that the role of PLP is the formation of the proper complex. These studies concerning the counteracting effect of progesterone on the inhibitory effect of β-oestradiol or ethynyloestradiol could be one of the future *in vivo* trials to the approach of studying adverse drug reactions in contraceptive users.

Department of Cancer Chemistry,
Medical Research Institute,
Alexandria University,
Alexandria, Egypt

S. M. EL-ZOGHBY
S. M. EL-SEWEDY
A. A. SAAD
M. H. MOSTAFA
S. M. EBIED
G. A. ABDEL-TAWAB

REFERENCES

1. J. M. Price, R. R. Brown and N. Yess, in *Advances in Metabolic Disorders* (Eds. R. Levine and R. Luft) Vol. 2. Academic Press, New York (1965).
2. P. Holtz and D. Palm, *Pharmac. Rev.* **16,** 113 (1964).
3. R. R. Brown, N. Yess, J. M. Price, H. Linkswiler, P. Swan and L. V. Hankes, *J. Nutr.* **87,** 419 (1965).
4. H. Linkswiler, *Am. J. clin. Nutr.* **20,** 547 (1967).
5. J. Kelsay, L. T. Miller and H. Linkswiler, *J. Nutr.* **97,** 27 (1968).
6. J. Baddiley, *Nature, Lond.* **170,** 711 (1952).
7. D. E. Metzler and E. E. Snell, *J. biol. Chem.* **198,** 353 (1952).
8. D. E. Metzler, *Fedn Proc.* **20** (10), 234 (1961).
9. S. M. El-Sewedy, G. A. Abdel-Tawab, S. M. El-Zoghby, R. Zeitoun, M. H. Mostafa and Sh. Shalaby, *Biochem. Pharmac.* **23,** 2557 (1974).
10. A. A. Saad, G. A. Abdel-Tawab, S. M. El-Zoghby, M. H. Mostafa and G. E. Moursi, *Biochem. Pharmac.* **23,** 999 (1974).
11. M. Mason and B. Manning, *Am. J. clin. Nutr.* **24,** 786 (1971).
12. I. P. Braidman and D. P. Rose, *Biochem. Pharmac.* **20,** 973 (1971).
13. D. P. Rose, R. R. Brown, *Biochim. biophys. Acta* **184,** 412 (1969).
14. D. E. Metzler, M. Ikawa and E. E. Snell, *J. Am. chem. Soc.* **76,** 648 (1954).

Biochemical Pharmacology, Vol. 25, pp. 2413–2415. Pergamon Press, 1976. Printed in Great Britain.

Inhibition of purine nucleotide biosynthesis by 3-deazaguanine, its nucleoside and 5′-nucleotide

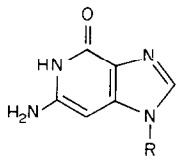
(*Received* 7 *November* 1975; *accepted* 26 *March* 1976)

The synthesis of 3-deazaguanine, 3-deazaguanosine, and 3-deazaguanylic acid (Fig. 1) was recently realized in our laboratories [1] as part of a continuing program to develop chemotherapeutically useful analogs of the naturally occurring purines with particular reference to broad spectrum antiviral agents. Previous studies reporting the antibacterial [2,3] antitumor [4,5] and antiviral [6,7] activity of certain 1- and 3-deazapurines have established the potential chemotherapeutic importance of such compounds. Indeed, 3-deazaguanine, together with the nucleoside and 5′-nucleotide, has demonstrated potent antiviral activity *in vitro* against a variety of DNA and RNA viruses [8] as well as activity *in vivo* against L1210 leukemia and adenocarcinoma 755 in mice [9].

A procedure has been developed by Snyder *et al.* [10], and utilized in our laboratories, in which the apparent activities of eight enzymes of purine nucleotide biosynthesis and interconversion can be calculated when Ehrlich ascites tumor cells are incubated *in vitro* with hypoxanthine [^{14}C]. Previous studies have linked the antagonism of this pathway with a number of compounds possessing antiviral and antitumor activity [11–13]. In particular, the enzyme IMP dehydrogenase, which occupies a key position in this pathway, is a common site of inhibition by many of these compounds. It is, therefore, the purpose of this communication to report the effects of 3-deazaguanine and its derivatives on purine nucleotide biosynthesis in Ehrlich ascites tumor cells.

