and R-enzymes could not be detected, but small traces of phosphorylase were present.

4. Maltose and maltotriose with some glucose are the main end products of the hydrolysis of amylose by these enzymes. Neither amylase hydrolyses these sugars, but maltotetraose is degraded by both enzymes.

5. A simple method for determination of α -amylase activity is described.

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The Free Amino-acids Occurring in the Body Tissues and Blood of the Rat and of the Cow

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Previous to the work reported here, few papers have been published showing the distribution of free amino-acids among the tissues of any one animal. Awapara, Landau & Fuerst (1950) have reported on the free amino-acids in rat tissues, while Gordon (1949) has investigated the intracellular fluid of calf embryo muscle and compared its free amino-acid composition with that of adult ox muscle and adult ox serum.

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EXPERIMENTAL

Total N. Micro-Kjeldahl estimations were carried out on the fresh tissue.

Preparation of protein-free filtrates. These were prepared from rat and cow tissues, blood and urine. The rats used in the experiments were of no specified age, but were mature males of approx. 400 g. body wt. They were slaughtered by dislocation of the neck and the organs removed as quickly as possible. Cow tissues were obtained from the slaughterhouse and were removed from the body as soon as possible after the death of the animal. They were then packed in ice for transport to the laboratory where they were immediately

minced and extracted. The whole process was always completed within 2 hr. of slaughter. For the preparation of protein-free filtrates the methods of Awapara (1948) and of Roberts & Frankel (1949) were used for cow and rat tissues respectively. It was necessary with such tissues as muscle, heart and lung in the cow to pass them through an ice-cold mincer before homogenization in the Waring Blender.

An examination of both blood cells and plasma was carried out in the following manner. Fresh blood was withdrawn from the dorsal aorta of the rat or the jugular vein of the cow, heparin being present in the receiving vessel to prevent clotting. The blood was centrifuged as soon as possible after collection. The plasma and cells were pipetted off separately and the cells haemolysed with an equal volume of distilled water. Proteins in the plasma and haemolysed cells were precipitated in several different ways. Ethanol precipitation was effected by treating 1 ml. of plasma with 10 ml. of 95% ethanol, allowing to stand for 15 min. and then centrifuging down the precipitate. The ethanol was removed from the supernatant by evaporation in a current of warm air. The residue was taken up in a known volume of water, desalted in the apparatus of Consden, Gordon & Martin (1947) and quantities equivalent to 250 μ l. of blood cells and plasma were applied to chromatograms. Ultrafiltration and tungstic acid precipitation (Hier & Bergeim, 1945) were also tried. On comparing the results obtained by these three methods the ethanol precipitation was found to produce the cleanest chromatograms. However, it appeared that with this method of precipitation losses of amino-acids occurred and glutathione especially was in smaller concentration when compared with ultrafiltration.

Urine was collected from cows in milk and young calves still receiving an all-milk diet. A few samples were also obtained from heifers of 6 and 12 months of age. Provided a small enough quantity (about 15 μ l.) was placed on the chromatogram, no interference from salts was encountered.

Chromatographic techniques. Methods of chromatography were similar to those described by Dent (1948). One-dimensional chromatograms were run in butanol-acetic acid on Whatman no. 4 paper. Markers of histidine, tyrosine and arginine were run side by side with various concentrations of the extract. The paper was then dried and the presence of histidine, tyrosine and arginine established by means of specific colour reactions.

Whatman no. 1 papers (20 $\times$ 20 cm.) were used for small chromatograms with the frame of Datta, Dent & Harris (1950). Two-dimensional runs in phenol and the collidine mixture (collidine/lutidine/water: 1/1/1) were carried out, with NH_3 in the phenol tank and diethylamine in the collidine tank. Various concentrations of the extract equivalent to 0.5, 1.0 and 1.5 mg. N of the fresh tissue were applied to chromatograms. The squares were then sprayed with 0.1% ninhydrin in *n*-butanol and heated at 100° for 1 min.

Large two-dimensional chromatograms were run on Whatman no. 4 (18 $\times$ 22 in.) paper in phenol and collidine mixture, the phenol developing along the 18 in. edge and the collidine/lutidine following at right angles in the longer direction. A quantity of extract, equivalent to 5 mg. N in fresh tissue was applied.

Hydrolysis of extracts. The extract was hydrolysed with 6N-HCl for 15 hr. at 100° in a closed glass phial. The hydrolysate was taken to dryness in a current of hot air, taken up

in a known volume of water and desalted. Quantities of extract equivalent to 0.5, 1.0 and 1.5 mg. N in the fresh tissue were then run on small chromatograms.

