roots is inhibited by dinitrophenol; and that the uptake of chloride by carrot disks is inhibited by dinitrophenol and other respiratory inhibitors (Robertson, Wilkins & Weeks, 1951). In addition, these latter workers showed that the release of electrolyte accumulated by slices of carrot is increased by dinitrophenol. There are, therefore, close similarities between the uptake of inorganic ions in these systems and the uptake of amino acids by slices of carrot. Further, potassium chloride and potassium phosphate inhibit the uptake of L-histidine by this tissue. It is, therefore, possible that in carrot tissue inorganic ions and amino acids are taken up by the same mechanism. In contrast, Arisz (1953) found that phosphate does not interfere with the movement of asparagine along the tentacles of *Drosera*.

The carrot and bacterial systems seem to remove amino acids completely from the external medium, whereas the animal systems establish a concentration difference with a steady state between amino acids in the tissue and the medium. Further, the plant system studied above does not show the same high specificity for uptake of the L-isomers of the amino acids as do certain animal systems (Agar *et al.* 1954; Christensen, Riggs, Fischer & Palatine, 1952). For this reason there seems to be a difference between plant and animal systems.

SUMMARY

1. The conditions determining the uptake of amino acids by slices of carrot have been investigated.

2. Slices of carrot take up amino acids from the medium and establish a large concentration gradient in favour of the tissue. The process of uptake of both D- and L-isomers is inhibited by 2:4-dinitrophenol and by cyanide. The process, therefore, requires energy from respiration.

3. The rate of uptake of L-histidine was greater than that of D-histidine, although for phenylalanine, valine and leucine there was usually no significant difference between the amounts of the optical isomers taken up during 5 hr.

4. The inorganic salts potassium chloride and potassium phosphate (solutions at pH 6.0–8.0) inhibited the uptake of L-histidine. L-Histidine and L-arginine mutually inhibit the uptake of each other.

One of us (L.M.B.) held the Sir John and Lady Higgins Research Scholarship from the University of Melbourne during the course of this investigation.

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The Volatile Fatty Acids of Bovine-Muscle and -Liver Phospholipids

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It has long been known that C₄–C₁₂ volatile fatty acids with an even number of carbon atoms normally occur to the extent of approximately 6% by weight in the glycerides of milk fats (Hilditch, 1947), and more recently [James & Popják, unpublished observations quoted by Popják (1952)] this series has been extended to include acetic acid. Larger amounts of C₂–C₆ saturated volatile fatty acids

(12.9% by weight) occur in the triglycerides of the female reproductive organs of *Ascaris lumbricoides* (Fairbairn, 1955).

The development of a suitable micro-analytical technique for determining volatile fatty acids (James & Martin, 1952) has made possible the detection of smaller amounts of these acids in lipids from other sources, and it has already been shown

that acetic and propionic acids are present in the glyceride fat of liver [James & Popják, unpublished observations quoted by Popják (1952)]. A consecutive series of volatile fatty acids from C_2 to C_{10} has been found in ox perinephric fat (Hansen & McInnes, 1954), and with analyses of mutton fat this series has been extended to include formic, isobutyric, isovaleric and α -methylbutyric acids (McInnes, Hansen & Jessop, 1956).

Available analyses of animal phospholipids (Hilditch, 1947) indicate that the component fatty acids are 16 carbon atoms or more in chain length, although small amounts of *n*-tetradecanoic and *n*-tetradecenoic acids were found in ox-liver phospholipids (Hilditch & Shorland, 1937). However, Stevens & Chaikoff (1951) fed ^{14}C -labelled dodecanoic and tetradecanoic acids to rats and found that these fatty acids can be incorporated into the phospholipid molecule. An analysis of bovine-muscle phospholipids (Hawke, 1955) showed the presence of small amounts of volatile fatty acids, and this work, now described in detail, has been extended to include the volatile fatty acids of bovine-liver phospholipids.

EXPERIMENTAL

Methods

Total nitrogen. Total N was determined by the micro-Kjeldahl method described by Ma & Zuazaga (1942).

Phosphorus. Phosphorus was determined after digestion with $HClO_4$ by the method of Allen (1940).

