ON DEAMIDIZATION*

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PRELIMINARY.

When we consider the fate of ingested protein in the organism, we find that its cleavage products, consisting of polypeptides and amino acids, are present in the intestine as the result of digestion. In the course of absorption we lose sight of them, nor have attempts to find them in the blood proved very successful, though it must be remembered that Howell¹ claims, by the use of the most modern methods, to have demonstrated their presence. When the nitrogen of ingested protein again appears, it is almost wholly in the form of urea in the urine. The reappearance of the ingested nitrogen does not necessarily imply the complete metabolism of the protein itself. Indeed, Voit has shown that the nitrogen of the protein is rapidly excreted, while the metabolism of the nitrogen free moiety, as estimated by the CO₂ output, is spread over many hours. As a yielder of energy, nitrogen cannot be regarded as being of much importance. The dynamic value of an amino acid is only slightly lessened by the removal of the amino group. Further, the organism has great difficulty in storing nitrogen; an increased intake of nitrogen leads only to a temporary retention, and nitrogenous equilibrium is quickly re-established.

In attempting to establish nitrogenous equilibrium by feeding dogs with the minimum amount of protein, Michaud² emphasised the importance of the chemical composition of the protein as indicated by the relative amounts of the various amino acids it contains. He found that the protein minimum was lowest when the composition of the protein fed corresponded most closely with that of the animal's own tissues, proving apparently that only appropriate 'Bausteine' were used in

*In all that follows the term deamidization is used to express the fact that ammonia makes its appearance as a result of the action of living tissue on an amino acid or the amide of such an amino acid; strictly speaking the latter is a deamidization and the former a desamination.

building up the living flesh. In the conditions of his experiments the protein was manifestly all needed for synthesis. In the well-fed animal, however, protein is probably used largely for purely dynamic purposes, and in this case deamidization may well play an important part in protein metabolism.

Whatever the immediate precursors of urea may be, it has been abundantly proved that ammonia is converted into urea in the liver. The ammonia liberated from the amino acids produced in the course of digestion will share a like fate, leaving the non-nitrogenous remainder available for the energy demands of the organism.

Investigation of the literature in this field has shown that there is much indirect evidence available in support of this deamidization theory.

Abderhalden and Terruchi³ found that subcutaneous injection of the dipeptides, glycyl-glycin and alanyl-alanin in dogs led to a large increase in the excretion of urea in the urine. Stollte⁴ injected amino acids into the blood stream of rabbits, and found that glycocoll and leucin were practically all excreted as urea. Asparagin, glutaminic acid and alanin were partly excreted as urea and partly as unchanged monamino acid.

Salaskin⁵ perfused the liver with blood to which amino acids had been added, and found that glycocoll, leucin and asparagin could be largely recovered from the blood as urea or an amide-like body. Various observers have carried out feeding experiments on animals with aromatic amino acids, and have succeeded in isolating the deamidized aromatic acid from the urine. Flatau⁶ found in rabbits that o. tyrosin was converted into o. oxyphenyl acetic acid, and m. tyrosin was converted into m. oxyphenylpyruvic acid. Chlorphenylalanin also appeared abundantly in the urine as chlorphenylpyruvic acid. Friedmann⁷ fed dogs with p. chlorphenylalanin, which, after conversion into p. chlorphenylacetic acid, paired with glycocoll, and reappeared in the urine as chlorphenaceturic acid. Finally, Neubauer and Fischer⁸ perfused a dog's liver with phenylamino acetic acid, and found that phenylglyoxylic acid had taken its place in the blood at the end of the experiment.

In alcaptonuria interesting evidence is also obtained of a deamidization occurring in the side chain of the aromatic amino acids. Tyrosin and phenylalanin are both excreted in the urine as homogentisinic acid, or 1, 4 dioxypyhenylacetic acid, the presence of the aromatic nucleus in this condition apparently interfering with a further oxidation of the side-chain. It is interesting to note that there has been a loss of one

carbon atom in the side chain in addition to that of the amino group. Abderhalden⁹ fed alcaptonuric patients with polypeptides containing tyrosin and phenylalanin, and found an increase in homogentisinic acid corresponding in amount to the aromatic amino acid present in the polypeptides. Neubauer and Falta¹⁰ found that phenylalanin in alcaptonuria was first converted into phenyl α lactic acid.

Further evidence in favour of deamidization of amino acids is found in the capacity of the liver to form acetone and diacetic acid on perfusion with certain amino acids.

As regards direct *in vitro* evidence, there is not much available. Experiments which have been carried out have consisted as a rule of digesting tissue to which amino acids have been added. Jacoby¹¹, experimenting with autolytic enzymes, found an increase in ammonia on digesting glycocoll with liver extract. During the course of autolysis he found that a loosening of the tightly-bound nitrogen of the amino acids occurred, with a resulting increase in amide nitrogen (urea?).

Loewi¹² on repeating Jacoby's work also found that glycocoll added to an alcohol liver extract was converted into a urea-like body. He concluded that amino acids were readily attacked by ferments, and gave rise to bodies containing loosely bound nitrogen which were closely related to urea.

Gonnermann¹³ incubated certain amides—formamide, acetamide and succinamide—with liver and kidney emulsions, and found that saponification occurred, the free acid being liberated. The organs sometimes differed in their action, e.g., acetamide and succinamide are split by liver, but not by kidney. Lastly, Lang¹⁴ claimed that deamidization occurred in most of the organs and tissues of the body, and Furth and Friedmann¹⁵ carried out a few experiments to investigate the extent of asparagin splitting by organ ferments. They used Lang's method, and found that liver, spleen, muscle, kidney, lung, brain and intestinal mucous membrane all split amide nitrogen from asparagin in autolytic experiments. The intestinal mucosa evinced the greatest activity.

