and by inorganic orthophosphate. Added Na^+, K^+, Cl^- and SO_4^{2-} ions in the range 1–10 mM are without effect, and citrate, oxalate and ethylenediaminetetraacetate protect mitochondria from swelling.

4. The effects of the nucleotides, Mg^{2+} and Mn^{2+} ions on the water content of mitochondria are also appreciable in a medium consisting of only 40 mM sucrose. In this medium AMP is more effective than adenosine diphosphate (ADP), which is better than ATP, apparently because of the inhibitory effect of the inorganic phosphate liberated during incubation. All three nucleotides are broken down to adenosine and inorganic phosphate, and this breakdown is more rapid in the presence of Mg^{2+}, Mn^{2+} and Ca^{2+} ions either alone or in mixtures.

5. Adenosine 3′-phosphate, inosine 5′-phosphate, guanosine 5′-phosphate, uridine 5′-phosphate, flavin mononucleotide, adenosine and inosine were without effect on the water content of mitochondria.

6. It is concluded that the agents which prevent swelling act on the structure of the mitochondria in ways which are not yet understood.

We wish to thank Professor H. A. Krebs, F.R.S., for his interest in this work, which was aided by a grant from the Rockefeller Foundation.

REFERENCES

Bartley, W. & Davies, R. E. (1952). *Biochem. J.* **52**, xx.
Bartley, W. & Davies, R. E. (1954). *Biochem. J.* **57**, 37.
Berenblum, I. & Chain, E. (1938). *Biochem. J.* **32**, 295.
Brenner-Holzach, O. & Raaflaub, J. (1954). *Helv. physiol. acta*, **12**, 242.
Craigie, J. (1949). *Brit. J. Cancer*, **3**, 439.
Deutsch, A. & Nilson, R. (1953). *Acta chem. scand.* **7**, 1288.
Ernster, L., Lindberg, O. & Löw, H. (1955). *Nature, Lond.*, **175**, 168.
Ernster, L. & Löw, H. (1955). *Exp. Cell Res.* (suppl.), **3**, 133.
Fonnesu, A. & Davies, R. E. (1955). *Biochem. J.* **61**, vi.
Harman, J. W. & Feigelson, M. (1952). *Exp. Cell Res.* **3**, 509.
Hunter, F. E. jun. & Ford, L. (1955). *J. biol. Chem.* **216**, 357.
Krebs, H. A. & Hems, R. (1953). *Biochim. biophys. Acta*, **12**, 172.
LePage, G. A. (1949). *Biochem. Prep.* **1**, 5.
Lindberg, O. & Ernster, L. (1954). *Nature, Lond.*, **173**, 1038.
Macfarlane, M. G. & Spencer, A. G. (1953). *Biochem. J.* **54**, 569.
Ostern, P. (1932). *Biochem. Z.* **254**, 65.
Price, C. A. & Davies, R. E. (1954). *Biochem. J.* **58**, xvii.
Price, C. A., Fonnesu, A. & Davies, R. E. (1956). *Biochem. J.* **64**, 754.
Raaflaub, J. (1952). *Communications, 2nd Int. Congr. Biochem., Paris*, p. 41.
Raaflaub, J. (1953*a*). *Helv. physiol. acta*, **11**, 142.
Raaflaub, J. (1953*b*). *Helv. physiol. acta*, **11**, 157.
Schneider, W. C. & Hogeboom, G. H. (1951). *Cancer Res.* **11**, 1.
Slater, E. C. & Cleland, K. W. (1953). *Biochem. J.* **55**, 566.
Stanbury, S. W. & Mudge, G. H. (1953). *Proc. Soc. exp. Biol., N.Y.*, **82**, 675.

