of glyoxylate, or oxalate, in the presence of acetyl-coenzyme A.

8. It is suggested that during growth on oxalate, the oxalate, possibly in the form of oxalyl-coenzyme A, is reduced to glyoxylate, which is taken to be the precursor of glycine and phosphoglycerate. The phosphoglycerate formed then enters established routes of biosynthesis.

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The Interconversion of Glutamic Acid and Aspartic Acid in Respiring Tissues

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In previous papers (Krebs, 1950; Ternier, Eggleston & Krebs, 1950) it was briefly reported that glutamic acid, when added in excess to respiring animal-tissue preparations, is rapidly converted into aspartic acid, and that aspartic acid, when added in excess, is converted into glutamic acid. Qualitatively these interconversions are not unexpected: both amino acids are oxidized in animal tissues through the tricarboxylic acid cycle and

therefore form α -oxoglutarate and oxaloacetate as intermediates. As animal tissues all contain aspartic-glutamic transaminase, any excess of the added amino acids may be expected to transaminate with the appropriate α -oxo acid arising as an intermediate. Starting with an excess of glutamate, a trace quantity of oxaloacetate present in the tissue, or formed from glutamate, would thus be expected to react as follows:

Analogously, starting with an excess of aspartate, a trace quantity of α -oxoglutarate and an endogenous substrate such as lactate, the expected sequence is:

In this case a source of acetyl-coenzyme A, such as lactate or a triose phosphate equivalent, a C_2 -fragment of a fatty acid or second molecule of aspartate is required.

Whilst, then, qualitatively the interconversion of glutamate and aspartate is expected, the extent to which this occurs cannot be predicted because several other pathways, especially complete oxidation, are open to both α -oxoglutarate and oxaloacetate, and no information is available on the rate at which transamination occurs at the low concentrations of the α -oxodicarboxylic acids in animal tissues.

The data presented in this paper indicate that, under some conditions, transamination rather than complete oxidation of the α -oxo acids is the preferred route. Although the steady-state concentration of the α -oxo acids is very low, transamination occurs very readily.

METHODS

Manometric determination of aspartic acid. The manometric method of Crawford (1957) employing the specific aspartic decarboxylase of *Nocardia globerula* (National Collection of Industrial Bacteria, Teddington, Middlesex, no. 8852) was used. This enzyme converts L-aspartate into α -alanine. The aspartic decarboxylase of this organism was found to be much more active (by a factor of about 10) in washed suspensions than in the acetone-dried powder recommended by Crawford. On the other hand, washed suspensions, in contrast with acetone-dried powders, absorb O_2 under test conditions when added to tissue extracts. Although the rate of consumption of O_2 is very low, the error introduced by it can be significant but can be avoided by anaerobic conditions. The specificity of acetone-dried powder and washed suspensions appears to be equal, as identical aspartate values were obtained with both preparations in an analysis of casein hydrolysate. Asparagine did not react.

The yield of the organism was greatly increased when growth conditions were aerobic. The organism was therefore grown in Roux bottles containing 2 g. of peptone, 0.25 g. of Difco-yeast extract, 1 g. of glucose, 3 g. of agar and 100 ml. of water. The flasks were inoculated with a (5 ml.) liquid subculture, containing 0.5% of yeast extract, 0.9% of NaCl, 0.2% of sodium citrate and 0.1% of glucose. After 18 hr. at 30°, about 4.5 g. of wet cells was obtained from one Roux bottle. The cells were washed three times on the centrifuge with 0.9% NaCl and finally suspended in 4 vol. of 0.9% NaCl. Of this suspension, 0.5 ml. liberated the theoretical amount of CO_2 from 20 μ moles of aspartic

acid in 20–40 min. Q_{CO_2} during the initial stages was about 180. The stock suspension was stored at -15° . Repeated thawing and freezing did not reduce the activity of the decarboxylase.

Replacing glucose by hydrolysed casein did not affect the yield of cells or the activity of the enzyme. Addition of asparagine to the growth medium reduced the decarboxylase activity, especially in the early stages of incubation.

For the determination of aspartate, up to 3 ml. of the solution to be tested was measured into the main compartment of a conical Warburg vessel, together with 0.3 ml. of 3M-acetate buffer, pH 5.0. The side arm contained 0.5 ml. of the *Nocardia* suspension and 0.1 ml. of the acetate buffer; the centre well contained phosphorus and the gas space N_2 . The bath temperature was 37°.