Lang's (*loc. cit.*) work was received practically without criticism, owing no doubt to the fact that it fitted in admirably with many known facts in the metabolism of protein, such as the rapid appearance in the form of urea of the nitrogen of protein or amino acid, when introduced by mouth into the organism. In view of the importance of such a link in the downward metabolism of the amino acids, it seemed well worth while to repeat and extend some of Lang's work, especially as his claim that

the process is carried on to a significant extent in intermediate protein metabolism, is based on rather slender evidence.

To test the capacity of the tissues to liberate ammonia from amino acids *in vitro* he carried out a large number of experiments with various organ emulsions, incubating them at 38-40° C. for longer or shorter intervals with certain amino acids and other bodies containing the amide group. The organs examined were liver, pancreas, spleen, kidney, testis, adrenal, lymph gland and muscle tissue. Glycocoll, tyrosin, aspartic acid, asparagin, cystin, leucin, phenylalanin, acetamide, glutamin, glycosamin, urea and uric acid were the substances investigated. Lang's more important results were as follows:—

The total amide nitrogen of asparagin and glutamin was split off by all the organs; a small portion of the amino nitrogen as well was apparently liberated by the liver.

Glycocoll was split to a less extent by kidney, liver, testis and adrenal, and abundantly by pancreas and intestine.

He further noted that fresh aseptic tissue was more active than tissue incubated under antiseptic precautions, e.g., the fresh intestinal mucous membrane of a dog split an amount of glycocoll in one to two hours, equal to that split in twelve days by the incubated mucous membrane incubated in presence of an antiseptic.

The figures quoted from Lang in the following table (Table I) are concerned only with liver and intestine, as those are the organs which have been used in the present series of experiments.

It is largely on the value obtained for the ammonia liberated from glycocoll in Test 21 that Lang bases his claim for the importance of deamidization in intermediate proteid metabolism, i.e., in one and a half hours as much ammonia is liberated in the aseptic test as is liberated at the end of twelve days' incubation in presence of an antiseptic.

PRESENT INVESTIGATION

I. *Liver*

The first question to be answered was as to what extent deamidization could be demonstrated in an organ emulsion to which an amino acid or an amide of an amino acid had been added.

The method followed in carrying out the present series of experiments closely resembles that used by Lang. The organ, unless obtained from

TABLE I

Remarks	Days incubation	CONTROL		ASPARAGIN		GLYCOCOLL		LEUCIN	
		Mg. NH_3N in 50 g. organ	Mg. NH_3N in 50 g.	Mg. NH_3N liberated from Asp. Asparagin added	Mg. NH_3N in 50 g.	Mg. NH_3N split from Glyc.	Mg. NH_3N in 50 g. Glyc.	Mg. NH_3N split from Leucin	Percentage of T.N. of Leucin
14. Ox intestine	12	36.68	—	—	50.24	13.56	14.5	—	—
15. Ox gut 60 g.	9	29.96	61.6	31.64	—	—	—	—	—
16. " 100 g.	26	46.78	(47.96)	1.48 (Aspartic Acid)	43.56	2.92	—	—	—
1. Ox Liver 150 g.	1½	57.12	—	—	56.2	0.92	—	—	—
2. " 50 g. A	5	45.16	—	—	49.28	4.12	3.1	—	—
" 50 g. B	9	91.6	—	—	85.8	5.8	—	—	—
3. " 45 g.	18	32.2	84	51.8	39.2	7	12.5	—	—
" " "	25	38.73	—	—	41.9	3.17	5.5	—	—
" " "	32	46	—	—	49	3	5.3	—	—
" " "	33	44.29	98	53.71	—	—	—	—	—
4. " 459	6½	34.41	—	—	—	—	—	—	—
" " "	12	38.26	—	—	—	—	—	11.4	23.7
5. " 50 g.	13	36.4	79.8	73.7	38.8	2.4	3.2	—	—
" " "	23	59.08	—	—	60.8	1.72	2.3	—	—
" " "	31	62.32	—	—	62.32	0	—	—	—
		CONTROL		ASPARAGIN		GLYCOCOLL		Percentage T.N.	
		Mg. NH_3H in 50 g.	Hours incubated	Mg. NH_3N in 50 g.	Mg. NH_3N in 50 g.	Mg. NH_3N in 50 g.	Mg. NH_3N split		
20. Dog Liver 60 g. + 100 blood	...	...	1½	—	20.2	15.05	—	—	—
21. 27 g. liver + 50 blood	...	...	1½	3.92	—	15.12	11.2	15	15
" " "	...	...	2½	5.68	—	15.51	9.83	13	13
22. 50 g. liver + 50 blood	...	...	1½	5.96	—	—	—	—	—

an animal killed in the laboratory, was brought as quickly as possible from the slaughter-house (*circa* half an hour) and was immediately minced finely by a mincing machine.

A number of tests each weighing 50 grams were then weighed into a series of glass-stoppered jars; 100 c.c. of 0.9 per cent. sodium chloride solution was added to each test, with 2 c.c. toluol. To every test, save those serving as controls, an addition of 0.3 to 1 gram of an amino acid or amide was made. All the tests were well shaken, and after being overlaid with toluol were incubated at 38° C. for periods varying from days to weeks, and even months.

To ensure antisepsis, Lang used a shaking machine; this was frequently omitted by me, as Meltzer¹⁶ has stated that enzymes could be destroyed by continued shaking. The tests were shaken by hand for five to fifteen minutes, when the shaking machine was omitted, and were then overlaid with toluol and incubated as usual.