The Metabolism of Butylated Hydroxyanisole in the Rabbit

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(*Received* 15 *February* 1956)

This paper deals with the metabolism in the rabbit of a substance known as butylated hydroxyanisole (BHA) that is used commercially as an antioxidant in edible fats (Kraybill *et al.* 1949). The material is a mixture of two isomers, 85% of 2-*tert.*-butyl-4-methoxyphenol (I) and 15% of 3-*tert.*-butyl-4-methoxyphenol (II). Although studies of the conventional type on acute and chronic toxicity have been made (see Wilder & Kraybill, 1948; Lehman, Fitzhugh, Nelson & Woodward, 1951; Graham, Teed & Grice, 1954) no studies on metabolism have been reported. The metabolism of the commercial mixture and of the pure isomers has been examined and the major metabolites have been isolated and characterized.

EXPERIMENTAL

Animals, diet and dosage. The rabbits used were does of the New Zealand white strain, weighing 1·6–2·3 kg. They were maintained on a standard pellet diet (wheat, 40%; pollard, 33%; bran, 27%) and were kept singly in metabolism cages designed to permit the separate collection of

urine and faeces. Water and food were supplied *ad lib.* and the urine was collected daily. Doses of 0·25, 0·5 or 1·0 g. of BHA were dissolved in 9 ml. of olive oil and administered by stomach tube. In some experiments 1 g. of L-cystine or sodium sulphite was dissolved in water and given by stomach tube immediately after the olive oil solution. The rabbits usually refused food during the 24 hr. after receiving single doses of 1 g. of BHA.

Materials. BHA (Sústane) was obtained from the Universal Oil Products Company, Riverside, Illinois, U.S.A., and the isomers I and II were given by Dr J. A. Chenicek of this firm. The following were purchased: *tert.*-butylquinol (Eastman Organic Chemicals, Rochester, N.Y.), *p*-methoxyphenol and quinol (British Drug Houses Ltd., London) and β-glucuronidase (bacterial) (Sigma Chemical Co., St Louis, Missouri). *tert.*-Butyl-*p*-benzoquinone (m.p. 53°) was prepared by Dr G. M. Richardson (Nutrition Research Department) by the oxidation of *tert.*-butylquinol with potassium bromate in 92% yield after the method of McCoy (1937).

Analytical methods

All spectrophotometric measurements were made with a Unicam SP. 350 Model D.G. spectrophotometer.

Free and total phenols. (*a*) *Folin & Ciocalteu method.* Phenols were determined on diluted rabbit urine by the Folin & Ciocalteu (1927) method. The final concentration of phenol was adjusted to the range 0·3–1·5 mM and the developed colour was measured at 700 Å. The calibration curves of optical density against concentration for phenol, quinol, BHA, the isomers I and II and 2-*tert.*-butyl-4-methoxyphenyl glucosiduronic acid (after acid hydrolysis) gave the same line for the range 0·3–1·5 mM. (*b*) *Chloroimide method.* The chloroimide reaction of Gibbs (1927) as modified by Mahon & Chapman (1951) was applied to diluted rabbit urine. The 2:6-dichloroquinone-4-chloroimide was used as a 0·1% solution in ethanol, and the blue colour developed at pH 9·4 and measured at 620 Å. A calibration curve was prepared with BHA in the range 0·1–0·45 mM. Isomer (I) had about twice the colour value of isomer (II) with this reagent.

Glucuronides. The naphtharesorcinol method, as modified by Bray, Humphris, Thorpe, White & Wood (1952*a*), was used for the estimation of total glucuronic acids. Urine was diluted to give a glucuronide concentration between 0·5 and 2·0 mM and readings were made at 600 Å. A calibration curve was prepared by using 2-*tert.*-butyl-4-methoxyphenyl glucosiduronic acid over the range given above. Urines were tested for ester glucuronides with Fehling's and Benedict's solutions, but none was found.

Ethereal sulphates. These were determined by Sperber's (1948) modification of the turbidimetric method. The calibration curve was made with K_2SO_4 and readings were taken in the range 0·0–1·0 mg. of SO_3.

Mercapturic acids. Stekol's (1936) method (iodine consumption after hydrolysis by alkali) as modified by Parke & Williams (1951) was used.

Paper chromatography. The phenolic compounds were separated on Whatman no. 1 filter-paper sheets by the descending method, with the solvent systems of Table 1. In all cases the solvent front was run for 30–40 cm.

