oxidation system. Similarly the presence of catalase in micro-organisms does not protect them from the toxic effect of H_2O_2 added as such or formed in the medium as a result of certain oxidation reactions. It is therefore unlikely that the main function of catalase consists in the decomposition of H_2O_2 into O_2 and water in order to protect cells from its toxic effect.

6. The catalysis of coupled oxidation by means of H_2O_2 formed in a primary oxidation is a more likely biological property of catalase. In fact, the

concentration of catalase in liver tissues appears to be about 1000 times higher than that required for the catalytic decomposition of H_2O_2 , and is of the same order as is found to be necessary for catalysis of coupled oxidation.

7. So far only methanol, ethanol, *n*-propanol, isobutanol, β -amino-ethanol and ethylene glycol have been shown to undergo coupled oxidation catalyzed by catalase *in vitro*. It is possible that *in vivo* other biologically important compounds may undergo similar oxidations.

REFERENCES

- Bingold, K. (1933). *Klin. Wschr.* **12**, 1201.
 Birkinshaw, J. H. & Raistrick, H. (1943). *J. biol. Chem.* **148**, 459.
 Brooks, J. (1929). *Biochem. J.* **23**, 1391.
 Brooks, J. (1935). *Proc. Roy. Soc. B*, **118**, 560.
 Coulthard, C. E., Michaelis, R., Short, W. F., Sykes, G., Skrimshire, G. E. H., Standfast, A. F. B., Birkinshaw, J. H. & Raistrick, H. (1942). *Nature, Lond.*, **150**, 634.
 Dixon, M. (1925). *Biochem. J.* **19**, 507.
 Dixon, M. (1926). *Biochem. J.* **20**, 703.
 Holmberg, C. G. (1939). *Biochem. J.* **33**, 1901.
 Keilin, D. & Hartree, E. F. (1936). *Proc. Roy. Soc. B*, **119**, 141.
 Keilin, D. & Hartree, E. F. (1945). *Biochem. J.* **39**, 148.
 Lipmann, F. & Owen, C. R. (1943). *Science*, **98**, 246.
 Loew, O. (1901). *Rep. U.S. Dep. Agric.* no. 68, p. 1.
 Lwoff, A. & Morel, M. (1942). *Ann. Inst. Pasteur*, **68**, 323.
 Negelein, E. & Brömel, H. (1939). *Biochem. Z.* **300**, 225.
 Neill, J. M. & Hastings, A. B. (1925). *J. biol. Chem.* **63**, 479.
 Schönbein, C. (1863a). *J. prakt. Chem.* **98**, 323.
 Schönbein, C. (1863b). *Verh. naturf. Ges. Basel*, **3**, 697.
 Sevag, M. G. & Maiweg, L. (1934). *J. exp. Med.* **60**, 95.
 Sumner, J. B. & Dounce, A. L. (1937). *J. biol. Chem.* **121**, 417.
 Thénard, L. J. (1811). *Ann. Chim. (Phys.)*, **11**, 85.

The Urinary Partition of Sulphur in Rats Treated with Aromatic Hydrocarbons, with Special Reference to Growth Retardation

By L. A. ELSON, F. GOULDEN AND F. L. WARREN, *Chester Beatty Research Institute, Royal Cancer Hospital (Free), Fulham Road, London, S.W. 3*

(Received 30 May 1945)

Haddow, Scott & Scott (1937) found that certain carcinogenic hydrocarbons produced immediate, constant, and long-continued reduction in the growth rate of young rats, while some non-carcinogenic hydrocarbons did not show this effect. White & White (1939) produced growth retardation in rats by the incorporation of methylcholanthrene, 3:4-benzpyrene, or pyrene in the diet. Later the same workers (1940) observed no difference between the growth effects produced by single intraperitoneal injections of carcinogenic or non-carcinogenic hydrocarbons; both types retarded growth.