At the end of the incubation period the tests were acidified with acetic acid, and after the addition of 40 c.c. of a 5 per cent. tannic acid solution they were heated, with continual stirring, until coagulation occurred. On cooling, the precipitate and fluid were made up to 400 or 500 c.c.; half of the filtrate for this was taken for the estimation of the ammonia, which was carried out *in vacuo* at a temperature of 38-40° C. Lang used magnesia for driving off the ammonia, but Kruger and Reich¹⁷ point out that magnesia causes a gradual liberation of ammonia from certain nitrogenous bodies. Grafe's¹⁸ recommendation was therefore adopted, and a mixture of sodium carbonate and sodium chloride was substituted for the magnesium oxide.

A number of tests prepared according to this method were incubated in the presence of an antiseptic (toluol) for periods varying from a few days to three or four months. Liver was the organ first investigated, as previous work, such as that of Salaskin, Stolte, and others, had shown that the liver was the site of some kind of deamidization. The amino acids used were glycocoll, leucin, alanin, and the amide asparagin. A glance at the table below (Table II) will show that in every case ammonia has been liberated from asparagin in fair amount, but in the case of glycocoll the liberation has been much less marked, and in some cases has even been absent.

The next question was how far the antiseptic used inhibited the action of the deamidizing enzyme; in other words, in a fresh organ emulsion incubated for several hours without an antiseptic, is the amount

TABLE II

	Days incubated	CONTROL Mg. NH_3N in 50 g.	Mg. NH_3N in 50 g.	ASPARAGIN		GLYCOCOLL		Percentage of T.N. of Glyc.
				Mg. NH_3N split from Asp.	Percentage of T.N. of Asp.	Mg. NH_3N in 50 g.	Mg. NH_3N split from Glyc.	
1. Ox Liver 50 g. ...	5½	24	39	15	32.1	—	—	—
2. Sheep Liver 50 g. ...	11	25	49	24	25	—	—	—
6. Dog Liver 50 g. ...	19	48	—	—	—	75	27	14.6
	9	28	—	—	—	36	8	4.3
	21	24	—	—	—	32	8	4.3
7. Foetal Sheep Liver 50 g.	7	30	66	36	87.1	—	—	—
	17	29	—	—	—	32	3	3.2
5. Foetal Dog Liver 50 g. ...	6½	25	42	17	36.4	—	—	—
16. Human Foetal Liver 50 g.	12	22	29	7	25	—	—	—
19. Human Liver 50 g. ...	12	37	—	—	—	36	—1	—
	Months							
	3½	60.4	72.8	12.4	44.3	—	—	—
25. Ox Liver 50 g. ...	3½	56	—	—	—	59	3	5.3
" " 50 g. ...	3½							
17. Rabbit Liver 30 g. ...	13	14.3	21.6	7.3	20	—	—	—
12. Sheep (Gut + Liver) 50 g.	7	26	48	22	78	26	0	—
" " 50 g. ...	7	21	38	17	60.7	23	2	3.5
" " 50 g. ...	19	29	49.9	20.9	74.7	29	0	—
" " Liver 50 g. ...	9	35	52	17	60.7	37	2	3.5
15. Ox Liver 50 g. ...	66	26.3	51.5	25.2	90.3	—	—	—

of ammonia liberated from added amino acids greater or less than in an antiseptic test incubated for days instead of hours? Lang laid very great stress on the rapidity of deamidization in the 'aseptic' tests; he was satisfied that bacterial action could be disregarded if proper aseptic precautions were observed, and if the incubation period were not prolonged ($1\frac{1}{2}$ to $2\frac{1}{2}$ hours). It may be remarked that there is no record of any cultures being taken to test the success of his precautions. As the result of these series of tests with the fresh organ incubated aseptically, Lang maintains that the deamidizing enzyme is retarded in its action by toluol, and that deamidization in the tissues of the organism is much more extensive than a consideration of the amount of liberated ammonia in the antiseptic experiments would indicate.

Some tests were prepared in the usual way, but without the addition of any antiseptic; the results are tabulated below in Tables III and IV. The instruments and vessels were sterilized, and the tests were incubated from $1\frac{1}{2}$ to 5 hours; they were not shaken continuously for reasons given above. In the serial tests (No. 3, Table III), in which the first tests were incubated for $1\frac{1}{2}$ hours, and the others for $3\frac{1}{2}$ hours, an addition of toluol was made to the latter at the end of incubation, as it was impossible to work up all tests on the same day at the end of incubation.

These tests (*b* and *c*) were kept at laboratory temperature (7° C. to 14° C.) until they could be dealt with. As a result it was found that not only did the ammonia liberated increase in the latter tests, but the ammonia in the controls increased also; evidently autolytic and deamidizing processes were still active at a temperature much lower than 38° C., and in the presence of an antiseptic. In No. 6, Table III, the same thing is evident, although in this case the tests were placed in the refrigerator immediately after incubation. In order to stop further ferment action the tests in all subsequent experiments were always heated immediately after incubation till the protein was coagulated.

TABLE III

			CONTROL		ASPARAGIN	
			Hours incubated	Mg. NH_3N in 50 g.	Mg. NH_3N in 50 g.	NH_3N split from Asp.
3.	6.5.10 Ox Liver 50g.	(a)	$1\frac{1}{2}$	3.6	5.2	1.6
	7.5.10 "	(b)	$3\frac{1}{2}$	12	19	7
	9.5.10 "	(c)	$3\frac{1}{2}$	21	40	19
4.	13.5.10 Dog Liver	(a)	3	14	15	1
	15.5.10 "	(b)	3	14	20	6
6.	28.5.10 Dog Liver	(a)	$4\frac{1}{2}$	15	16	1
	30.5.10 "	(b)	$4\frac{1}{2}$	16	21	5
	33.5.10 "	(c)	$4\frac{1}{2}$	30	49	19
						Percentage of T.N. of Asp.
						3.4
						15
						47.2
						1
						6.4
						2
						10.7
						38.5

A comparison of Lang's table (Table I, Experiments 20, 21 and 22, with Experiments 3, 4 and 6, Table III) shows that Lang obtained higher values for the liberated ammonia than I was able to get. As he used defibrinated blood in these 'aseptic' tests, it was thought that the absence of this in my experiments might explain the lower figures obtained.