Effect of acid on BHA, tert.*-butylquinol and* p*-methoxyphenol*

Butylated hydroxyanisole. BHA (5 g.) suspended in 5N-HCl (100 ml.) was boiled under reflux for 2 hr. The mixture was then steam-distilled for 2 hr. and the distillate extracted with light petroleum, b.p. 40–60° (2 × 50 ml.) and then with peroxide-free ether (2 × 50 ml.). The non-volatile residue was extracted with *n*-butanol (2 × 50 ml.). All three extracts were evaporated almost to dryness *in vacuo* and the resultant concentrates examined by paper chromatography with solvent system *A* (Table 1). The light-petroleum extract contained most of the BHA and some *p*-methoxyphenol, and the ether extract some BHA and *p*-methoxyphenol; the butanol extract contained *tert.*-butylquinol, quinol and traces of BHA and *p*-methoxyphenol. The bulk of the concentrates was run on strip chromatograms, the individual bands were located under a u.v. lamp and each was eluted with methanol. From these eluates, *tert.*-butylquinol, *p*-methoxyphenol and quinol were isolated and identified by m.p. and mixed m.p. (quinol as the diacetate). From 5 g. of BHA, 150 mg. of *tert.*-butylquinol and 50 mg. of quinol were obtained. The *p*-methoxyphenol was distributed throughout all the fractions, but, on the basis of the intensity of spots on the chromatograms, the amount appeared to be of the same order as that of *tert.*-butylquinol.

tert.-*Butylquinol.* A portion (5 g.) was suspended in 5N-HCl (100 ml.) and boiled under reflux for 2 hr. After cooling, the mixture was extracted with *n*-butanol (2 × 50 ml.). Quinol and unchanged *tert.*-butylquinol were identified in this extract by paper chromatography with solvent systems *A* and *C* (Table 1).

Table 1. *R_F values of BHA isomers and degradation products*

Solvent mixtures (by vol.): *A*, light petroleum (b.p. 80–100°)–*n*-butanol–formic acid (85%) (5:1:5); *B*, light petroleum (b.p. 40–60°)–formic acid (85%) (2:1); *C*, benzene–acetic acid–water (4:5:1).

Compound	R_F in solvent mixture			Colour with diazotized sulphanilic acid followed by 20% Na_2CO_3
	A	*B*	*C*	
2-*tert.*-Butyl-4-methoxyphenol	0·85	0·7	0·9–1·0	Orange
3-*tert.*-Butyl-4-methoxyphenol	0·9	0·8	0·9–1·0	Orange
tert.-Butylquinol	0·4	0·15	0·7	Purplish black fading rapidly*
tert.-Butyl-*p*-benzoquinone	0·9–1·0	0·9–1·0	0·9–1·0	No colour
p-Methoxyphenol	0·3	0·3	0·8	Purple–red, blue edges, fading
Quinol	0·0	0·0	0·7	Green changing to dark grey*

* Ammoniacal $AgNO_3$ gives a characteristic black with quinols.

p-*Methoxyphenol.* A sample (5 g.) was treated in the same way as *tert.*-butylquinol. Quinol and *p*-methoxyphenol were detected.

Purity of commercial BHA

An initial chromatographic examination of the BHA used did not show the presence of any impurity, but traces of *p*-methoxyphenol were detected after steam-distillation. BHA (5 g.) was suspended in water (100 ml.) and steam-distilled for 2 hr. The non-volatile residue was extracted with *n*-butanol (50 ml.) and the concentrate examined chromatographically with solvent system *A*. BHA and *p*-methoxyphenol (2·5 mg. isolated) were present but no quinol or *tert.*-butylquinol was found.

Isolation and characterization of glucuronides

All melting points are uncorrected.