White & White concluded, on the basis of their own results and from a consideration of data available in the literature for bromobenzene, naphthalene, and anthracene, that the growth retardation produced by the hydrocarbons is due to a specific deficiency of the sulphur-containing amino-acids

imposed on the animals by an increased requirement for organic sulphur, in the form of cystine or methionine, for detoxication mechanisms that involve the formation of mercapturic acids. They emphasized the desirability of actual isolation of the postulated mercapturic acids in order to demonstrate conclusively the relation between growth effects and sulphur metabolism.

Stekol (1943) showed that the addition of bromobenzene to the diet of rats produces, in effect, a decrease in available protein proportional to the mercapturic acid excreted. Growth retardation does not occur on a high protein diet but increases as the protein content is progressively lowered. Bromobenzene, composition of diet, and the degree of growth retardation are quantitatively related.

The mechanism of the growth retardation produced by these hydrocarbons is important, since

some of them are capable of retarding the growth of neoplasms (Haddow, 1935; Haddow & Robinson, 1937). It appeared probable that some light might be thrown on the possible significance of sulphur metabolism in this connexion by a quantitative examination of the sulphur partition in the urine of rats treated with selected aromatic hydrocarbons. In particular, the formation of mercapturic acids should be apparent from estimations of the neutral sulphur fraction. The experiments described in the present paper were designed to investigate this point. At the same time they afforded a comparison of the relative growth-retarding effects of aromatic hydrocarbons of differing structures and complexity.

MATERIALS AND METHODS

Animals. All experiments were carried out on male rats. During the experimental period the animals were maintained on a standard diet of the following composition: national wheatmeal flour (85% extraction) 72%, casein 11%, milk powder (full cream) 8%, Bemax (Vitamins Ltd.) 2.5%, margarine 3%, CaCO₃ 1%, salt mixture (Glaxo Laboratories Ltd.) 1%, cod-liver oil 1.5%. This diet contains approximately 25% of protein and is rich in sulphur (230 mg./100 g.). Rats grow well on it, the daily weight increases for male rats being 3–4 g. Groups of ten rats were kept in metabolism cages and supplied with this diet and water *ad libitum*. Each group was weighed daily throughout the experiments. At the beginning of the control period each rat received 1 ml. of arachis oil by intraperitoneal injection. During the week following this control injection four estimations of the daily excretion of inorganic sulphate, ethereal sulphate, and neutral sulphur were made. At the end of the control period the hydrocarbon was administered in arachis oil by the intraperitoneal route. The volume of oil was always 1 ml., and this contained the appropriate amount of hydrocarbon in solution or as a fine suspension. Daily estimations of urinary sulphur partition were continued for varying periods after this injection. In addition to the sulphur estimations a determination of glucuronic acid was also carried out on each specimen of urine by a method based on that described by Maughan, Evelyn & Browne (1938).

Sulphur determinations. These were carried out by the procedure described for human urines (Peters & Van Slyke,

1932). At first the attempts to apply this volumetric benzidine method to rat urines gave disappointing results, although it worked well with human urine. It was found that the introduction of small but important changes in the usual procedure led to a method that gave reproducible results. Careful attention to the following points was found to be necessary.

(a) The use of a more dilute aqueous acetone (75 ml. acetone diluted to 100 ml.) for washing the precipitated benzidine sulphate rather than the 80% or higher concentrations usually recommended was arrived at after many trials. It was found that the higher concentrations of acetone tended to precipitate benzidine hydrochloride on the filter and led to values for sulphur that were too high. Acetone in approximately 75% (v/v) concentration is safe in this respect and may be freely used for washing, since it does not dissolve any appreciable amount of precipitate.

(b) The rat urine was diluted tenfold and 5 ml. portions used for estimations. If larger volumes (or higher concentrations) are used, the end-point of the total sulphate estimation gives difficulty owing to the presence of brown colouring matter. The use of small volumes has the additional advantage that, provided a brisk ebullition is maintained, the precipitate dissolves during the course of the titration and it is unnecessary to break up the benzidine sulphate mechanically.

(c) Whatman no. 50 filter papers are more satisfactory in use than paper-pulp filters.

(d) In determining total sulphur it is essential to use a large excess of Benedict's sulphur reagent. The proportion of neutral sulphur is higher in rat than in human urine.

As an example of the use of the method the results of a study of normal male rats are shown in Table 1.