In Experiments 11 and 15, Table IV, an addition of 50 c.c. defibrinated blood to each test in 11, and of 25 c.c. in 15, was made.

These tests incubated with defibrinated blood do show on the whole a more active deamidization, probably because of a closer relation to vital conditions. In most of the subsequent experiments, therefore, an addition of 25 c.c. defibrinated blood was made to each test.

Part played by Bacteria. As gelatine cultures taken from the 'aseptic' tests at the end of incubation invariably gave positive results, it was of the greatest importance to ascertain the possible part played by bacteria in deamidization. It has been proved that a liberation of ammonia does occur when putrefaction organisms are incubated with amino acids. In a recent publication, Brasch¹⁹ showed that the *B. putrificus* deamidizes glycocoll and alanin, yielding acetic and propionic acid in their place, respectively. In the more complex amino acid, aspartic acid, formic, propionic and succinic acid appeared, and not merely the products of a simple deamidization.

It might be urged that the presence of an antiseptic in the tests is sufficient to prevent bacterial action. Gelatine stab cultures were made in Experiments 12, 16, 17 and 19, Table II, with negative result, but it must be remembered that a negative culture does not prove the absence of bacteria.

The simplest way of investigating the part played by bacteria in the deamidization of asparagin in an organ emulsion is to compare the amount of ammonia liberated at the end of incubation in a test containing both enzyme and bacteria with the amount liberated in a test containing bacteria only. The absence of the enzyme in the latter case can be assured by heating the tests to 100° C. Subsequent inoculation provides the bacteria.

In accordance with this, in Experiments 21, 22, 23 and 24, Table V, a series of tests, A, B, C and D, each consisting of 50 grams liver tissue, were prepared in the usual way. A and C were used as controls, no addition of any kind being made. To B, 0.3 gram of asparagin was added; C and D were immediately boiled to destroy the enzyme, and were then

TABLE IV

	Hours incubation	CONTROL		ASPARAGIN		GLYCOCOLL		LEUCIN		ALANIN	
		Mg. NH_4N in 50 g.	Mg. NH_4N in 50 g.	Mg. NH_4N in 50 g. split from Asp.	Percentage of T.N. of Asp.	Mg. NH_4N in 50 g. split from Glyc.	Percentage of T.N. of Glyc.	Mg. NH_4N in 50 g. split from Leucin	Percentage of T.N. of Leucin	Mg. NH_4N in 50 g. split from Alanin	
9. Rabbit liver	...	12	16	4	8.5	—	—	—	—	—	—
10. Ox liver	...	10	18	8	17.1	—	—	—	—	—	—
11. Ox liver + 50 c.c. defib. blood	(1) 2½	15	25	10	21.4	1	—	—	—	—	—
15. Ox liver	(2) 4½	19	32	13	27.8	0	—	—	—	—	—
25 c.c. defib. blood	(1) 2	11.3	18	6.7	11.9	2.4	—	—	—	—	—
26. Ox liver	(2) 4½	12.6	22.54	9.94	17.7	1.5	—	—	—	—	—
27. Ox liver	...	15.9	22.6	6.7	11.9	—	—	—	—	—	—
28. Ox liver + lactic acid	(a) 4	17.8	—	—	—	0.4	—	20	air bubbled through tests	—	—
		18.8	26.6	7.8	14.1	—	—	1.2	air bubbled through tests	—	—
33. Sheep liver	(b) 2	22.8	26.4	3.6	6.5	—	—	—	—	—	—
34. " "	3	12	15.5	3.5	6.2	—	—	1.4	2	18.2	0.4
40. Young dog liver	...	18.6	—	—	—	—	—	—	—	14	0.2
41. " "	...	14.2	—	—	—	—	—	—	—	—	—
43. " "	...	13.9	19.54	5.64	10	—	—	—	—	—	—
47. Ox liver	2½	16.98	—	—	—	17.37	0.39	—	—	—	—
48. Ox liver	3	11.7	—	—	—	12.7	1	—	—	—	—
	3½	13.4	20.6	7.2	4.9	15.6	2.2	15.6	6.8	continuous shaking	—

N.B.—The tests in 48 were shaken continuously during incubation; the values obtained for the liberated ammonia are not greater than some of those found in tests incubated in the usual way.

allowed to cool; A and B were incubated three hours. From each test 2 c.c. fluid were removed for the inoculation of C and D. A and B were then immediately worked up in the usual way.

After inoculation, C and D were incubated for $1\frac{1}{2}$ hours to give the bacteria a start; 0.3 gram asparagin was then added to D, and a further incubation of three hours followed, at the end of which time the tests were worked up.

Here there is evidence of a deamidization of asparagin due to bacterial action alone, the largest amount of ammonia liberated being 2 mgm. in Experiment 22; the smallest amount set free, 0.2 mgm., is found in Experiment 24. On the other hand, the combined action of enzyme and bacteria liberated 6 mgm. ammonia nitrogen in 23, but only 1.7 mgm. in 24.

Possibly the conditions were made too favourable for the bacteria in 22, they had $1\frac{3}{4}$ hours' incubation before the addition of asparagin was made. As a matter of fact, in Experiments 20 and 24, gelatine cultures taken from each test at the end of incubation showed colonies on the third day in the case of the C and D tests and not until the fifth day in that of the A and B tests.

In Experiments 23 and 24, two additional tests, E and F, were prepared. They were treated in exactly the same way as C and D, with this exception, that they were not heated to 100° C., i.e., they were allowed to stand at laboratory temperature, and were inoculated at the same time and in the same way as C and D, the result being that they contained active enzyme *plus* a double dose of bacteria. The addition of asparagin to F was made immediately before incubation—E served as a control. Positive and abundant gelatine growths were obtained from each test of the four series at the end of this incubation period.

