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

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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-

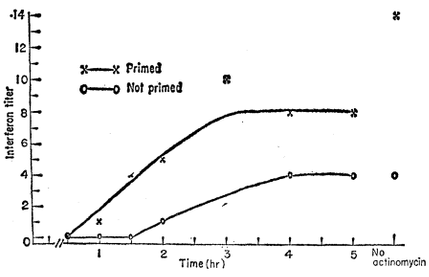

Fig. 1. Effect of prior treatment with interferon on interferon messenger RNA formation. Procedures in text. Virus added at zero time. Abscissa: interval after infection that actinomycin was added. For comparison, interferon titers with no added actinomycin are shown.

night, at 4°C, to inactivate the virus. It was assayed on primary chick embryo cells by a plaque reduction method, with 30 plaque-forming units of vesicular stomatitis virus (VSV). The reciprocal of the dilution of an interferon preparation that gave a 50 percent reduction in the number of plaques was considered the titer of that preparation. Control fluid was obtained by comparable *p*H treatment of allantoic fluid from uninfected eggs. Chikungunya virus, grown in mouse brain, was assayed by a plaque method on rat embryo cells. Any mouse interferon in this virus preparation would have no effect on the chick cells used in our studies. We have shown that this strain of Chikungunya virus grows very little, if at all, in chick embryo tissue culture; but it yields detectable interferon within about 2 hours and maximum titers by about 6 hours after infection (*3*).

Interferon appeared in higher concentration, and probably earlier, in the medium of those cells that received prior treatment with interferon (Table 1). The statistical significance of the data is enhanced by three experiments with comparable results. The possibility had to be considered that some of the interferon found was not newly synthesized, but represented the release of interferon absorbed during the priming part of the experiment. Two considerations rule out this possibility. Our observations (*6*) reveal that little, if any, interferon is absorbed by cells during the treatment of cells by interferon. Second, advantage was taken of the fact that actinomycin D blocks interferon formation, to show that the interferon found was newly synthesized (Table 1). Even 2 hours after infection, the earliest time examined in any of our experiments, the interferon-primed cells were producing larger amounts of interferon than the unprimed ones.

To test whether prior treatment of cells with interferon leads to an earlier production of messenger RNA for interferon, the following experiments were performed. Chick embryo cells were exposed overnight to interferon or control fluid. The fluids were removed; the cells were washed and exposed to Chikungunya virus to induce the formation of new interferon. At the time of addition of virus, and at subsequent times, actinomycin D was added to different batches of cells to block any further formation of messenger RNA. The cultures were incubated for a total of 6 hours, and the fluids were assayed for interferon (Fig. 1). As in previous experiments (*3*), the synthesis of messenger RNA for interferon began slightly later than 1½ hours after infection, and was completed by 2½ hours after infection. Prior treatment with interferon led, in different experiments, to a decrease by 1 to 1½ hours in the time required for the first appearance of messenger RNA, as reflected in loss of sensitivity to actinomycin. Statistical analysis of these data, along with two other experiments, with the Sign test (*8*), indicated that the possibility that these differences were due to chance are less than 1 part in 500 ($P < .002$).

There are two different, but related, viewpoints from which one should consider the preceding observations. The first is related to the action of the interferon system on the subsequent action of the virus, and the second to the induction of interferon formation by the cell upon stimulation with virus.

In connection with the effect of prior treatment with interferon on the action of virus it should be noted that Chikungunya virus ordinarily induces the formation of messenger RNA for interferon within 2 hours after infection. Since prior treatment of the cells with interferon affects this inducing capacity of the virus, it follows that the action of interferon occurs earlier than 2 hours after infection in this system. Indeed, since messenger RNA is being made in primed cells by 1 hour after infection, it follows that interferon is affecting an event in virus infection not later than 1 hour after addition of the virus. This early time of action is in accord with other of our observations in which the suppression of cell RNA synthesis induced by Mengo virus within 20 minutes after infection is delayed by prior treatment with interferon (*2*). Comparable results on cell RNA cut-off have been obtained with Sindbis virus (*7*).

Table 1. The effect of prior treatment of chick embryo cells with interferon on subsequent interferon yield. Three-day-old chick embryo cells in 60-mm petri dishes were used. Each group represents the fluid from three dishes. The odd-numbered groups of cells receive 40 units of interferon in 0.7 ml, the even-numbered ones an equal volume of control fluid. The cells were incubated overnight at 36°C in an atmosphere of 95 percent air and 5 percent CO_2, and the medium was thoroughly removed. The cells were washed once, and exposed either to $10^{7.5}$ PFU of Chikungunya virus in 0.5 ml of Eagle's medium containing 1 percent calf serum (multiplicity, 10) or to medium without virus. Actinomycin (0.5 μg/ml) was added to groups V and VI, at the same time as the virus. Groups VII and VIII received no virus. After 20 minutes of exposure to virus or control fluid, 4 ml of medium were added to all dishes. At the indicated time after infection, the fluids were harvested and the interferon titer was measured. V, virus; A, actinomycin.

Group	Treatment	Addition	Time (hr)	Titer
I	Interferon	V	6	20
II	Control	V	6	10
III	Interferon	V	2	5
IV	Control	V	2	1
V	Interferon	V+A	6	<1
VI	Control	V+A	6	<1
VII	Interferon	None	6	<1
VIII	Control	None	6	<1

The second viewpoint mentioned above is concerned with the induction of interferon formation. The information for interferon synthesis appears to reside in the host cell genome, and is not expressed until some early event in virus replication leads to derepression. The prior treatment with interferon acts in such a way as to accelerate the derepression induced by the virus. This acceleration could be due to an effect of interferon on the cell genome, acting in some way as to facilitate derepression by the virus. Another possible way to explain the accelerated derepression in the interferon-primed cells is to hypothesize that these cells modify the infecting virus so that the virus is a better inducer of interferon formation. The first possibility involves an effect on host genome, the second, an effect on that part of the cell that modifies the inducing virus.

