Biochemical Pharmacology, Vol. 22, pp. 561–566. Pergamon Press, 1973. Printed in Great Britain.

EFFECT OF 6-SUBSTITUTED BENZO[a]PYRENE DERIVATIVES UPON MOUSE LIVER

FRANCIS DEWHURST and DAVID A. KITCHEN
School of Biology, City of Leicester Polytechnic, P.O. Box 143, Leicester LE1 9BH, England

(*Received* 11 *January* 1972; *accepted* 25 *September* 1972)

Abstract—Benzo[a]pyrene, 6-hydroxymethylbenzo[a]pyrene, 6-methylbenzo[a]pyrene, benzo[a]pyrene-6-carboxaldehyde, benzo[a]pyrene-6-carbonamide, benzo[a]pyrene-6-carbonitrile and 6-bromobenzo[a]pyrene all produced a fall in the liver glycogen level in mice for 2 days after injection. No effect on liver wet weight could be observed over a 9-day period. The first four compounds were also found to be without effect upon liver RNA, DNA and protein content and did not alter the histological appearance of the livers. The fall in glycogen, which appears to be a general response to inducers of microsomal drug metabolizing enzymes, was discussed. It does not appear to be associated either with depressed production of adrenal corticosteroids or with increased release of adrenaline. Measurement of hepatic glycogen levels did not appear to be a good method of investigating induction of drug metabolizing enzymes.

A NUMBER of inhibitors of microsomal drug metabolizing enzymes, particularly SKF 525-A, have been shown[1] to produce a fall in hepatic glycogen levels. Adrenaline lowered hepatic glycogen levels and microsomal metabolism of hexobarbital and chlorpromazine[2] whilst noradrenaline inhibited hexobarbital, aminopyrine and aniline metabolism and lowered hepatic glycogen concentration.[3] Kato and Gillette[4] confirmed the effect of adrenaline on the metabolism of hexobarbital and aminopyrine in male rats, which had been used in all previous studies, but found that the effect did not occur in the female rat. These observations, the facts that new-born animals have low levels of hepatic glycogen[5] and of drug metabolizing enzymes,[6] and observations on alloxan diabetic animals,[4,7,8] animals with rapidly regenerating hepatic tissues[9,10] and on starving animals[11] lead to the belief that low microsomal drug metabolizing activity was linked with low glycogen levels in liver. In studies on animals treated with phenobarbitone, which increases drug metabolizing enzyme activity, instead of an increase in hepatic glycogen, a fall in lead-staining granules, believed to be glycogen, was observed in electron micrographs.[12] A fall in chemically determined glycogen to about 60 per cent of the control level was also observed.[13] Due to the high standard deviations involved this fall was not statistically significant however. SKF 525-A has a biphasic action on drug metabolism in mice,[14] initially acting as an inhibitor but later producing increased metabolism. Wooles and McPhillips showed that the action of SKF 525-A in inhibiting drug metabolism appeared to be independent of the lowering of glycogen levels. This implies that the fall in glycogen may be associated with induction of drug metabolizing enzyme activity.

It is well established that adrenal corticosteroids can raise liver glycogen levels[16] and this forms the basis of a biological assay for glucocorticoids.[17] In previous studies on the biological properties of 6-substituted benzo[a]pyrene derivatives[18] we observed

stimulation of drug metabolizing enzyme activity by a range of compounds one of which showed evidence of causing release of adrenal hydrocortisone. In view of this and the previous studies on drug metabolizing enzyme activity and liver glycogen levels, we investigated the effect of these benzo[a]pyrene derivatives upon glycogen levels. To provide a firmer basis for the comparison of changes, in the chemical composition of liver, DNA and RNA content and total liver protein were determined and livers subjected to histological examination in the case of some of the compounds.

MATERIALS AND METHODS

The 6-substituted benzo[a]pyrene derivatives were synthesized as described by Dewhurst and Kitchen.[19] Female Schofield mice weighing 20–25 g were injected intraperitoneally with 0·5 ml of arachis oil solution. Controls received arachis oil only whilst experimental animals received 1 mg of test compound, except for the 6-hydroxymethyl and 6-carboxaldehyde compounds which were given in 0·5 mg doses. Animals were always injected and killed at the same time each day to eliminate diurnal variation. Normally two experimental groups were run with each control group. Animals were killed by cervical dislocation, taking four at a time from each group. The livers were rapidly removed, blotted dry, weighed, individually homogenized in ice-cold distilled water and each homogenate made up to a final volume of 20 ml.