Amino nitrogen. Amino N was determined in the acid hydrolysate by the Van Slyke manometric method after removal of the fatty acids by extraction with light petroleum.

Choline. Choline was determined by the colorimetric method described by Entenman, Taurog & Chaikoff (1944) after hydrolysis of the fat with saturated $Ba(OH)_2$ (Glick, 1944).

Sphingosine and dihydrosphingosine. Sphingosine and dihydrosphingosine were determined according to the method of McKibbin & Taylor (1949b) except that the acid hydrolysate of the phospholipid was evaporated to dryness under vacuum before extracting the sphingosine and dihydrosphingosine with $CHCl_3$. The $CHCl_3$ extract was then washed several times with water and the water washings were added to the hydrolysate insoluble in $CHCl_3$. Clear $CHCl_3$ extracts were obtained without recourse to centrifuging. Sphingosine and dihydrosphingosine were determined in the $CHCl_3$ extract as N.

Volatile fatty acids. These were determined by the chromatographic procedure of James & Martin (1952) after hydrolysis of the phospholipids.

Muscle phospholipid (24.22 g.) was hydrolysed by refluxing in a stream of N_2 with 100 ml. of ethanol containing 1% (v/v) of H_2SO_4 , and then with approx. 13% (w/v) KOH in ethanol, for 3 and 5 hr. respectively. After removal of the unsaponified material by extraction with ether, the hydrolysate was acidified with dilute H_2SO_4 and the volatile fatty acids were separated from the higher fatty acids by steam-distillation, 13.5 g. of non-volatile fatty acids remaining.

The ethanol used in the hydrolyses had been refluxed with KOH and freshly distilled. The steam-volatile material was neutralized with 2.17 m-equiv. of alkali and the soaps were concentrated to approx. 1.0 ml., but owing to the presence of higher fatty acids the soaps became solid at room temperature. The soaps were redissolved in water, $MgSO_4$ was added, and the mixture acidified and redistilled by the procedure suggested by Friedemann (1938). The volatile fatty acids were neutralized with 0.1 N-NaOH, and when the soaps were concentrated to 1.0 ml. they remained in solution at room temperature. In each analysis 0.1 ml. of soap was acidified and applied to the gas-liquid chromatogram (see Table 5) by the method suggested by James & Martin (1952).

Liver phospholipid (37.0 g.) was hydrolysed in the above manner, and after removing the ethanol the unsaponified material (0.3 g.) was extracted with ether in the usual way. The soaps were acidified with dilute H_2SO_4 and steam-distilled. The steam distillate (approx. 1 l.) required 1.78 m-equiv. of alkali for neutralization; 1.22 m-equiv. of alkali was required after a Friedemann distillation had been carried out.

In chromatographing the volatile fatty acids from liver phospholipids a different technique was used in applying the acids to the column. The sodium soaps of the volatile fatty acids were concentrated to 7.0 ml., and 2.5 ml. of this soap solution was evaporated to dryness at 90° in an extraction vessel (McInnes, 1956). Sodium-dried and distilled ether (2 ml.) was added to the dry soaps, which were then acidified with 0.3 ml. of 4N- $KHSO_4$. Anhydrous Na_2SO_4 (1.0 g.) was added, the mixture thoroughly ground, and the ether decanted into a 5 ml. flask. Extractions with smaller volumes of ether were repeated several times, with grinding at each extraction, and the extracts made up to 5 ml.

Portions of the ether extract were applied to a modified chromatogram tube (McInnes, 1956) by means of an Agla micrometer syringe. Formic and acetic acids were determined with 0.15 ml. of the ether extract, and the other volatile acids were determined with 0.5–1.0 ml. of the ether extract.

The columns, packed into a tube of 2 mm. internal diameter, consisted of 5.0 g. of Celite 545 (Johns Manville Co. Ltd.) with 2.0 g. of silicone MS550 (Hopkin and Williams Ltd.) containing 0.2 g. of stearic acid as the liquid phase. The acids from formic to valeric were determined at 100°, and the acids from α -methylbutyric to nonanoic were determined at 137°.