An important biological implication of these observations is the enhanced and earlier protection afforded to cells exposed to priming doses of interferon. In the intact animal or in tissue culture, increased amounts of interferon might

become available earlier in those cells that receive a priming dose of interferon before becoming infected by the spreading infection. In some cases, the early availability of larger amounts of interferon may be sufficient to abort the infection.

Data comparable to some of the foregoing have been obtained independently by Friedman, who also showed that the enhancement phenomenon requires protein synthesis (*9*).

HILTON B. LEVY
CHARLES E. BUCKLER
SAMUEL BARON

Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014

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18 February 1966

Human Visual Acuity Measured with Colored Test Objects

Abstract. *Visual acuity was measured with a grating test object in which alternating bars were matched in brightness but differed in wavelength. If the wavelength difference between adjacent bars was great enough, acuity scores were obtained which were as high as those obtained with test objects in which there was a large brightness difference between adjacent bars.*

Visual acuity is a measure of an observer's ability to perceive fine detail. Most experimental, and all clinical, tests of visual acuity use stimulus patterns in which there is a brightness contrast between the test object and the background from which it must be discriminated. Indeed, visual acuity is often treated as if it were a special case of brightness discrimination. This is not unrealistic since, in everyday life, brightness gradients are almost certainly the most important cues for visual discrimination. It has been reported that acuity is degraded when the test object and its background are equally bright but differ in hue (*1*); however, several recent studies (*2*) indicate that this may not be true when saturated stimuli are used. We have tested this proposition by measuring acuity with highly (but unequally) saturated monochromatic test objects which differ from their backgrounds in wavelength but not in brightness.

Foveal visual acuity was measured with a grating target which appeared to the observer as a series of equally wide bars filling a 1-degree-circular aperture centered in a 30-degree achromatic surround. Even-numbered bars were illuminated by one optical system, and odd bars by another similar system. Each system included filters and a grating monochromator, set for a nominal bandpass of 10 nm, so that the wavelength and intensity of the two sets of bars could be independently varied. The width of the bars could be changed optically to subtend visual angles between 0.5 and 2.0 minutes per bar, permitting measurement of acuity from 0.5 min^{-1} to 2.0 min^{-1} (20/40 to 20/10 in Snellen notation). The gratings were presented in Maxwellian view, with the observer's head position maintained by use of a forehead rest and dental impression block. Correcting optics and a 1.5-mm artificial pupil were used to reduce axial chromatic aberration. The following data were obtained from two emmetropic observers who had clinically normal color vision and whose photopic brightness matches resembled the photopic "standard observer."

Individual brightness-matching functions were obtained between 422 and 680 nm by step-by-step matching of stimuli which were separated by 5 nm. The luminance of the resulting stimuli could be approximately matched by a 7.5-millilambert extended source. These brightness matches were then used to equate all the acuity test objects and backgrounds. During a series of observations the wavelength and intensity of one set of bars remained fixed. These bars will be arbitrarily referred to as background. The other bars, which alternate with the background, were varied in wavelength and will be called the test objects.

Acuity thresholds were obtained by a modified method of adjustment (*3*). The grating was initially presented below threshold, and the observer increased the width of the bars until they were visible. The observer was required to detect the presence of the grating, but not to identify the hues of the bars. Figure 1 shows how acuity changes as a function of wavelength separation between test object and background. As expected, acuity is poor when only a small wavelength separation exists between adjacent bars. As wavelength separation is increased, acuity improves until it reaches a maximum of about 1.3 min^{-1}. This maximum is essentially the same as the observer's acuity measured with a conventional test grating made of adjacent black and white bars. An intentional brightness mismatch between adjacent colored bars improves acuity only when the wavelength separation between bars is small; once maximum acuity has been reached no further improvement can be effected by introducing either a small (0.1 log unit) brightness mismatch or by completely occluding one set of bars. We note parenthetically that examination of a number of these records did not reveal any consistent unusual effects when test object and background were illuminated with complementary wavelengths.

As a check on the method of adjustment acuity, thresholds were de-

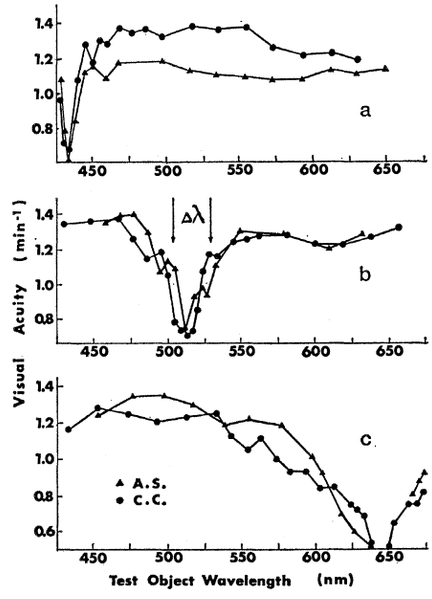

Fig. 1. Visual acuity measured with gratings in which adjacent bars are equally bright but differ in wavelength. In (*a*) the background bars were 430 nm; in (*b*), 520 nm; and in (*c*), 650 nm.