These results are consistent with the hypothesis that the oxidative deamination of amitryptiline might occur only after demethylation of the drug.

Cattedra di Farmacologia e Farmacognosia,
Università di Pavia,
27100 *Pavia, Italy*

R. MAFFEI FACINO
G. SANTAGOSTINO
G. L. CORONA

REFERENCES

1. R. MAFFEI FACINO and G. L. CORONA, *Il Farmaco, Ed. Prat.* **23**, 366 (1968).
2. G. L. CORONA, R. MAFFEI FACINO, *Biochem. Pharmac.* **17**, 2045 (1968).
3. R. MAFFEI FACINO, G. L. CORONA, *J. Pharm. Sci.* **58**, 764 (1969).
4. E. STAHL, *Thin-Layer Chromatography* (Ed. E. STAHL), n. 52, p. 490. Springer Verlag, Berlin (1965).
5. E. STAHL, *Thin-Layer Chromatography* (Ed. E. STAHL), n. 22, p. 487. Springer Verlag, Berlin (1965).
6. E. STAHL, *Thin-Layer Chromatography* (Ed. E. STAHL), n. 141, p. 500. Springer Verlag, Berlin (1965).
7. T. KARIYONE and Y. HASHIMOTO, *Nature, Lond.* **168**, 511 (1951).
8. E. STAHL, *Thin-Layer Chromatography* (Ed. E. STAHL), n. 133, p. 500. Springer Verlag, Berlin (1965).
9. E. STAHL, *Thin-Layer Chromatography* (Ed. E. STAHL), n. 61, p. 491. Springer Verlag, Berlin (1965).
10. E. STAHL, *Thin-Layer Chromatography* (Ed. E. STAHL), n. 60, p. 491. Springer Verlag, Berlin (1965).
11. H. B. HUCKER, *Pharmacologist* **4**, 171 (1962).
12. A. H. BECKETT, M. A. BEAVEN and A. E. ROBINSON, *Biochem. Pharmac.* **12**, 782 (1963).
13. S. O. WINTHROP and M. A. DAVIS, *J. Am. Chem. Soc.* **80**, 4331 (1958).

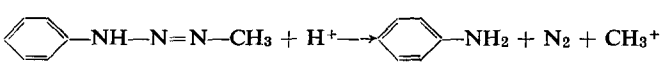
Biochemical Pharmacology, Vol. 19, pp. 1505–1508. Pergamon Press. 1970. Printed in Great Britain

Mechanism of carcinogenesis with 1-aryl-3,3-dialkyltriazenes—II *In vitro*-alkylation of guanosine, RNA and DNA with aryl-monoalkyltriazenes to form 7-alkylguanine

(*Received* 14 *July* 1969; *accepted* 7 *October* 1969)

1-ARYL-3-MONOALKYLTRIAZENES act as typical alkylating agents towards guanosine, RNA and DNA. Reaction *in vitro* resulted in the formation of 7-alkylguanine.

In part I of this series[1] a mechanism has been proposed for the carcinogenic action[2] of 1-aryl-3,3-dialkyltriazenes. Compounds of this type are *N*-dealkylated by microsomal fraction of the liver to form the corresponding aldehyde and 1-aryl-3-monoalkyltriazenes as proximate carcinogens. Aryl-monoalkyltriazenes are alkylating agents[3–5] (Equation 1):



We therefore proposed the alkylation of biopolymers as the first step in carcinogenesis by aryldialkyltriazenes.[1] To verify this hypothesis, the reaction *in vitro* of phenylmonomethyl- and -monoethyltriazene with guanosine, RNA and DNA has been investigated.

MATERIALS AND METHODS

Guanosine (SCHUCHARDT), RNA (SERVA) from yeast, and DNA, highly polymerized, (SERVA) from salmon sperm, were obtained from commercial sources. Tetramethylurea (MERCK) was distilled before use.

