From the results reported in the present paper there seems to be no relation between the changes in total iron contents and those of flavines, as a depletion of the first, following intravenous OT precedes depletion of the second, and an uptake of iron apparently occurs during the depletion of flavines.

Further investigation is expected to show the meaning of these results.

Institute of Pharmacology Medical faculty,
University of Sarajevo,
Sarajevo, Yugoslavia

P. STERN
E. HASANAGIĆ
Z. FUKS

REFERENCES

1. P. C. BUCY, *J. Neuropath. exp. Neurol.* **1,** 224 (1942).
2. A. BLAŽEVIĆ, S. HADŽOVIĆ and P. STERN, *Iugosl. Physiol. Pharmacol. Acta* **1,** 63 (1965).
3. E. A. SPIEGEL, H. T. WYCIS, E. G. SZEKELY and H. SPULER, in *Extrapyramidal System and Neuroleptics* (Ed. J. M. BORDEREAU). Editions Psychiatriques, Montreal p. 255, 1961.
4. H. SPATZ, *Z. ges. Neurol. Psychiatr.* **77,** 261 (1922).
5. P. B. DIESEL and M. TAUBERT, *Verhandl. dtsch. Ges. Path.* **38,** 321 (1954).
6. S. HADŽOVIĆ, B. NIKOLIN and P. STERN, *J. Neurochem.* **12,** 98 (1965).
7. P. STERN and E. HASANAGIĆ, *J. Neurochem.* **14,** 1129 (1967).
8. E. HASANAGIĆ and P. STERN, *Life Sci.* **7,** 921 (1968).
9. A. L. FOY, H. L. WILLIAMS, S. CORTELL and M. E. CONRAD, *Anal. Biochem.* **18,** 559 (1967).
10. O. A. BESSEY, O. H. LOWRY and R. H. LOVE, *J. biol. Chem.* **180,** 755 (1949).

Biochemical Pharmacology, Vol. 18, pp. 942–945. Pergamon Press. 1969. Printed in Great Britain

The effect of benzo[a]pyrene derivatives upon drug metabolising enzyme activities

(*Received* 26 *August* 1968; *accepted* 20 *November* 1968)

MANY polycyclic aromatic hydrocarbons are known to induce, in rodents, the synthesis of the so-called "Drug Metabolising Enzymes", a group of apparently related adaptive microsomal enzymes found predominantly in the liver.[1] Recently a number of reports have appeared concerning the induction of "drug metabolising enzymes" by substituted derivatives of aromatic polycyclic hydrocarbons and of related heterocyclic systems.[2–4] It seemed at one time that ability to induce these enzymes was associated with carcinogenicity but recent work, including that quoted above, shows that this is not necessarily so. In view of this and the fact that under a number of circumstances it could be therapeutically desirable to stimulate the activity of these "drug metabolising enzymes", in man and his domestic animals; information upon the effect of substituent groups, in polycyclic aromatic systems, on the induction of adaptive microsomal enzymes is of great interest.

In the course of a systematic investigation of the relationship between substituents and biological properties in benzo[a]pyrene derivatives we studied the effect of a variety of 6-substituted compounds (see formula) upon adaptive microsomal enzymes. Benzo[a]pyrene itself, (R=H), 6-formylbenzo[a]-pyrene, (R=CHO), 6-bromobenzo[a]pyrene (R=Br), 6-chlorobenzo[a]pyrene (R=Cl), 6-methyl-benzo[a]pyrene ($R=CH_3$), 6-hydroxymethyl benzo[a]pyrene ($R=CH_2OH$), benzo[a]pyrene-6-amide ($R=CONH_2$) and benzo[a]pyrene-6-nitrile (R=CN) were examined. We wish to report the effect of these compounds upon the duration of zoxazolamine paralysis and hexobarbital hypnosis in mice. It appears that two different adaptive microsomal enzyme systems are involved in the metabolism of zoxazolamine and hexobarbital, as administration of benzo[a]pyrene increases the rate of zoxazola-mine metabolism, hence shortening the paralysis time, but does not alter hexobarbital metabolism. In contrast to the behaviour of benzo[a]pyrene phenobarbital stimulates both zoxazolamine and

hexobarbital metabolism.[5] The types of metabolites produced from zoxazolamine[6] and hexobarbital[7] appear to be different also.

Female Schofield mice weighing 20–25 g maintained on a standard laboratory pellet diet and water, were used. Groups of ten animals were used per compound. The animals were each injected intraperitoneally (i.p.) with 0·5 ml of arachis oil containing 1 mg of benzo[a]pyrene or derivative, except for the controls, which received arachis oil only, and the hydroxymethyl group, which received 0·5 mg of compound in 0·5 ml of arachis oil. In the case of the hydroxymethyl compound a dose of 1 mg per mouse produced some fatalities. Twenty-four hr after injection with the arachis oil solutions zoxazolamine or hexobarbitone sodium, both 3 mg per mouse, were injected. The zoxazolamine solution was prepared by dissolving 300 mg of zoxazolamine (McNeil Laboratories) in 3·6 ml of N hydrochloric acid and making up to 15 ml with 0·9% sodium chloride solution to give a final concentration of 3 mg in 0·15 ml. The hexobarbital solution was prepared by dissolving 300 mg of hexobarbitone sodium (Bayer Products Ltd.) in 0·9% sodium chloride solution and making up to 15 ml to give a final concentration of 3 mg in 0·15 ml. Paralysis and hypnosis times were estimated by measuring the time between injection and the animals regaining the ability to right itself three times in 30 sec.