The results of these experiments show that the bacteria present are only responsible for a minor part of the ammonia liberated from asparagin.

In connection with bacterial deamidization, it is interesting to note that when two gelatine cultures of three days' growth (obtained at the end of incubation from the control and asparagin test, respectively, in Experiment 15, Table IV) were each incubated for two hours with 0.15 gram asparagin; 3.8 mgm. ammonia nitrogen were liberated from the first, and 3.6 mgm. from the second culture. Unfortunately the absence of a third culture as a control, to which no addition of asparagin had been made, leaves it uncertain whether the whole of the ammonia nitrogen

liberated came from the asparagin. The technique was the same as in the case of an organ emulsion.

TABLE V

	Hours incubation	CONTROL		ASPARAGIN	
		Mg. NH_3N in 50 g.	Mg. NH_3N in 50 g.	Mg. NH_3N split from Asp.	Percentage of T.N. of Asp. liberated
21. Sheep liver, 50 g., Enzyme intact, A and B	3	8.4	13.7	5.3	18.9
Bacteria only present, Enzyme destroyed, C and D	3½	9.5	10.6	1.1	3.8
24. Sheep liver, Enzyme intact, A and B	3	15.8	17.9	2.1	7.5
Bacteria present, Enzyme destroyed, C and D	3	11.2	11.4	0.2	0.7
Bacteria present, Enzyme intact, E and F	3	14.8	16.5	1.7	6
22. Ox liver, Enzyme intact, A and B	3	11.7	14.8	3.1	11
„ „ destroyed, C and D	3	10	12	2	7
23. Ox liver, Enzyme intact, A and B	3	9.9	15.9	6	21.4
„ „ destroyed, C and D	3	10.9	12.3	1.4	5
„ „ intact, E and F	3	15.2	18.9	3.7	13.2

In view of the facts shown in Table V, bacterial deamidization has been ignored in the subsequent investigations, which have been almost entirely carried out with fresh organ emulsion incubated without the presence of any antiseptic for two to four hours. The results detailed in Table IV obtained in this manner are, in my opinion, of greater value in considering intravital metabolic processes than those obtained from antiseptic organ emulsions at the end of one or more weeks.

Other Factors

Oxygen Supply. It was thought that a better supply of oxygen to the tissues might increase deamidization activity, and in tests 26 and 28 (Table IV) air was bubbled through the emulsion during incubation (the air leaving the digest flask was passed then through decinormal acid to collect any ammonia liberated). No appreciable increase of deamidization was observed.

Presence of Lactic Acid. In view of the results of Schryver,²⁰ who demonstrated, when working on the autolysis of the liver, that there was an increase of rapidity of breakdown (increased activity of ferment) if the tests were acidified with lactic acid, Experiment 38, Table IV was carried out using the usual methods except that the tests were acidified to the extent of 0.1 per cent. with lactic acid. There was no increased deamidization.

Optical Activity. As Abderhalden²¹ found that organ extracts and pancreatic juice only liberated from certain racemic polypeptides the optically active amino acids which occur naturally in protein, the question of optical activity had to be considered. In this connection it is interesting to note that the expressed juice of liver, placenta and kidney, according to Bergell and Brugsch,²² can cleave dl leucinamid and dl alanyl-alanin into the active components. They find that this is not due to autolytic activity, for the fresher the juice the more active is the cleavage.

The material I used was an abiuret pancreatic digest, very kindly given to me by Dr. Cathcart. As a considerable amount of ammonia was present in this digest, it was neutralised with acetic acid and to obviate the necessity of making an ammonia estimation in it, an amount of this digest, containing 0.296 gram nitrogen (Kjeldahl), was added to Test 39 (Table VI) *before* digestion, and an equal amount to the control test *at the end* of incubation. The result obtained showed but a very small increase of ammonia.

A second experiment (46) was carried out with a freshly prepared casein digest, which had been digested with pancreatin for a month. Before use the digest was precipitated by means of phosphotungstic acid, the excess of the precipitant being removed with barium carbonate. 100 c.c. of the filtrate thus obtained, containing 10 mgm. ammonia nitrogen and 170 mgm. T.N. was then added to 50 gram ox liver. As this test contained at the end of incubation only 9.1 mgm. of ammonia nitrogen more than the amount of ammonia nitrogen in the control test, it is evident that there has been no deamidization of the digest.

TABLE VI

				CONTROL	DIGEST	
				Mg. NH ₃ N in	Mg. NH ₃ N in	Mg. NH ₃ N
				50 g.	50 g.	liberated
				Hours of incubation		
39.	Ox Liver	...	...	4	91	5.2
46.	Ox Liver	...	...	3	11.7	9.1
				3	11.6	lost
						—

II. *Intestinal Mucosa*

A consideration of the following facts led to an investigation of the deamidizing action of the intestinal mucous membrane. Nencki, Pawlow and Zaleski found the ammonia content of the portal blood constantly higher than that of arterial blood. They found also an increased amount

of ammonia in the intestinal mucosa after flesh feeding compared with the amount present at the end of two days' starvation. These results were confirmed by Horodynski, Salaskin and Zaleski.²³ Cohnheim²⁴ found an increase of ammonia at the end of six hours' digestion in the blood bathing a fish's intestine, which he had previously filled with a solution of an amino acid (aspartic acid), proving that a deamidization had occurred. He obtained the following figures:—

9 mgm. ammonia were liberated from 0.25 gram aspartic acid; and

15 mgm. ammonia were liberated from 0.23 gram lysin chloride.

This would suggest that an active deamidization had taken place in the intestinal mucosa.

Lang carried out three experiments with ox intestinal mucosa, with the results shown in Table I.