Isolation of barium (2-tert.-*butyl*-4-*methoxyphenyl glucosid*)*uronate.* The 24 hr. urine (73 ml. from the total of 81 ml.) excreted by a rabbit given 1 g. of 2-*tert.*-butyl-4-methoxyphenol was diluted to 100 ml., 50% (w/v) barium acetate (4 ml.) added, the pH adjusted to 9–10 with ammonia and the mixture then filtered through a kieselguhr pad. The filtrate was evaporated *in vacuo* below 40° to 25 ml. and the crystals which separated were collected. The mother liquor and washings were concentrated to 15 ml. and a second crop of crystals was obtained (total yield, 1·196 g. hydrate = 1·02 g. anhydrous). Recrystallization from hot water yielded long white needles of *barium* (2-tert.-*butyl*-4-*methoxyphenyl glucosid*)*uronate*, m.p. 221° (decomp.), which lost 3·5 H_2O on vacuum-drying over conc. H_2SO_4. (Found: C, 48·1; H, 5·4; OMe, 7·3; Ba, 15·9. $C_{34}H_{46}O_{16}Ba$ requires C, 48·2; H, 5·5; OMe, 7·3; Ba, 16·2%.)

The 24 hr. urine (55 ml.) from a rabbit given 1 g. of BHA was treated as described above (yield of glucuronide as hydrate, 0·82 g.). The recrystallized barium salt had m.p. 216° (decomp.) and lost 3·5 H_2O on vacuum-drying over conc. H_2SO_4. (Found: C, 48·0; H, 5·4; OMe, 8·0; Ba, 16·0%.)

Isolation of barium (3-tert.-*butyl*-4-*methoxyphenyl glucosid*)*uronate.* The 24 hr. urine (57 ml.) from a rabbit given 1 g. of 3-*tert.*-butyl-4-methoxyphenol was treated as described above and produced a gum (yield 100–200 mg.), which crystallized slowly from water to yield small white rosettes of *barium* (3-tert.-*butyl*-4-*methoxyphenyl gucosid*)-*uronate*, m.p. above 350°. (Found: C, 48·2; H, 5·7; OMe, 7·9; Ba, 15·9. $C_{34}H_{46}O_{16}Ba$ requires C, 48·2; H, 5·5; OMe, 7·3; Ba, 16·2%.) This isomer was not isolated from urine of rabbits given BHA.

Preparation of 2-tert.-*butyl*-4-*methoxyphenyl glucosiduronic acid.* The barium salt (500 mg.) was suspended in water (2 ml.) and 1·5 equiv. of HCl added, when the barium salt dissolved. The free glucosiduronic acid which separated was filtered off and washed with cold water. The small white needles of 2-tert.-*butyl*-4-*methoxyphenyl glucosiduronic acid* had m.p. 168·5–169°, $[\alpha]_D^{20}$ – 67·5° in water (*c*, 1·0). (Found: C, 57·5; H, 6·3. $C_{17}H_{24}O_8$ requires C, 57·3; H, 6·8%.)

An attempt to prepare the triacetate of 2-*tert.*-butyl-4-methoxyphenyl glucosiduronic acid produced a syrup which failed to crystallize.

Acid hydrolysis of barium (2-tert.-*butyl*-4-*methoxyphenyl glucosid*)*uronate.* The barium salt (125 mg.) was suspended in 5 N-HCl (25 ml.) and boiled under reflux for 2 hr. Samples were taken for analysis by the Folin & Ciocalteu and chloroimide methods and for paper chromatography. The Folin & Ciocalteu method gave a quantitative recovery of phenols, but with the chloroimide method only 48% of the expected phenolic material was found. Chromatographic analysis of the acid hydrolysate with solvent systems *A* and *C* showed the presence of 2-*tert.*-butyl-4-methoxyphenol, *p*-methoxyphenol, *tert.*-butylquinol, quinol and at least ten unidentified spots which could be seen under u.v. light or after spraying with diazotized sulphanilic acid.

The barium salt (500 mg.) was dissolved in 5 N-H_2SO_4 (10 ml.) and the solution steam-distilled for 2 hr. The distillates (2 × 200 ml.) were shaken with light petroleum, b.p. 40–60° (0·1 vol.). The extract was separated and the solvent was allowed to evaporate. The white material (yield 100 mg., 56%) crystallized from hot water as long fine needles, m.p. 60–61°, and was identified as 2-*tert.*-butyl-4-methoxyphenol by mixed m.p.