The glycogen content was determined by the anthrone method of Roe and Dailey[20] using glucose for the standard curve. Protein was determined by the method of Itzhaki and Gill[21] using bovine serum albumin as a standard. The DNA was extracted from the homogenate by a modification of the Schmidt–Thannhauser procedure,[22] and determined by the method of Byvoet[23] using salmon sperm DNA (Sigma Chemical Co.) as standard. RNA content was measured by the method of Byvoet[23] correcting for the DNA content and using highly polymerized yeast RNA (British Drug Houses Ltd.) as standard. Groups of 5 mice per compound were killed after 1, 3 and 9 days, the livers removed, placed in Bouins fixative, embedded in paraffin wax and sections cut and stained with eosin and haematoxylin.

RESULTS

The DNA, RNA and protein content of the livers showed no consistent changes for up to 9 days after treatment with benzo[a]pyrene, benzo[a]pyrene-6-carboxaldehyde, 6-methylbenzo[a]pyrene and 6-hydroxymethylbenzo[a]pyrene. Table 1 gives the results obtained with the first two of these compounds and similar results were obtained with the other two. Histological examination revealed no obvious morphological differences between control and experimental sections. In view of the lack of any significant observable effect on DNA, RNA, protein content and histological appearance these parameters were not examined with 6-bromobenzo[a]pyrene, benzo-[a]pyrene-6-carbonitrile and benzo[a]pyrene-6-carbonamide. None of the compounds produced any change in liver wet weight and typical results are included in Table 1. All the compounds produced a fall in liver glycogen content for the first 2 days after treatment. The levels returned to normal by day 3 and were still normal after day 9. Typical results are shown in Figs. 1, 2 and 3. The depression in the liver glycogen level was statistically significant, $P > 0{\cdot}05$, in every case except with 6-methylbenzo[a]-pyrene and with 6-hydroxymethylbenzo[a]pyrene on the first day. The statistical

TABLE 1. EFFECT OF BENZO[a]PYRENE AND BENZO[a]PYRENE-6-CARBOXALDEHYDE UPON MOUSE LIVER

Days after injection	1	2	3	4	7	9
Wet weight of liver in gram						
Control	1·38 ± 0·08	1·44 ± 0·12	1·41 ± 0·13	1·47 ± 0·22	1·56 ± 0·08	1·58 ± 0·06
Benzo[a]pyrene	1·35 ± 0·25	1·35 ± 0·31	1·32 ± 0·16	1·48 ± 0·19	1·60 ± 0·20	1·47 ± 0·20
Benzo[a]pyrene-6-carboxaldehyde	1·50 ± 0·18	1·37 ± 0·28	1·50 ± 0·25	1·44 ± 0·21	1·52 ± 0·20	1·72 ± 0·22
Protein content of liver mg/g wet weight of liver						
Control	121 ± 13·9	130 ± 19·1	120 ± 19·0	126 ± 10·4	121 ± 20·6	129 ± 19·2
Benzo[a]pyrene	132 ± 18·7	120 ± 18·7	119 ± 21·2	118 ± 20·5	115 ± 12·2	128 ± 16·9
Benzo[a]pyrene-6-carboxaldehyde	124 ± 9·7	130 ± 21·9	116 ± 14·0	119 ± 12·1	122 ± 18·0	126 ± 17·8
RNA content of liver mg/g wet weight of liver						
Control	10·76 ± 2·04	9·91 ± 1·00	9·91 ± 0·84	10·62 ± 1·03	10·60 ± 1·30	9·63 ± 1·34
Benzo[a]pyrene	10·57 ± 1·68	9·91 ± 1·20	9·79 ± 0·84	10·22 ± 1·20	10·47 ± 1·76	10·07 ± 1·47
Benzo[a]pyrene-6-carboxaldehyde	10·56 ± 2·06	10·13 ± 1·56	9·88 ± 1·05	10·66 ± 0·85	10·41 ± 0·42	9·93 ± 1·12
DNA content of liver mg/g wet weight of liver						
Control	1·57 ± 0·30	1·94 ± 0·26	1·34 ± 0·30	1·46 ± 0·10	1·04 ± 0·10	1·22 ± 0·07
Benzo[a]pyrene	1·53 ± 0·32	1·82 ± 0·46	1·39 ± 0·19	1·63 ± 0·45	1·05 ± 0·17	1·21 ± 0·15
Benzo[a]pyrene-6-carboxaldehyde	1·26 ± 0·29	1·67 ± 0·34	1·37 ± 0·17	1·38 ± 0·49	1·15 ± 0·13	1·09 ± 0·26