I carried out a series of experiments detailed in Table VII. In Test 12, 50 grams sheep intestine, incubated antiseptically for seven days, liberated 17 mgm. ammonia nitrogen from asparagin, and 2 mgm. ammonia nitrogen from glycocoll. 50 grams of sheep liver liberated a similar amount of ammonia nitrogen from asparagin and glycocoll in nine days.

In the aseptic tests with mucous membrane there is always a small amount of ammonia nitrogen liberated from asparagin, the maximum amount in three hours' incubation being 2.8 mgm. Contrasting this with the amount liberated by 50 grams liver in the same time, we find, in Experiment 15, Table IV, a maximum of 9.94 mgm. The deamidization of asparagin in the aseptic tests carried out in the manner already described is more active apparently in the case of liver than in that of intestinal mucosa.

Possible co-ferment. The possibility of a co-ferment action was considered in the same series, 25 grams liver and 25 grams mucous membrane being incubated together. There is, perhaps, evidence of a slightly increased activity in the mixed test with asparagin, but it is not enough to postulate the existence of a co-ferment.

It was thought that a more active deamidization might be found at the height of digestion, and for this reason, in Experiment 40, Table VII, a young dog was killed seven hours after a large meat meal. The intestinal mucous membrane was scraped off the muscular coat and finely chopped. It was then incubated with a mixture of 50 c.c. defibrinated blood and 100 c.c. Locke's solution, and air was bubbled through the tests during the four hours of incubation; 4.7 mgm.

TABLE VII

INTESTINAL MUCOSA		CONTROL		ASPARAGIN		GLYCOCOLL		LEUCIN	
		Mg. NH ₃ N in 50 g.	Mg. NH ₃ N in 50 g.	Mg. NH ₃ N split from Asp.	Percentage of T.N. of Asp.	Mg. NH ₃ N in 50 g.	Percentage of T.N. of Glyc.	Mg. NH ₃ N split from Leucin	Mg. NH ₃ N split from Leucin
12. Sheep 50 g.	...	(1) 7	21	38	17	60.7	23	2	3.5
Antiseptic—		(2) 19	29	40.9	20.9	74	—	—	—
Aseptic—		Hours							
29. Ox gut 50 g.	...	(1) 3	12.1	14.7	2.6	4.6	11.3	—0.8	—
		(2) 3	15.4	18.2	2.8	5	—	14.6	—0.8
37. Sheep 50 g.	...	...	2	9.9	12.3	4.2	—	—	—
40. Puppy—fed	...	...	4	34.3	—	—	39	4.7	8.4
41. " fed	...	...	3½	19.9	1.3	2.3	—	—	—
42. " 25 g., fasting	...	...	3¾	26.2	0.5	0.8	—	—	—
43. Fasting ...	...	...	3	15.7	—	—	15.91	0.21	0.3

TABLE VIII

Sheep	Incuba- ted	CONTROL.		ASPARAGIN		GLYCOCOLL	
		Mg. NH ₃ N in 50 g.	Mg. NH ₃ N in 50 g.	Mg. NH ₃ N split from A.p.	Mg. NH ₃ N in 50 g.	Mg. NH ₃ N split from Glyc.	
Gut + Liver aa 25 g. ...	7	26	48	22	26	—	
Antiseptic—							
Gut 50 g.	7	21	38	17	23	2	
Liver 50 g.	9	35	52	17	37	2	
Gut 50 g.	19	29	49.9	20.0	29.4	0	
Sheep	Hours Incuba- ed	LEUCIN					
Aseptic—							
(37) Gut + Liver aa 25 g. ...	4	22.4	28	5.4	23.2	0.8	
Gut 34 g.	2	9.9	12.3	2.4	—	—	
Liver 50 g.	2	14.2	—	—	19.4	0.2	

ammonia nitrogen were liberated from glycocoll. Unfortunately the amount of mucous membrane was only sufficient for two tests—the control and the glycocoll test.

In Experiment 41 two young dogs were killed five hours after a meat meal, whilst Experiments 42 and 43 were carried out with similar material obtained twenty-five hours and twenty-seven hours, respectively, after a meat meal.

As a result of these experiments it was found that under the conditions present in one case (Experiment 40) deamidization of glycocoll is certainly greater at the height of digestion, but the experiments are too few in number to dogmatise upon. The extraordinarily large amount of ammonia present in the control of Experiment 40 is in accordance with the known facts as to the large ammonia content of the intestinal mucosa at the height of digestion.

Deamidization in various Species and in Foetal States. As metabolism varies in certain particulars in different species of animals, the question may be asked whether any marked differences are found in the deamidizing capacity of the liver of the various animals used, namely, dog, rabbit, sheep and ox. The experiments were not carried out with this definite point in view, but on looking through the tables it will be seen that the most active splitting of glycocoll occurs in the sheep (Experiments I and VII); no other definite variations are noticed.

It is interesting to note that in the foetal livers (Experiments 5, 7 and 21, Table II) of varying ages examined, quite an active splitting of asparagin has occurred. The presence of tissue enzymes in the embryo

is a well-established fact. Amongst other workers in this field may be mentioned Mendell and Leavenworth,²⁵ who carried out a series of investigations on embryo pigs. They found an active autolytic enzyme in the liver, and demonstrated the early presence of lipase in liver and intestine, although the activity of the latter was less pronounced than in the full-grown animal.

In considering the question of how far the amount of ammonia indicates the extent of deamidization, there are three possibilities as to the fate of the liberated ammonia:—

- (1) It may be converted into urea in the liver tests.
- (2) It may be fixed in some way by the tissue.
- (3) It appears as such.

