of the functional groups on the toxicity of the metabolites. In no instance did any alteration in the structure of Rubratoxin B lead to an increase in toxicity and the results show that all the functional groups of this poly-functional molecule contribute to its toxicity.

Acknowledgement—We wish to acknowledge the advice of Professor G. Brownlee of King's College, London, also to thank Mr. R. T. Bone for his technical assistance and Dr. J. K. Sutherland for samples of Glauconic and Byssochlamic acids.

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Biochemical Pharmacology, Vol. 19, pp. 615–617. Pergamon Press. 1970. Printed in Great Britain

The effect of prolonged pretreatment with 6-substituted benzo [a] pyrene derivatives upon zoxazolamine paralysis times in mice

(*Received* 9 *June* 1969; *accepted* 12 *August* 1969)

IT HAS recently been shown[1] that pretreatment of mice with a single dose of a number of 6-substituted benzo [a] pyrene derivatives caused a shortening of the zoxazolamine paralysis time 24 hr later. In the case of 6-hydroxymethyl-benzo [a] pyrene, a marked prolongation was observed whilst benzo-[a] pyrene-6-carboxaldehyde was without effect. It seemed worth examining the effect of repeated pretreatment with benzo [a] pyrene derivatives upon zoxazolamine paralysis time. Accordingly groups of ten mice were injected with either arachis oil or 6-substituted benzo [a] pyrene derivatives in arachis oil and 72 hr and 96 hr later further doses given. A further 24 hr after the final dose zoxazolamine was administered and the paralysis time determined. The dose levels and experimental procedures have been described previously.[1]

It was observed that one mouse in the benzo [a] pyrene-6-carboxaldehyde group and two in the 6-methylbenzo [a] pyrene group died during the pretreatment period. A further three mice died during the determination of the zoxazolamine paralysis times. Two of these mice were in the 6-hydroxymethyl group and one was in the 6-carboxaldehyde group. Before death these animals had shown symptoms consistent with pulmonary oedema after treatment with zoxazolamine. A number of other animals in the groups pretreated with methyl, hydroxymethyl and carboxaldehyde derivatives had also shown signs of respiratory distress during paralysis by zoxazolamine. These symptoms were not observed in controls or in animals treated with other benzo [a] pyrene derivatives. Upon post mortem examination of all the animals used, marked fluid retention in the abdominal cavity was observed in animals treated with methyl, hydroxymethyl and carboxaldehyde derivatives of benzo [a] pyrene. This fluid retention was not observed with the controls, with benzo [a] pyrene itself or with any of the other benzo [a] pyrene derivatives described in this paper.

The effects of the benzo [a] pyrene derivatives upon the zoxazolamine paralysis times are summarised in the table. It was found that 6 hydroxymethyl-benzo [a] pyrene produced a very highly significant prolongation of the zoxazolamine paralysis time as found previously[1] when the paralysis time was measured 24 hr after treatment with a single dose of the compound. However a very highly significant prolongation was also observed with 6-methylbenzo [a] pyrene (which had reduced the paralysis time to 48 per cent of the control value 24 hr after a single dose) and with benzo [a] pyrene-6-carboxaldehyde (which had not altered the paralysis time 24 hr after a single dose). These results would appear

TABLE 1. THE EFFECT OF PROLONGED PRETREATMENT WITH 6-SUBSTITUTED BENZO [a] PYRENE DERIVATIVES UPON ZOXAZOLAMINE PARALYSIS TIMES IN MICE

Compound	Mean† paralysis time (min)	Range	Significance	Percentage of control
Control*	43·8 ± 14·1	23·5–65·0	—	—
Benzo [a] pyrene	24·9 ± 11·3	8·0–45·0	H. S.	57·0
6 Methylbenzo [a] pyrene	82·3 ± 33·7	45·0–150·0	V. H. S.	187·8
6 Hydroxymethylbenzo [a] pyrene	78·1 ± 20·0	60·0–127·5	V. H. S.	178·0
Benzo [a] pyrene -6-carboxaldehyde	107·3 ± 36·3	73·0–150·0	V. H. S.	245·0
Control*	43·6 ± 13·8	25·0–66·5	—	—
6 bromobenzo [a] pyrene	37·1 ± 7·5	27·0–46·0	S	85·1
Benzo [a] pyrene -6-carbonamide	17·9 ± 0·6	17·5–18·5	V. H. S.	41·2
Benzo [a] pyrene -6-carbonitrile	32·3 ± 6·1	21·5–41·0	S	74·1

*The experiments were carried out on two separate batches of animals. The results for the control for each group are listed in the table with the relevant experimental groups.

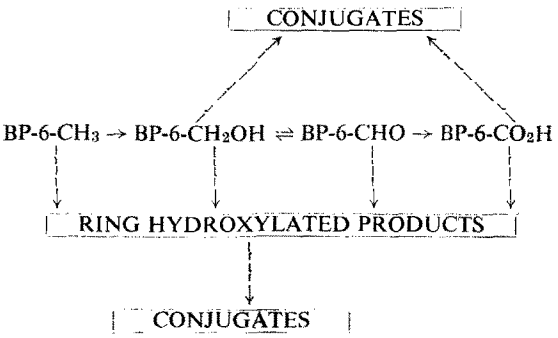
†Ten animals used in all groups for paralysis time determinations except hydroxymethyl (six mice) and carboxaldehyde (eight mice).

S = Significant, P 0·05–0·02; H. S. = Highly Significant, P 0·02–0·01; V. H. S. = Very Highly Significant, P < 0·01.

to be consistent with the view that the effect is due to the formation of a metabolite which is capable of producing *in vivo* inhibition of liver microsomal "Drug Metabolising" enzyme activity. The similarity of the toxicity of the three compounds and the fluid retention produced is noteworthy.

The nature of the metabolite is not certain as all three could be converted into a variety of related metabolites as shown in the diagram. All the interconversions shown are quite possible from a consideration of the known metabolism of other compounds.[2] Conjugate formation from carboxyl, alcoholic hydroxyl and phenolic hydroxyl groups (Ring hydroxylated products) might be expected

with glucuronic acid, sulphuric acid and amino acids etc. From a consideration of these metabolic interconversions it can be seen that if one of the compounds is responsible, in its own right, for the observed effects that compound must be the hydroxymethyl derivative.



Recently Buu-Hoi[3] has found that pretreatment with benzo [a] pyrene 6 carboxaldehyde produced a shortening of the zoxazolamine paralysis time in rats 24 hr after dosing. This contrasts with our own previous observations[1] and with the work reported here. However we have used mice rather than rats which may explain the difference. We have also observed that samples of benzo [a] pyrene 6 carboxaldehyde prepared by the literature method[4] may contain significant amounts of benzo [a] pyrene itself (unpublished observations).

The fact that the 6 methyl and 6 carboxaldehyde derivatives produce a prolongation of the zoxazolamine time only after repeated dosing over several days may be explicable in one of two ways. An active metabolite formed in relatively small amounts may gradually accumulate or the stimulation of microsomal enzymes by the first dose might lead to enhanced conversion of a subsequent dose into an active metabolite. Further work is in progress to try to illuminate this problem.

Acknowledgements—We wish to thank McNeil laboratories for the generous gift of a sample of zoxazolamine.

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