The failure to detect volatile fatty acids higher than C_6 was probably due to the trouble encountered when operating the chromatograms for long periods at 137°. The columns were not stable at this temperature, and owing to loss of stearic acid from the columns the indicator solution in the titration cell became extremely turbid after several hours. This affected the sensitivity of the titration of the volatile fatty acids with higher molecular weights in particular, and this fact, together with the loss of column efficiency, would probably mean that small amounts of acid would not be titrated. In an attempt to overcome this, the stearic acid in the liquid phase was replaced by arachidic acid, and although much less arachidic acid was stripped from the column and the titration cell became much less turbid, the resolving power, as measured by the separation of C_2 – C_3 acids, had decreased at the conclusion of a 14 hr. run, and consequently arachidic acid was not used in these experiments. It appears that loss of acid from the liquid phase is

not the only reason for the lower resolving power of the column after continuous operation at 137° for longer than 10 hr.

Materials

Preparation of bovine-muscle phospholipid. Muscle (2085 g.) from the hind-quarter of a Jersey cow was trimmed free of all visible fat, finely minced and added to approximately 15 l. of boiling ethanol. This stage was reached approximately 1 hr. after slaughter.

The tissue was dried with ethanol and extracted with ethanol-ether mixtures (3:1 and 1:3, v/v) in a Soxhlet-type glass extractor. After these extractions the dry residue was ground in a Waring Blender and re-extracted with ethanol-ether (1:3, v/v) for three days, which removed only 3.0 g. of material. The total extracts were combined and the solvent was removed. Ether was added to the extract and the warmed solution filtered through a Whatman no. 4 paper in a Büchner funnel. The soluble material weighed 734 g. The solvents used in the extraction and purification procedures had been purified in the laboratory. As far as possible operations were carried out in an atmosphere of N₂ and the extracted lipids kept in a vacuum between operations. Solvents saturated with N₂ were used in the chromatographic procedures and the columns were operated under a slight N₂ pressure.

Purification of lipid extract. Numerous washing procedures designed to remove impurities soluble in water from the solvent extracts of tissue lipids are in common use, and the efficiency of one of these, that is, washing a solution of the crude extract in light petroleum several times with water (Shorland, Bruce & Jessop, 1952), was compared with two chromatographic methods which have been found effective for egg-yolk phospholipids (Lea & Rhodes, 1953). In the latter methods the impurities in the phospholipid were removed on a column of cellulose or cation-exchange resin (Zeo-Karb 215, the Permutit Co. Ltd., London). An impure phospholipid from bovine muscle, prepared in this laboratory, was applied to the columns as a 10% (v/v) CHCl₃ solution and the columns were eluted with CHCl₃ (80 vol.) equilibrated with ethanol (20 vol.) and water (2.5 vol.).

Table 1 shows that partitioning between non-aqueous and aqueous phases on a cellulose column lowered the N:P ratio considerably more than extraction with water, although in subsequent experiments the N:P ratio of a different preparation of muscle phospholipid (Table 4) was not lowered to the same extent. The lower ratio after passing the crude phospholipid through a cation-exchange column may indicate that some decomposition has taken place, which is, perhaps, not unexpected, having regard to the acidic nature of the column. Assuming for the purpose of calcula-

tion that the N impurity (approximately 0.8%) was present as alanine, this would account for 5% of the 7% weight loss recorded in the purification by a cellulose-column. The weight loss, therefore, corresponded approximately to the loss of contaminants. In later experiments the CHCl₃-ethanol-water mixture was replaced by CHCl₃ equilibrated with water (Smith, 1954).

The presence of amino acids as impurities in the extracted phospholipids was demonstrated by means of paper chromatography with the water-poor phase of CHCl₃-ethanol-water (80:20:2.5, by vol.) as solvent (Bevan, Gregory, Malkin & Poole, 1951). The amino acids were detected as slow-moving spots by spraying with ninhydrin in butanol. These spots were not present when phospholipids that had been purified by cellulose were chromatographed on paper.