The hydrocarbons investigated were: benzene, naphthalene, phenanthrene, anthracene, 1:2-benzanthracene, 1:2:5:6-dibenzanthracene, 3:4-benzpyrene, pyrene, and chrysene. Preliminary purification through the picrate or by chromatographic adsorption was carried out where necessary.

RESULTS

The daily excretions/100 g. rat of inorganic sulphate, ethereal sulphate, and neutral sulphur are shown in Figs. 1–3, both during a preliminary control period and after injection of the hydrocarbons. In Fig. 4 the effects of the various hydrocarbons on the growth

Table 1. *The daily excretion of inorganic sulphate, ethereal sulphate and neutral sulphur in the urine of normal male rats*

(Data for urinary sulphur are expressed as mg. of sulphur/day. Highest and lowest figures are given in parentheses.)								
No. of determinations	Body wt. (g.)	Inorganic sulphate		Ethereal sulphate		Neutral sulphur		
		per rat	per 100 g. rat	per rat	per 100 g. rat	per rat	per 100 g. rat	
30	80–100	2.7 (1.2–3.9)	3.0 (1.4–4.4)	0.21 (0–0.40)	0.24 (0–0.50)	0.9 (0.4–1.8)	1.0 (0.5–1.9)	
18	100–120	2.9 (1.8–3.9)	2.6 (1.6–3.6)	0.22 (0–0.36)	0.20 (0–0.35)	1.6 (0.8–2.6)	1.4 (0.6–2.2)	
20	120–140	3.3 (1.7–4.7)	2.6 (1.4–3.5)	0.23 (0–0.40)	0.18 (0–0.30)	1.4 (0.6–2.6)	1.1 (0.5–2.1)	

rate of the rats are illustrated. Increased excretions of ethereal sulphate, neutral sulphur, and glucuronic acid, together with the corresponding growth rates, are shown in Table 2.

glucuronic acid. The growth rate fell to about 60 % of its control value.

1:2-Benzanthracene. 100 mg./100 g. produced a small increase in the excretion levels of neutral

Table 2. *The effect of intraperitoneal injections of aromatic hydrocarbons on the excretion of urinary sulphur and glucuronic acid and on the rate of growth of male rats*

(Only increased excretions are indicated for sulphur and glucuronic acid. Growth rates marked * are the steady rates reached after a short period of weight loss.)

Hydrocarbon	Ethereal sulphate	Neutral sulphur	Glucuronic acid	Growth rate (g./day)	
				Control period	After injection
Benzene	+++	-	-	4.3	4.3
Naphthalene (46 mg.)	++	++++	++++	3.9	4.9
Naphthalene (86 mg.)	+	+++++	+++++	3.0	3.0*
Phenanthrene	+++	++	++	4.4	2.6*
Anthracene	-	+	+++	4.7	2.8
1:2-Benzanthracene	-	+	+	3.5	1.6
1:2:5:6-Dibenzanthracene	-	+-	-	3.3	0.0
3:4-Benzpyrene	-	-	+	3.6	0.0
Pyrene	+++	++	++	4.3	3.1*
Chrysene	-	-	-	4.1	2.0*

Results with the individual hydrocarbons are summarized under the name of the hydrocarbon in question.

Benzene. Rats receiving 86 mg./100 g. body weight showed a decreased excretion of inorganic sulphate with concomitant rise in that of ethereal sulphate during the 24 hr. following injection; excretions of both forms rapidly regained their normal values. Excretions of neutral sulphur and of glucuronic acid were not significantly affected and no growth retardation was observed.

Naphthalene. This hydrocarbon was administered at two dose levels (46 and 86 mg./100 g.). The higher dose led to the death of four of ten rats during the week following the injection, whereas the smaller quantity was well tolerated. In both experiments there was a considerable decrease in the excretion of inorganic sulphate and a moderate rise in the ethereal sulphate in the urine. Excretion of glucuronide was also increased, but the most striking effect was the high output of neutral sulphur. The lower dose of naphthalene did not retard the growth of the rats. The higher dose killed four of ten rats within 72 hr., but, in spite of this, the surviving animals resumed their normal growth rate within 48 hr. after injection.

