensively by Herbst and co-workers[1–4] who suggested that the first steps in the reaction led to the formation of a Schiff base with a carboxyl group on each of the carbon atoms adjacent to the central nitrogen atom. It was concluded by these workers that the mechanism of this reaction involves the decarboxylation of an intermediate Schiff base-like compound, followed by a simultaneous shift of the double bond and addition of a proton from the medium to a carbonium ion.

Metzler and Snell[5] have reported another type of reversible non-enzymatic transamination reaction between pyridoxal and most amino acids in aqueous media at 100° C. Metzler *et al.*[6] have further shown that pyridoxamine and many amino acids undergo rapid transamination with glyoxylic acid at pH 5 and at 80°–100° C. to yield glycine.

Recently Nakada and Weinhouse[7] observed a rapid, non-enzymatic conversion of glyoxylate to glycine in presence of various amino acids. The reaction proceeded at room temperature and in the physiological pH range.

In a preliminary note, Giri and Kalyankar[8] briefly reported their observations that several amino acids in presence of α-ketoglutarate and pyruvate gave rise to glutamic acid and alanine respectively, when a mixture of the amino acid and keto acid was spotted on filter-paper and dried at temperatures higher than 80° C. Later studies[9] revealed that dicarboxylic amino acids like glutamic and aspartic acids underwent decarboxylation on filter-paper when heated in the presence of keto acids. A detailed investigation was therefore undertaken to furnish more precise information regarding the role of cellulose in the reaction, the effect of variables like temperature and pH of the reaction mixture and also the influence

of metal ions and moisture on the reaction. In the present communication are recorded the results of these studies. A tentative mechanism has also been proposed to explain the concomitant decarboxylation of amino acids.

Experimental

The amino acids used in the present investigation were commercial products (Nutritional Biochemicals Corporation, Ohio). They were found to be chromatographically pure and were used without further purification. α-Ketoglutarate was obtained from Hoffmann La Roche, Switzerland. Sodium pyruvate was prepared from freshly distilled pyruvic acid.

The reactants and products of the reaction were separated by the circular paper chromatographic technique.[10, 11] The amino acids were estimated by the method described by Giri *et al.*[12, 13] For identifying β-alanine and γ-amino butyric acid among the products, the method described by Crumpler and Dent[14] was found to be useful. The mixture to be analysed was spotted on the paper in the usual way. The filter-paper was then lightly dusted with finely powdered basic copper carbonate along the path of movement of amino acids. The chromatogram was then developed with phenol-water solvent and the colour developed with ninhydrin. Only amino acids which are not of the α-type survive this treatment with copper carbonate and can thus be identified comparatively easily.

Results

Several amino acids were tested for their ability to transaminate in presence of keto acids when heated on filter-paper at higher temperatures (Table I). As was reported earlier,[8] the reaction was unspecific as most of the amino acids acted as effective amino group donors, β-alanine, γ-aminobutyric acid and proline being the exceptions.

It was observed that asparagine and glutamine were quite active in undergoing transamination reactions under the conditions described above. The transfer of amino groups from these amides were direct without the intermediate formation of the corresponding acids, namely, aspartic and glutamic acids.

Cystine, when heated alone on filter-paper at temperatures above 90° C., gave a product which was identified as α-alanine by its relative positions in chromatograms developed with different solvents.

The reaction was more complex when methionine was used as the amino donor. When methionine was heated in the presence of keto acids at temperatures above 100° C. an unidentified band was observed which occupied the position of leucines on chromatograms developed with butanol-acetic acid-water solvent. Formation of methionine sulphoxide was also observed.

Effect of different temperatures

The influence of temperature on the transamination reaction with various amino acids, as amino group donors was investigated. The data showing the

TABLE I

Survey of Amino Acids taking part in Non-enzymatic Transamination with α-keto acids

The reaction mixtures consisted of 0·1 ml. of 0·1 M amino acid, 0·1 ml. of 0·1 M solution of sodium salt of the keto acid and 0·8 ml. of M/15 potassium phosphate solution (pH 5·2). In the case of cystine and tryptophan more dilute solutions were prepared. 0·2 ml. of cystine (5 mg./ml.) and tryptophan (4 mg./ml.) were taken. The volume in all cases were made up to 1 ml. with potassium phosphate solution.

20 µl. aliquots of the mixtures were spotted on the circumference of a circle (5 cm. dia.) drawn from the centre of a filter-paper circle (24 cm. diameter). After air-drying, the filter-paper was kept at 100° C. for 30 minutes. The chromatograms were then irrigated as usual with butanol-acetic acid-water (40 : 10 : 50).