Separation of glycerides and phospholipids. The ease of separation of glycerides and phospholipids varies according to the origin of the material, and it is possibly dependent upon the nature of the component fatty acids present. Whereas in the separation of glycerides and phospholipids in egg lipids, fractional precipitation at 0° in acetone and ethanol saturated with MgCl₂ is effective (Hawke & Lea, 1953), in the present experiment a greater number of precipitations resulted only in a partial fractionation. A further separation of glyceride and phospholipids was obtained with silicic acid.

(a) *Acetone fractionation.* First, successive acetone separation made in the usual manner (cf. Shorland *et al.* 1952) gave two practically pure phospholipid and glyceride fractions (P, 3.9 and 0.03%, respectively) and a large mixed fraction containing approx. 8% of phospholipid (P, 0.32%) (Table 2).

Further phospholipid was precipitated from an acetone solution of this mixture of glyceride and phospholipid by adding a few ml. of ethanol saturated with MgCl₂ (cf. Fairbairn, 1945). The Mg²⁺ and Cl⁻ ions were removed from the precipitate by passing the lipid through a Bio-Deminolite column (2 parts of De-Acidite FF and 1 part of Zeo-Karb 225). The solvent used was CHCl₃-water-ethanol (1:3:7, by vol.) (Macpherson, 1954). In the present case, however, not all the lipid precipitated by MgCl₂ was soluble in this solvent. The insoluble lipid was found to be almost pure glyceride and was added to the final glyceride fraction.

The three fractions obtained from acetone fractionation weighed 107.4, 44.0 and 479.5 g. and contained 3.90, 2.23 and 0.14% of P, respectively.

(b) *Separation on silicic acid columns.* The fraction containing 2.23% of P (44.0 g.) obtained from the above fractionation with acetone was passed through a column of activated silicic acid (chromatographic grade, L. Light and Co. Ltd.) according to the method of Borgström (1952) and

Table 1. *Purification of muscle phospholipids*

See Materials section for details.

Method	Before purification			After purification			Wt. recovered (%)
	N (%)	P (%)	N:P atomic ratio	N (%)	P (%)	N:P atomic ratio	
Extraction with water	2.51	3.62	1.53	2.47	3.65	1.46	99.8
Cellulose column	2.51	3.62	1.53	1.73	3.79	1.01	93.0
Cation-exchange resin	2.51	3.62	1.53	1.46	3.68	0.88	93.7

was separated into two fractions containing 1.03% of P (18 g.) and 3.04% of P (26 g.). The latter fraction was added to the 'phospholipid' fraction obtained from the acetone fractionation, and this combined 'phospholipid' fraction was subsequently purified on silicic acid prepared in the laboratory from sodium silicate (Isherwood, 1946).

In one typical purification 28.12 g. of the phospholipid was added as a 10% solution in CHCl_3 to a column containing 230 g. of silicic acid, and allowed to enter the column. A small volume of CHCl_3 was used to wash down the walls of the glass tube before elution was commenced. In order to obtain reasonable flow rates (1–2 ml./min.) 50 g. of Celite

Table 2. *Acetone fractionation of the glycerides and phospholipids of bovine muscle*

Insoluble

'Phospholipid'
(107.4 g., 3.9% of P)

Soluble

561.5 g. (0.309% of P)
cooled to 0°
in acetone

Insoluble in acetone at 0°
sol. at 20° (85.9 g., 0.03% of P)

Soluble (0.32% of P)
6 l. of acetone
+ 6 ml. of satd. MgCl_2
in ethanol

Insoluble

Bio-Deminrolit
column

Mixed fraction
(44.0 g., 2.23% of P)

Soluble

3 l. of acetone
+ 6 ml. of satd. MgCl_2

Insoluble

Insoluble

Soluble
(0.216% of P)
2 l. of acetone
+ 6 ml. of satd. MgCl_2
in ethanol

Insoluble

Insoluble

Soluble
(393.6 g.,
0.16% of P)

'Glyceride'
(479.5 g., 0.14% of P)

545, which had been washed with conc. HCl, was mixed with the silicic acid, and the column packed as a slurry in CHCl_3 . The column was operated under a slight pressure of N_2 . Elution of the column with CHCl_3 gave 3.08 g. of lipid (10.97% of the original sample). Further elution with methanol gave 24.22 g. of lipid (86.1% of the original sample). No phosphorus was detected in the CHCl_3 eluate, and the methanol eluate contained 3.91% of P. The final

product was a golden-brown solid which gave at room temperature a slightly cloudy solution with light petroleum and a clear solution with ethyl ether.

