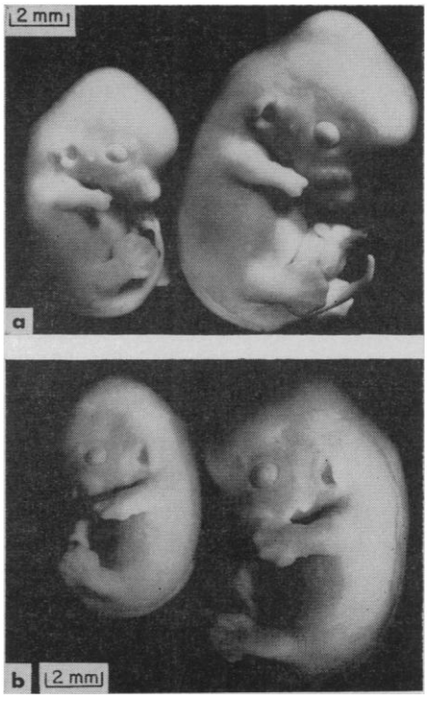

Fig. 3. Fetus of a mouse at 14 days (left) and a rat at 16 days (right) from mothers given acetazolamide during pregnancy. (*a*) Right side, digits 4 and 5 missing from hand. (*b*) Left side, no deformity.

The high dose required to produce teratogenesis in the rat is not well tolerated and results in a reduction in food intake (20 percent in the rats fed on the diet containing 0.6 percent concentrations of the drug), and the usual weight gain during pregnancy is greatly depressed.

In a study designed to evaluate the effect of a high and sustained but tolerated dose of acetazolamide on the reproductive process, male and female rats were fed a diet containing 0.1 percent of the compound. After 6 weeks of being fed on this diet, the rats were mated. Two or three weeks after the pups from the first litter were weaned, the rats were remated and the pups from the second breeding cycle were raised to weaning age, at which time the study was terminated. The diet containing the drug was administered throughout the entire test and the average drug intake varied from 40 to 60 mg per kilogram of body weight per day. Table 2 shows that acetazolamide had no demonstrable effect on male or female fertility or on lactation of pregnant females or on the offspring of treated parents.

Although acetazolamide has been widely used as a diuretic in pregnant humans (*1*), there are no reports of its proven or suspected teratogenicity in this species. This is reasonable if one compares the minimum teratogenic dose for the rat (more than 100 mg kg^{-1} day^{-1}) with the usual human therapeutic dose (5 mg kg^{-1} day^{-1}) and especially if one considers the very steep dose-response relationship (Table 1). This kind of postaxial arm defect is rare in man, and it is not the kind of deformity that can be easily overlooked. It is of interest that in man ulnar defects are reported to be predominantly right-sided (*2*).

The specificity of localization of this defect appears to be unique among either induced or genetically determined deformities. This specificity is manifest not only in a right-sided localization but also in an involvement of only the postaxial border of the forelimb without other skeletal or visceral abnormalities.

There are two similar deformities which should be mentioned. Searle (*3*) described a mutation of the mouse of the "luxate-luxoid" group, "postaxial hemimelia," which affects the postaxial side of the forelimb and sometimes also the hind limb. However, there is always a scapular defect and both sexes are sterile. The right forelimb and the left hindlimb tend to be more severely deformed.

Dagg (*4*) found that 5-fluorouracil given to pregnant mice could result in a number of malformations, including a reduction in the number of toes of the hind feet. The number of toes missing was a function of dose and when one toe was missing it was always the most postaxial (5th) toe. However, when two toes were missing these were digits 1 and 5.

The mechanism by which acetazolamide produces deformity is not clear. It has been postulated that spontaneous postaxial hemimelia is due to a defect in the induction of limb elements by the apical ectodermal ridge (*2*, *5*). Results of preliminary work we have done on rat embryos are consistent with this idea in that the defect is first visible at a stage of development during which the inductive action of the apical ectodermal ridge is taking place [Christie stage 22 (*6*), 12½ days after mating], but we have not made a detailed examination of the limb bud at this stage.

The only pharmacological activity ascribed to acetazolamide has been carbonic anhydrase inhibition, and we are not aware of any postulated role of carbonic anhydrase in limb development. However, it should be kept in mind that the exceedingly large amounts of acetazolamide required may produce other, as yet undiscovered, pharmacological effects.

Acetazolamide should be useful as a tool for the investigation of limb morphogenesis.

