and the 5 control animals each had an average survival time of 2.6 days after the detectable onset of disease. These data indicate that impure preparations of Helenine such as were available had marked prophylactic activity against poliomyelitis in monkeys but no apparent therapeutic activity.

Discussion. Helenine, like M5-8450(6), was found to be an effective prophylactic against poliomyelitis in monkeys. In contrast to its effectiveness in monkeys, Helenine did not alter significantly the incidence of paralysis in mice, although it did appear to prolong the incubation period. A species difference in the response of the host to this material seems unlikely in view of the effectiveness of Helenine against other neurotropic viruses in mice(2,3). One can speculate that the observed effects may have represented type specific differences in susceptibility between types I and II poliomyelitis viruses. Type differences have been encountered in susceptibility to inactivation with merthiolate in the 1954 poliomyelitis vaccine(10) and have been reported to occur *in vitro* with certain chemical agents(11). On the other hand, differences in effectiveness also could have been due to differences in reactivity of the whole host-virus complex; Mahoney virus in the monkey versus MEF_1 poliomyelitis virus in the mouse. A direct comparison of Helenine and M5-8450 is not possible with these data owing to the differences in dosage employed. The data presented do not justify any conclusion as to the relative effectiveness of the two materials, but seem to be a further indication of their apparent similarity(4,7). Additional studies of the antiviral action of such penicillium filtrates are planned to obtain information regarding their activity and mechanism of action.

Summary. Helenine, an antiviral agent derived from *P. funiculosum,* was found to have marked prophylactic activity in cynomolgus monkeys infected with poliomyelitis virus.

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Received April 19, 1956. P.S.E.B.M., 1956, v92.

Protection by Sodium Hydrosulfite Against X-Ray-Induced Mitotic Inhibition in Grasshopper Neuroblast.* (22438)

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It is well known that a reduced oxygen tension during irradiation causes a corresponding reduction in sensitivity to ionizing radiations. The literature on this phenomenon at the cellular level has been reviewed by Giles(1) (chromosome breakage), Muller(2) (mutations), and Gray(3). Recovery from X-ray-induced mitotic inhibition in grasshopper neuroblasts as affected by oxygen tension during irradiation of the intact egg was observed by Gaulden, Nix, and Moshman(4). They found that at 64 r the delay of mitosis was detectably decreased when embryos *in vivo* were irradiated *in vacuo*. At lower doses (3.5 and 8 r) the recovery of mitotic activity appeared to be independent of the oxygen tension

* This work was performed under contract No. W-7405-eng-26 for U. S. Atomic Energy Commission.

around the eggs during irradiation. The small "oxygen effect" at the high dose and the lack of a detectable one at low doses may be due to the inefficiency of a vacuum in removing dissolved oxygen from the fluids of the egg. To eliminate oxygen by a vacuum or by changing the gaseous atmosphere around embryos from which the protecting egg membranes have been removed is not feasible with present technics, because osmotic changes resulting from the evaporation of water interfere with the determination of the X-ray-induced effect. Sodium hydrosulfite is very efficient in protecting bacteria against the lethal effects of X rays(5,6) and in reducing the frequency of radiation-induced chromosome aberrations(7,8). Therefore, sodium hydrosulfite, which readily combines with molecular oxygen in solution(9), was used in the following experiments to remove the oxygen dissolved in the medium.