1-Phenyl-3-monomethyltriazene was synthesized from diazotised aniline and methylamine,[3] m.p. 37°; λ_{max}273 nm, log ϵ 4·19. 1-Phenyl-3-monoethyltriazene (II) was obtained by the same method, m.p. 29–30° (lit.[3] 31°) λ_{max}276 nm, log ϵ 4·08.

Ultraviolet absorption spectra were measured with a Zeiss DMR 21 recording spectrophotometer. Authentic 7-methylguanine resulted from the action of dimethylsulphate on guanosine and subsequent acid hydrolysis.[6] 7-Ethylguanine[7] was a gift from Dr. F. W. Krüger, Heidelberg. Thin-layer chromatography was carried out on cellulose pre-coated sheets (MERCK). Solvents used were: (A) methanol: hydrochloric acid (15%):water (7:2:1) and (B) 3% NH_4Cl.

Reaction with guanosine

Guanosine (100 mg) was dissolved in 0·1 N acetic acid (15 ml) and tetramethylurea (TMU) (8 ml) Phenyl-monoalkyltriazene (500 mg), dissolved in TMU (2 ml), was added dropwise and stirring at room temperature continued for 15 hr. Solvents were then removed *in vacuo* on a rotating evaporator at 85°. The residue was hydrolysed for 1 hr at 100° after addition of water (30 ml). After removal of solvent 0·1 N potassium carbonate solution (3 ml) was added and filtered. One ml of this solution was used for column chromatography.

Reaction with DNA and RNA

DNA (100 mg) was dissolved in water (15 ml) and TMU (8 ml) and treated with phenyl-monoalkyltriazene (500 mg) in TMU (2 ml). The experiment was then continued as with guanosine. RNA (100 mg) was dissolved in phosphate buffer, pH 7·4 (15 ml) and then treated as described above.

Column chromatography

Digests of the reaction products were separated on a column (1 × 85 cm) of Sephadex G-10 (PHARMACIA) according to Sweetman and Nyhan.[8] The column was eluted with 0·05 M NaH_2PO_4 adjusted to pH 7·0 with NaOH.

RESULTS AND DISCUSSION

Reaction of 1-phenyl-3-monomethyl- (I) and monoethyltriazenes (II) respectively with guanosine RNA and DNA resulted in the formation of 7-methyl- and 7-ethylguanine. These are the expected reaction products if I and II react as alkylating agents. Elution chromatography, absorption spectroscopy and thin-layer chromatography were used for identification of the reaction products.

Tetramethylurea was added to the reaction medium for two reasons: (1) To ensure a homogeneous reaction medium because the monoalkyltriazenes are only very slightly soluble in aqueous medium. (2) Tetramethylurea is known to be a favorable solvent for alkylations.[9] Formamide or dimethylformamide has been used for similar reasons in the methylation of viral RNA.[10]

Hydrolysis of alkylated guanosine, RNA and DNA with water at 100° was used as suggested for the elimination of alkylated bases from DNA by Lawley.[11] We have found that the reaction can also be applied to alkylated guanosine[12] and to RNA (Fig. 1). The result with RNA is particularly remarkable in that no guanine and only little adenine was seen after column chromatography, the only base observed in quantity being 7-methylguanine, though no quantitative implications are claimed.

The hydrolysed reaction products were separated by column chromatography on Sephadex G-10.[8] Two typical elution diagrams are shown in Fig. 1. Similar results were obtained for the methylation of guanosine and DNA and for the ethylation of guanosine and RNA. Addition of authentic 7-methyl- and 7-ethylguanine, respectively, to the hydrolysis products did not produce new peaks but only increased the peak heights of fractions after 125 and 145 ml eluate.