Oxidation of extracts. The extract was oxidized according to the method of Dent (1948). Quantities of extract equivalent to 0.5, 1.0 and 1.5 mg. N of the fresh tissue were then run on small chromatograms.

Fig. 1. 'Map of spots' showing those substances reacting with ninhydrin and occurring in the various biological extracts.

A	Glycine	Q	Proline
B	Serine	R	γ -Aminobutyric acid
C	Glutamic acid	S	Lysine
D	Aspartic acid	T	Histidine
E	α -Alanine	U	Ethanolaminephosphoric acid
F	Threonine	V	Cystine
G	Glutamine	W	Unknown—above left of taurine
H	β -Alanine and glyceryl-phosphorylethanol-amine	X	'Fast-phenol'
I	Taurine	Y	Carnosine
J	α -Aminobutyric acid	Z	'Cow-spot'
K	Valine	2	Cysteic acid
L	Leucines	3	Glutathione
M	Phenylalanine	4	Unknown—left of α -alanine
N	Tyrosine	5	Chloride
O	Methionine sulphone	6	Arginine
P	Ethanolamine		

RESULTS

Table 1 shows those amino-acids which were found in the separate tissues of the rat and the cow after the various treatments, while Fig. 1 gives a map of the amino-acids and other ninhydrin-reacting substances.

An examination of the results obtained with the rat tissues reveals that the following ninhydrin-reacting substances occur in the free state in all tissues: glycine, glutamic acid, aspartic acid, α -alanine, glutathione, glutamine, taurine, leucines, serine, lysine, valine, proline, threonine, tyrosine,

Table 1. *Free amino-acids in an ethanol extract of rat and cow tissues*

(* = after hydrolysis of ethanol extract; † = after oxidation of ethanol extract; + + +, dense; + +, medium; +, small; ?, doubtful; -, none.)

	Brain	Lung	Heart	Liver	Muscle	Kidney	Spleen	Blood cells	Blood plasma
Rat									
Glycine	+++	+++	++	+++	+++	+++	+++	+++	+++
α-Alanine	++	++	++	+++	+++	++	++	++	++
Serine	++	+	+	+	++	++	++	++	++
Threonine	+	+	+	+	+	+	+	+	++
Valine	+	+	+	+	+	++	++	++	++
Leucines	+	+	+	+	+	++	++	++	++
Phenylalanine	+	+	+	+	+	+	+	+	++
Tyrosine	?	++	?	+	++	+	++	+	+
Proline	+	+	+	+	+	+	+	+	+
Aspartic acid	+	++	++	++	+	++	++	++	++
Glutamic acid	+++	++	+++	++	++	+++	+++	++	++
Histidine	+	+	+	++	++	+	?	+	-
Arginine	-	-	-	-	-	??	-	+	+
Lysine	+	+	+	+	+	+	+	++	++
β-Alanine	+	+	+	??	+++	++	+	-	+
α-Aminobutyric acid	-	-	-	+	-	-	-	+	+
γ-Aminobutyric acid	++	+	+	+	-	??	??	+	+
Taurine	+++	+++	+++	+++	+++	+++	+++	+	+
Methionine sulphone	-	++	++	-	-	++	++	++	++
Cysteic acid	++	++	++	++	-	++	++	++	-
Glutamine	+++	++	+++	+++	+++	++	++	+	+
Glutathione	+++	++	+	+++	++	++	++	++	-
Histamine	-	-	-	-	-	-	-	-	-
1-(3)-Methylhistidine	-	-	-	-	+++	-	-	-	-
Carnosine	-	-	-	-	+++	-	-	-	-
Ethanolamine	-	-	-	+	-	-	-	??	-
Ethanolaminephosphoric acid	++	++	+	++	?	+++	+++	-	-
Glycerolphosphorylethanolamine	-	-	-	++	-	-	-	-	-
Unknown—left of α-alanine	-	-	-	-	-	-	-	-	-
Unknown—above left of taurine	-	-	-	-	-	-	-	-	-
Cow									
Glycine	++	+++	++	+++	++	+++	+++	+++	+++
α-Alanine	++	++	+++	+++	+++	++	++	++	++
Serine	++	++	++	++	++	++	++	++	++
Threonine	++	-	-	-	-	+	++	+	+
Valine	++	++	++	++	++	++	++	++	++
Leucines	++	++	++	++	++	++	++	+++	++
Phenylalanine	+	+	++	-	-	+	+	+	+
Tyrosine	++	++	++	+	-	++	+	+	+
Proline	+	++	+	+	+	++	+	++	+
Aspartic acid	++	++	++	++	++	++	++	+	+
Glutamic acid	+++	++	+++	+++	++	+++	+++	++	++
Histidine	++	+	++	++	++	++	+	++	++
Arginine	-	-	-	-	-	+	?	-	+
Lysine	+	++	++	+	++	++	+	++	+
β-Alanine	+	+	++	++	++	+	+	++	+
α-Aminobutyric acid	+	+	-	++	+	+	+	+	++
γ-Aminobutyric acid	++	++	+	+	-	+	+	+	+
Taurine	++	++	++	++	++	++	++	+	++
Methionine sulphone	++	-	++	++	++	++	++	++	++
Cysteic acid	++	-	-	++	-	++	++	++	-
Glutamine	+++	+	+++	++	++	+	++	+	++
Glutathione	++	++	++	++	++	++	++	++	-
Histamine	-	-	+	+	-	-	-	-	-
1-(3)-Methylhistidine	-	-	-	-	++	-	-	-	-
Carnosine	-	-	-	-	++	-	-	-	-
Ethanolamine	+	-	-	++	-	+	+	-	-
Ethanolaminephosphoric acid	++	++	++	++	+	++	++	+	-
Glycerolphosphorylethanolamine	+	-	-	++	-	++	+	-	-
Unknown—left of α-alanine	-	-	-	-	+	-	-	-	-
Unknown—above left of taurine	+	-	+	++	+	+	+	++	++