Phenanthrene. Injection of 100 mg./100 g. led to a decrease in inorganic sulphate excretion and increases in ethereal sulphate, neutral sulphur, and glucuronic acid in the urine. A fall in weight during the 24 hr. after injection was succeeded by a period during which growth was less than normal.

Anthracene. With 100 mg./100 g. there was no increased ethereal sulphate excretion but a moderately increased output of neutral sulphur and

sulphur and of glucuronic acid without change in the excretion of ethereal sulphate. The growth retardation was greater than that produced by the same dose of anthracene.

1:2:5:6-Dibenzanthracene. The rats tolerated 100 mg./100 g., only two of ten animals dying within 10 days of the injection. During this period no significant change from the normal excretions was observed except a transient fall in inorganic sulphate and a very small rise in neutral sulphur output. Growth of the rats was completely arrested, and the animals maintained a constant weight.

3:4-Benzpyrene. 100 mg./100 g. had no effect on the urinary sulphur excretion apart from inducing a fall in the output of inorganic sulphate. The level of excretion of glucuronic acid was higher after injection than during the control period. Complete cessation of growth was observed in these animals after injection.

Pyrene. Rats injected with 100 mg./100 g. showed a sharp fall in inorganic sulphate excretion accompanied by relatively large increases in output of ethereal sulphate, neutral sulphur, and glucuronic acid. A considerable fall in body weight occurred during the 4 days immediately following the injection; this decline was then abruptly arrested and was followed by a resumption of growth at 75 % of the normal rate.

Chrysene. 100 mg./100 g. produced no significant change in sulphur or glucuronic acid excretion. A fall in body weight occurred immediately after injection and persisted for 48 hr. Growth was then resumed at about half the rate of the pre-injection period.

DISCUSSION

The metabolism of aromatic hydrocarbons. The results may be briefly considered in the light of the

experiments are only capable of revealing urinary metabolites of the substances administered when the latter undergo rapid metabolism and excretion. The constant excretion of very small amounts of a

Fig. 1.

Fig. 1. Excretion of inorganic sulphate in the urine of male rats after single intraperitoneal injections of aromatic hydrocarbons. Average value during control period is shown by broken line; the actual values represented by these lines are shown in parentheses. The day of injection is shown by the arrow on the abscissa.

Fig. 2.

Fig. 2. Excretion of ethereal sulphate in the urine of rats after single intraperitoneal injections of aromatic hydrocarbons. Average value during control period is shown by broken line; the actual values represented by these lines are shown in parentheses. The day of injection is shown by the arrow on the abscissa.

data already available on the metabolism of some of the hydrocarbons used in this work. It must be emphasized that the methods used in the present

metabolite of a substance undergoing slow absorption and metabolism would be undetectable, as would the presence of minor metabolites. This is

well shown in the case of benzene, where the rapid formation and excretion of ethereal sulphate is clearly demonstrated (Fig. 2), while the estimations

shown in the present experiments, was expected from the fact that in some species naphthalene is known to yield some naphthol (Lesnik, 1888). The most striking feature, however, is the speed and extent of mercapturic acid formation as shown by the large rise in the excretion of neutral sulphur immediately following injection of the hydrocarbon. Bourne & Young (1934) isolated naphthylmercapturic acid from the urine of rabbits fed a diet containing naphthalene, and Stekol (1937) showed that the same substance is a metabolite of this hydrocarbon in the rat.

Fig. 3. Excretion of neutral sulphur in the urine of rats after single intraperitoneal injections of aromatic hydrocarbons. Average value during control period is shown by broken line: the actual values represented by these lines are shown in parentheses. The day of injection is shown by the arrow on the abscissa.

of neutral sulphur excretion (Fig. 3) give no indication of the formation of very small quantities of a mercapturic acid which has been isolated by Zbarsky & Young (1943).

The increased excretion of ethereal sulphate and of glucuronide after naphthalene administration,

Fig. 4. Effect of single intraperitoneal injections of aromatic hydrocarbons on the rate of growth of male rats. Day of injection of the hydrocarbon is indicated by an arrow.