The 7-alkylguanines were further identified by u.v.-absorption spectroscopy at pH 1, 7 and 13. Both the methyl and ethyl derivatives showed identical spectra with maxima at 249 nm (pH 1); 214, 247 and 283 nm (pH 7); and 279 nm (pH 13). Minima were observed at 226 (pH 1), 234 and 260 (pH 7) and 256 nm (pH 13). These spectra are characteristic for 7-alkylguanines.[13]

Thin-layer chromatography of the alkylguanine fractions on cellulose gave R_f values of 0·19 (solvent A) and 0·33 (solvent B) for the methylated products and R_f values of 0·28 (solvent A) and 0·42 (solvent B) for the ethylation products. Authentic 7-methyl- and 7-ethylguanine had identical. R_f values.

It was not attempted to look for possible minor alkylation products of purines and pyrimidines Qualitative identification of the main alkylation products was the aim of this investigation. We therefore can give no data on differences in the alkylation rate between RNA and DNA *in vitro*. However, assuming quantitative removal of alkylated base from RNA and DNA, a comparison of extinction coefficients indicates a yield on methylation of approximately twice that on ethylation. Studies with other methylating and ethylating agents are known to have given similar results.[14]

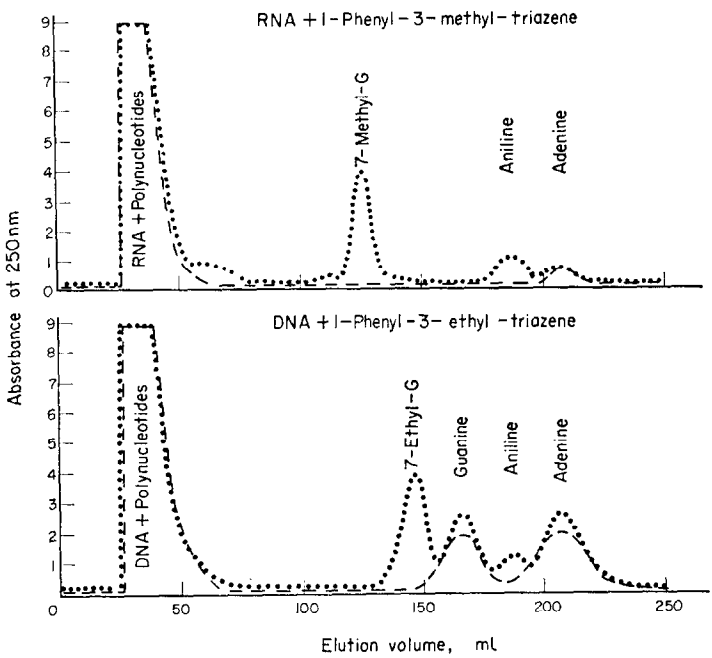


FIG. 1. Distribution of products arising from the hydrolysis of RNA and DNA with water at 100° for 1 hr before (– – –) and after (. . . . .) alkylation with either 1-phenyl-3-methyl-triazene or 1-phenyl-3-ethyl-triazene as eluted from Sephadex G-10 with 0·05 M NaH_2PO_4 brought to pH 7.

Most of the aniline formed according to equation 1 is removed by steam distillation on evaporating the solvents after hydrolysis. Residual aniline is eluted from the column after 185 ml and can easily be identified.

The results presented show that phenylmonoalkyltriazenes act as typical alkylating agents toward guanine in nucleic acids and that 7-alkylguanine is formed as with other alkylating agents. This result supports the hypothesis that carcinogenic aryldialkyltriazenes are metabolized *in vivo* by *N*-dealkylation[1] and that the monoalkyl-triazenes formed act as alkylating agents *in vivo*.

In vivo results with ^{14}C-methyl labelled 1-phenyl-3,3-dimethyltriazene will be reported shortly.[15]

Acknowledgements—Our work was supported by the Deutsche Forschungsgemeinschaft. We thank Prof. Dr. H. Druckrey for his interest and many discussions.