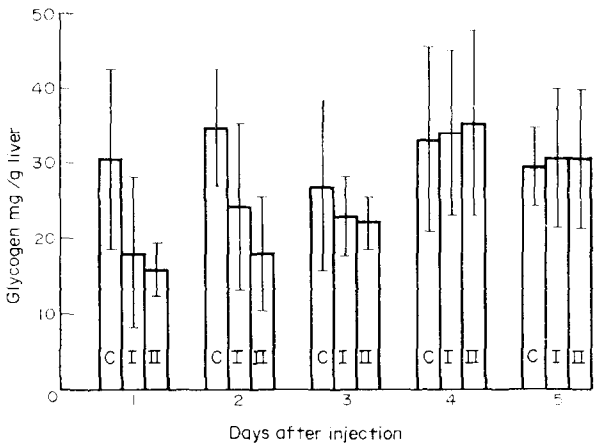

FIG. 1. Liver glycogen ±S.E.M. after treatment with, (I) 6-methylbenzo[a]pyrene and, (II) 6-hydroxymethylbenzo[a]pyrene. Control values = C.

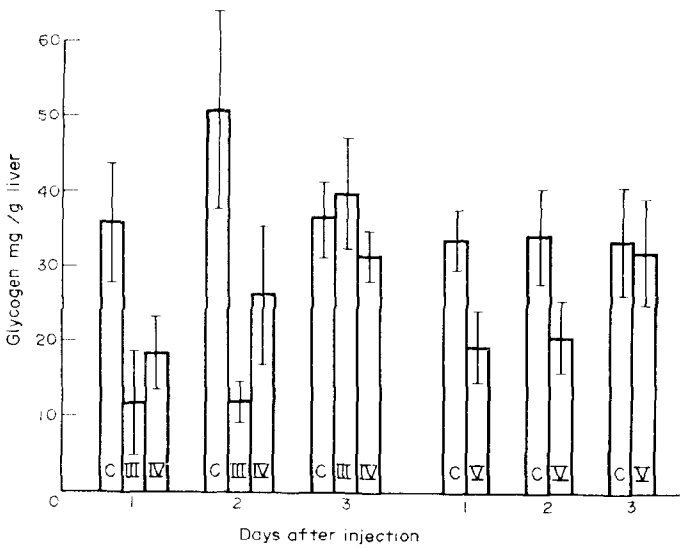

FIG. 2. Liver glycogen ±S.E.M. after treatment with, (III) 6-bromobenzo[a]pyrene, (IV) benzo[a]pyrene-6-carbonitrile and, (V) benzo[a]pyrene-6-carbonamide. Control values = C.

significance of the results was determined using Student's *t*-test. The statistically non-significant falls were of the same order as the significant but the standard errors were larger. It is thus reasonable to state that all the compounds reduced liver glycogen levels for 2 full days after injection.

DISCUSSION

It is well established that phenobarbitone, a potent inducer of microsomal drug metabolizing enzymes, produces considerable increases in liver weight, protein, DNA and RNA content[24] but the same authors found the polycyclic aromatic hydrocarbon 3-methylcholanthrene produces only small increases, if any. These effects reach their

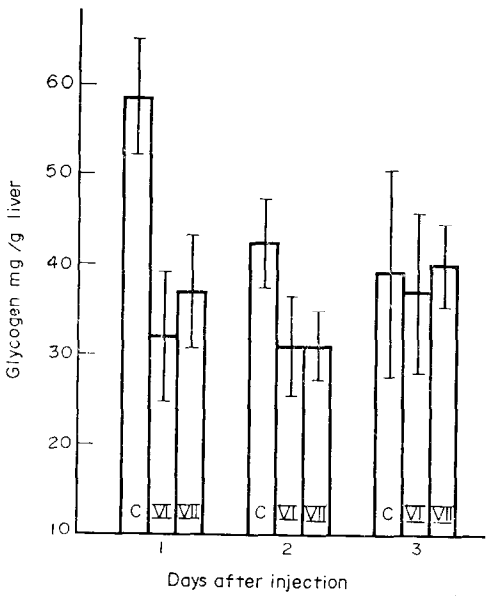

FIG. 3. Liver glycogen ±S.E.M. after treatment with, (VI) benzo[a]pyrene and, (VII) benzo[a]pyrene-6-carboxyaldehde. Control values = C.

maximum in 5–10 days. In our study all the polycyclic aromatic hydrocarbon derivatives failed to produce a statistically significant change in these parameters. It is clear that whilst both phenobarbitone and polycyclic aromatics induce drug metabolizing enzymes there are profound differences in response to these compounds. It is of interest that Mycek[25] has shown that phenobarbitone inhibits liver microsomal ribonuclease, but 3-methylcholanthrene was without effect.