The synthesis of 3-deazaguanine, 3-deazaguanosine and 3-deazaguanylic acid, as well as the 7-ribosyl derivative of 3-deazaguanine, has been reported [1]. Hypoxanthine [^{14}C] (51 mCi/m-mole, 0.1 mCi/ml) was obtained from ICN Pharmaceuticals, Isotope and Nuclear Division, Irvine, Calif. Ribonucleotide markers were obtained from Sigma Chemical Co., St. Louis, Mo., and PEI-cellulose chromatography plates from Brinkman Instruments Inc., Los Angeles, Calif. Ehrlich ascites tumor cells were the generous gift of Dr. J. Frank Henderson, University of Alberta, Edmonton, Alberta, Canada. Procedures for the maintenance, preparation and incubation of the cells have been described [10,14]. The apparent activity of each of the enzymes in the pathway is determined as the sum of the radioactive substrate (hypoxanthine [^{14}C]) in all metabolites further along in the pathway. Table 1 gives the arithmetic sums denoting each of the reactions together with typical control values in parentheses. The incorporation of radioactivity into NAD was not included in these calculations because of the low amount (less than 100 counts/min) found in this product. By comparison of the control and drug-treated cells, the per cent inhibition of each of the reactions can be calculated after a correction is made for inhibition of the reactions prior to the one under consideration. A complete analysis of these computations can be found in Ref. 10.

IMP dehydrogenase (IMP:NAD-oxidoreductase, EC 1.2.1.14) was partially purified from *E. coli. B.* and assayed



(a) R = H
(b) R = β-D-ribose
(c) R = β-D-ribose-5′-phosphate

Fig. 1. Structures of (a) 3-deazaguanine, (b) 3-deazaguanosine and (c) 3- deazaguanylic acid.

Table 1. Summations representing the apparent enzyme activities in the biosynthesis of purine nucleotides from hypoxanthine [^{14}C]

Enzyme	Summation (cpm)
Hypoxanthine-phosphoribosyl transferase	AMP + ADP + ATP + IMP + XMP + GMP + GDP + GTP (10,755)
AMP synthetase + lyase	AMP + ADP + ATP (9,200)
AMP kinase	ADP + ATP (8,824)
ADP kinase	ATP (7,526)
IMP dehydrogenase	XMP + GMP + GDP + GTP (1,165)
GMP synthetase	GMP + GDP + GTP (1,117)
GMP kinase	GDP + GTP (1,049)
GDP kinase	GTP (668)

as described previously [11]. It was found in those studies that the bacterial enzyme preparation was more active and stable to storage than that prepared from Ehrlich ascites cells, while possessing similar kinetic properties and activity toward nucleotide inhibitors. Purine nucleoside phosphorylase was assayed in cell-free extracts of Ehrlich ascites tumor cells prepared from about 5 g of packed cells which were suspended in 10 ml of 0.01 M potassium phosphate buffer, pH 7.5, and homogenized at top speed in the 50-ml cell of a Sorvall omni-mixer for 2 min at 5°. Microscopic examination indicated almost complete cell breakage under these conditions. The homogenate was centrifuged at 25,000*g* for 30 min and the supernatant used as a source of the phosphorylase. Protein concentrations were determined by the method of Lowry *et al.* [15]. Reactions were carried out by incubating 200–300 mg protein with 250 nmoles substrate in 5 ml of 0.01 M potassium phosphate buffer at 37° for 18–20 hr. Reaction mixtures which had been preheated to 60°, 4 min prior to incubation at 37°, were used as controls. Phosphorolysis was determined by noting the marked u.v. spectral shift of either the 7- or 9-ribosyl derivative of 3-deazaguanine relative to the free base (see Ref. 1).