Enzymic hydrolysis of 2-tert.-*butyl*-4-*methoxyphenyl glucosiduronic acid.* A suspension of 50 Sigma units of β-glucuronidase in 0·5 ml. of sodium acetate buffer at pH 4·5 was added to 1·0 ml. of 0·7 mM neutralized 2-*tert.*-butyl-4-methoxyphenyl glucosiduronic acid in acetate buffer and 3·5 ml. of the acetate buffer. The mixture was kept at 37° and the degree of hydrolysis was determined at intervals by the Folin & Ciocalteu and the chloroimide methods. At a final substrate concentration of 0·14 mM, hydrolysis was complete in 4 hr., but with 0·28 mM, 53% of the glucuronide was hydrolysed in 4 hr.

Ethereal sulphates. An attempt to isolate the ethereal sulphates by the 5-aminoacridine method (Dodgson, Rose & Spencer, 1955) was not successful.

RESULTS

Excretion of normal metabolites. The mean daily excretions ± S.D. by undosed rabbits were: free phenols, 0·613 ± 0·188; combined phenols, 0·344 ± 0·086; glucuronides, 0·364 ± 0·169; ethereal sulphates, 0·205 ± 0·119 m-moles.

Metabolites of BHA and its isomers

Glucuronides. After the administration of 1 g. of BHA there is a considerable increase in the urinary excretion of both combined phenols and glucuronides that accounts for the excretion of almost half the dose as the phenolic glucuronide. This glucuronide excretion was not further increased by the daily administration of 10 ml. of 10% glucose solution by stomach tube immediately after the BHA (cf. Südhof, 1952). When the pure isomers of BHA were given separately the glucuronide excretions were the same as obtained with the commercial mixture. The isolation and identification of the glucuronides have been described above. It is probable that these glucuronides have the usual -β-D-configuration, and this is supported by the hydrolysis of 2-*tert.*-butyl-4-methoxyphenyl glucosiduronic acid by β-glucuronidase.

Ethereal sulphates. About 9 % of a dose of 1 g. of BHA or either of the pure isomers was excreted as ethereal sulphates. The extent of ethereal sulphate excretion after BHA could be varied experimentally. When a rabbit was maintained for some weeks on a low-sulphur diet it showed no increase in ethereal sulphate excretion after dosage with BHA. The administration of 1 g. of sodium sulphite by stomach tube along with the BHA resulted in an excretion of as much as 20 % of the BHA in the form of ethereal sulphate (cf. Bray, Humphris, Thorpe, White & Wood, 1952*b*). L-Cystine (1·0 g.) had little if any effect on the sulphate excretion. The figures in Tables 2–4 are for animals maintained on the standard diet described above.

Free phenols. After a dose of 1 g. of commercial BHA or either of the isomers the increase in excretion of free phenols corresponded to about 6 % of the dose.

Mercapturic acids. No consistent increase in mercapturic acid excretion was found.

The results of determinations of metabolites are summarized in Table 2 which shows that about 60 % of the administered dose is accounted for.

Table 2. *Metabolites excreted in rabbit urine in 24 hr. after administration of 1 g. of BHA*

Results expressed as mean percentage of the dose (±S.D.) with number of experiments in brackets.

Free phenols	5·8±5·1 (8)
Combined phenols	55·8±7·6 (8)
Glucuronides	46·4±9·8 (8)
Ethereal sulphates	9·2±3·2 (5)

Effect of varying the dose of BHA

The effect of lowering the dose level from 1·0 to 0·5 g. and then to 0·25 g. on the excretion of glucuronides, combined phenols and ethereal sulphates is shown in Table 3.

Effect of repeated doses of BHA

Between four and seven daily doses of 1 g. of BHA were lethal to rabbits. In Table 4 the recovery of the metabolites of BHA is set out for three experiments in which the rabbit was given daily doses of 1 g. and for one experiment in which the daily dose was 0·5 g. In all these experiments the animals died.