Forschergruppe Praeventivmedizin am Max-Planck-Institut fur Immunobiologie, Freiburg/Br., Germany

R. PREUSSMANN
A. V. HODENBERG

REFERENCES

1. R. PREUSSMANN, A. v. HODENBERG and H. HENGY, *Biochem. Pharmac.* **18**, 1 (1969).
2. H. DRUCKERY, S. IVANKOVIC and R. PREUSSMANN, *Naturwissenschaften* **54**, 171 (1967).
3. O. DIMROTH, *Ber. dt. chem. Ges.* **36**, 909 (1903); **38**, 670 (1905).
4. E. H. WHITE and H. SCHERRER, *Tetrahedron Lett.* 785 (1961).
5. R. PREUSSMANN, H. SCHNEIDER and F. EPPLE, *Arzneimittelforsch.* **19**, 1059 (1969).
6. J. W. JONES and R. K. ROBINS, *J. Am. chem. Soc.* **85**, 193 (1963).
7. P. BROOKES and P. D. LAWLEY, *J. chem. Soc.* 3923 (1961).
8. L. SWEETMAN and W. L. NYHAN, *J. Chromat.* **32**, 662 (1968).
9. A. LÜTTRINGHAUS and H. W. DIRKSEN, *Angew. Chem.* **75**, 1059 (1963).
10. B. SINGER and H. FRAENKEL-CONRAT, *Biochemistry* **8**, 3260 (1969).
11. P. D. LAWLEY, *Nature, Lond.* **218**, 580 (1968).
12. R. PREUSSMANN, H. DRUCKREY, S. IVANKOVIC and A. v. HODENBERG, *Ann. N.Y. Acad. Sci.*, in press.
13. B. C. PAL, *Biochemistry* **1**, 558 (1962).
14. E. KRIEK and P. EMMELOT, *Biochim. biophys. Acta* **91**, 59 (1964).
15. F. W. KRUEGER and R. PREUSSMANN, in preparation.

Biochemical Pharmacology, Vol. 19, pp. 1508–1512. Pergamon Press. 1970. Printed in Great Britain

Hydrocortisone induction of tyrosine aminotransferase activity in genetically obese and diabetic mice—Effects of a multiple dosage schedule*

(*Received* 2 *May* 1969; *accepted* 29 *August* 1969)

THE ACTIVITY of liver L-tyrosine: 2-oxoglutarate aminotransferase, TA (EC 2.6.1.5) increases several-fold after the administration of hydrocortisone.[1] This enzyme system is one of the first to be induced by the hormonal action of glucocorticoids.[2] The rapid induction effect is attributed primarily to the rapid turnover rate of the enzyme protein.[3] When hydrocortisone is administered as a single intraperitoneal injection, the enzyme activity increases to a maximum in several hours and then declines rapidly to basal levels. Multiple injections of hydrocortisone maintain the induced enzyme activity for longer periods of time. In the multiple injection dosage schedule, smaller amounts of hydrocortisone result in the same or higher levels of induced TA activity.[4] The multiple injection schedule, with the total dose subdivided into small doses, is a more effective method of inducing and maintaining TA activity compared to a single large dose.

The present report deals with the hydrocortisone induction of liver TA activity in genetically obese and diabetic mice of the C57BL/6J-*ob* and C57BL/Ks-*db* strains using a multiple injection dosage schedule. Previous studies indicated that hydrocortisone was less effective in the induction of liver TA activity in C57BL/6J-*ob* (genetically obese) mice than in the normal littermates.[5] These results demonstrate that the induction of liver TA activity in the obese mice was approximately the same by either the single or multiple dosage schedule, indicating possibly an insensitivity of this enzyme system in the mutants to glucocorticoids. Other possible explanations are proposed. Similar results of

*This investigation was supported in part by an allocation from the NIH General Research Support Grant FR 05545 from the Division of Research Resources to The Jackson Laboratory and in part by Institutional Grant IN-19 from the American Cancer Society to The Jackson Laboratory.