Table 2 contains a breakdown of the various enzymes involved in purine nucleotide biosynthesis and the effects of 3-deazaguanine and its derivatives on the activities of enzymes in Ehrlich ascites *in vitro*. For comparison, ribavirin which is a known inhibitor of some of the enzymes in this pathway [12] was tested in parallel. In all four cases, IMP dehydrogenase and hypoxanthine-guanine phosphoribosyl transferase (HGPRT) appear to be the principle sites of inhibition, although ribavirin exerts some effect on GDP kinase as well. The relative effectiveness of the base, nucleoside, and 5′-nucleotide in inhibiting IMP dehydrogenase may or may not be significant since the relative permeabilities of these compounds to the cell have to be taken into account. The fact that all three compounds do show a similar pattern of inhibition suggests that they may be metabolized to a common active derivative. The most likely inhibitor of IMP dehydrogenase would be the 5′-nucleotide, since both guanylic acid and ribavirin-5′-phosphate are inhibitors of the isolated enzyme. The relative I_{50} values (concentration required for 50 per cent inhibition) in the same experiment, performed in duplicate, were: guanylic acid, 7.3×10^{-5} M; ribavirin-5′-phosphate, 1.7×10^{-7} M; and 3-deazaguanylic acid, 1.2×10^{-6} M. 3-Deazaguanylic acid, though 10-fold less active than ribavirin-5′-phosphate, is still about 60 times more effective than guanylic acid as an inhibitor of this enzyme. Formation of the 5′-nucleotide would most likely be accomplished through phosphoribosylation of the free base. This would offer a possible explanation for the inhibition of HGPRT in the ascites cell assay as a substrate competition between 3-deazaguanine and the [^{14}C]-hypoxanthine. Phosphorylation of the nucleoside to the 5′-nucleotide is a less likely route, since the presence of an inosine-guanosine kinase has only been demonstrated in Ehrlich ascites cells which are lacking hypoxanthine-guanine phosphoribosyl transferase [16]. The activity of 3-deazaguanosine would most likely be the result of phosphorolytic (or hy-

Table 2. Inhibition of purine nucleotide biosynthesis in Ehrlich ascites tumor cells by 3-deazaguanine and related compounds*

Reaction	% Inhibition by: 3-Deaza-guanine	3-Deaza-guanosine	3-Deaza-guanylic acid	Ribavirin
Hypoxanthine-guanine phosphoribosyl transferase	4	37	32	38
AMP synthetase + lyase	0	0	0	0
AMP kinase	0	0	6.2	0
ADP kinase	0	0	0	0
IMP dehydrogenase	37	43	60	70
GMP synthetase	0	1.2	10	5.6
GMP kinase	0	0.2	5.9	0
GDP kinase	7.8	0	7.7	32

* Ehrlich ascites tumor cells, approximately 6×10^6 cells/ml, were incubated at 37°, for 20 min in 0.1 ml of Fischer's medium containing 25 mM sodium phosphate buffer, pH 7.4, with and without 1 mM of the indicated compounds. Incubations were carried out in triplicate. Hypoxanthine [^{14}C] was then added to a final concentration of 0.1 mM and the incubation continued for 60 min. The apparent enzyme activities were calculated according to the summation in Table 1 and the inhibition of these activities was determined according to the method described in Ref. 10.

Table 3. Enzymic phosphorolysis of 3-deazaguanosine and 7-ribosyl-3-deazaguanine by cell-free extracts of Ehrlich ascites tumor cells

Compound	Reaction conditions	λmax*	Product identity	Analysis
3-Deazaguanosine	60°, 4 min +37°, 18 hr	270,298	3-Deazaguanosine	No reaction
3-Deazaguanosine	37°, 18 hr	258,300	3-Deazaguanine	Complete phosphorolysis
7-Ribosyl-3-deazaguanine	60°, 4 min + 37°, 18 hr	317,258	7-Ribosyl-3-deazaguanine	No reaction
7-Ribosyl-3-deazaguanine	37°, 18 hr	317,258	7-Ribosyl-3-deazaguanine	No reaction

* Refer to [1] for u.v. spectral properties of the nucleosides and the free base. The reaction conditions are described in the text.

drolytic) cleavage to release the free base followed by subsequent phosphoribosylation to form the 5'-nucleotide. When 3-deazaguanosine was incubated with a cell-free extract of Ehrlich ascites (Table 3), u.v. spectral analysis revealed that, indeed, the nucleoside was cleaved to the free base, and further that the activity is specific for the 9-ribosyl linkage to the base. Thin-layer chromatographic analysis of the reaction mixtures was consistent with the results reported in Table 3.