DISCUSSION

In the early stages of this work the conventional procedure of ether extraction of rabbit urine to remove free phenols, followed by acid hydrolysis and further ether extraction of the liberated phenols (cf. Bray & Thorpe, 1954), was used. In the first ether extract, the free phenol fraction, only traces of BHA and phenolic materials normally found in rabbit urine were identified by paper chromatography. The second ether extract, made after acid hydrolysis, contained *tert.*-butylquinol, traces of BHA and considerable amounts of a yellow substance which was separated by paper chromatography. This compound was isolated and its ultraviolet absorption was that of *tert.*-butyl-*p*-benzoquinone. The presence of this quinone in the conjugated fraction and its absence from the free phenol fraction suggests that it was formed during the

Table 3. *Effect of size of dose of BHA on excretion of metabolites*

Results expressed as mean percentage of the dose from four experiments.

Dose (g.)	Free phenols	Glucuronides	Ethereal sulphates	Total
0·25	18·9	84·4	18·1	121·4
0·5	4·4	60·2	12·3	76·9
1·0	3·3	38·9	9·7	51·9

Table 4. *Excretion of metabolites after repeated doses of BHA*

Results expressed as percentage of the dose (single animals).

Dose level (g.)	Free phenols	Combined phenols	Glucuronides	Ethereal sulphates	Total
3 × 1·0	0·0	39·7	36·5	1·3	37·8
4 × 1·0	2·1	48·0	30·2	4·0	36·3
7 × 1·0	1·4	61·5	47·5	13·9	62·8
11 × 0·5	0·0	58·6	50·5	3·3	53·8

hydrolysis and extraction processes. By the usual hydrolytic procedure of boiling the urine with 5N-HCl for 2 hr. BHA breaks down and 1% has been isolated as quinol, 3% as *tert.*-butylquinol and 3% as *p*-methoxyphenol. Similarly, *p*-methoxyphenol and *tert.*-butylquinol break down to give quinol. Thus both the *-O*-methyl and the *-C-tert.*-butyl groups can be removed under the conditions used in acid hydrolysis. This breakdown of BHA in acid is small in comparison with the breakdown of the phenolic moiety of 2-*tert.*-butyl-4-methoxyphenyl glucosiduronic acid when this is hydrolysed for 2 hr. in 5N-HCl at 100°. This hydrolysate contained more than ten phenolic substances (mainly unidentified) and much less 2-*tert.*-butyl-4-methoxyphenol than expected. Some measure of this chemical breakdown was obtained by comparing analytical data for the hydrolysate. The Folin & Ciocalteu method is relatively non-specific and gave a quantitative recovery of phenols. The chloroimide reagent, on the other hand, is relatively specific and reacts with BHA but not with all its breakdown products; in the hydrolysate this reagent gave a recovery of only 48% of phenols. When this glucuronide was hydrolysed with β-glucuronidase both the Folin & Ciocalteu and chloroimide methods gave identical recoveries. This showed that in the acid hydrolysate at least half of the phenolic material was no longer 2-*tert.*-butyl-4-methoxyphenol.

The extensive breakdown of the phenolic moiety of 2-*tert.*-butyl-4-methoxyphenyl glucosiduronic acid on acid hydrolysis provides an explanation of the findings made when the conventional ether extraction methods were used. The use of these methods of analysis might have led to the conclusion that the phenols found after acid hydrolysis were originally present as conjugates, when in fact they were produced by the chemical degradation of 2-*tert.*-butyl-4-methoxyphenyl glucosiduronic acid. The production of artifacts by acid hydrolysis during isolation of sterols from human urine has been described (cf. Marrian, 1951; Jones & Stitch, 1953; Brown, 1955).