Amino acid added	Glutamic acid formed from α-ketoglutarate by transamination γ	α-alanine formed from pyruvic acid γ
DL-Serine ..	175	75
DL-Valine ..	130	140
L-Histidine HCl ..	120	135
DL-Lysine HCl ..	97	52
DL-Alanine ..	80	..
DL-Threonine ..	..	65
L-Tryptophan ..	42	50
DL-Methionine ..	46	58
L-Cystine ..	82	125
L-Glutamine ..	126	85
L-Asparagine ..	132	80
L-Glutamic acid ..	..	120
DL-Aspartic acid ..	65	70
L-Arginine HCl ..	85	85
Glycine ..	Traces	55
L-Leucine ..	210	200
DL-Phenyl alanine ..	80	110
L-Tryosine ..	85	130

relationship of the extent of transamination and the temperature of the reaction are presented in Table II.

It was found that the amino acids were destroyed to some extent when heated alone on filter-paper at temperatures above 100° C. As the products of the

TABLE II

Influence of Temperature on the Non-Enzymatic Transamination Reaction

The reaction mixtures were the same as given in Table I. After 20 μl. aliquots of the mixtures were spotted, the filter-paper was kept at a particular temperature for 30 minutes. The chromatograms were then irrigated with butanol-acetic acid-water and subsequently the amino acids were estimated as usual.

Amino donor	Amino acid estimated in the total reaction mixture	Concentration of amino acid in the total reaction mixture				
		80° C.	90° C.	100° C.	110° C.	125° C.
Aspartic acid..	Aspartic acid (control)	1·30 mg.	1·05 mg.	1·05 mg.	0·90 mg.	0·40 mg.
	Aspartic acid when heated in presence of α-ketoglutarate ..	1·30 mg.	0·95 mg.	0·95 mg.	0·75 mg.	0·55 mg.
	Aspartic acid when heated in presence of pyruvate	1·15 mg.	0·80 mg.	0·75 mg.	0·70 mg.	0·50 mg.
	Glutamic acid formed from α-ketoglutarate	33 γ	65 γ	65 γ	130 γ	33 γ
	α-Alanine formed from pyruvate ..	20 γ	70 γ	70 γ	160 γ	140 γ
	β-Alanine formed by decarboxylation in presence of α-ketoglutarate	..	..	..	60 γ	50 γ
	β-Alanine in presence of pyruvate ..	70 γ	80 γ	160 γ	70 γ	95 γ
Glutamic acid	Glutamic acid (control) ..	1·40 mg.	1·00 mg.	0·80 mg.	0·40 mg.	0·12 mg.
	Glutamic acid when heated in presence of α-ketoglutarate ..	1·25 mg.	0·90 mg.	0·70 mg.	0·30 mg.	90 γ

TABLE II—(*Contd.*)

Amino donor	Amino acid estimated in the total reaction mixture	Concentration of amino acid in the total reaction mixture				
		80° C.	90° C.	100° C.	110° C.	125° C.
Glutamic acid	Glutamic acid when heated in presence of pyruvate	1·16 mg.	0·78 mg.	0·65 mg.	0·30 mg.	90 γ
	α-alanine formed from pyruvate ..	50 γ	80 γ	120 γ	85 γ	60 γ
	γ-aminobutyric acid formed by decarboxylation of glutamic acid in the presence of α-ketoglutarate ..	..	..	30 γ	33 γ	33 γ
	γ-aminobutyric acid formed by decarboxylation of glutamic acid in the presence of pyruvate ..	33 γ	70 γ	90 γ	170 γ	35 γ
Valine ..	Valine (control)	1·10 mg.	0·97 mg.	0·94 mg.	0·88 mg.	..
	Valine when heated in presence of α-ketoglutarate	0·76 mg.	0·63 mg.	0·53 mg.	0·52 mg.	..
	Valine when heated in presence of pyruvate	0·77 mg.	0·60 mg.	0·49 mg.	0·45 mg.	..
	Glutamic acid formed in presence of α-ketoglutarate	30 γ	75 γ	130 γ	110 γ	..
	α-alanine formed in presence of pyruvate	25 γ	45 γ	140 γ	150 γ	..

reaction are destroyed at higher temperatures, an increase in the rate of formation of the products with increasing temperatures was not detectable in all cases. The dicarboxylic amino acids were more susceptible to thermal destruction than the mono-carboxylic acids.

During the course of this work, it was observed that some of the amino acids underwent decarboxylation in the presence of keto acids when heated at about 90° C. under the conditions described above. For instance, glutamic acid when heated on filter-paper with α-ketoglutarate or pyruvate gave a product which was identified as γ-aminobutyric acid. The product was not immobilised on chromatograms dusted with basic copper carbonate and developed with phenol-water as solvent, which proves that it is not an α-amino acid. The chromatograms were sprayed with 1 : 2 naphthoquinone-4-sulfonate reagent as described by Giri and Nagabhushanam.[15] The new band which occupied the position of γ-aminobutyric acid was coloured pale green. For confirming the product as γ-aminobutyric acid, two-dimensional chromatograms were run with the solvent butanol-acetic acid-water (40 : 10 : 50) followed by pyridine water (80 : 20).