Preparation of bovine-liver phospholipid. Trimmed bovine liver (6575 g.) from a 5-year-old Aberdeen Angus-Hereford ox that had been fasted for several days was finely minced and placed in 11 l. of boiling ethanol. This stage was reached approximately 1 hr. after slaughter. Refluxing was

Table 3. *Scheme for the fractionation of the glycerides and phospholipids of bovine liver*

continued for 1 hr., and then the ethanol was removed under vacuum. After cooling, the partially dried liver was re-minced.

Twelve solvent extractions were made. At each stage the minced liver was shaken with solvent at room temperature for approximately 4 hr., and then allowed to stand overnight. The solvent was separated from the liver tissue by filtration through Whatman no. 1 paper in a Büchner funnel. The first four extractions were made with 95% ethanol (approx. 3 l.), the next three with ethanol-ether (3:1, v/v), the next three with ethanol-ether (1:3, v/v) and the final two with ether alone.

The extracted lipid was purified on a cellulose column in an atmosphere of N_2 with $CHCl_3$ equilibrated with water as eluting solvent. This purification reduced the N content from 2.29 to 1.69% and the N:P atomic ratio from 2.19 to 1.61.

Separation of glycerides and phospholipids. The procedure adopted was similar to that used to separate glyceride and phospholipids from bovine muscle, except that the use of $MgCl_2$ in acetone was discontinued. The scheme in Table 3 outlines the method used for the separation of glycerides and phospholipids in bovine liver. This method was adopted in the light of experience gained in the separation of glycerides and phospholipids in bovine muscle. The final product was a brown solid which had a solubility similar to that of muscle phospholipid in both light petroleum and ethyl ether.

RESULTS AND DISCUSSION

Composition of the bovine-muscle and bovine-liver phospholipids

The muscle tissue consisted of 3.2% of lipid, of which 0.57% was phospholipid, whilst the bovine liver had 6.9% of lipid, of which 3.99% was phospholipid. Table 4 gives the composition of the phospholipids in terms of the components containing nitrogen which were obtained after hydrolysis.

It can be calculated that phosphatidylcholine, phosphatidylethanolamine plus phosphatidylserine, and sphingomyelin formed, respectively, 56, 32 and 12 moles % of the identified nitrogen of the muscle phospholipid; the same components formed, respectively, 58, 24 and 18 moles % of the identified nitrogen of the liver phospholipid. The nature of the 'excess' nitrogen is not known, but it cannot be amino nitrogen and therefore would probably not be detectable with a ninhydrin spray.

The only figures available for the phospholipids of bovine muscle (Kaucher, Galbraith, Button & Williams, 1943) give 55.9–57.8% for lecithin and 36.4–37.4% for 'cephalin'. In livers of dogs (McKibbin & Taylor, 1949*a*) and rats (Artom, 1945) the choline N:total N ratios were 0.449–0.639.

Volatile fatty acids of muscle and liver phospholipids

In muscle phospholipids the normal volatile fatty acids from C_1 to C_8 (excluding C_7) and isovaleric acid were present (Table 5), whereas in liver phospholipids the normal acids from C_1 to C_9 , isobutyric, isovaleric, α -methylbutyric and an unidentified acid, having an R_f value between those of n -hexanoic and n -heptanoic acids, were present (Table 6). The volatile fatty acids formed a larger proportion of the total fatty acids in liver than in muscle, and this may account for the wider range of acids identified in the former. The volatile fatty acids comprised 0.68 and 1.28 moles % of the total fatty acids in muscle and liver phospholipids, respectively.

In the present work, as well as in several recent studies, e.g. on blood lipids (Annison, 1954) and depot fat (McInnes *et al.* 1956), an appreciable proportion of the volatile fatty acids is formic acid (28.7 moles % and 24.7 moles % in liver and muscle, respectively).