With 2-*tert.*-butyl-4-methoxyphenol the insolubility of the barium salt of the glucuronide allowed the isolation of 85% of the total glucuronide as this salt (1·02 g. isolated from 1·20 g. as indicated by glucuronide analysis). This sets an upper limit to any glucuronide formed from a modified form of this isomer of less than 15% of the administered dose. With BHA the independent estimations of glucuronides plus ethereal sulphates gave a recovery of 55·6% of the dose, which agrees closely with the 55·8% for the combined phenols. The amount of undetected phenolic material in urine after a 1 g. dose of BHA is not likely to exceed the errors in determinations as given in Table 2, i.e. the amount is likely to be less than 152 mg. This comparatively large figure is a measure of the variation found when increases in glucuronide or phenol excretion above basal levels are determined by non-specific methods. These methods provide a useful indication of what metabolites may be present, and a rough measure of the amounts to be expected. Thus a figure of 46·4% of a 1 g. dose excreted as glucuronide (Table 2) is derived from an increase of 464 ± 98 mg. above a normal daily excretion of 71 ± 29. The utility of the method is greatly diminished where the increase above basal levels is small, e.g. 5·8% excreted as free phenol is based on an increase of 0·328 ± 0·280 m-mole above the basal level of 0·613 ± 0·188. These difficulties are still greater when low dose levels are given.

This account of the metabolism of BHA is part of a study of an additive for human food. The identification of the main metabolites as glucuronides, and the rise in the proportion excreted as glucuronides after single doses from 39 to 60 to 84% as the dose is lowered from 1 to 0·5 to 0·25 g. is reassuring. Less satisfactory are the findings that recoveries of glucuronides are less complete after repeated doses and that four or five daily doses of 1 g. to a rabbit have cumulative and lethal effects. This shows the need for further studies of the metabolism of BHA at much lower dose levels; but, for this, methods other than those used in this paper will have to be developed. These methods might include chromatographic separation of the conjugates themselves and the use of enzymes to hydrolyse the conjugates either in the urine or after separation.

SUMMARY

1. The metabolism of the antioxidant, butylated hydroxyanisole (BHA), and of its component isomers, 2- and 3-*tert.*-butyl-4-methoxyphenol has been studied in the rabbit.

2. After 1 g. of BHA by mouth, rabbits excreted 46% as glucuronides, 9% as ethereal sulphates and 6% as free phenols. The recovery of glucuronide was 60% of a 0·5 g. dose, and 84% of a 0·25 g. dose.

3. After repeated doses of 1 and 0·5 g., recovery of BHA as glucuronide was lower than after single doses.

4. The glucuronides of the two BHA isomers have been isolated from the urine as barium (2-*tert.*-butyl-4-methoxyphenyl glucosid)uronate and barium (3-*tert.*-butyl-4-methoxyphenyl glucosid)-uronate.

5. BHA is unstable on heating in 5N-HCl and breaks down to *tert.*-butylquinol, *p*-methoxyphenol and quinol.

6. Hydrolysis of 2-*tert.*-butyl-4-methoxyphenyl glucosiduronic acid with 5N-HCl results in the

destruction of at least half of the 2-*tert.*-butyl-4-methoxyphenol and the production of more than a dozen other phenolic compounds.

The authors desire to thank Dr A. D. Campbell, Chemistry Department, University of Otago, who did the microanalyses.