The increased excretion of ethereal sulphate and of glucuronic acid by the rats injected with anthracene was expected from the results of Boyland & Levi (1935, 1936*a, b*). These workers showed that the metabolites of anthracene in the rat included 1:2-dihydroxy-1:2-dihydroanthracene, a glucuronide of this substance, and 1-anthrylmercapturic acid.

Bergel & Pschorr (1903) showed that phenanthrylglucuronic acid probably arises in rabbits treated with phenanthrene. The increased excretion of glucuronide in rats injected with this hydrocarbon might be accounted for by the presence of this metabolite in the urine. The increased output of neutral sulphur points to mercapturic acid formation. A marked difference in the metabolic routes of anthracene and of phenanthrene is indicated by the increased excretion of ethereal sulphate following injection of phenanthrene. This might be

explained by the use of both sulphate and glucuronide in the detoxication of the phenolic group of the phenanthrol, while the alcoholic hydroxyl of 1:2-dihydroxy-1:2-dihydroanthracene is combined exclusively with glucuronic acid.

The results of the present experiments on 1:2-benzanthracene, 1:2:5:6-dibenzanthracene, 3:4-benzpyrene, and chrysene, throw little new light on possible conjugated urinary metabolites of these hydrocarbons. The estimations indicate that the quantities of such metabolites, if they exist at all, are very small, and that attempts at their isolation are unlikely to be profitable unless large amounts of urine from animals receiving high doses of these hydrocarbons are used.

The relatively large response in output of ethereal sulphate, neutral sulphur, and glucuronide, in the rats injected with pyrene, offers more promise for experiments on the isolation of metabolites of this hydrocarbon: this point is being further investigated. During the period of weight loss immediately following injection of this substance it was observed that the animals were suffering from severe diarrhoea. Berenblum & Schoental (1943) identified 3:4-benzpyrene-5:8-quinone in the faeces of rats injected with 3:4-benzpyrene. The diarrhoea caused by injections of pyrene might be produced by excretion into the gut of a pyrene quinone, or a hydroxypyrene quinone, with purgative properties. A preliminary examination of faeces from pyrene-injected rats has given evidence for the presence of one or more pyrene quinone derivatives.

The connexion between sulphur metabolism and growth retardation. It is apparent from the curves for average growth rate before and after administration of the hydrocarbons shown in Fig. 4 that three types of response may occur. In the first type the rats continue to show a daily increase in weight even after injection of the hydrocarbon: these daily increases may be equal to those during the control period or they may be less. This type of response is obtained in animals treated with benzene, naphthalene in low doses, anthracene, and 1:2-benzanthracene. In the second type the rats respond to the injection by showing a fall in body weight lasting for several days. After this short period of weight loss, growth is resumed either at a normal or at a retarded rate. Examples of this type occurred in the rats treated with larger doses of naphthalene, phenanthrene, chrysene, and pyrene. The third type encountered in the present experiments may be considered to be a special case of the first type. After injection of 3:4-benzpyrene or of 1:2:5:6-dibenzanthracene there was immediate complete cessation of growth, and the animals maintained a constant weight for a prolonged period.

The immediate, constant, and long-continued reduction in growth rate that Haddow *et al.* (1937)

observed with certain carcinogenic hydrocarbons is well shown by the results with the two carcinogens used in the present experiments. The retardation of growth with 3:4-benzpyrene and with 1:2:5:6-dibenzanthracene was greater and persisted longer than that caused by any of the other seven hydrocarbons investigated. According to the views of White & White (1939) this retardation is caused by a lowering of the supply of sulphur-containing amino-acids (essentially cystine or methionine) available for growth, owing to the additional requirements for these acids for metabolism and elimination of the hydrocarbons. An examination of the results for excretion of neutral sulphur shown in Fig. 3 indicates, however, the improbability of such an explanation. Neither with 3:4-benzpyrene nor with 1:2:5:6-dibenzanthracene is there any evidence for the excretion of a mercapturic acid. On the other hand, injection of naphthalene (46 mg./100 g.) is followed by the excretion of large amounts of neutral sulphur, presumably a mercapturic acid, without influencing the rate of growth of the rats. Even with doses of naphthalene that produced toxic symptoms leading to death in some of the animals, and induced a very high neutral sulphur output in the survivors, growth, after a short period of weight loss followed by recovery, continued at an unretarded rate.