In summary, these studies show that 3-deazaguanine can interfere with the biosynthesis of purine nucleotides in Ehrlich ascites tumor cells, most probably through the inhibition of IMP dehydrogenase by the 5'-nucleotide. At the concentration used in these studies (1 mM), 3-deazaguanine is also about 50 per cent cytotoxic to KB tumor cell cultures. This, then, would offer one means of interfering with tumor cell growth through consequent inhibition of nucleic acid synthesis. However, direct interference with nucleic acid synthesis either by incorporation or interference with polymerase activities is also possible if metabolism to the triphosphate does occur. Further studies on the actual metabolism of 3-deazaguanine in tumor cells is obviously required to determine the primary mode of action of this compound.

Acknowledgement—I would like to thank Dr. Dan Cook for the 3-deazaguanine and derivatives used in these studies.

ICN Pharmaceuticals, Inc., Nucleic Acid Research Institute, Irvine, Calif. 92715, *U.S.A.*

DAVID G. STREETER
HAO HAO P. KOYAMA

REFERENCES

1. P. D. Cook, R. J. Rousseau, A. M. Mian, P. Dea, R. B. Meyer, Jr., J. T. Witkowski and R. K. Robins, *J. Am. chem. Soc.* **98**, 1492 (1976).
2. G. W. Kidder and V. C. Dewey, *Archs. Biochem. Biophys.* **66**, 486 (1967).
3. K. B. deRoos and C. A. Salemink, *Recl. Trav. chim. Pays-Bas Belg.* **90**, 1166 (1971).
4. J. A. May, Jr. and L. B. Townsend, *One hundred and sixty-seventh National Meeting of the Am. Chem. Soc.* (Abstr. No. 55), Los Angeles, Calif., March 1974.
5. J. A. Montgomery and K. Hewson, *J. med. Chem.* **9**, 105 (1066).
6. P. C. Jain, S. K. Chatterjee and M. Amand, *Indian J. Chem.* **1**, 30 (1965).
7. O. P. Babbar and B. L. Chowbury, *J. scient. ind. Res.* **21c**, 312 (1962).
8. L. B. Allen, J. H. Huffman, R. B. Meyer, Jr., P. D. Cook, J. T. Witkowski, L. N. Simon, R. K. Robins and R. W. Sidwell, *Fifteenth Interscience Conference Antimicrob. Agents Chemother.* (Abstr. No. 245), Washington, D.C., September 1975.
9. T. A. Khwaja, L. Kigwana, R. B. Meyer, Jr. and R. K. Robins, *Proc. Am. Ass. Cancer Res.* **16**, 162 (1975).
10. F. F. Snyder, J. F. Henderson and D. A. Cook, *Biochem. Pharmac.* **21**, 2351 (1972).
11. D. G. Streeter, J. T. Witkowski, G. P. Khare, R. W. Sidwell, R. J. Bauer, R. K. Robins and L. N. Simon, *Proc. natn. Acad. Sci. U.S.A.* **70**, 1174 (1973).
12. C. M. Smith, L. J. Fontenelle, H. Muzik, A. R. Paterson, H. Unger, L. W. Brox and J. F. Henderson, *Biochem. Pharmac.* **23**, 2737 (1974).
13. T. J. Franklin and J. M. Cook, *Biochem. J.* **113**, 515 (1969).
14. G. W. Crabtree and J. F. Henderson, *Cancer Res.* **31**, 985 (1971).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
16. K. J. Pierre and G. A. LePage, *Proc. Soc. exp. Biol. Med.* **127**, 432 (1968).

Biochemical Pharmacology, Vol. 25, pp. 2415–2417. Pergamon Press, 1976. Printed in Great Britain.

Allylisopropylacetamide preferentially interacts with the phenobarbital-inducible form of rat hepatic microsomal P-450

(*Received* 4 *November* 1974; *accepted* 6 *May* 1976)

Cytochrome P-450 is the terminal oxidase of a number of mammalian microsomal electron transport systems which metabolize a variety of compounds, including steroid hormones, fatty acids, and a variety of xenobiotics [1]. Several studies provide nearly overwhelming evidence that rat liver microsomes contain several distinct forms of this cytochrome [2–5], thus offering an explanation for the observable broad specificity of this enzyme system. How-