Animal glycerophospholipids from many species and tissues are characterized by the presence of appreciable and often very high proportions of C_{20} and C_{22} highly unsaturated acids, e.g. see Lovern & Olley (1953), whereas these acids frequently appear to be absent from the corresponding depot fats. Moreover, Sinclair (1935) demonstrated the presence of a selective mechanism in rats for the incorporation and retention of highly unsaturated fatty acids in phospholipids.

Hilditch & Maddison (1941) concluded that volatile fatty acids were absent from the phospholipids of milk fats, whereas it was already known that appreciable proportions of volatile fatty acids are present in the fatty acids of the triglycerides. It appeared probable, therefore, that the low-molecular-weight acids were selectively excluded from

Table 4. *Composition of bovine-muscle and bovine-liver phospholipids*

Phospholipid source	N (%)	P (%)	N:P atomic ratio	Choline N (%)	Choline N Identified N
Bovine muscle	2.38	3.91	1.35	1.04	0.62
Bovine liver	2.26	3.10	1.61	1.02	0.67
Phospholipid source	Amino N (%)	Amino N Identified N	Sphingosine N (%)	Sphingosine N Identified N	
Bovine muscle	0.54	0.32	0.11	0.06	
Bovine liver	0.36	0.24	0.13	0.09	

the animal phospholipids. However, the present work shows that the amounts of volatile fatty acids in animal phospholipids and animal depot fats (McInnes *et al.* 1956) are of the same order. It is already known (Stevens & Chaikoff, 1951) that dodecanoic and tetradecanoic acids can be incorporated into tissue phospholipids when fed to rats. This shows that these acids can be utilized in the synthesis of phospholipids, and now it has been found that trace amounts of the volatile fatty acids do in fact form natural constituents of the tissue phospholipids. It is probable that a reinvestigation of the phospholipids of milk fat by the techniques now available would establish the presence of volatile fatty acids.

In considering the possible source of the volatile fatty acids in ruminants, Annison & Pennington

(1954) working with sheep's rumen have shown that volatile fatty acids present in the rumen can be absorbed into the blood, and it is presumed that these acids will be, in part at least, utilized in the tissues.

SUMMARY

1. Animal lipids have been separated into glycerides and phospholipids by acetone precipitation and chromatographic methods.

2. It has been shown by means of gas-liquid chromatography that the volatile fatty acids comprise 0.68 and 1.28 moles % of the total fatty acids in bovine muscle and bovine-liver phospholipids, respectively.

3. In muscle phospholipids all the normal acids from C_1 to C_8 , except C_7 , have been detected. In addition, isovaleric acid was present.

4. All the normal acids from C_1 to C_9 , and, in addition, isobutyric, isovaleric and α -methylbutyric acids, have been detected in liver phospholipids.

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Table 5. *Volatile fatty acids in phospholipids of bovine muscle*

In 13.5 g. of fatty acids (mean mol.wt. 279.5) there were present 400 μ -equiv. of volatile fatty acids. Results are calculated for 2.42 g. of muscle phospholipids.

Acid	Amount present (μ -equiv.)	Moles of acid (% of fatty acids of phospholipids)	Moles of acid (% of volatile fatty acids)
Formic	9.91	0.168	24.7
Acetic	21.65	0.367	54.0
Propionic	1.70	0.029	4.2
n-Butyric	0.99	0.017	2.5
isoValeric	0.43	0.007	1.1
n-Valeric	0.14	0.002	0.4
n-Hexanoic	1.13	0.019	2.8
n-Octanoic	4.10	0.069	10.4

Table 6. *Volatile fatty acids in phospholipids of bovine liver*

In 26.6 g. of fatty acids (mean mol.wt. 283.7) there were present 1188 μ -equiv. of volatile fatty acids. Results are calculated for 2.64 g. of liver phospholipids.