The conversion of ammonia into urea by the liver *in vivo* is a well-established fact; in the case of organ emulsion, however, added ammonia only disappears to a limited extent. For example the following:— Table IX shows that in Experiments 44, 45 and 47, 3·54, 6·52 and 4 mgm. ammonia nitrogen (in percentage amount 12, 23 and 8), respectively, disappeared during incubation for three hours with 50 grams liver tissue.

TABLE IX

	Hours incubated	CONTROL		AMMONIA	
		Mg. NH ₃ N in 50 g.	Mg. NH ₃ N in 50 g.	Mg. NH ₃ not recovered	Percentage of T.N. not recovered
44. 50 g. Ox Liver 25 c.c. Ammon. Acet. Solution added to one test containing 27·82 mg. N.	3	12·4	36·68	3·54	12
45. 50 g. Ox Liver + 25 c.c. defibrinated blood Ammon. Acet. = 27·82 mg. N.	3	12·2	33·5	6·52	23
47. 50 g. Ox Liver, Ammon. Acet. = 50 mg. N.	3	13	59	4	8

Further insight into the fate of ammonia in these digest experiments could only be gained by quantitative estimations of urea or of nitrogen content of an alcohol extract carried out at the end of incubation. Difficulty was found in extracting the evaporated filtrate obtained with the alcohol-ether mixture or amyl alcohol, as the addition of these at once made the residue very sticky, rendering satisfactory extraction impossible. Folin's method could not be used owing to the presence of considerable amounts of sugar in the organ extract. The same objection applies to Pfluger-Schöndorff's method. The second possibility as to the

fate of ammonia is rendered not unlikely by the extremely interesting work of Kowalevsky and Marchewicz²⁶ on the capacity of the tissue to remove an excess of ammonia from the blood. Both points are of extreme interest and importance in connection with the present research, and are now being further investigated. The results will be given in a later communication.

With regard to the amino acids investigated in the present experiments, it is quite evident that more ammonia is liberated from asparagin than from any of the others. Glycocoll is split to a considerable extent in the antiseptic tests, but only to a small extent in the aseptic tests. Leucin and alanin were used only in the aseptic tests; no ammonia was ever found to have been liberated from alanin, but small amounts were set free in some of the leucin tests. It is quite evident that the amide nitrogen attached to the carboxyl group of asparagin is more readily liberated than the amide nitrogen occupying the α position to the carboxyl group of the other amino acids and of asparagin itself.

Having considered the effect of various organ emulsions on amino acids with regard to deamidization, it is of interest to observe the behaviour of these amino acids in the living organism itself. How soon and to what extent does their nitrogen appear in the urine as urea?

Levene and Kober²⁷ fed a dog on a standard diet of low nitrogen value. When they added glycocoll to this diet they found that its nitrogen was removed from the body in the form of urea within twenty-four hours. Dr. Cathcart, in an investigation (not yet published) on the rate of absorption of digest products and simple amino acids from the small intestine, found also a very rapid excretion of the nitrogen, mostly as urea.

To carry out a somewhat similar experiment, a dog of 21.900 kilos was fed for some days on a diet consisting of 300 grs. meal and 500 c.c. milk, representing a caloric intake of 50 calories per kilo. On the four test days, which were separated by intervals of a few days, the bladder was emptied by catheter at 9.30 a.m. The urine was next collected by catheter at 10.30 a.m., and thereafter at intervals of two hours until 6.30 p.m.

On two test days 200 c.c. of water were given alone at 10.30 a.m., by means of the stomach tube. On the other two test days 10 grams of glycocoll and 10 grams of asparagin, respectively, dissolved in 200 c.c. of water, were given in a similar manner at 10.30 a.m. On all four days

the animal fasted. The distribution of total nitrogen ammonia and urea in the samples of urine thus obtained was investigated. The total nitrogen was estimated by Kjeldahl's method, the ammonia and urea by Folin's methods.

The results are detailed in Tables X and XI.

Table XI gives the total amount of urea nitrogen excreted in the eight hours following the ingestion of the 200 c.c. of water alone. The mean of this amount for the two fast days is subtracted from the amount of urea similarly excreted on the glycocoll and asparagin day respectively. In this way it is found that 96 per cent. of the ingested glycocoll nitrogen in the present experiment is excreted within eight hours in the form of urea, while 63 per cent. of the ingested asparagin is similarly excreted within eight hours. This result is in marked contrast with the results of the *in vitro* experiments, as in the latter ammonia is liberated more rapidly from asparagin than from glycocoll, whereas in the living organism glycocoll is apparently metabolised more rapidly than asparagin.

TABLE X

	Vol. of Urine	NH ₃ N gms.	Urea N gms.	Total N gms.	
Fast Day I—					
10.30—12.30 ...	95/100	0.06	0.386	0.578	
12.30—2.30 ...	40/60	0.032	0.199	0.231	
2.30—4.30 ...	70/80	0.033	0.419	0.532	
4.30—6.30 ...	20/50	0.028	0.291	0.353	
		0.150	1.295	1.694	T.N. excreted within 8 hours after ingestion of 200 c.c. water at 10.30 a.m.
Glycocoll Day—					
10.30—12.30 ...	38/110	0.032	0.747	0.856	
12.30—2.30 ...	40/100	0.026	0.910	0.988	
2.30—4.30 ...	28/100	0.029	0.766	0.855	
4.30—6.30 ...	22/100	0.029	0.709	0.781	
		0.116	3.132	3.480	T.N. excreted within 8 hours after ingestion of 10 g. Glycocoll in 200 c.c. water at 10.30 a.m.
Asparagin Day—					
10.30—12.30 ...	60/100	0.031	0.453	0.606	
12.30—2.30 ...	62/100	0.026	0.589	0.758	
2.30—4.30 ...	28/100	0.022	0.519	0.627	
4.30—6.30 ...	26/100	0.021	0.501	0.562	
		0.100	2.062	2.553	T.N. excreted within 8 hours after ingestion of 10 g. Asparagin in 200 c.c. water at 10.30 a.m.
Fast Day II—					
10.30—12.30 ...	85/100	0.022	0.389	0.434	
12.30—2.30 ...	45/100	0.017	0.344	0.383	
2.30—4.30 ...	44/100	0.016	0.335	0.399	
4.30—6.30 ...	23/100	0.007	0.280	0.305	
		0.062	1.348	1.521	T.N. excreted within 8 hours after ingestion of 200 c.c. water at 10.30 a.m.