The fact that induction of liver drug metabolizing enzyme activity was accompanied in every case with a fall in liver glycogen fits in with the observations previously reported for phenobarbitone and for SKF 525-A. It is interesting that foetal glycogen levels in mice are high and that birth is associated with a sharp and massive fall followed by a small rise and then another fall between a week and a fortnight after birth.[16] It is about the time of this second fall that liver drug metabolizing enzyme activities start to rise. It appears that increased synthesis of microsomal drug metabolizing enzymes is associated with a fall in liver glycogen. The control of liver glycogen levels is complex and the fall produced by inducers cannot, as a consequence, be used as a test for enzyme induction. The high standard deviations on liver glycogen determinations is a further drawback to the use of glycogen measurements in the study of enzyme induction.

The fact that 6-hydroxymethylbenzo[a]pyrene produces a fall in liver glycogen demands comment in view of the evidence that this compound causes release of adrenal corticosteroids. The tendency for liver glycogen to fall during enzyme induction appears to be stronger than the power of hydrocortisone to cause increased synthesis of liver glycogen. The mechanism for the depression of liver glycogen is not clear but in view of the effects of adrenaline etc. in depressing microsomal drug metabolizing enzyme activity[2–4] the explanation clearly does not lie in the release of

such compounds in response to the hydrocarbons. The observations on the 6-hydroxymethyl compound also indicate that a depression in adrenal corticosteroid production is not the explanation either.

REFERENCES

1. L. A. ROGERS, R. L. DIXON and J. R. FOUTS, *Biochem. Pharmac.* **12,** 341 (1963).
2. J. R. FOUTS, *Adv. Enzyme Reg.* **1,** 225 (1963).
3. R. L. DIXON, L. A. ROGERS and J. R. FOUTS, *Biochem. Pharmac.* **13,** 623 (1964).
4. R. KATO and J. R. GILLETTE, *J. Pharmac. exp. Ther.* **150,** 285 (1965).
5. H. H. SHELLEY, *Br. med. Bull.* **17,** 137 (1961).
6. J. R. FOUTS and R. H. ADAMSON, *Science* **129,** 897 (1959).
7. R. L. DIXON, L. G. HART and J. R. FOUTS, *J. Pharmac. exp. Ther.* **133,** 7 (1961).
8. J. R. GILLETTE, *Ann. N.Y. Acad. Sci.* **123,** 42 (1965).
9. J. R. FOUTS, R. L. DIXON and R. W. SHULTICE, *Biochem. Pharmac.* **7,** 265 (1961).
10. A VON DER DECKEN and T. HULTIN, *Expl. Cell. Res.* **14,** 88 (1958).
11. R. L. DIXON, R. W. SHULTICE and J. R. FOUTS, *Proc. Soc. exp. Biol. N.Y.* **103,** 333 (1960).
12. J. R. FOUTS and L. A. ROGERS, *J. Pharmac. exp. Ther.* **147,** 112 (1965).
13. H. REMMER and H. J. MERKER, in *Procceedings of the Seond International Pharmacological Meeting* (Eds. B. B. BRODIE and J. R. GILLETTE) Vol. 4, p. 299. Pergamon Press, Oxford (1965).
14. D. M. SERRONE and J. M. FUJIMOTO, *Biochem. Pharmac.* **2,** 609 (1962).
15. W. R. WOOLES and J. J. MCPHILLIPS, *Proc. Soc. exp. Biol. Med.* **121,** 399 (1966).
16. A. F. BURTON, R. M. GREENALL and R. W. TURNELL, *Can. J. Biochem.* **48,** 178 (1970).
17. E. H. VENNING, V. E. KAZMIN and J. C. BELL, *Endocrinology* **38,** 79 (1946).
18. F. DEWHURST and D. A. KITCHEN, *Biochem. Pharmac.*, to be published.
19. F. DEWHURST and D. A. KITCHEN, *J. Chem. Soc.* **1,** 256 (1972).
20. J. H. ROE and R. E. DAILEY, *Analyt. Biochem.* **15,** 245 (1966).
21. R. F. ITZHAKI and D. M. GILL, *Analyt. Biochem.* **9,** 401 (1964).
22. H. N. MUNRO and A. FLECK, *Analyst.* **91,** 84 (1966).
23. P. BYVOET, *Analyt. Biochem.* **15,** 31 (1966).
24. W. KUNZ, G. SCHAUDE, H. SCHIMASSEK, W. SCHMID and M. SIESS, *Proc. Eur. Soc.* **7,** 138 (1966).
25. M. J. MYCEK, *Biochem. Pharmac.* **20,** 325 (1971).