histidine, phenylalanine and ethanolaminephosphoric acid.

The cow tissues show several unknown spots and in muscle a ninhydrin-positive substance appears to the left of α -alanine. This may be identical with the spot which Gordon (1949) has reported as methionine sulphoxide. However, it withstood oxidation with H_2O_2 in the presence of ammonium molybdate, while Dent (1948) has stated that methionine sulphoxide will be converted into the sulphone under these conditions. After hydrolysis of a muscle extract a green spot in the position of methylhistidine was present. This would suggest that the peptide anserine was present in the ethanol extract, though owing to the large amount of carnosine present this would not be seen. Zapp & Wilson (1938) have demonstrated the presence of both anserine and carnosine in ox muscle and have shown that there is almost twice as much carnosine as anserine in most muscles.

In cow liver the unknown spot (Fig. 1, spot *H*) appears to be the same as that demonstrated by Campbell (1951) in pig liver. On hydrolysis of the latter, an ethanolamine spot appears, and the unknown has been identified as glycerylphosphoryl-ethanolamine (Campbell & Work, 1952). This material was also seen in small amounts in brain, kidney and spleen. Brain tissue showed a large amount of γ -aminobutyric acid. Although this amino-acid also occurs in other tissues it does not do so in such relatively large quantities. In the spleen tissue examined there appeared to be some relationship between threonine and the unknown spot situated in a position above-left of taurine. Sometimes on the small chromatograms a threonine spot was visible, yet when the same material was run on a large chromatogram the unknown spot was seen and no threonine spot. It is not yet known whether it is threonine varying its position in the collidine mixture or whether the threonine is changed during running to a different form. The unknown spot was seen on both hydrolysed and unhydrolysed extracts and has been noted on both large and small chromatograms.

A small sharply defined spot was often seen on the bottom left-hand corner of the chromatogram, (Fig. 1, spot *X*) on the oxidized and ethanol extract squares. It may be referred to as 'fast-phenol' as it moves very little in the collidine mixture. It has been found in most tissues and in blood, but has not been seen after hydrolysis. It may be identical with the 'nephrosis-peptide' mentioned by Dent (1948) but has not been further studied. It has been noted in spleen, brain, liver, kidney, heart, blood cells and plasma but not in lung and muscle extracts. There is also a commonly occurring purple spot in position 5 (Fig. 1). This is due to the presence of chloride in the extract, which reacts with the diethylamine in the

collidine tank and then gives a positive ninhydrin reaction.

The Pauly reaction with lung tissue extract showed a strong spot for tyrosine with only a weak spot for histidine. This was in direct opposition to the general finding for all other tissues tested and for blood cells and plasma, where histidine was present in greater amount. Table 2 has been constructed giving numerical values to the depth of colour obtained with equivalent amounts of different tissues, as judged by eye. A further spot given by the Pauly reaction in heart and liver was probably due to histamine.