Materials and methods. Embryos of the grasshopper *Chortophaga viridifasciata* (De Geer) at a stage comparable to 14 days of development at 26°C were dissected in a medium described by Shaw(10). The embryos were pretreated by transfer from the dissecting medium into paraffin chambers filled with medium that contained freshly prepared sodium hydrosulfite. The chambers were sealed with cover glasses to maintain antisepsis and to prevent the diffusion of oxygen from the air into the medium. Within 3-5 minutes after immersion into the pretreatment medium, the embryos were irradiated through the cover glass (0.14 mm) with 8 or 32 r of X rays. A General Electric Maxitron 250 X-ray machine was used at 120 kvp, 25 ma with 3½ mm of Al additional filtration. The target distance averaged 60 cm. The dose rate was 32 r per minute. Within 2 minutes after irradiation the embryos (both irradiated and control) were transferred to a large volume of the dissecting medium to remove the sodium hydrosulfite and its decomposition products. The medium remains isotonic with the embryos when they are kept in a relatively large volume. Hanging-drop cultures were then prepared from the X-rayed and control embryos according to the technics described by Gaulden, Nix, and Moshman(4) and Carlson, Hollaender, and Gaulden(11). The medium used in hanging drops was slightly more dilute than that used for dissection and exposure, in order to compensate for an increase in solute concentration caused by evaporation of water from the medium placed on the cover glass during the preparation of the hanging-drop cultures. The mitotic activity of cultures was determined by counting the number of neuroblasts in mid-mitosis (prometaphase, metaphase, and anaphase) in 6 segments of the embryo (first and second maxillary, first, second, and third thoracic, and first abdominal segments). These segments contain approximately 240 neuroblasts. In all cases, the mitotic activity for each group is the arithmetic mean of the values obtained from 4 embryos. Mitotic ratio is the ratio of the total number of mid-mitotic cells of an irradiated group to those of its control. An analysis of variance† of the raw data was performed to determine whether there was any significant difference between the various groups with respect to the level of mitotic activity. It was found that the use of the new medium(10) and the technic described for handling embryos reduced the fluctuations in mitotic activity between counting periods and between embryos in controls. Thus significant differences could be detected between groups with only 4 embryos per group instead of the 8-16 used by previous workers. Mid-mitotic figures in the cultures were counted at 22-minute intervals. The validity of these values as an index of mitotic activity is discussed by Gaulden and Kokomoor(12). If the duration of mid-mitosis (22 minutes) is not altered by the treatment, each cell that passes through this phase will be counted only once. The treatments used in these experiments caused only a slight prolongation of mid-mitosis before, and occasionally during, the first counting period.

Results. 8 r with no pretreatment. A dose of 8 r of X rays causes about 75% inhibition of mitosis (a mitotic ratio of 0.25) by the 66-minute count (Fig. 1). The mitotic ratio

† The author is indebted to Dr. A. W. Kimball and Mr. George Atta for statistical analysis of the data.

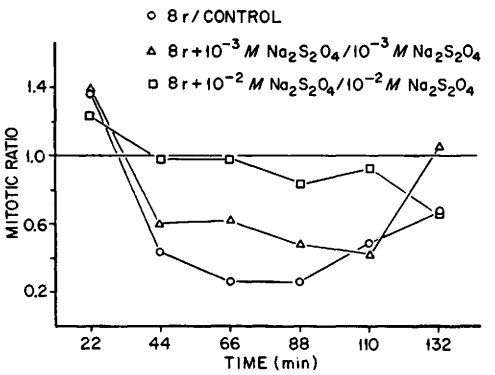

FIG. 1. Effect of concentration of sodium hydrosulfite in pretreatment on mitotic ratio of grasshopper neuroblast cultures.

remains low during the 88-minute count and recovery begins during the 110-minute count. By the time of the 132-minute count the mitotic ratio is 0.67.

Pretreatment with 10^{-3} M sodium hydrosulfite. Short periods of treatment with sodium hydrosulfite arrest mitosis temporarily during the prometaphase-metaphase period. These stages progress again normally if the hydrosulfite is removed within several minutes. After release from blockage by prolonged treatment with sodium hydrosulfite, the rate of progression of the prophase stages into prometaphase and metaphase is considerably slowed. The magnitude of this change in rate is directly dependent on the length of the period of treatment. As the treatment period is lengthened, other abnormalities appear after the release of the cells from the mitotic block; the structure of and rate of formation of the spindle and the condensation of the chromosomes becomes abnormal. A very short treatment with a concentration of 2 x 10^{-2} M sodium hydrosulfite produces the same effects as the prolonged treatment at lower concentrations. The highest usable concentration was 10^{-2} M.