Manometric determination of glutamic acid. The decarboxylase method with the glutamic decarboxylase of *Clostridium welchii*, strain S.R. 12, was used (see Krebs, 1950). The values obtained by this method are too high when both aspartic acid and α -oxo acids are present (see Müller & Leuthardt, 1950; Meister, Sober & Tice, 1951). Very low concentrations of pyruvate or α -oxoglutarate suffice to cause a decarboxylation of aspartic acid to α -alanine. This difficulty was overcome by removing aspartic acid first with *Nocardia* suspensions and carrying out the glutamate determination on the material in which aspartate had been determined.

Chromatographic determination of amino acids. The methods of Clayton & Strong (1954) and of Meyer (1957) were used. After incubation, the tissue suspension was added to an equal volume of boiling ethanol. The mixture was heated at 100° for a further 5 min. and then cooled and centrifuged at 25 000 g for 30 min. Samples of the supernatant fluid and standard solutions of amino acids were applied 8 cm. from one end of strips of Whatman no. 1 (chromatography grade) paper about 30 cm. long. They were placed 4 cm. apart and contained from 0.5 to 2.0 μ g. of amino nitrogen. The chromatograms were developed with a descending solvent (Clayton & Strong, 1954) for about 8 hr.

Ninhydrin solution (Meyer, 1957) was prepared weekly by mixing a freshly prepared solution of 0.3 g. of ninhydrin in 100 ml. of 95% ethanol with a solution of 0.02 g. of ethylenediaminetetra-acetic acid in 6.5 ml. of water. After the papers were dried for 1 hr. in a stream of air at room temperature, the ninhydrin solution was sprayed lightly on to the paper. The chromatogram was heated at 110° until the coloured spots could just be detected (about 3 min.).

Each spot was cut out, divided into small pieces and placed in 10 ml. stoppered tubes with 3 ml. of ninhydrin reagent. The tubes were heated at 50° with occasional shaking for 45 min. The extinction of the resultant solution was measured at 578 m μ .

The chromatographic method was used in all experiments on mitochondria. In the experiment recorded in Table 2 manometric methods were also used.

Determination of α -oxo acids. α -Oxoglutarate was estimated as the 2:4-dinitrophenylhydrazone by the method of El Hawary & Thompson (1953), and oxaloacetate according to Kalnitsky & Tapley (1958).

Preparation and incubation of tissue homogenates. Tissue from a freshly killed animal was cooled in ice and homogenized with 4 vol. of 0.155M-KCl in a stainless-steel apparatus (type Potter & Elvehjem, 1936). For the incubation, 4 ml. of homogenate was measured into the main compartment of a large (50 ml.) conical Warburg vessel, together with 0.8 ml. of 0.1M-potassium phosphate buffer, pH 7.4, 0.8 ml. of 0.02M-MgCl₂, 0.8 ml. of 0.02M-adenosine triphosphate (ATP), varying quantities of 0.2, 0.4 or 0.75M-sodium glutamate and sufficient 0.155M-KCl to bring the volume to 8 ml. The centre well contained 0.3 ml. of 2N-NaOH and the gas space O₂. The vessels were shaken in a Warburg bath at 37° and the O₂ consumption was measured manometrically. At the end of the incubation period (1 hr.), the enzymic reaction was stopped by the addition of 2 ml. of 2N-HCl.

Micro-organisms. *Escherichia coli*, N.C.I.B. no. 8571, was grown at 30° for 24 hr. in a medium containing 1% of Lab-Lemco, 0.5% of NaCl, 0.5% of Difco-yeast extract, 2% of Tryptone, 2% of glucose and 3% of agar. *Alcaligenes faecalis* was grown as described by Krebs, Whittam & Hems (1957). The cells were washed twice on the centrifuge with water and finally resuspended in approx. 6 vol. of water. For measurement of the metabolism of glutamate, 2 ml. of washed cells (approx. 18 mg. dry wt.) was measured into

the main compartment of a conical Warburg vessel together with 1 ml. of 0.1M-potassium phosphate buffer, pH 7.4, and glutamate to a final concentration of 0.02M. The volume was made up to 4.5 ml. with water.