TABLE XI

Nitrogen eliminated in 8 hours following ingestion of water

		NH ₃ N	Urea N	Resid. N	Total N	Urea in in excess of fast days	Percentage of ingested N	Gms. N in 10 gms. amino acid added
Fast Day 1	...	0.150	1.295	1.321	0.249	1.694	—	—
Fast Day 2	...	0.062	1.348		0.111	1.521	—	—
Glycocoll Day	...	0.116	3.132	0.232	3.480	1.811	96	1.86
Asparagin Day	...	0.100	2.062	0.391	2.553	0.741	63	1.2

CONCLUSIONS

In agreement with Lang, it has been found that emulsions of liver and intestinal mucosa, when incubated with asparagin, glycocoll, leucin, or alanin, liberate ammonia except in the case of alanin, and that the total amount of ammonia so liberated is much greater in the case of asparagin than in that of glycocoll or leucin.

On the other hand, the presence of an antiseptic does not exert the marked inhibition of the deamidizing enzyme claimed by Lang, i.e., the fresh organ, incubated without an antiseptic, shows no striking increase in its deamidization of glycocoll.

There is a marked contrast between the fate of the nitrogen of asparagin and glycocoll, respectively, *in vivo* and *in vitro*. In the living organism the nitrogen of glycocoll appears practically quantitatively at the end of eight hours in the urine as urea, while only about 63 per cent. of the nitrogen of asparagin so appears in the same time.

In vitro, however, ammonia is liberated more quickly and in larger amount from asparagin than from glycocoll, i.e., *in vitro* the amide nitrogen attached to the carboxyl group is more readily liberated than the amide group in the α position.

Altogether the results of the investigation indicate that the method of studying the deamidizing action of the tissues adopted by Lang is by no means representative of the intravital changes in the body, and that far-reaching conclusions based upon them are not justified.

In conclusion, I wish to express my best thanks to Professor Noël Paton for much helpful advice, and to Dr. E. P. Cathcart, at whose instigation and under whose direction the research has been carried out. The work was begun during my tenure of a McCunn Scholarship.

REFERENCES

1. Howell, *Amer. Journ. Physiol.*, Vol. XVII, p. 273, 1906.
2. Michaud, *Zeit. f. physiol. Chem.*, Vol. LIX, p. 405, 1909.
3. Abderhalden and Terruchi, *Zeit. f. physiol. Chem.*, Vol. XLVII, p. 159, 1906.
4. Stollte, *Hof. Beit.*, Vol. V, p. 15, 1904.
5. Salaskin, *Zeit. f. physiol. Chem.*, Vol. XXV, p. 125, 1898.
6. Flatow, *Zeit. f. physiol. Chem.*, Vol. LXIV, p. 267, 1910.
7. Friedmann, *Bioch. Zeit.*, Vol. XXVII, p. 97, 1910.
8. Neubauer and Fischer, *Zeit. f. physiol. Chem.*, Vol. LXVII, p. 230, 1910.
9. Abderhalden, Bloch and Rona, *Zeit. f. physiol. Chem.*, Vol. LII, p. 434, 1907.
10. Neubauer, Otto and Faltz, *Zeit. f. physiol. Chem.*, Vol. XLII, p. 81, 1904.
11. Jacoby, *Zeit. f. physiol. Chem.*, Vol. XXX, p. 149, 1900.
12. Loewi, *Zeit. f. physiol. Chem.*, Vol. XXV, p. 511, 1898.
13. Gonnermann, *Pfl. Archiv.*, Vol. LXXXIX, p. 493, 1902; Vol. XCV, p. 278, 1903.
14. Lang, *Hof. Beit.*, Vol. VIII.
15. Furth and Friedmann, *Bioch. Zeit.*, Vol. XXVI, p. 435, 1910.
16. Shaklee and Meltzer, *Amer. Jour. Physiol.*, Vol. XXIII, 1908; Vol. XXIX.
17. Kruger and Reich, *Zeit. f. physiol. Chem.*, Vol. XXXIX, p. 165, 1903.
18. Grafe, *Zeit. f. physiol. Chem.*, Vol. XLVIII, p. 300, 1906.
19. Brasch, *Bioch. Zeit.*, Vol. XXII, p. 402, 1909.
20. Schryver, *Bioch. Jour.*, Vol. I, p. 123, 1906.
21. Fischer and Abderhalden, *Zeit. f. physiol. Chem.*, Vol. XLVI, p. 52, 1905.
22. Bergell and Brugsch, *Zeit. f. physiol. Chem.*, Vol. LXVII, p. 97, 1910.
23. Horodynski, Salaskin and Zaleski, *Zeit. f. physiol. Chem.*, Vol. XXXV, p. 246, 1902.
24. Cohnheim, *Zeit. f. physiol. Chem.*, Vol. LIX, p. 239, 1909.
25. Mendell and Leavenworth, *Amer. Jour. Physiol.*, Vol. XXI, pp. 67 and 95, 1908.
26. Kowalevsky and Marchewicz, *Bioch. Zeit.*, Vol. IV, p. 196, 1907.
27. Levene and Kober, *Amer. Jour. Physiol.*, Vol. XXIII, p. 325, 1909.
28. Levene and Meyer, *Amer. Jour. Physiol.*, Vol. XXV, p. 214, 1909.