8 r with 10^{-3} M sodium hydrosulfite pretreatment. There is no statistically significant difference in the number of neuroblasts in mid-mitosis of irradiated embryos pretreated with 10^{-3} M sodium hydrosulfite and those which received 8 r alone. Because of the 5-minute pretreatment with sodium hydrosulfite, however, the mitotic activity of

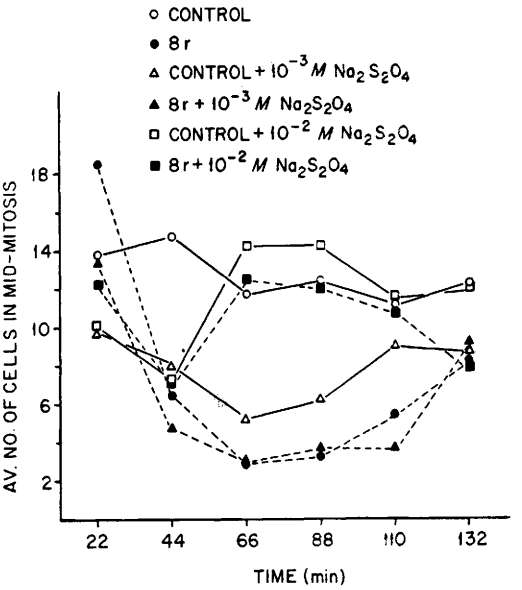

FIG. 2. Effects of X-rays on mitotic activity of grasshopper neuroblast in pretreated and untreated cultures.

the 10^{-3} M hydrosulfite controls is lower than in the untreated controls (Fig. 2). If sodium hydrosulfite at this concentration has both a toxic and a protective effect of equal magnitude, they will compensate for each other and the result will be no change in mitotic activity of irradiated cultures as compared with that of untreated irradiated cultures. Since the mitotic activity of the 10^{-3} M sodium hydrosulfite controls is lower than that of the untreated controls, the mitotic ratio (Fig. 1) of the 8 r plus 10^{-3} M sodium hydrosulfite experiment is higher than in the 8 r experiment with no pretreatment. Comparison of mitotic ratios shows an apparent protective effect of the 10^{-3} M concentration.

8 r with 10^{-2} M sodium hydrosulfite pretreatment. Fig. 2 shows that the depression of mitotic activity of both the X-rayed and of the control cells occurred only during the 44-minute count. This depression, which was due to the toxic effect of the sodium hydrosulfite pretreatment, was less than in the 10^{-3} M experiment because the pretreatment time was reduced to 3-4 minutes. There were no statistically significant differences between the X-ray-treated and the controls in this experiment. This is reflected in the mitotic ratio

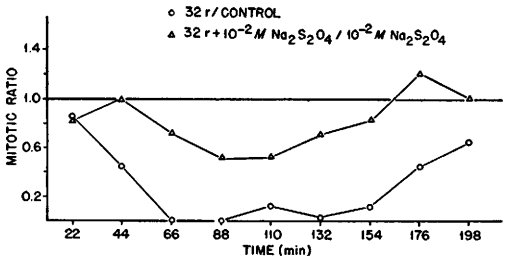

FIG. 3. Effect of pretreatment on mitotic ratio of X-irradiated cultures of grasshopper neuroblasts.

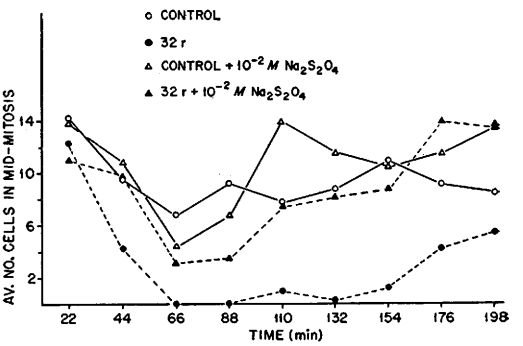

FIG. 4. Effects of X-rays on mitotic activity of grasshopper neuroblasts in untreated and pretreated cultures.

(Fig. 1), which is essentially 1.0 at most of the counts. Thus the pretreatment has almost completely prevented the mitotic inhibition usually caused by 8 r of X rays.

32 r with no pretreatment. A dose of 32 r causes complete inhibition of mitosis by the 66-minute count (Fig. 3) and it remains almost complete through the 132-minute count. By the 154-minute count, recovery begins and the cultures show about 65% of the mitotic activity of the controls (mitotic ratio of 0.65) by the 198-minute count.

