containing L-lactate instead of D-lactate.

A lactate dehydrogenase specific for NAD and D-lactate was also found in *Mindeniella*. Extracts from both organisms had similar activity in the pyruvate assay, and in both cases high pyruvate concentrations were slightly inhibitory. However, the reverse reaction, with NAD and D-lactate, proceeded very slowly in the presence of the *Mindeniella* enzyme. A twofold faster reaction was obtained when the 3-acetylpyridine analog of NAD was used in place of the natural coenzyme. In the case of the *Sapromyces* enzyme, the analog was no more effective than NAD. A third difference between the enzymes from *Sapromyces* and *Mindeniella* was evident. When the two extracts were run in the same starch gel, that from *Mindeniella* showed a single anodic band of lactate dehydrogenase activity 2.7 cm from the application slit, whereas that from *Sapromyces* showed a cathodic band.

Further characterization of the *Mindeniella* enzyme is in progress. In addition, preliminary studies indicate that representatives of two other genera in the order Leptomitales, *Rhipidium* and *Aqualinderella*, also contain NAD-dependent D-lactate dehydrogenase.

The D-lactate dehydrogenase has been regarded as uncommon. L-Lactate dehydrogenase (*7*), on the other hand, is widely distributed and is probably responsible for lactate production by all animal tissues, many bacteria (*8, 9*), and some vascular plants (*10*). The D-lactate dehydrogenase is probably responsible for lactate production in certain lactic acid bacteria (*8*), in some species of *Chlorella* (*11*), and throughout the order Leptomitales. NAD-dependent D-lactate dehydrogenase has also been detected in a very few other microorganisms, for example, the hyphomycete *Piricularia* (*12*), the cellular slime mold *Polysphondylium* (*13*), and the photosynthetic flagellate *Euglena* (*14*), but in these cases the biological function of the enzyme has not been determined.

FRANK H. GLEASON
RICHARD A. NOLAN
Department of Botany,
University of California, Berkeley
ALLAN C. WILSON
Department of Biochemistry,
University of California, Berkeley
RALPH EMERSON
Department of Botany,
University of California, Berkeley

References and Notes

1. J. W. Foster, *Chemical Activities of Fungi* (Academic Press, New York, 1949), pp. 282–295; E. C. Cantino, *Am. J. Botany* **36**, 95 (1949); J. M. Crasemann, *ibid.* **41**, 302 (1954); C. G. Golueke, *J. Bacteriol.* **74**, 337 (1957); M. Margulies and W. Vishniac, *ibid.* **81**, 1 (1961).
2. The flavoprotein lactate dehydrogenases comprise a class of enzymes entirely distinct from the NAD-dependent lactate dehydrogenases. These flavo-enzymes are widely distributed in microorganisms. Their biological function may be to permit the utilization of D- or L-lactic acid as a carbon and energy source, usually during aerobic growth. They do not seem to be involved in lactate production in vivo.
3. E. C. Cantino and J. S. Lovett, *Physiol. Plantarum* **13**, 450 (1960).
4. Number 1.1.1.28 in *Enzyme Nomenclature, Recommendations of the International Union of Biochemistry* (Elsevier, New York, 1965).
5. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
6. I. H. Fine and L. A. Costello, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1963), vol. 6, pp. 958–972.
7. Number 1.1.1.27 in *Enzyme Nomenclature* (see *4*).
8. D. Dennis and N. O. Kaplan, *J. Biol. Chem.* **235**, 810 (1960).
9. A. Yoshida, *Biochim. Biophys. Acta* **99**, 66 (1965); E. M. Tarmy and N. O. Kaplan, *Biochem. Biophys. Res. Commun.* **21**, 379 (1965).
10. F. A. Loewus and H. A. Stafford, *J. Biol. Chem.* **235**, 3317 (1960).
11. O. Warburg, H. Klotsch, G. Krippahl, *Z. Naturforsch.* **12b**, 722 (1957); R. A. Lewin, Ed., *Physiology and Biochemistry of Algae* (Academic Press, New York, 1962).
12. K. Yamada, H. Yamada, Y. Takesue, S. Tanaka, *J. Biochem. Tokyo.* **50**, 72 (1961).
13. R. Garland and N. O. Kaplan, personal communication.
14. C. A. Price, *Biochem. J.* **82**, 61 (1961).
15 Supported in part by a grant to A.C.W. from NSF.

17 March 1966

Mutagenicity of Cycasin Aglycone (Methylazoxymethanol), a Naturally Occurring Carcinogen

Abstract. *Methylazoxymethanol, a carcinogenic and hepatotoxic methylating agent prepared from cycad plants, has been found to be a good mutagen in* Salmonella typhimurium.