Preparation of mitochondria. Rat- and pigeon-liver mitochondria were prepared according to the method of Werkheiser & Bartley (1957), except that the dissolved CO₂ was not removed from the sucrose solution. Rat-brain mitochondria were prepared by the method of Bellamy (1959) and pigeon-breast-muscle mitochondria according to the method of Chappell & Perry (1953). The mitochondria were finally suspended in 0.25M-sucrose. Each cup contained 2 ml. of mitochondrial suspension (about 400 mg. wet wt.); 0.4 ml. of 0.1M-potassium phosphate buffer, pH 7.4; 0.4 ml. of 0.02M-MgCl₂; 0.4 ml. of 0.02M-ATP, varying quantities of 0.4M-sodium glutamate and sufficient 0.25M-sucrose solution to bring the volume to 4.5 ml. The centre well contained 0.2 ml. of 2N-NaOH, the gas space air. The bath temperature was 25°.

RESULTS

Formation of aspartate from glutamate. Glutamate was added to homogenates of liver, breast muscle and kidney cortex and the oxygen consumption and the changes in the concentrations of glutamate and aspartate were measured (Tables 1-4). The addition of glutamate increased the

Table 1. *Effect of glutamate concentration on the conversion of glutamate into aspartate in rat-liver homogenate*

For experimental conditions see text. Results refer to μ moles in 8 ml. of suspension. Before addition of glutamate 8 ml. contained 8.8 μ moles of glutamate, 4.8 μ moles of aspartate and 4.6 μ moles of α -oxoglutarate. Glutamic acid and aspartic acid were determined manometrically.

	μ moles				
Glutamate added	—	152	304	456	608
Glutamate found	9.9	94	237	349	519
Aspartate found	2.7	64.0	77.5	72.4	64.0
α -Oxoglutarate found	2.3	9.1	7.7	20.4	16.0
Changes:					
O ₂	-71	-200	-192	-183	-156
Glutamate	+ 1.1	- 67	- 76	-116	- 98
Aspartate	- 2.1	+ 59.2	+ 72.7	+ 67.6	+ 59.2
α -Oxoglutarate	- 2.3	+ 4.5	+ 3.1	+ 15.8	+ 11.4

Table 2. *Effect of glutamate concentration on the conversion of glutamate into aspartate in homogenate of pigeon-breast muscle*

For experimental conditions see text. Results refer to μ moles in 8 ml. of suspension. Before addition of glutamate 8 ml. contained 5.1 μ moles of glutamate and 2.2 μ moles of aspartate. Amino acids were estimated manometrically (m) and chromatographically (c). The calculation of the changes is based on the manometric determinations.

determinations.		μ moles							
Glutamate added	—	152		304		456		608	
	(m)	(m)	(c)	(m)	(c)	(m)	(c)	(m)	(c)
Glutamate found	1.4	60.8	53.9	212	194	386	342	538	536
Aspartate found	0.6	70.4	72.7	98.2	83.7	60.8	56.5	49.0	47.5
Changes:									
O ₂	- 95	- 189		- 161		- 139		- 150	
Glutamate	- 3.7	- 96		- 97		- 75		- 75	
Aspartate	- 1.6	+ 68.2		+ 96.0		+ 58.6		+ 46.8	

Table 3. *Effect of glutamate concentration on the conversion of glutamate into aspartate in homogenate of pigeon liver*

For experimental conditions see text. Results refer to μ moles in 8 ml. of suspension. Before addition of glutamate 8 ml. contained 12.9 μ moles of glutamate and 5.6 μ moles of aspartate. Glutamic acid and aspartic acid were determined manometrically.

	μ moles				
Glutamate added	—	152	304	456	608
Glutamate found	7.5	78	194	418	574
Aspartate found	5.6	65.0	95.5	31.2	19.6
Changes:					
O ₂	- 90	- 224	- 184	- 129	- 136
Glutamate	- 5.4	- 87	- 123	- 51	- 47
Aspartate	0	+ 59.4	+ 89.9	+ 25.6	+ 14.0

Table 4. *Effect of glutamate concentration on the conversion of glutamate into aspartate in homogenate of sheep-kidney cortex*

For experimental conditions see text. Results refer to μ moles in 8 ml. of suspension. Before addition of glutamate, 8 ml. contained 3.6 μ moles of glutamate and less than 0.2 μ mole of aspartate. Glutamic acid and aspartic acid were determined manometrically.