W. M. Layton, Jr.
D. W. Hallesy
Experimental Therapeutics Research,
Lederle Laboratories,
Pearl River, New York

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27 May 1965

Thymine Photoproducts but not Thymine Dimers Found in Ultraviolet-Irradiated Bacterial Spores

Abstract. Bacillus megaterium *spores labeled with tritiated thymidine were irradiated with monochromatic ultraviolet light, and the DNA of the spores was analyzed for thymine-containing products. No thymine dimers were observed, but three other thymine photoproducts were found. The unknown products of radiation were produced in vitro by irradiation of DNA that had been dried in the presence of various salts.*

Ultraviolet irradiation of vegetative cells of various organisms and of DNA in solution forms dimers between adjacent thymine residues in DNA strands (*1*). Formation of dimers accounts for much of the failure of ultraviolet-irradiated DNA to prime DNA synthesis (*2*) and for the loss in transforming activity of irradiated *Haemophilus influenzae* DNA (*3*). The lethal effects of ultraviolet light on cells (*4*) has been attributed to the formation of dimers.

The resistance of bacterial spores to the deleterious effects of such agents as ultraviolet light, ionizing radiation, and

heat has often been attributed to a state of dryness within the spore. Various mechanisms for achieving this state have been reviewed and proposed by Lewis *et al.* (*5*). If the DNA in a spore is "dry," the resistance of the spore to ultraviolet may arise from the fact that one-tenth as many dimers are produced by irradiation of dry DNA as are made by irradiating wet DNA (*6, 7*).

Spores of *Bacillus megaterium* labeled with tritiated thymidine were obtained by the following procedure. A liver extract medium (*8*) [1 percent "Liver Fraction B" (Wilson and Co.) in 10 m*M* potassium phosphate, *p*H 6.5] containing methyl-H^3-thymidine was inoculated with a dilute suspension of heat-shocked spores (60°C, 15 minutes). The culture was shaken for 36 hours at 30°C, harvested by centrifugation, and washed twice in 50 m*M* potassium phosphate, *p*H 4.5, and then at least three times with water. The final pellet, consisting of more than 95 percent free spores, was suspended in distilled water at a concentration of 2×10^7 per milliliter and irradiated at 2650 Å, obtained with a large quartz-prism monochromator. The average radiation intensity was 2.7×10^3 erg mm^{-2} min^{-1}. The H^3 content of the suspension was 20,000 to 30,000 count min^{-1} ml^{-1}. After irradiation, the spores were collected by centrifugation. They were broken in a dental amalgamator with glass beads and a glass ball (*9*), and the DNA was extracted by a phenol method (*10*). The extracted DNA was hydrolyzed in 97 percent formic acid at 175°C, and radioactive products were separated by descending paper chromatography on Whatman No. 1 paper with a mixture of *n*-butanol, acetic acid, and H_2O (80:12:30 by volume) (*11*). The chromatograms were cut into appropriate strips and eluted with water, and the radioactivity from each strip was measured in a liquid-scintillation counter with a dioxane-naphthalene scintillator. Chromatograms of hydrolyzates of DNA from unirradiated spores contained less than 0.1 percent of the total radioactivity, in regions other than thymine.

Figure 1 shows a graph of a chromatogram of the hydrolyzed DNA of spores irradiated with 5×10^4 erg/mm^2 together with a similar graph of hydrolyzed *B. megaterium* vegetative cells irradiated with a much smaller dose (2×10^3 erg/mm^2). The amount of thymine dimer is negligible in the spores compared with that in the vegetative cells. The small amount of radioactivity in the thymine dimer region may be accounted for by the few percent of vegetative cells present in the spore preparation.

However, unidentified photoproducts (*a, b,* and *c*) were present in the hydrolyzed DNA of irradiated spores. At 5×10^4 erg/mm^2 photoproducts *b* and *c* contain some 30 percent of the spores' thymine. Our measurements show that, at doses (1000 to 2000 erg/mm^2) yielding 100-percent survival of colony-forming ability, the unknown protoproducts (*b* and *c*) total 1 to 2 percent of the spores' thymine, or some 50,000 thymine residues per spore. We have succeeded in producing large amounts (12 percent of the total thymine) of the photoproducts *b* and *c* in vitro by irradiating DNA (from *Escherichia coli*) that had been dried in the presence of various salts—NaCl, or the calcium salt of 2,6-dicarboxypyridine. A photoproduct with similar chromatographic properties is formed in half this amount in *E. coli* DNA dried from 0.02*M* tris-chloride buffer