This type of decarboxylation of the amino acid in presence of keto acids was not observed by Herbst and coworkers in the case of the acid-catalysed transamination reactions studied by them.

In a similar manner, aspartic acid when heated with keto acids, gave β-alanine which was identified by its characteristic colour, its relative positions on chromatograms developed with different solvents (*e.g.*, phenol saturated with water, pyridine-water, etc.) and also by the fact that it was not immobilized on chromatograms dusted with basic copper carbonate.

Arginine in presence of pyruvate gave in addition to alanine an unidentified band which gave a positive Sakaguchi test showing that it contains an intact guanidino group.

In the case of other amino acids also, several unidentified bands were obtained, which may, in all probability be the decarboxylation products of the corresponding amino acids. The extent of decarboxylation was more when pyruvic acid was used instead of α-ketoglutarate.

The amounts of glutamic acid and aspartic acid formed by the non-enzymatic transamination reaction do not in all cases represent the extent to which the reaction has proceeded, for, glutamic acid and alanine themselves are degraded easily both in the presence and absence of keto-acids at temperatures above 90° C.

Effect of Time of Incubation on the Course of the Transamination Reaction

The effect of time of incubation was studied by heating the solutions after spotting on paper at 90° C., because at this temperature, the transamination reaction proceeds readily and other undesirable degradative changes of the reactants and other products are minimized. It can be seen from the results given in Table III that the amino acids when heated alone on filter-paper even at 90° C. for more than 30 minutes are destroyed to a considerable extent.

TABLE III

Effect of Time of Incubation on the course of the Transamination Reaction

The reaction mixtures were the same as used previously. The spots on the filter-paper were always air-dried before starting the experiment. Temperature of Reaction: 90° C.

Amino donor	Amino acid estimated in the total reaction mixture	Concentration of amino acid in the total reaction mixture				
		15 min.	30 min.	1 hr.	2 hrs.	3 hrs.
Glutamic acid	Glutamic acid (control)	1·15 mg.	1·00 mg.	0·83 mg.	0·46 mg.	0·45 mg.
	Glutamic acid when heated in presence of α-ketoglutarate ..	1·15 mg.	0·90 mg.	0·85 mg.	0·40 mg.	0·40 mg.
	Glutamic acid when heated in presence of pyruvate	1·00 mg.	0·78 mg.	0·78 mg.	0·40 mg.	0·25 mg.
	α-alanine formed from pyruvate ..	80 γ	80 γ	70 γ	130 γ	120 γ
	γ-amino butyric acid formed in presence of α-ketoglutarate ..	Traces	Traces	Traces	20 γ	40 γ
	γ-amino butyric acid formed in presence of pyruvate	65 γ	65 γ	70 γ	70 γ	60 γ
Aspartic acid ..	Aspartic acid (control)	1·15 mg.	1·10 mg.	0·95 mg.	0·95 mg.	0·85 mg.
	Aspartic acid when heated in presence of α-ketoglutarate ..	1·05 mg.	0·95 mg.	0·85 mg.	0·80 mg.	0·70 mg.
	Aspartic acid when heated in presence of pyruvate	0·85 mg.	0·80 mg.	0·70 mg.	0·60 mg.	0·60 mg.
	Glutamic acid formed from α-ketoglutarate	40 γ	65 γ	65 γ	65 γ	80 γ
	α-alanine formed from pyruvate ..	60 γ	70 γ	80 γ	120 γ	65 γ
	β-alanine formed by decarboxylation in presence of pyruvate	60 γ	80 γ	145 γ	115 γ	120 γ

Therefore, for further studies on the factors affecting the transamination reaction, the reaction was carried out at 95° and the time of heating was restricted to 30 minutes. In most cases there was a progressive increase in the concentration of the products upto a period of two hours.

The Effect of pH

The influence of pH and the presence of different buffers in the reaction mixture on the transamination reaction was tested. An examination of the results summarized in Table IV will reveal that the rate of transamination reaction is maximum at acidic pH and that the presence of phosphate definitely favours the reaction.

TABLE IV

Effect of pH on the Non-enzymatic Transamination between α-amino butyric acid and α-keto acids

The reaction mixture was compounded as follows : 0·1 ml. of M/10 α-amino butyric acid, 0·1 ml. of M/10 solution of the sodium salt of the keto acid and 0·8 ml. of the appropriate buffer. After 20 μl. aliquots were spotted on the filter-paper, it was dried in a current of air and subsequently heated at 90° C. for 30 minutes.

pH	Buffer used	Glutamic acid formed from α-ketoglutarate γ	α-Alanine formed from pyruvate γ
3·7	M/5 Acetate	110	98
4·6	M/5 Acetate	100	102
5·2	M/15 KH_2PO_4 solution ..	230	172
7·0	0·04 M Veronal—HCl ..	150	130
7·7	M/15 Phosphate Buffer ..	130	90
7·6	0·05 M Borax—HCl ..	102	70
8·8	0·04 M Veronal—HCl ..	55	49
9·3	M/5 KH_2PO_4—NaOH ..	65	52

In contrast to the reactions reported by Herbst and collaborators[1-4] the transamination reaction under the conditions specified above proceeds even at highly alkaline pH although the velocity of the reaction is somewhat reduced.