The benzo[a]pyrene derivatives used were synthesized by us and analysed correctly. They appeared to contain no detectable amounts of the parent hydrocarbon. Details of the synthesis of these compounds and our investigation of their purity together with further information upon some of their other biological properties will be published at a later date.

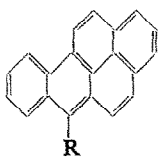

FIG. 1.

The observed hypnosis and paralysis times are recorded in Tables 1 and 2. Probabilities were calculated by means of "Students" *t*-test. The amide, chloro and bromo derivatives were investigated some time after the other compounds and as a consequence two "control" values for animals treated with arachis oil only are included in the tables. The "experimental" and "control" results in each of the two groups of compounds were obtained at the same time and using animals from the same batch.

It can be seen (Table 1) that none of the compounds appeared to stimulate significantly hexobarbital metabolism, as measured by the hypnosis time. The hydroxymethyl derivative produced a highly significant increase in hypnosis time indicating, most probably, depression of the rate of hexobarbital metabolism. In the case of zoxazolamine paralysis times (Table 2) most of the compounds appear to stimulate zoxazolamine metabolism as measured by paralysis times. The effectiveness of the compounds in shortening paralysis times is $CONH_2 > H \approx Me \approx Br. > Cl \approx CN$ The 6 formyl derivative produced no apparent effect whilst the 6 hydroxymethyl gave a very highly significant prolongation of the paralysis time. It would probably not be wise to attach too much significance to the order of effectiveness of these compounds in shortening paralysis times as the standard deviations of the paralysis times are quite large. It does appear however that the parent aromatic polycyclic hydrocarbon system can be substituted with a range of electron donating or withdrawing groups without loss of the power to induce the synthesis of certain "drug metabolising enzymes". This is of potential value as, generally speaking, substituents, other than amino and alkyl, appear to decrease the carcinogenicity of polycyclic aromatic hydrocarbons. Information on the effect of substituents upon carcinogenicity in polycyclic aromatic systems is rather scanty however[8,9] and testing procedures in some of the older reports leave much to be desired.

The effect of the hydroxymethyl derivative is of great interest particularly in view of the fact that Buu Hoi *et al.*[3] have demonstrated a prolongation of zoxazolamine paralysis times after treatment with a carboxyl substituted indophenazine. It is quite reasonable to suppose that the hydroxymethyl might be metabolised to carboxyl as this is the metabolic fate of benzyl alcohol and of a number of related compounds.[10] Buu Hoi *et al.*[4] have observed some formyl derivatives to be as active as the

TABLE 1. THE EFFECT OF PRETREATMENT WITH 6 SUBSTITUTED BENZO[a]PYRENES UPON THE HEXOBARBITAL HYPNOSIS TIME FOR MICE

Compound injected	Mean hypnosis time (min)	Range	Standard deviation	Significance	% of Control
		Experiment I			
"Control"	60·7	47·5–81·0	±10·3	—	—
Benzo[a]pyrene	58·4	40·0–89·5	±13·5	N.S.	96
6-Methyl benzo[a]pyrene	59·7	40·5–99·5	±18·6	N.S.	98
6-Hydroxymethyl benzo[a]pyrene	104	67·3–193·0	±43·8	H.S.	171
6-Formyl benzo[a]pyrene	73·4	41·5–147·5	±29·3	N.S.	121
Benzo[a]pyrene-6-nitrile	68·9	41·5–96·0	±17·5	N.S.	113
		Experiment II			
"Control"	45·0	30·5–57·0	±10·3	—	—
Benzo[a]pyrene-6-amide	59·9	29·0–79·5	±16·8	N.S.	133
6-Bromo benzo[a]pyrene	42·1	23·5–58·5	±10·2	N.S.	94
6-Chloro benzo[a]pyrene	45·9	36·5–54·5	±7·0	N.S.	102

N.S. = Not significant P > 0·05
S. = Significant P = 0·05–0·02
H.S. = Highly significant P = 0·02–0·001
V.H.S. = Very highly significant P < 0·001