Table 2. *Concentration of histidine and tyrosine in blood and tissues of the cow*

(Values comparative, as judged by eye.)

	Histidine	Tyrosine
Muscle	10	—
Spleen	1	1
Heart	5	2
Brain	6	1
Liver	2	2
Kidney	2	2
Lung	1	6
Blood cells	6	1
Blood plasma	1	1

The main difference noted between blood cells and plasma was the absence of glutathione in the latter. Ethanolaminephosphoric acid was present in traces in the cells but was not seen in plasma. Different samples of blood showed variation in the proportions of different amino-acids. Taurine was sometimes present only in traces in the cells and plasma, while at other times there were high concentrations. Aspartic acid always seemed to be present in small amount.

Undesalted cow urine gave two ninhydrin-reacting spots with 20 μ l. of urine. Glycine appeared as a weak spot with an unknown spot, to be termed 'cow-spot', in a position on the chromatogram above phenylalanine and giving a purple colour (Fig. 1, spot *Z*). On desalting the urine this unknown spot disappeared and the glycine spot appeared larger in size and deeper in colour. Attempts were made to concentrate the spot by cutting it out from large sheets, but the material was extremely labile and often disappeared during the process. When calf urines were examined, the 'cow-spot' was not present but the urine contained the following amino-acids: glycine, glutamic acid, taurine, α -alanine and glutamine. Aspartic acid was found in some calf urines. The urine of 6- and 12-month-old heifers contained the 'cow-spot' so that it is probably of dietary origin, its absence from the calf urine being due to the all-milk diet. The 'cow-spot' was present in all cow urines analysed, but owing to its lability it was not studied from the point

of view of breed of animal, time of year when it appeared, effect of diet, etc. When the urine was stored for some time after collection the 'cow-spot' again disappeared and the glycine spot only was present.

DISCUSSION

Although many individual tissues have been examined for their free amino-acids only two papers have come to the notice of the author where several different tissues of any one animal have been studied. The paper by Awapara *et al.* (1950) on the free amino-acids of rat tissues includes many results which are at variance with the work reported here. It is unnecessary to discuss all the differences, but it may perhaps be relevant to mention the most widely divergent results. Awapara *et al.* (1950) detected no serine, valine, lysine, histidine or carnosine in muscle. They did, however, find methionine and hydroxyproline. It is noticeable that they detected no leucines in any tissue, but found methionine in all of them. One may suppose that they have mistaken the leucine spot for methionine, for both amino-acids run to almost identical positions on a phenol-collidine chromatogram. The methionine can only be definitely identified by oxidizing it to its sulphone (Dent,

1948). Awapara *et al.* also state in their paper that the appearance of β -alanine and histidine upon acid hydrolysis of liver and skeletal-muscle extracts points to the existence of carnosine in these organs. It is possible that on the chromatograms of the unhydrolysed extract they have mistaken the carnosine for hydroxyproline since both give brownish yellow spots with ninhydrin, though the carnosine spot will go green in colour if heated to 105° (Dent, 1948).

The analyses of Gordon (1949) of ox muscle and ox serum agree quite satisfactorily with the results obtained in the present work. Arginine, tyrosine and phenylalanine were, however, not detected in the concentration of muscle extract applied to the chromatograms. Gordon also found tryptophan in the ox serum which he examined. This amino-acid was not recognized in the present work in any tissue. It is possible that loss of tryptophan occurred at some stage during the preparation of the extract.

SUMMARY

The free amino-acids occurring in various tissues and body fluids of the rat and cow have been detected using the technique of paper partition chromatography.

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The Fate of Intravenously Administered Coproporphyrin III in Normal and Lead-treated Rabbits

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Abnormally large amounts of coproporphyrin, mainly of type III, are usually found in the urine of mammals to which salts of lead have been administered. A three-carboxyl porphyrin has also been observed (Nicholas & Rimington, 1951; Weatherall & Comfort, 1952), and the latter authors have found evidence of a porphyrin containing five carboxyl groups. The quantity of coproporphyrin in the

faeces is little or not at all increased, at least in man (Kark & Meiklejohn, 1941) and rabbits (Fischer & Duesberg, 1932; Weatherall, 1948 and unpublished observations; Schwartz & Zagaria, 1951), and the coproporphyrin which is present is predominantly of type I (Dobriner, 1937*a*; Vigliani & Libowitzky, 1937; Watson, 1937), as in normal subjects. The abnormal excretion of coproporphyrin is therefore