Acid	Amount present (μ -equiv.)	Moles of acid (% of fatty acids of phospholipids)	Moles of acid (% of volatile fatty acids)
Formic	24.54	0.367	28.7
Acetic	51.31	0.768	60.0
Propionic	5.38	0.081	6.3
isoButyric	0.52	0.008	0.6
n-Butyric	0.52	0.008	0.6
isoValeric	0.62	0.009	0.7
n-Valeric	0.46	0.007	0.5
α -Methylbutyric	0.34	0.005	0.4
n-Hexanoic	0.57	0.009	0.7
Unknown	0.23	0.003	0.3
n-Heptanoic	0.17	0.003	0.2
n-Octanoic	0.60	0.009	0.7
n-Nonanoic	0.28	0.004	0.3

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The Effect of Organic Solvents on Mammalian Histidine Decarboxylase

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Mammalian histidine decarboxylase has been described by Werle and his colleagues (Werle, 1940, 1942; Werle & Heitzer, 1938; Werle & Herrmann, 1937; Werle & Krautzun, 1938; Werle & Koch, 1949) and by Holtz (1941; see also Holtz & Credner, 1944). This enzyme is present in certain mammalian tissues and may be responsible for the formation of histamine from histidine absorbed into the circulation from the gut. Its distribution and possible significance in tissue metabolism have been discussed by Waton (1956).

While testing for potentiators of the histidine-decarboxylase activity of rabbit-kidney tissue, Waton (1956) found that chloroform and benzene considerably increased histamine production from histidine. Enzyme potentiation by an organic solvent has been described for the choline acetylase of brain tissue. Stedman & Stedman (1939), Mann, Quastel & Tennenbaum (1939) and Feldberg (1945) reported that formation of acetylcholine by ox and rat brain was greatest when the incubation medium was 100% ether or chloroform. In the present case, the potentiation of histidine-decarboxylase activity was produced in an aqueous medium containing only a trace of organic solvent. Experiments were therefore performed to elucidate the mechanism of this action.

METHODS

Source of enzyme

Rabbit-kidney histidine decarboxylase was used. The rabbit kidneys were removed and dropped into 0.9% NaCl. They were freed from all extraneous tissue, cut into small pieces and minced in a Latapie mincer. Tissue extracts were

prepared by grinding the minced tissue for 10 min. in a mortar with 1 g. of silver sand and 10 ml. of 0.9% NaCl/g. of tissue. The mixture was centrifuged at 2500 g for 10 min. and the supernatant fluid used for some of the experiments.

In other experiments an extract of rabbit kidney was prepared by homogenizing the minced tissue with 0.25 M sucrose solution in a Potter-Elvehjem homogenizer for 10 min. at 0°. The homogenate was successively centrifuged at 2500, 37000 and 90000 g for 10 min., 45 min. and 1 hr., respectively. The minced tissue and a measured volume of each supernatant fluid were incubated in the presence and absence of benzene. The final supernatant would be free from all particulate cell matter and would contain only substances in colloidal suspension or in solution (Schneider & Hogeboom, 1950).

In a control experiment it was shown that the production of histamine from histidine by rabbit kidney was unaffected by changing from Tyrode's solution and buffer mixture to 0.25 M sucrose-buffer mixture.

Method of incubation

The minced tissue (0.4 g.) and 6 ml. of Tyrode's solution, or 4 ml. of tissue extract and 2 ml. of Tyrode's solution, were placed in a 25 ml. conical flask together with 3 ml. of 0.2 M Na_2HPO_4 solution to buffer at pH 8.2 (glass electrode), and 1 ml. of neutralized histidine solution (15 mg./ml.). The mixture was incubated at 38° for 3 hr. with mechanical agitation.

To show the effect of CHCl_3 and benzene, one drop (about 20 mg.) of one of these substances was added (a) before incubation, (b) after incubation, (c) before incubation, no histidine in the incubation mixture, and (d) before incubation, no enzyme preparation present. Any difference between (a) and (b) would show a potentiating or inhibitory effect. A positive value for (c) would represent either free tissue histamine or histamine liberated by the solvent from bound histamine, and a positive value for (d) would represent histamine produced by an interaction of the reagents.

Duplicate experiments were carried out on each enzyme preparation, and the experiment was repeated with the tissue of another animal.

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