TABLE 2. THE EFFECT OF PRETREATMENT WITH 6 SUBSTITUTED BENZO[a]PYRENES UPON THE ZOXAZOLAMINE PARALYSIS TIME FOR MICE

Compound injected	Mean paralysis time (min)	Range	Standard deviation	Significance	% of Control
		Experiment I			
'Control'	57·9	45·0–81·5	±12·3	—	—
Benzo[a]pyrene	27·6	15·0–42·0	±9·5	V.H.S.	46
6-Methyl benzo[a]pyrene	28·4	11·5–38·0	±7·0	V.H.S.	48
6-Hydroxymethyl benzo[a]pyrene	111·8	70·0–157·5	±31·5	V.H.S.	187
6-Formyl benzo[a]pyrene	57·9	41·0–86·0	±16·1	N.S.	100
Benzo[a]pyrene-6-nitrile	36·5	28·5–44·0	±5·0	V.H.S.	61
		Experiment II			
'Control'	48·7	29·0–115·0	±24·7	—	—
Benzo[a]pyrene-6-amide	17·9	7·5–23·0	±4·9	V.H.S.	37
6-Bromo benzo[a]pyrene	22·7	13·5–46·0	±10·0	H.S.	47
6-Chloro benzo[a]pyrene	28·0	16·5–40·0	±6·69	S.	58

N.S. = Not significant P > 0·05
S. = Significant P = 0·05–0·02
H.S. = Highly significant P = 0·02–0·001
V.H.S. = Very highly significant P < 0·001

parent compound (Naphthacene) in stimulating zoxazolamine metabolism. This does not fit in too well with our observation of the apparent ineffectiveness of the 6 formyl derivative of benzo[a]pyrene. However Buu-Hoi *et al.* used rats in their work and we have used mice. Our choice of animals was governed by the nature of some of the other biological properties we wished to investigate. It was clearly desirable to use the same species for all our work so that a meaningful comparison of the results could be made.

It is of interest that aromatic aldehydes are normally metabolised to acids.[11] The rates of metabolism in the mouse and the rat could be markedly different. The apparent lack of effect of 6 formyl benzo[a]-pyrene could arise from a combination of stimulation by the parent formyl compound coupled with inhibition produced by a metabolite. In this context the methyl, amide and to a lesser extent nitrile derivatives might all be expected to give carboxyl derivatives on metabolism.[12] Examination of Table 1, in which inhibition of drug metabolising systems would show up clearly; reveals that hypnosis times are prolonged, but not significantly, for formyl, nitrile and amide derivatives. In the case of the amide, if an inhibitory metabolite is formed then the inducing effect of the amide itself must be very great (see Table 2). It must be pointed out that the inhibitory effect is not necessarily a direct one but may operate via an indirect mechanism such as producing adrenal damage. This matter is being investigated further and attempts are being made to prepare the 6 carboxyl derivative of benzo[a]pyrene

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

Department of Pharmacy,
Leicester Regional College of Technology,
The Newarke,
Leicester

F. DEWHURST
D. A. KITCHEN

REFERENCES

1. J. C. ARCOS, A. H. CONNEY and N. P. BUU-HOI, *J. biol. Chem.* **236**, 1291 (1961).
2. N. P. BUU-HOI and HIEN DO-PHUOC, *Naturwiss.* **53**, 435 (1966).
3. N. P. BUU-HOI, HIEN DO-PHUOC and G. SAINT-RUF, *Compt. rend.* **264**, 2414 (1967).
4. N. P. BUU-HOI and HIEN DO-PHUOC, *Z. Naturforsch.* **22** B, 532 (1967).
5. A. H. CONNEY, C. DAVISON, R. GASTEL and J. J. BURNS, *J. Pharmac. exp. Ther.* **130**, 1 (1960).
6. A. H. CONNEY, N. TROUSOF and J. J. BURNS, *J. Pharmac. exp. Ther.* **128**, 333 (1960).
7. J. R. COOPER and B. B. BRODIE, *J. Pharmac. exp. Ther.* **114**, 409 (1955).
8. D. B. CLAYSON, *Chemical Carcinogenesis*, pp. 135, 201. Churchill, London (1962).
9. W. C. HUEPER and W. D. CONWAY, *Chemical Carcinogenesis and Cancers*, pp. 197, 258. Thomas. Illinois (1964).
10. R. T. WILLIAMS, *Detoxication Mechanisms*, p. 318. Chapman and Hall, London, 2nd edn. (1959).
11. R. T. WILLIAMS, *Detoxication Mechanisms*, p. 332. Chapman and Hall, London, 2nd edn. (1959).
12. R. T. WILLIAMS, *Detoxication Mechanisms*, pp. 194, 156, 407. Chapman and Hall, London, 2nd edn. (1959).

Biochemical Pharmacology, Vol. 18, pp. 945–947. Pergamon Press. 1969. Printed in Great Britain

A comparison of hepatic drug-metabolizing enzyme activity in the germ-free and conventional rat*

(*Received* 25 *October* 1967; *accepted* 4 *October* 1968)

THE OXIDATIVE and reductive pathways of hepatic microsomal drug metabolism are generally deficient

* This study was supported in part by Grant HD-03074 from the National Institutes of Health.