Role of Moisture in the Reaction

In a review article on transamination reactions, Herbst[2] has stated that addition of alkali in sufficient quantity to convert the keto acids into their salts effected

complete inhibition of the reaction. In confirmation of this observation, it was found by us that no transamination occurred when a mixture of amino acids and the sodium salt of pyruvic acid in aqueous solution (pH adjusted to 7·4) was boiled for as long a period as 2 hours. If, however, the same reaction mixture was spotted on a filter-paper and the spot dried at about 80° C. for 30 minutes, the formation of alanine could be proved by chromatographic procedure.[9] This led to the investigation of the effect of traces of moisture and also cellulose on the non-enzymatic transamination reaction (Table V).

It was observed during our preliminary experiments[9] that no transamination took place in presence of water and that the reaction proceeded in the dry state only in the presence of cellulose.

Further experiments were carried out to see whether the addition of traces of water on to the filter-paper itself will in any way help in accelerating the rate of reaction (Table V).

TABLE V

Effect of Moisture on the Progress of the Tansamination Reaction between iso-leucine and α-keto acids

The reaction mixture consisted of 0·1 ml. of M/10 solution of iso-leucine, 0·1 ml. of M/10 solution of the sodium salt of keto acid and 0·8 ml. of M/15 potassium (KH_2PO_4) solution. A mixture of 0·1 ml. isoleucine and 0·9 ml. KH_2PO_4 solution was kept as control.

20 μl. aliquots of the reaction mixture were spotted in triplicate on the circumference of a small circle drawn at the centre of a circular filter-paper. The paper was heated at 100° C. for 30 minutes. It was then removed and on spot Nos. 2 and 3, 20 μl. aliquots of water were spotted. After air-drying, the paper was again heated for 30 minutes at 100° C. The same process was repeated, but this time, 20μl. of water was spotted only on spot No. 3. The paper was heated again at 100° C. for 30 minutes. After cooling the chromatogram was developed with butanol-acetic acid-water and the products estimated.

The quantity of extra water added	Amino acid estimated	Concentration in the reaction mixture
Nil	Isoleucine (control) ..	1·18 mg.
	Glutamic acid formed from α-ketoglutarate ..	280 γ
	Alanine formed from pyruvate ..	240 γ
20 μl. water spotted	Isoleucine (control)	1·13 mg.
	Glutamic acid formed	300 γ
	Alanine formed	280 γ
20 μl. water spotted twice after 30 minutes interval	Isoleucine (control)	1·08 mg.
	Glutamic acid formed	320 γ
	Alanine formed	285 γ

The results show that the addition of water does have some effect in accelerating the rate of reaction although the reactants are destroyed to a certain extent.

The Effect of Area of the Spot on the Reaction

The results of the previous experiments suggested that the catalytic effect of cellulose might be due to the fact that the reactants are adsorbed by cellulose fibres providing a larger surface area for the reaction to proceed. It was, therefore, thought desirable to determine the effect of area of the spot on the transamination reaction.

TABLE VI

Effect of the Size of the Spot on the Non-enzymatic Transamination Reaction

The reaction mixture contained 0·1 ml. of M/10 α-aminobutyric acid, 0·1 ml. of M/10 sodium pyruvate and 0·8 ml. of M/15 potassium phosphate solution (pH 5·2). 20 μl. aliquots of the reaction mixture were spotted along the circumference of a small circle drawn on a circular filter-paper (25 cm. diam.). The spots were made to spread and occupy different areas by addition of different quantities of water on to the filter-paper before spotting the reaction mixture. After air-drying, the filter-paper was heated for 30 minutes at 95° C. Suitable controls were also run without the addition of pyruvate.

Diameter of the spot	Amino acid estimated	Concentration in the reaction mixture
1·4 cm.	α-aminobutyric acid (control)	0·96 mg.
	α-aminobutyric acid when heated in presence of pyruvate	0·63 mg.
	α-alanine formed from pyruvate	172 γ
1·7 cm.	α-aminobutyric acid (control)	0·97 mg.
	α-aminobutyric acid when heated in presence of pyruvate ..	0·63 mg.
	α-alanine formed	178 γ
2·0 cm.	α-aminobutyric acid (control)	0·97 mg.
	α-aminobutyric acid in presence of pyruvate ..	0·64 mg.
	α-alanine formed	175 γ

The results presented in Table VI show that the size of the spot does not have any appreciable effect on the rate of the reaction.

Effect of concentration of the Reactants

The data showing the effect of concentration of the reactants on the transamination are given in Table VII. It was observed that an increase in the concentration of the reactants caused a definite increase in the rate of the reaction.