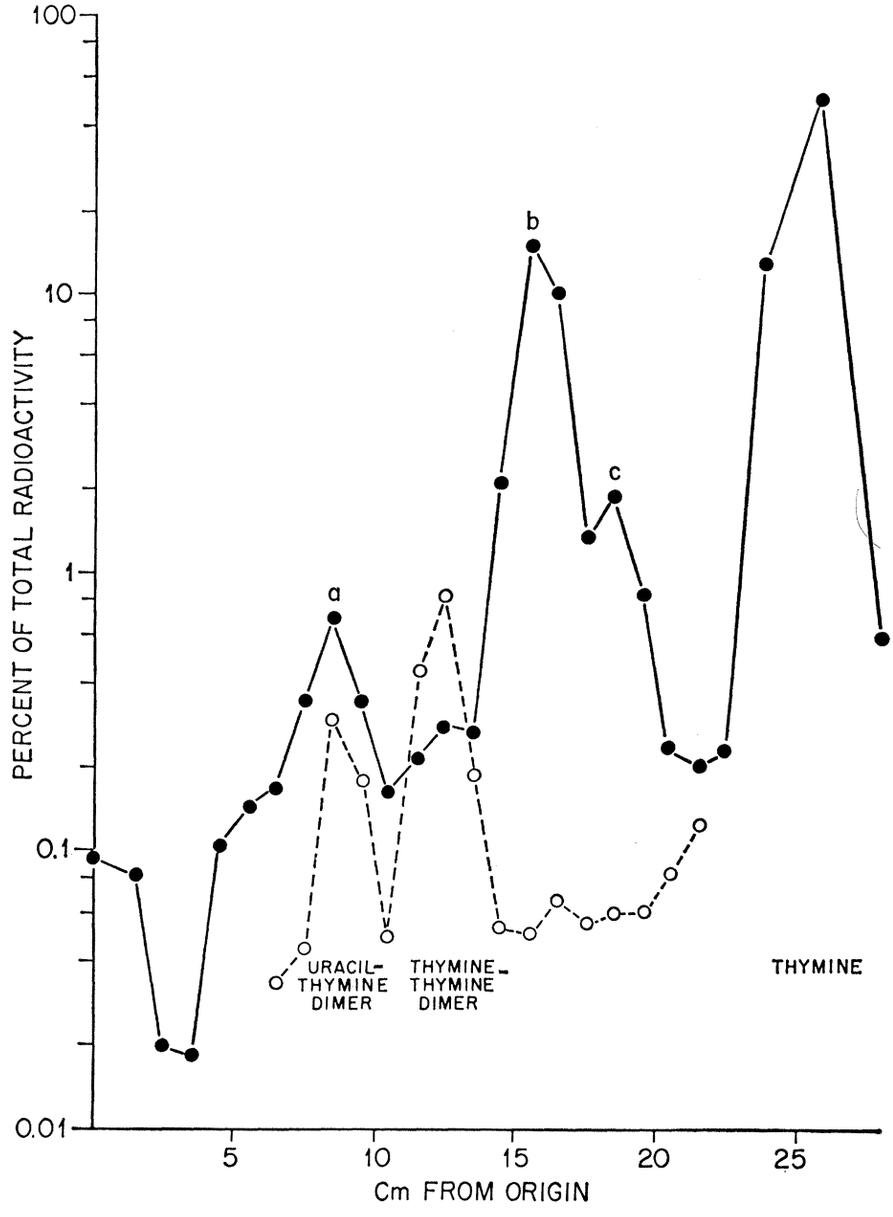

Fig. 1. Graph of chromatograms of hydrolyzed DNA from *Bacillus megaterium* spores (solid lines) and hydrolyzed *B. megaterium* vegetative cells (broken lines) irradiated at 2650 Å. Spores and cells labeled with tritiated thymidine. Spores were irradiated with 5×10^4 erg/mm^2; vegetative cells, 2×10^3 erg/mm^2. R_F's of photoproducts: *a* at 0.20, *b* at 0.38, *c* at 0.44, uracil-thymine dimer at 0.21, thymine-thymine dimer at 0.29, and thymine at 0.61. The small amount of radioactivity at the origin was also present in unirradiated samples.

in the absence of any other salts. Riklis reported a small amount of a thymine photoproduct with similar chromatographic properties in lyophilized DNA which had been irradiated in the dry state (*7*). One of the photoproducts (*a*), appearing in small amount, chromatographs in the region of the uracil-thymine dimer (*11*), but other evidence indicates that the photoproduct may not be this dimer. Smith (*12*) reported finding small amounts of thymine-containing photoproducts chromatographing in the region of our photoproducts (*b* and *c*) in DNA from *E. coli* irradiated in vivo and in an irradiated solution of polydeoxyadenylate-thymidylate.