The experiments described in this paper demonstrate, therefore, that (a) the growth retardation in rats caused by the administration of such hydrocarbons as 3:4-benzpyrene or 1:2:5:6-dibenzanthracene is not accompanied by the excretion of any appreciable amount of sulphur-containing derivatives of these hydrocarbons during the period of retarded growth, and that (b) on an adequate diet very considerable demands on the supply of sulphur-containing amino-acids for the purpose of detoxication can be met without retarding the growth of the animals.

These results are, apparently, contradictory to those obtained by White and his colleagues. An examination of the experimental method used by these authors, however, reveals that the results they obtained are by no means unequivocal. Their conclusions were based on the restoration of growth observed on feeding cystine or methionine to rats retarded in growth by the inclusion of hydrocarbons in their diet. Rats on the supplemented diet, but still ingesting hydrocarbon, showed about the same growth rate as when they were fed the unsupplemented diet free from hydrocarbon.

The amount of supplement used by White & White greatly exceeded the theoretical requirement for conversion of all the ingested hydrocarbon into mercapturic acid. Stekol (1943) showed that, in the case of bromobenzene, when due allowance is made for the sulphur required for mercapturic acid

formation, all the remaining sulphur in the diet is available for growth. Hence, in the experiments of White *et al.*, if the growth-inhibitory action of the ingested hydrocarbon has actually been completely annulled by the supplement, then the rats would be expected to show a higher growth rate when receiving the supplemented diet plus hydrocarbon than when on the unsupplemented diet free from hydrocarbon. The excess of the supplement above requirement for detoxication should be available for additional growth. Experiments by the present authors, which will be described in detail elsewhere, have shown that the growth rate of rats on White's basal diet is increased two to four times on addition of cystine or methionine.

Experiments on dimethylaminoazobenzene (White, 1940) and on iodoacetic acid (Stevenson & White, 1940) are open to the same criticism. It appears that, with the methods and criteria employed by these authors, any substance inducing growth inhibition *not* caused by deprivation of sulphur-containing amino-acids would show an apparent connexion with sulphur metabolism.

The results recorded in the present paper, together with a re-examination of the data of White *et al.*, show that the growth retardation produced by such hydrocarbons as 3:4-benzpyrene, methylcholanthrene, and 1:2:5:6-dibenzanthracene, cannot be attributed to the necessity for furnishing sulphur-containing amino-acids for detoxication at the expense of those available for body growth. The growth retardation must be produced by other causes, which are possibly also operative to a smaller extent in the retardation produced by anthracene, 1:2-benzanthracene, phenanthrene, or chrysene (Fig. 4). The cause appears to be more subtle than the mere sulphur deprivation that accounts for the whole of the growth-retarding effects of such mercapturic acid-forming substances as bromobenzene or naphthalene. Further search for the true mechanism is clearly important. An obvious possibility is inhibition of the anterior lobe of the hypophysis with consequent suppression of growth hormone secretion. Such an action is known to occur with oestrogens (Zondek, 1941). Experiments to test this hypothesis are being carried out. Alternatively, interference with any one of a number of vital enzyme systems by the hydrocarbons or their metabolic products could lead to partial or complete cessation of growth in an animal.

The demonstration that sulphur plays no major role in the elimination of such hydrocarbons as

3:4-benzpyrene renders more interesting the experiments of Crabtree (1944) on the inhibition of the carcinogenesis caused by this hydrocarbon. He showed that three classes of substances, which are structurally unrelated but have the common property of fixing labile sulphhydryl groups, all produce the effect of checking carcinogenic action. If sulphur is not involved in the elimination of the hydrocarbon this observation points, as Crabtree suggests, to the necessity for combination between the carcinogenic hydrocarbon and sulphhydryl at a primary stage in the biological action of the carcinogen. Competition for sulphhydryl groups between inhibitor and carcinogen might lead to complete suppression of the carcinogenic change.