TABLE VII

Effect of Concentration of the Reactants on the Transamination Reaction

The solutions of amino acids and keto acids were prepared in M/15 KH_2PO_4 solution (pH 5·2). The following reaction mixtures were compounded:—

1. 0·1 ml. α-aminobutyric acid (M/10); 0·1 ml. of sodium pyruvate solution (M/10); 0.3 ml. of M/15 KH_2PO_4 solution (pH 5·2); Total volume 0·5 ml.
2. 0·1 ml. α-aminobutyric acid; 0·1 ml. sodium pyruvate; 0·8 ml. KH_2PO_4 solution; Total volume 1·0 ml.
3. 0·1 ml. α-aminobutyric acid; 0·1 ml. pyruvate and 1·3 ml. of KH_2PO_4 solution. Total volume 1·5 ml.

Controls were set up without the addition of pyruvate, at all the three concentration levels. The reaction was carried out at 95° C. for 30 mins. Volume of the reaction mixture spotted in each case : 20 μl.

Reaction mixtures spotted	Amino acid estimated	Concentration in 20 μl. spotted γ
1	α-aminobutyric acid (control)	37
	α-aminobutyric acid when heated in presence of pyruvate	28
	α-alanine formed	9
2	α-aminobutyric acid (control)	19
	α-aminobutyric acid when heated in presence of pyruvate	15
	α-alanine formed	5
3	α-aminobutyric acid (control)	12
	α-aminobutyric acid in presence of pyruvate	11
	α-alanine formed	3

Influence of Metal Ions

Metzler and Snell[5] have reported that the reversible transamination between pyridoxal and most amino acids is catalysed by copper and iron salts. It was, therefore, thought worthwhile to investigate the effect of metallic ions on the new type of transamination reaction described in the foregoing pages. A general survey of the effect of various metallic ions could not be made because some of the metallic ions interfered with the colour reaction of amino acids with ninhydrin.[13] The results (Table VIII) clearly show that the presence of manganese, copper and cobalt ions suppresses the formation of alanine from pyruvic acid.

TABLE VIII

Influence of Metallic ions on the Non-enzymatic Transamination Reaction

The reaction mixtures contained 0·1 ml. α-aminobutyric acid, 0·1 ml. pyruvate and 0·3 ml. of the metal salt solution (2 mg. M^{++}/ml. where M represents the divalent metallic ion). Controls with metallic ions and without pyruvate. and without metallic ions with or without pyruvate were also carried out. The volume in all cases was made up to 1·0 ml. with distilled water and 20 μl. aliquots were spotted for carrying out the reaction. The papers were heated at 95° C. for 30 minutes.

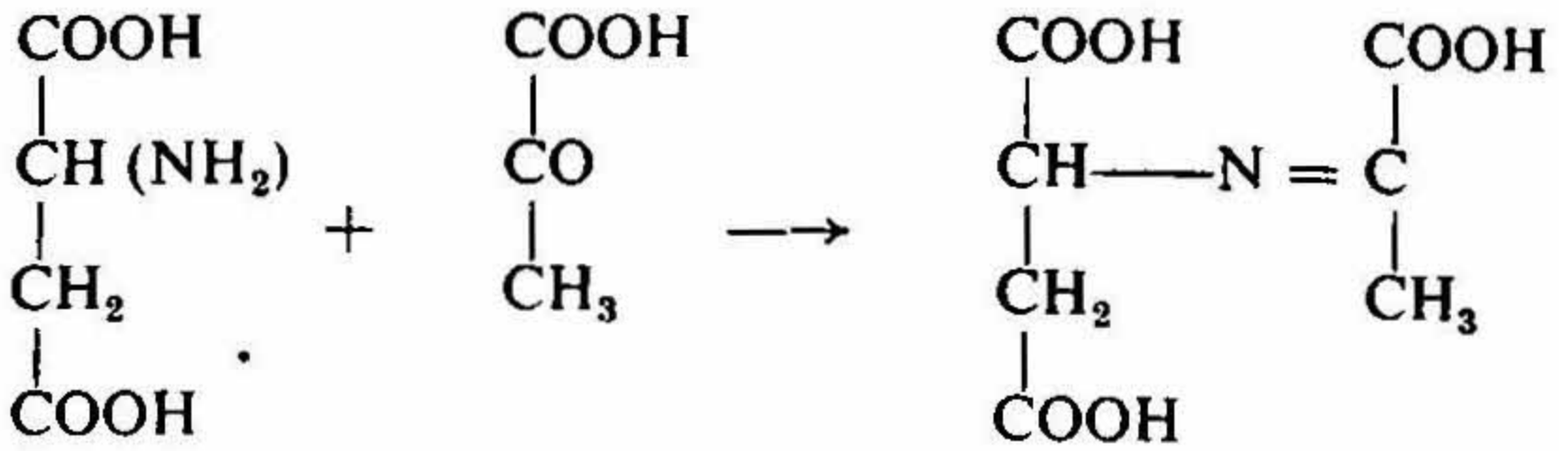
Ion present	Amino acid estimated	Concentration in the reaction mixture
Nil	α-amino butyric acid (control)	1·02 mg.
	α-aminobutyric acid when heated in presence of pyruvate	0·82 mg.
	α-Alanine formed from pyruvate ..	160 γ
Mn^{++}	α-Aminobutyric acid (control)	1·02 mg.
($MnSO_4$)	α-Aminobutyric acid in presence of pyruvate	0·90 mg.
	α-Alanine formed	95 γ
Co^{++}	α-Aminobutyric acid (control)	0·99 mg.
($CoCl_2$)	α-Aminobutyric acid in presence of pyruvate	0·90 mg.
	α-Alanine formed	55 γ
Cu^{++}	α-Aminobutyric acid (control)	0·90 mg.
($CuSO_4$. $5H_2O$)	α-Aminobutyric acid in presence of pyruvate	0·77 mg.
	α-Alanine formed	90 γ