	μ moles				
Glutamate added	—	76.5	153	316	632
Glutamate found	0.2	34.1	101.5	263	562
Aspartate found	6.2	42.6	55.3	61.6	70.1
Changes:					
O ₂	- 61.4	- 145	- 168	- 195	- 215
Glutamate	- 3.4	- 46	- 55	- 57	- 74
Aspartate	+ 6.2	+ 42.6	+ 55.3	+ 61.6	+ 70.1

oxygen consumption in all four tissues, the increases being greater with 150 and 300 μ moles of glutamate than with 450 and 600 μ moles, except in kidney cortex, where oxygen consumption continued to increase with the glutamate concentration. Much of the added glutamate was removed during the incubation and much aspartate was formed. Under several conditions, the glutamate oxidized was almost quantitatively recovered as aspartate, e.g. with rat liver and pigeon-breast muscle with 300 μ moles of glutamate and at all glutamate concentrations in kidney cortex. The total (uncorrected for endogenous) oxygen consumption was generally greater than expected from reaction (1) but in some experiments, e.g. when about 300 μ moles of glutamate was added to rat liver or muscle, the excess of oxygen used was small. This indicates that the main oxidative reaction which occurred was the oxidation of glutamate to aspartate according to reaction (1).

The level of α -oxoglutarate was measured at the end of the incubation in the experiment shown in Table 1. It increased in the presence of glutamate and was of the order of 1–2 mm.

The level of oxaloacetate was measured in another experiment and was below the capacity of the method, i.e. below 0.05 mm. The transamination between glutamate and oxaloacetate thus readily occurred at very low oxaloacetate concentrations.

Formation of glutamate from aspartate. Like glutamate, aspartate increased the rate of oxygen consumption of the four tissues (Tables 5–8). The amounts of aspartate removed generally rose with the aspartate concentration except in sheep-kidney cortex, where the rate of aspartate removal was not much affected by the concentration of aspartate. The maximum rates of aspartate removal were lower than those for glutamate, about half in rat liver, pigeon-breast muscle and sheep-kidney cortex and a quarter in pigeon liver. The amounts of glutamate formed on addition of aspartate were between 40 and 60 % of the aspartate removed. A value of 50 % would be expected if both the oxaloacetate and the acetyl-coenzyme A needed for the synthesis of glutamate were derived from aspartate. The total amounts of oxygen used were rather greater than those required according to reaction (2), save in rat liver at high aspartate concentrations. In this tissue, most of the total oxygen consumption was due to the oxidation of aspartate to glutamate; in kidney roughly 50 % accounted for this reaction. The α -oxoglutarate level at which ready transamination took place was measured in the kidney experiments and found to be about 2 μ moles in 8 ml., or 0.25 mm.

Conversion of glutamate into aspartate in mitochondria. As shown in Tables 9 and 10, a conversion of glutamate into aspartate also occurred in isolated mitochondria but the yield of aspartate was

Table 5. *Effect of aspartate concentration on the conversion of aspartate into glutamate in homogenate of rat liver*

For experimental conditions see text. Results refer to μ moles in 8 ml. of suspension. Before addition of aspartate 8 ml. contained 5.7 μ moles of glutamate and 5.3 μ moles of aspartate. Glutamic acid and aspartic acid were determined manometrically.

	μ moles				
Aspartate added	—	160	320	480	640
Aspartate found	4.1	150	294	425	590
Glutamate found	2.7	12.3	23.8	41.6	32.5
Changes:					
O ₂	-39.0	-67.0	-79.6	-80.2	-78.0
Aspartate	-1.2	-15	-31	-60	-55
Glutamate	-3.0	+6.6	+18.1	+35.9	+26.8

Table 6. *Effect of aspartate concentration on the conversion of aspartate into glutamate in homogenate of pigeon-breast muscle*

For experimental conditions see text. Results refer to μ moles in 8 ml. of suspension. Before addition of aspartate 8 ml. contained 5.5 μ moles of glutamate and 1.7 μ moles of aspartate. Glutamic acid and aspartic acid were determined manometrically.