SUMMARY

1. Modifications of the standard procedure for estimating urinary sulphur partition by the volumetric benzidine method are described, and the results of a study of sulphur excretion in normal male rats are given.

2. The excretion of inorganic sulphate, ethereal sulphate, neutral sulphur and glucuronic acid in the urine of male rats injected with benzene, naphthalene, phenanthrene, anthracene, 1:2-benzanthracene, 1:2:5:6-dibenzanthracene, 3:4-benzpyrene, pyrene, or chrysene was investigated. Observations on the effect of these hydrocarbons on the rate of body growth were also made.

3. No evidence of the excretion of mercapturic acids by rats treated with the carcinogens 3:4-benzpyrene and 1:2:5:6-dibenzanthracene was found. The retardation of growth produced by these hydrocarbons cannot be attributed to deprivation of sulphur-containing amino-acids.

4. A re-examination of the data of White and his colleagues, who arrived at conclusions contrary to the above, indicates that their conclusions are not unequivocal and that their results are in fact compatible with the absence of a connexion between the growth retardation produced by polycyclic hydrocarbons and sulphur metabolism.

We wish to thank Miss P. Hales for her assistance with the animals. One of us (F. L. W.) is indebted to the Sir Halley Stewart Trust for a Fellowship held during this work. Generous grants from the British Empire Cancer Campaign, the Anna Fuller Fund, the Jane Coffin Childs Memorial Fund, and facilities afforded by Imperial Chemical Industries Limited, have also assisted.

REFERENCES

- Berenblum, I. & Schoental, R. (1943). *Cancer Res.* **3**, 145.
Bergel, P. & Pschorr, R. (1903). *Hoppe-Seyl. Z.* **38**, 17.
Bourne, M. C. & Young, L. (1934). *Biochem. J.* **28**, 803.
Boyland, E. & Levi, A. A. (1935). *Biochem. J.* **29**, 2679.
Boyland, E. & Levi, A. A. (1936a). *Biochem. J.* **30**, 728.
Boyland, E. & Levi, A. A. (1936b). *Biochem. J.* **30**, 1225.
Crabtree, H. G. (1944). 41st *Ann. Rep.*, 1943-1944, *Imperial Cancer Research Fund*. London.

- Haddow, A. (1935). *Nature, Lond.*, **136**, 868.
 Haddow, A. & Robinson, A. M. (1937). *Proc. Roy. Soc. B*, **122**, 442.
 Haddow, A., Scott, C. M. & Scott, J. D. (1937). *Proc. Roy. Soc. B*, **122**, 477.
 Lesnik, M. (1888). *Arch. exp. Path. Pharmacol.* **24**, 167.
 Maughan, G. B., Evelyn, K. A. & Browne, J. S. L. (1938). *J. biol. Chem.* **126**, 567.
 Peters, J. P. & Van Slyke, D. D. (1932). *Quantitative Clinical Chemistry*, **2**, 894. Baltimore: Williams and Wilkins.
 Stekol, J. A. (1937). *J. biol. Chem.* **121**, 87.
 Stekol, J. A. (1943). *Arch. Biochem.* **2**, 151.
 Stevenson, E. S. & White, A. (1940). *J. biol. Chem.* **134**, 709.
 White, A. & White, J. (1940). *Yale J. Biol. Med.* **12**, 427.
 White, J. (1940). *J. nat. Cancer Inst.* **1**, 337.
 White, J. & White, A. (1939). *J. biol. Chem.* **131**, 149.
 Zbarsky, S. H. & Young, L. (1943). *J. biol. Chem.* **151**, 487.
 Zondek, B. (1941). *Clinical and Experimental Investigations on the Genital Functions and their Hormonal Regulation*, Chap. 3. Baltimore: Williams and Wilkins.