Cycasin (methylazoxymethanol-β-D-glucoside) is a carcinogenic and hepatotoxic compound occurring in plants of the family Cycadaceae. The frequent ingestion of various parts of these plants by people and domesticated animals in tropical parts of the world makes the compound of special interest (*1*). In its naturally occurring form cycasin is a β-glucoside, but there is mounting evidence that it is the aglycone methylazoxymethanol that is actually toxic and carcinogenic, and that deglucosylation must be performed by intestinal microorganisms before the toxicity can be manifested (*2*). Matsumoto and Higa (*3*) have demonstrated that methylazoxymethanol is a methylating agent, with 7-methlyguanine being formed by its reaction with DNA or RNA. The genetic damage resulting from such methylation should be re-

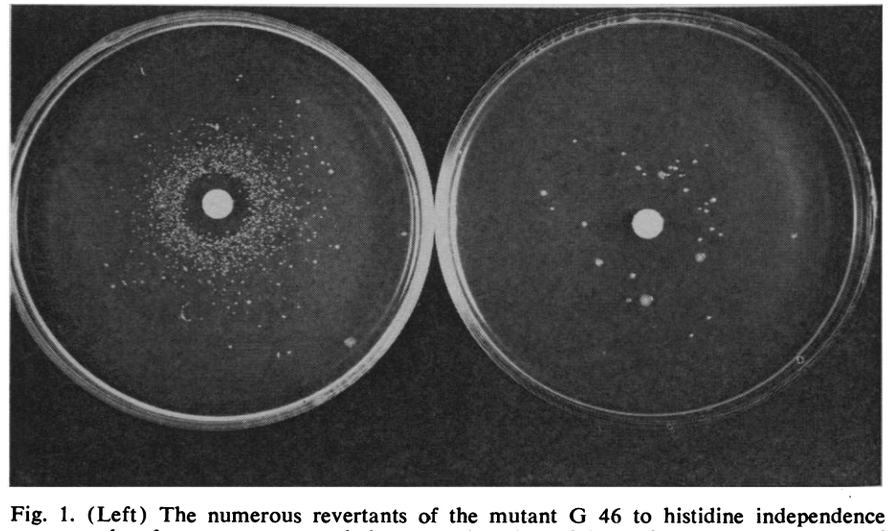

Fig. 1. (Left) The numerous revertants of the mutant G 46 to histidine independence as a result of exposure to methylazoxymethanol. (Right) The smaller number of revertants seen when less sensitive histidine-requiring mutants (C 496 in example shown) are placed under the same condition. The localization of mutants around the disc containing methylazoxymethanol is typical.

flected in an increased mutation rate in the presence of this aglycone. The following results will show that methylazoxymethanol is a good mutagen in the bacterium *Salmonella typhimurium*.

Methylazoxymethanol was prepared from crystalline cycasin which had been purified from the seeds of *Cycas circinalis*. Enzymatic deglucosylation (almond emulsin, Sigma Chemical Co.) and purification were carried out according to the method of Kobayashi and Matsumoto (*4*). The mutagenic activity of both cycasin and the aglycone was tested by measuring the frequency of reversion to histidine independence of several histidine-requiring mutants of *Salmonella* (*5*). The bacteria were exposed to these componds on petri plates which were prepared by mixing 0.2 ml of freshly grown cultures (2×10^9 bacteria per milliliter) of the mutants with 2 ml of 0.6-percent agar at 45°C. The soft agar, which contained a trace (0.20 μmole) of histidine as well as the bacterial inoculum, was then poured onto plates of a histidine-free minimal agar medium. The aglycone (1.5 mg), on a disc of absorbant filter paper, was introduced onto the surface of each plate after the agar had hardened.

In experiments with cycasin, 9 mg was applied to each disc, this amount being sufficient to release 3 mg of aglycone after deglucosylation. The trace of histidine present permits a small amount of growth so that zones of inhibition caused by some mutagens are visible around the discs. Revertant colonies usually could be seen clearly after 40 hours of incubation at 37°C, but on some plates the number of revertants increased between 40 and 70 hours, and revertants of one of the mutants used, C 151, did not appear until 70 hours. A control plate containing no cycasin or its aglycone was prepared for each of the mutants tested. The spontaneous reversion rate of the mutants was low (0 to 15 colonies per plate).