	μ moles				
Aspartate added	—	160	320	480	640
Aspartate found	2.6	138	285	429	596
Glutamate found	0.4	15.7	31.6	38.8	30.3
Changes:					
O ₂	-97	-146	-153	-147	-139
Aspartate	+0.9	-24	-37	-53	-46
Glutamate	-5.1	+10.2	+26.1	+33.3	+24.8

Table 7. *Effect of aspartate concentration on the conversion of aspartate into glutamate in homogenate of pigeon liver*

For experimental conditions see text. Results refer to μ moles in 8 ml. of suspension. Before addition of aspartate 8 ml. contained 5.7 μ moles of glutamate and 3.1 μ moles of aspartate. Glutamic acid and aspartic acid were determined manometrically.

	μ moles				
Aspartate added	—	160	320	480	640
Aspartate found	2.4	155	310	469	615
Glutamate found	1.3	17.8	13.2	14.2	21.6
Changes:					
O ₂	-55	-69	-76	-87	-109
Aspartate	-0.7	-8	-13	-14	-28
Glutamate	-4.4	+12.1	+7.5	+8.5	+15.9

Table 8. *Effect of aspartate concentration on the conversion of aspartate into glutamate in homogenate of sheep-kidney cortex*

For experimental conditions see text. Results refer to μ moles in 4 ml. of suspension. Before addition of aspartate, 4 ml. contained 3.1 μ moles of glutamate, 1.5 μ moles of aspartate and 0.2 μ mole of α -oxoglutarate. Glutamic acid and aspartic acid were determined manometrically.

	μ moles					
Aspartate added	—	75	150	300	450	600
Aspartate found	1.0	47.0	126	273	424	576
Glutamate found	0.8	19.9	20.4	21.0	19.0	16.0
α -Oxoglutarate found	0.2	1.3	0.9	1.2	1.0	1.0
Changes:						
O ₂	-34.2	-54.0	-62.0	-62.0	-66.0	-69.0
Aspartate	-0.5	-29.5	-25.5	-28.5	-27.5	-25.5
Glutamate	-2.3	+16.8	+17.3	+17.9	+15.9	+12.9
α -Oxoglutarate	0	+1.1	+0.7	+1.0	+0.8	+0.8

very low with mitochondria prepared from rat liver, pigeon liver and pigeon-breast muscle. In contrast, with rat-brain mitochondria most of the glutamate removed was recovered as aspartate, the yield reaching 91% when 80 μ moles of glutamate was added to 4.5 ml. of suspension (Table 9). Under these conditions, the changes observed are fairly close to those expected according to reaction (1). This finding indicates that the brain mitochondria possessed a high transaminase activity. The low yield of aspartate in liver and muscle mitochondria was presumably due to loss of transaminase in the washing process.

Müller & Leuthardt (1950), using paper chromatography, have already demonstrated qualitatively a conversion of glutamic acid into aspartic acid in rat-liver mitochondria.

Oxidation of glutamate by micro-organisms. As many bacteria can oxidize glutamic acid by the same route as animal tissue and possess transaminase activity, experiments were carried out to test whether a ready conversion of glutamate into aspartate occurs in bacteria.

E. coli and *A. faecalis* both readily oxidized added glutamate, as shown by an increased oxygen consumption and removal of glutamate, but no accumulation of aspartate occurred. The data on the oxygen consumption of glutamate removal indicated that most of the glutamate which was removed underwent complete oxidation. Thus

with the *Alcaligenes* strain, the suspension consumed 43.4 μ moles of oxygen with a removal of 8.8 μ moles of glutamate and a formation of less than 0.4 μ mole of aspartate.

It should be mentioned that Bovarnick & Miller (1950) found a conversion of glutamic acid into aspartic acid in typhus rickettsiae.

DISCUSSION

The experiments show that the four tissues tested readily interconvert glutamate and aspartate. The observations are in agreement with the assumption that glutamate yields oxaloacetate, and aspartate yields α -oxoglutarate, through the reactions of the tricarboxylic acid cycle, and that the α -oxo acids form the respective amino acids by transamination with the starting material [see also Müller & Leuthardt (1950) and Klingmüller (1955)]. The steady-state levels of α -oxoglutarate were of the order of 1 mM and those of oxaloacetate below 0.1 mM. Under many conditions, transamination was the metabolic reaction preferred to those of the tricarboxylic acid cycle.