DISCUSSION

From the analysis of the data presented above it can be concluded that the new type of transamination reaction reported in this communication is a dry reaction and takes place only in the presence of cellulose. However, it is just possible that traces of moisture may be necessary to start the reaction as the values in Table V indicate. This water may be made available in the form of 'bound water' present in cellulose.

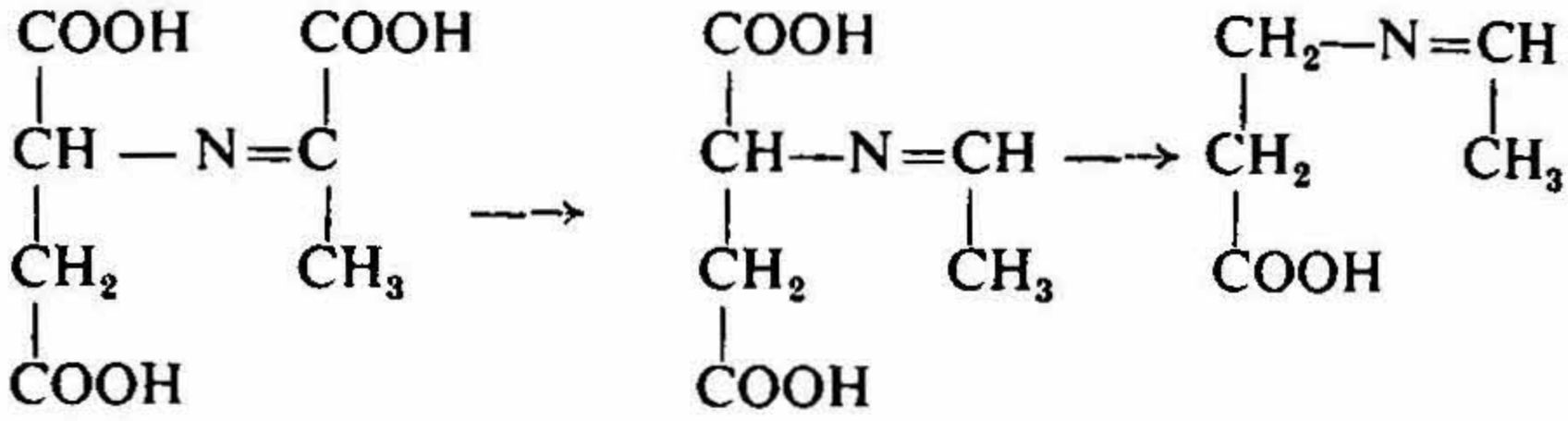
It was also observed in the course of the present investigation (Table II) that glutamic acid when heated at 100° C. in presence of α-keto acids (α-ketoglutarate or pyruvate) was decarboxylated giving γ-aminobutyric acid. In a similar manner, aspartic acid gave rise to β-alanine. It is possible to propose a mechanism for this

reaction in terms of the intermediate formation of a Schiff's base. Taking for instance the case of aspartic acid and pyruvic acid, the following intermediate is supposed to be formed:



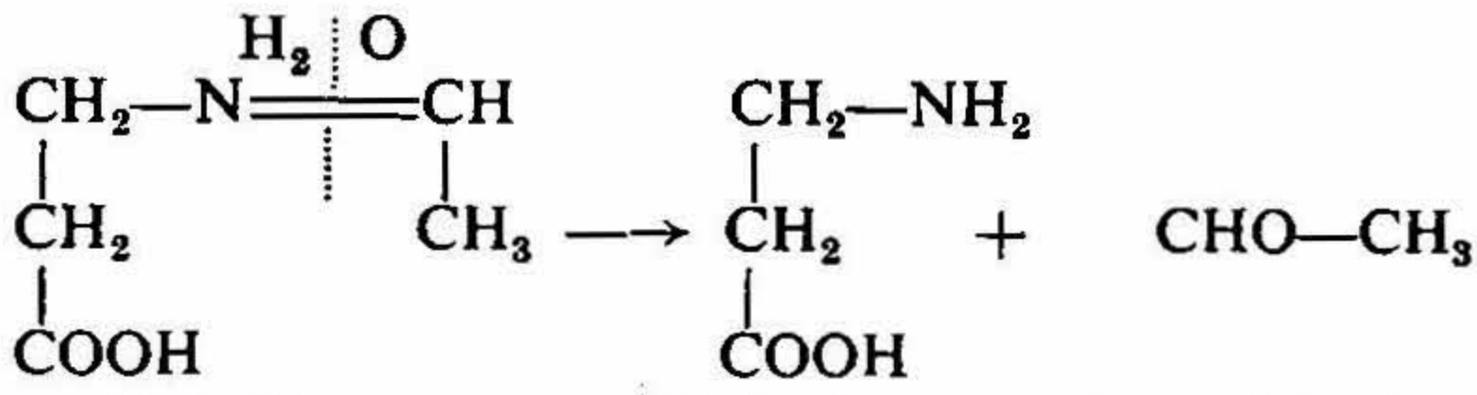
Aspartic acid Pyruvic acid Intermediate A

The intermediate compound being labile is visualized to be decarboxylated in two successive steps, as follows:



Intermediate A Intermediate B Intermediate C

The intermediate C is hydrolysed, probably by the action of water absorbed on the cellulose, giving β-alanine and acetaldehyde.



Intermediate C β-alanine Acetaldehyde

Acetaldehyde could not, however, be detected, due to its volatility at the conditions of our experiments.

In the absence of keto acids, β-alanine was not formed from aspartic acid. This indicates that decarboxylation occurs as a concomitant reaction along with transamination.

In this connection, it is interesting to cite the observation of Kating[16] that in the cells of *Endomycopsis vernalis* there is a close relationship between γ-amino-butyric acid and the coupling of transamination systems. He found that there was formation of glutamic acid when static cultures of *E. varnalis* were incubated with 1% solutions of γ-amino butyric acid and α-ketoglutaric acid. The formation of γ-aminobutyric acid by reverse transamination when α-ketoglutaric acid or pyruvic acid was added to glutamic acid solution was also demonstrated. Kating

has further pointed out that the necessity of addition of pyruvic acid or α-keto-glutaric acid for the formation of γ-amino butyric acid from glutamic acid after the glutamic acid decarboxylase activity has been completely inhibited by semi-carbazide, is in parallel with our findings[9] on the non-enzymatic formation of γ-aminobutyric acid from glutamic acid.

The studies on non-enzymatic transamination reactions in certain model systems have contributed not a little towards the unravelling of the intricate mechanism of the biological interconversion of keto and amino acids and there is no doubt that further investigation on the non-enzymatic reactions will furnish valuable information which may ultimately lead to a better understanding of similar reactions taking place in biological systems.

On a careful analysis of the results herein reported, it will be reasonable to suggest, that the mechanism of the transamination reaction in presence of cellulose reported in this paper might be different in some respects from that of the well-known transamination reaction of Herbst. It also appears to be somewhat different from that reported by Heyns and Walter[17] because in this case cellulose seems to have a definite role in the reaction.

It has long been known that neutral salt solutions become acidic when passed through filter-paper. Studies of this and related phenomena have shown that they result from the ability of cellulosic materials to engage in cation-exchange reactions, which are similar to those that occur in zeolites. It has been established that these exchange reactions are limited to cations[18] and that cellulosic products exchange metal ions for one another or for hydrogen-ions or hydrogen-ions for metal ions with a rapidity characteristic of inorganic reactions. This exchange capacity has been accounted for by acidic groups in the cellulose itself.[19]

It is probable that in the transaminations described above, this cation exchange capacity of cellulose may come into play, thus converting the sodium salts of keto acids to the free keto acids themselves and also altering the pH of the reaction. Subsequently, the reaction may proceed in a manner similar to that postulated by Herbst[2] and the bound water in cellulose may effect the hydrolysis of the intermediate Schiff's base.

The products of the reaction other than the amino acid corresponding to the keto acid added, are presumed to be an aldehyde and carbon dioxide. As the experiments are usually carried out with filter-paper on which microquantities of the reactants are spotted and the former heated at as high a temperature as 100° C., any aldehyde formed will immediately be removed from the system by its extreme volatility at the temperature of the reaction and hence will escape detection.

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ABSTRACTS

DEPARTMENT OF BIOCHEMISTRY

1. SANITATION OF WATERS AROUND TOWNS AND CITIES. S. C. Pillai and C. Anandeswara Sastry, *Surgical & Medical News*, 1955, **1**, 51–56.

Uncontrolled discharge of sewage and similar wastes into rivers and other watercourses leads to insanitary conditions, the basic cause of which is the depletion of oxygen from the waters. The available evidence on the de-oxygenation and re-oxygenation processes in polluted waters is reviewed in this article, and the need for further information is indicated for explaining the mechanism of oxygenation of polluted waters and of sewage during rapid purification by the activated sludge process.