The absence of thymine dimers in the DNA of irradiated spores is sufficient to explain their resistance to ultraviolet irradiation. However, the appearance of large amounts of unidentified photoproducts implies either that such products do not interfere with DNA synthesis or that the cells have a very efficient repair mechanism for dealing with the photoproducts. Certain data (*13*) indicate that the photoproducts do not remain in the DNA of spores during differentiation into vegetative cells. Our data show that during sporulation the physical state of the DNA within the cell is changed from that found either in vegetative cells or in solution, because normal thymine dimers are not found in irradiated spores.

J. E. DONNELLAN, JR.
R. B. SETLOW
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

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Interferon-Like Virus-Inhibitor Induced in Human Leukocytes by Phytohemagglutinin

Abstract. *Phytohemagglutinin, an extract of the kidney bean,* Phaseolus vulgaris, *induces in human leukocyte cultures an inhibitor of the cytopathic effects of Sindbis virus. The physicochemical and biological properties of this virus-inhibitor are similar to those of interferon induced by Newcastle disease virus, except for an instability at* p*H 2 and 10 and at 56°C.*

During attempts to culture peripheral leukocytes from the blood of persons with and without viral infections, phytohemagglutinin (PHA), an extract of the kidney bean (*Phaseolus vulgaris*) which agglutinates red blood cells in the preparation of cultures, induced synthesis, in leukocytes cultured from normal individuals, of a virus-inhibitor with interferon-like properties. Interferon production in virus-infected cultures of human leukocytes has been previously reported (*1*, *2*).

In this report the properties of the phytohemagglutinin-induced virus-inhibitor are described and compared with interferon induced in white blood cells by infection with Newcastle disease virus (NDV).

Cells were grown in Eagle's minimum essential medium supplemented with tryptose phosphate broth (4 percent) and fetal calf serum (10 percent). The concentration of sodium bicarbonate was 1.75 g/liter, and all cell-culture vessels were gassed with 5 percent CO_2 in air before incubation at 37°C. White blood cells were obtained from the venous blood of normal adults; the blood was placed in tubes containing phenol-free heparin (0.5 ml for each 15 ml blood). The tubes were incubated at 37°C for 50 to 60 minutes and then centrifuged at room temperature at 800 rev/min for 2 minutes. The supernatant leukocyte-rich plasma was then recentrifuged at 1600 rev/min for 8 minutes, and the pellet was suspended in growth medium by gentle pipetting. The leukocytes were counted in a hemocytometer, and the cells were diluted in growth medium to a final concentration of 2×10^6 cells per milliliter. Screw-cap culture tubes were each planted with 2 ml of the cell suspension, placed in a roller drum, and incubated at 37°C.

Three types of cell cultures were used for the interferon assay: (i) cells derived from a human fetal lung, grown in continuous culture, and used between the 10th and 25th passage; (ii) the BHK 21 clone 13 (C13) an established cell line derived from baby hamster kidneys (*3*); (iii) the 'L' cell strain of mouse fibroblasts derived from normal mouse skin (*4*).

Phytohemagglutinin P (5-ml bottles, Difco Co.) was dissolved in phosphate-buffered saline (100 ml) at room temperature and passed through a filter with a pore size of 600 mμ. The filtrate was stored at 4°C.

The specimens to be tested for interferon were diluted in growth medium, and 1 ml of each dilution was added to 1-day-old cultures of human fetal lung cells grown in incomplete monolayers in screw-cap tubes. After 20-hour incubation at 37°C, the cultures were washed once with 4 ml of phosphate-buffered saline. One milliliter of warm Eagle's medium was added to each tube, and then 1 ml of cold growth medium containing 5000 tissue culture infective doses ($TCID_{50}$) of Sindbis virus (Egypt AR 339 strain) was inoculated. This amount of virus produces gross cytopathic effects in cultures in 24 to 30 hours. Cultures were considered to be protected when there was less than 10-percent cytopathic effect at a time when control cultures exhibited more than 75-percent cytopathic effect.

Interferon titers are expressed as reciprocals of the highest dilution of the specimen, 1 ml of which protected cultures against challenge with Sindbis virus. No specimen was tested at less than 1:10 dilution.

To prepare Newcastle disease virus interferon white blood cell cultures were inoculated with 10^8 EID_{50} (egg infective doses) of virus (Hickman strain) and incubated for 48 hours. The media were then collected, and all infective virus was completely neutralized with hyperimmune guinea pig antiserum to the virus (*5*). The inhibitory property of this preparation could be attributed to interferon on the basis of (i) no reduction in the virus inhibitory titer of the supernatant after centrifugation at 105,000*g* for 3 hours; (ii) no reduction in inhibitory titer on acidifi-