Animal tissues contain relatively large quantities of free glutamic acid (2–10 μ moles/g.; Krebs, Eggleston & Hems, 1949) and relatively small amounts of aspartate (0.1–0.4 μ mole/g.; Krebs, 1950). The ready formation of aspartate from glutamate raises the question of the function of

Table 9. *Conversion of glutamate into aspartate in mitochondria*

For experimental conditions see text. Results refer to μ moles in 4.5 ml. of suspension. Before addition of glutamate, the mitochondrial suspension contained the following amounts of glutamate and aspartate: rat liver, 1.0, 0.4 μ mole; rat brain, 3.1, 2.4 μ moles; pigeon liver, 1.7, 0.7 μ mole; pigeon-breast muscle, 0.4, 0.4 μ mole.

	Rat liver		Rat brain		Pigeon liver		Pigeon-breast muscle	
Glutamate added	—	80	—	80	—	80	—	80
Glutamate found	4.6	56.5	0.7	43.4	2.1	65.0	0.8	70.8
Aspartate found	2.1	4.8	1.1	38.6	2.6	5.9	0.1	2.4
Changes:								
O ₂	-27.1	-61.4	-14.7	-60.2	-19.0	-40.3	-7.9	-25.0
Glutamate	+ 3.6	-24.5	- 2.4	-39.7	+ 0.4	-16.7	+0.4	- 9.6
Aspartate	+ 1.7	+ 4.4	- 1.3	+36.2	+ 1.9	+ 5.2	-0.3	+ 2.0

Table 10. *Effect of glutamate concentration on the conversion of glutamate into aspartate in rat-brain mitochondria*

For experimental conditions see text. Results refer to μ moles in 4.5 ml. of suspension. Before addition of glutamate, the mitochondrial suspension contained 2.7 μ moles of glutamate and 1.3 μ moles of aspartate.

	μ moles			
Glutamate added	—	80	160	240
Glutamate found	1.0	46.6	105	178
Aspartate found	0.5	34.7	44.1	52.2
Changes:				
O ₂	-18.3	-69.2	-86.0	-85.2
Glutamate	- 1.7	-36.1	-57.7	-64.7
Aspartate	- 0.8	+33.4	+42.8	+50.9

aspartate formed from glutamate. It is relevant in this context that the amino group of several compounds synthesized in the tissues is known to be derived from aspartate. Examples are: the formation of arginine from citrulline, a step in the synthesis of urea in the mammalian liver (Ratner, Petrack & Rochovansky, 1953; Ratner, Anslow & Petrack, 1953; Ratner & Petrack, 1956) and in the synthesis of guanidinoacetic acid in kidney cortex (Borsook & Dubnoff, 1941; Krebs, 1942); the amidation of 5-amino-4-carboxyiminazole ribotide to yield 5-amino-4-carboxy-amidoiminazole ribotide, one of the steps of the synthesis of uric acid in birds (Lukens & Buchanan, 1957; Miller, Lukens & Buchanan, 1957); the amination of inosinic acid, leading to adenylic acid (Carter & Cohen, 1955; Abrams & Bentley, 1955; Lieberman, 1956). The reactions investigated in this paper can account for the supply of the aspartate required for these syntheses.

Cravioto, Massieu & Izquierdo (1951) have shown that in insulin shock the glutamate concentration of the brain falls and the aspartate concentration rises. The changes of the aspartate and glutamate concentration were of the same order of magnitude and of opposite sign, and this indicates that in insulin shock the same reactions occur *in vivo* as are described in this paper as occurring in isolated brain mitochondria.

SUMMARY

1. Homogenates of rat and pigeon liver, pigeon-breast muscle and sheep-kidney cortex convert added glutamate into aspartate. Under some conditions, the amounts of glutamate removed and aspartate formed are approximately equal. The homogenates also convert aspartate into glutamate, the amounts of glutamate formed being of the order of 50 % of the amounts of aspartate removed. In many cases, reactions (1) and (2) account for most of the oxygen consumption in the presence of glutamate or aspartate.

2. An almost stoichiometric conversion of glutamate into aspartate also occurs in rat-brain mitochondria. In mitochondria of rat and pigeon liver and of pigeon-breast muscle, the yield of aspartate was only 20–30 % of the glutamate removed.

3. Washed suspension of *Escherichia coli* and

Alcaligenes faecalis did not accumulate aspartate on addition of glutamate.

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