Cycasin neither inhibited nor caused reversion in any of the mutants. This suggests that the *Salmonella* lack the necessary deglucosylating enzyme. In contrast, methylazoxymethanol caused all but two (C 120 and C 207) of the ten different mutants tested to revert. Most sensitive was G 46 (Fig. 1), with hundreds of revertant colonies appearing; C 50 and D 130 were also quite sensitive, with about 100 colonies appearing on each plate. Only 20 to 40 colonies (3 to 5 times the number seen on control plates) appeared in tests with the other mutants (for example, C 496, C 527, and C 151). Zones of inhibition, 3 cm in diameter, surrounded the discs of the methylazoxymethanol. A mutagenic effect resulted in the obvious localization of the revertants just outside the zone of inhibition (Fig. 1). These results were reproduced two to four times with each of the mutants.

Most of the mutants tested are known to have a histidine requirement because of the substitution of a single base in one of the genes coding for the enzymes of histidine biosynthesis (*6*). These mutants are revertible with a variety of alkylating agents, and many are suppressible if amber and ochre suppressors are introduced. The mutant C 207, however, appears to be a reading-frame error (*6*), and alkylating agents including methylazoxymethanol do not cause it to revert.

It is likely that in aqueous solution methylazoxymethanol breaks down, forming diazomethane, which is a well-known methylating agent (*7*), mutagen (*8*), and carcinogen (*9*). Structurally related nitrosamides and nitrosamines, many of which are mutagenic, hepatotoxic, and carcinogenic, are also thought to be converted enzymatically and spontaneously to diazomethane (*10*).

Genetic damage, presumably by methylazoxymethanol, has also been described by Teas *et al.* (*11*) who showed that chromosome breakage occurred in onion-root tips treated with cycasin. Although it has not been demonstrated, mutation may occur in animals and humans ingesting cycasin, and mechanisms can be imagined by which both the carcinogenicity and hepatotoxicity of methylazoxymethanol could be consequences of the alkylation of DNA and RNA.

DAVID W. E. SMITH
Laboratory of Experimental Pathology, National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland

References and Notes

1. The toxicity of cycads was the subject of a conference reported in *Fed. Proc.* **23**, 1337 (1964).
2. G. L. Laqueur, *ibid.*, p. 1386.
3. H. Matsumoto and H. H. Higa, *Biochem. J.* **98**, 20c (1966).
4. A. Kobayashi and H. Matsumoto, *Arch. Biochem. Biophys.* **110**, 373 (1965).
5. P. E. Hartman, J. C. Loper, D. Serman, *J. Gen. Microbiol.* **22**, 323 (1960); P. E. Hartman, Z. Hartman, D. Serman, *ibid.*, p. 354; C. E. Kirchner, *J. Mol. Biol.* **2**, 331 (1960).
6. H. Whitfield, R. G. Martin, B. N. Ames, *Fed. Proc.* **25**, part 1, 337 (1966).
7. O. M. Friedman, G. N. Mahapatra, B. Dash, R. Stevenson, *Biochem. Biophys. Acta* **103**, 286 (1965).
8. K. A. Jensen, I. Kirk, G. Kølmark, M. Westergaard, *Cold Spring Harbor Symp. Quant. Biol.* **16**, 245 (1951).
9. R. Scohental, *Nature* **188**, 420 (1960).
10. H. Marquardt, F. K. Zimmerman, R. Schwaier, *Z. Vererbungslehre* **95**, 82 (1964); H. Marquardt, *German Med. Monthly* (Engl. ed.) **10**, 107 (1965); P. N. Magee and R. Schoental, *Brit. Med. Bull.* **20**, 102 (1964).
11. H. J. Teas, H. Sax, K. Sax, *Science* **149**, 541 (1965).
12. I thank Miss JoAnn Holmes for technical assistance and Dr. B. N. Ames and Dr. G. L. Laqueur for helpful discussions.

17 February 1966

Effect of Interferon on Early Interferon Production

Abstract. *Chick embryo cells given prior treatment with interferon make new interferon earlier and in larger quantities upon stimulation with Chikungunya virus than cells not so treated. By the criterion of loss of sensitivity to actinomycin, the time needed for formation of messenger RNA for interferon was decreased in the primed cells. Thus interferon affects virus action within 1 hour after infection.*

Interferon exerts its antiviral action very early in virus replication, probably before viral RNA is made (*1*, *2*). In at least one virus-cell system, a very early event in virus infection leads to the derepression of host-cell genome function, leading to the formation of interferon (*3*). We now report a relation between the action and formation of interferon: namely, the effect of prior treatment of cells with interferon on the subsequent formation of interferon by the cells upon exposure to virus. It has been reported (*4*) that such prior treatment may increase or decrease the amount of interferon formed. These previous studies dealt with periods late in the course of viral growth. We now present a somewhat more detailed examination of an early enhancement phenomenon.

Chicken interferon was prepared by intraallantoic infection of 11-day-old embryonated eggs with the NWS strain of influenza A (*5*). The harvested allantoic fluid was kept at *p*H 2 over-