The Antibacterial Action of 4:6-Dimethoxytoluquinone and its Fate in the Animal Body

By G. E. GLOCK, R. H. THORP (*The Wellcome Foundation Ltd.*), J. UNGAR (*Glaxo Laboratories Ltd.*)
 AND R. WIEN (*Mayer and Baker Ltd.*)

(Received 30 May 1945)

During the examination of a series of derivatives of fumigatin, 3-hydroxy-4-methoxytoluquinone, Oxford (1942) noted that while toluquinone was much less bacteriostatic, certain other quinones, particularly 4:6-dimethoxytoluquinone (synthesized: Anslow, Ashley & Raistrick, 1938; Aulin & Erdtman, 1938), were more bacteriostatic than fumigatin.

We have investigated the biological properties of the latter quinone for its possible use as a therapeutic agent by local or systemic administration. It was found at an early stage that the compound was inactivated in the presence of blood and it did not give any protection to mice against certain pathogenic organisms. Since we were, however, engaged in research on similar compounds we studied the mode of its deactivation and detoxication in the animal body, as a possible guide for further chemical work in the preparation of more active compounds.

After the completion of the work described in this paper a publication by Barber (1944) appeared on the antibacterial action of 4:6-dimethoxytoluquinone. She found that it had a powerful bacteriostatic action against *Staphylococcus aureus* and *Streptococcus pyogenes* in broth, but owing to its greatly decreased activity in the presence of blood or serum, and to the markedly antileucocytic properties of the compound, it was contra-indicated as a therapeutic agent. Our results agree with these findings.

EXPERIMENTAL

ACTION AGAINST BACTERIA

In vitro experiments

The following organisms were used: *Streptococcus pyogenes*, *Staphylococcus aureus*, *Diplococcus pneumoniae* (Type 1), *Ps. aeruginosa*, *Proteus vulgaris*, *Esch. coli*, *Cl. perfringens*.

Different types of culture media were used:

(a) *Synthetic media*. The synthetic media used were evolved from work described by McIlwain, Fildes, Gladstone & Knight (1939) on the nutrients essential for the growth of bacteria.

For staphylococci a medium was used consisting of: NaCl, 5.0 g.; K_2HPO_4 , 1.0 g.; KH_2PO_4 , 1.0 g.; $MgSO_4$, 0.2 g.; $CaCl_2$, 0.1 g.; ammonium lactate, 5.0 g.; acid-hydrolyzed casein, 1.0 g.; distilled water to 1 l.

For streptococci, the following medium was used: K_2HPO_4 , 0.5 g.; KH_2PO_4 , 0.5 g.; $MgSO_4 \cdot 7H_2O$, 0.2 g.; NaCl, 0.1 g.; $FeSO_4 \cdot 7H_2O$, 0.01 g.; $MnSO_4 \cdot 4H_2O$, 0.01 g.; glucose, 20.0 g.; sodium acetate, 6.0 g.; adenine, 20 mg.; riboflavin, 0.2 mg.; calcium *DL*-pantothenate, 0.2 mg.; nicotinic acid, 0.2 mg.; pyridoxine, 0.2 mg.; biotin, 0.0001 mg.; distilled water to 1 l. This medium was Seitz-filtered before use, and 1% of acid-hydrolyzed casein was then added. For the other organisms the medium used was as follows: glycerol, 10.0 g.; NaCl, 5.0 g.; $CaCl_2$, 0.1 g.; $MgSO_4$, 0.5 g.; K_2HPO_4 , 2.0 g.; ammonium lactate, 6.0 g.; acid-hydrolyzed casein, 1.0 g.; distilled water to 1 l.

(b) *Papain digest broth*, containing 1% (w/v) glucose.

(c) *Papain digest broth*, with the addition of 10% (v/v) normal horse serum.

(d) *Normal human defibrinated blood*, used in Fleming's slide-cell technique.

Testing of anti-bacterial activity. Serial dilutions of 4:6-dimethoxytoluquinone or the corresponding quinol were added to the tubes containing the media. In making dilutions of 4:6-dimethoxytoluquinone, suitable precautions were taken to prevent oxidation.

The tubes of medium + 4:6-dimethoxyquinone dilutions were inoculated with one standard drop of a saline suspension of a 24 hr. agar culture, containing about 100×10^6 organisms/ml., and were incubated at 37°. The results were read after 24 and 48 hr. for bacteriostatic effects; the tubes