2. DEPLETION OF OXYGEN FROM POLLUTED WATERS. C. Anandeswara Sastry and S. C. Pillai, *Science & Culture*, 1955, **21**, 37–39.

The paper relates to the nature and extent of depletion of oxygen from waters polluted with different forms of organic matter. The experimental observations given in the paper show that depletion of oxygen from waters polluted with organic wastes is largely due to the associated microbial activity and that materials carrying more bacteria deplete more dissolved oxygen.

3. SOME ASPECTS OF DISPOSAL OF INDUSTRIAL WASTE WATERS. S. C. Pillai and C. Anandeswara Sastry, *Surgical & Medical News*, 1955, **1**, 59–64.

In this article the polluting character of various trade effluents and the methods employed in different parts of the world for their treatment and disposal are discussed and a possible line of future development is indicated.

PHARMACOLOGY LABORATORY

1. ON THE CONSTITUTION OF BONE SALT AND TRICALCIUM PHOSPHATE—PARTS I AND II. T. K. Wadhwani. *J. of the Indian Chem. Soc.*, 1954, **31**, 359–65.

Study has been made of the available data about the physical and chemical aspects of calcium phosphate, and about the physical, chemical and biochemical aspects of bone salt. In the light of the study thus made, it has been concluded that though both bone salt and calcium phosphate give an X-Ray spectrogram of an apatite, their composition is variable, and is essentially determined by the composition of the liquid phase and by the conditions under which these are precipitated. In view of the functions and behaviour of the bone salt *in vivo*, and of the behaviour of bone salt and calcium phosphate *in vitro*, it has been further concluded that both these substances consist of two parts, the labile and the non-labile.

The data are presented about (i) the nature of the exchange reaction between a solution of sodium fluoride and calcium phosphate, (ii) the mechanism of phosphate adsorption by bone salt and calcium phosphate, and (iii) the nature of anions in bone salt and calcium phosphate.

It has been shown that the manner in which the anions of calcium phosphate and bone salt react with the fluoride of the liquid phase can be mathematically represented by the Freundlich adsorption isotherm, and that the adsorption of phosphate by bone salt and calcium phosphate is regarded as ionic, involving the exchange of phosphate with the anions of these substances and that all the anions in these substances, at least theoretically, by the process of repeated equilibration, can be replaced with fluoride in the manner that can approximately be denoted by the Freundlich adsorption isotherm.

2. STUDIES ON THE CONTROL OF FERTILITY. M. Sirsi, *Souvenir, Mysore Med. Assoc.*, 1954.

The available data on the physiology of reproduction, the biochemical reactions involved about the metabolism of spermatozoa and ovum are reviewed in detail. The use of enzyme inhibitors like hespiridin, and anti-vitamin E factors in natural materials are suggested for practical use. Their limitations in use has also been indicated.

3. A METHOD OF BIOLOGICAL STANDARDISATION OF CRUDE TOTAL ALKALOIDS OF *Rauwolfia serpentina*. C. N. Shaw and M. Sirsi, *Curr. Sci.*, 1955, **24**, 39.

Crude extracts of *R. serpentina* alkaloids have been assayed on the seminal vesicle of the rat for the evaluation of sympatholytic action. This test has been standardised to give consistent qualitative and quantitative results.

4. OXYSPORIN, A NEW ANTIBIOTIC FROM *Fusarium oxysporum* SCHLECHT. M. O. Tirunarayanan and M. Sirsi, *Curr. Sci.*, 1955, **24**, 162.

Oxysporin, the antibiotic principle from *F. oxysporum*, has been shown to have the same antitubercular activity as streptomycin *in vitro*. Further studies on the chemistry and pharmacology are in progress.

FERMENTATION TECHNOLOGY LABORATORY

1. EFFECT OF CHLOROMYCETIN SUPPLEMENTATION ON THE TRANSAMINASE ACTIVITY OF THE SILK WORM *Bombyx mori* L. (Mrs.) M. B. Shyamala and J. V. Bhat, *J. Sci. & Industrial Research*, 1955, **14 C**, 97–99.

In this paper some data are presented on the effect of supplementation of chloromycetin on the transaminases in the tissues of the silk worm. Transaminase activity was studied in hæmolymph and in the tissue extracts of silk glands and intestines. Circular paper chromatographic technique was adopted for enzyme studies. By employing the same technique, glutamic acid was quantitatively estimated in 20 ml. aliquots spotted at different intervals of time. The results show the intestinal extracts from the control batch of worms exhibiting high transaminase activity as compared to the hæmolymph which showed little activity. In the chloromycetin fed silk worms, on the other hand, the transaminase activity is even more high in the intestines and the hæmolymph, whereas the silk glands show little increase in activity. The increased transaminase activity in the hæmolymph of the chloromycetin fed silk worms is very significant and it has been surmised from the results that the hæmolymph probably has a very important function in the metabolism of the silk worm.

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