REFERENCES

Bray, H. G., Humphris, B. G., Thorpe, W. V., White, K. & Wood, P. B. (1952*a*). *Biochem. J.* **52**, 412.
Bray, H. G., Humphris, B. G., Thorpe, W. V., White, K. & Wood, P. B. (1952*b*). *Biochem. J.* **52**, 419.
Bray, H. G. & Thorpe, W. V. (1954). In *Methods of Biochemical Analysis*, vol. 1, p. 27. Ed. by Glick, D. New York: Interscience Publishers Inc.
Brown, J. B. (1955). *Biochem. J.* **60**, 185.
Dodgson, K. S., Rose, F. A. & Spencer, B. (1955). *Biochem. J.* **60**, 346.
Folin, O. & Ciocalteu, V. (1927). *J. biol. Chem.* **73**, 627.
Gibbs, H. D. (1927). *J. biol. Chem.* **72**, 649.
Graham, W. D., Teed, H. & Grice, H. C. (1954). *J. Pharm., Lond.*, **6**, 534.
Jones, J. K. N. & Stitch, S. R. (1953). *Biochem. J.* **53**, 679.
Kraybill, H. R., Dugan, L. R. jun., Beadle, B. W., Vibrans, F. C., Swartz, V. & Rezabek, H. (1949). *J. Amer. Oil Chem. Soc.* **26**, 1.
Lehman, A. J., Fitzhugh, O. G., Nelson, A. A. & Woodward, G. (1951). *Advanc. Food Res.* **3**, 197.
McCoy, H. N. (1937). *J. chem. Educ.* **14**, 494.
Mahon, J. H. & Chapman, R. A. (1951). *Analyt. Chem.* **23**, 1116, 1120.
Marrian, G. F. (1951). *J. Endocrin.* **7**, lxix.
Parke, D. V. & Williams, R. T. (1951). *Biochem. J.* **48**, 624.
Sperber, I. (1948). *J. biol. Chem.* **172**, 441.
Stekol, J. A. (1936). *J. biol. Chem.* **113**, 279.
Südhof, H. (1952). *Hoppe-Seyl. Z.* **290**, 72.
Wilder, O. H. M. & Kraybill, H. R. (1948). *Summary of Toxicity Studies on Butylated Hydroxy Anisole*, pp. 1–5. Chicago: American Meat Foundation, University of Chicago.

Nomenclature of Enzymes of Fatty Acid Metabolism

The Second International Conference on Biochemical Problems of Lipids was held between 27 and 30 July 1955, at the University of Ghent, Belgium. A special meeting* was convened at this Conference to discuss problems of enzyme nomenclature in the fatty acid field and the following recommendations were made.

Principles of nomenclature

A. It is recommended that these enzymes should be named according to the systematic chemical nomenclature for the substrate or substrates acted upon and the overall reaction catalysed. The mechanism of the reaction need not be implicit in the name of the enzyme, since present-day concepts of reaction mechanism may change.

B. It is desirable to follow unwritten conventions where these already exist. Thus, while it might appear desirable to denote enzymes according to the favoured equilibrium of the reactions, the convention already exists that all enzymes catalysing reduction or oxidation should be called dehydrogenases, whether the equilibrium of the reaction favours reduction or oxidation.

C. Since fatty acids are generally metabolized in combination with coenzyme A, it is considered unnecessary to include coenzyme A in the terminology. However, since the substrate is not the free acid, it is recommended that the name of the acid radical be used to denote the substrate.

Examples: Butyryl dehydrogenase (an enzyme acting on butyryl-coenzyme A); butyric dehydrogenase (an enzyme acting on free butyric acid).

D. Most of the enzymes of fatty acid metabolism act upon several substrates derived from fatty acids of differing chain length. It is recommended that the enzyme be named after that chain length for which it shows the highest affinity, or, if one optimum chain length cannot be defined, then after the range of chain lengths involved.

E. In all instances where free coenzyme A may be considered to be a substrate of the reaction (either explicity or implicitly), the term denoting the function of the enzyme should be preceded by the prefix 'thio'.

Individual enzymes of fatty acid metabolism

Individually the enzymes concerned in fatty acid metabolism are considered under five headings according to the reactions they catalyse. These are (1) activation, (2) condensation and cleavage, (3) reduction and oxidation, (4) hydration and dehydration and (5) transfer reactions.

(1) *Activating enzymes.* The reaction catalysed by these enzymes can be represented† as

$$\text{Fatty acid} + \text{ATP} + \text{CoA} \rightleftharpoons \text{fatty acyl-CoA} + \text{PP}_i + \text{AMP}.$$

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† The following abbreviations are used in this paper: ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; CoA, coenzyme A; PP_i, inorganic pyrophosphate; DPN and DPNH, oxidized and reduced diphosphopyridine nucleotide respectively; FAD and $FADH_2$, oxidized and reduced flavine-adenine dinucleotide respectively.