32 r with 10^{-2} M sodium hydrosulfite. The mitotic activity of the X-ray-treated cultures is about 70% at the 66-minute count (Fig. 3). However, Fig. 4 shows that both the X-rayed and the control cells had a depressed mitotic activity during the time of the first 3 counts. This inhibition was probably due to the toxicity of the hydrosulfite pretreatment, since the unirradiated hydrosulfite-treated cells show the compensatory rise in mitotic activity during the 88- and 110-minute counts, which is the typical pattern of recovery from a temporary inhibition of mitosis. The mitotic ratio (Fig. 3) shows that the maximum inhibition was only about 50%, and that recovery was complete (when the mitotic ratio equals 1.0) between the 154- and 176-minute counts. There is no statistically significant difference in the shape of the curves of the mitotic ratios for the untreated and the pretreated (Fig. 3). Their heights or levels of mitotic activity, however, are significantly different at the 5% level. Although recovery begins sooner in the cultures receiving the sodium hydrosulfite pretreatment, the rate of return of mitotic activity is apparently the same in cultures that have received the pretreatment as in those that have not. These findings may be interpreted as meaning that the recovery of mitotic activity of the pretreated cultures occurs sooner because there is less radiation-induced mitotic inhibition, and not because the initial rate of recovery is increased.

Discussion. Since sodium hydrosulfite reacts readily with molecular oxygen in solution, the reduction of the radiation effect by sodium hydrosulfite is probably due to a decrease in the quantity of the oxidizing radicals initially produced by the radiations. Gray (3) has pointed out that the presence of oxygen is capable of quadrupling the oxidative yield of X rays through the production of HO_2 radicals, which have the oxidizing equivalent of 3 OH radicals. The reduction of the amount of oxidizing radicals may account for the prevention of the mitotic inhibition induced by 8 r, for at this dose the manifestation of the radiation effect is essentially completely prevented by the hydrosulfite pretreatment. The primary damage responsible for mitotic inhibition at this dose level may therefore be due to the oxidation of intracellular components by HO_2 or similar oxidizing radicals whose formation is dependent on the presence of oxygen during irradiation. At 32 r, since the reduction of the radiation effect by the pretreatment is only partial, the inhibition of mitosis may be only partly caused by the actions of oxidizing radicals.

Gaulden, Nix, and Moshman(4) observed approximately a 50% reduction of mitotic activity with 3.5 r. In the experiments re-

ported here, the reduction of the mitotic activity caused by 32 r in 10^{-2} M sodium hydrosulfite-pretreated cultures is only 50% as contrasted with 100% inhibition in the absence of the pretreatment. This difference is significant at the 5% level. Therefore, the dose-reduction factor is about 8. [The reduction of the total oxidizing power of the radicals is, at most, only a factor of 4. The complete removal of oxygen from the medium by hydrosulfite pretreatment causes a fourfold reduction of the radiation effect as measured by the change in survival of X-irradiated bacteria (5,13)]. At 8 r with the same hydrosulfite pretreatment, the protection is essentially complete. If the dose-reduction factor is 8, then the effective dose is only 1 r. The effect of a dose as small as 1 r would be very difficult to detect. Gaulden, Nix, and Moshman(4) observed only slightly less mitotic delay when intact eggs were irradiated with 64 r *in vacuo*, as in contrast with irradiation in air. A low dose-reduction factor may therefore have been responsible for the lack of detection of a protective effect of the vacuum at the lower doses (3.5 and 8 r). Thus the magnitude of the dose-reduction factor can account for the lack of a detectable protective effect by vacuum at low doses in the work of Gaulden, Nix, and Moshman(4) and for the large protective effect of sodium hydrosulfite pretreatment observed at 8 r presented here.

The dose-reduction factor was, however, actually determined at one dose level (32 r) only. At 8 r, the dose-reduction factor cannot be determined because the residual radiation effect in pretreated cultures is, if present at all, too small to be evaluated. Thus it will require further experiments at other dose levels to be able to decide if the dose-reduction factor is constant with changes in dose. This is of interest because the constancy or lack of constancy of the dose-reduction factor at different doses may offer a clue to the degree of complexity of the primary effect of radiations in causing mitotic inhibition.

Summary. In neuroblast cultures of the embryo of the grasshopper *Chortophaga viridifasciata* (De Geer) protection against X-ray-induced mitotic inhibition is conferred by pretreatment with sodium hydrosulfite. A concentration of 10^{-2} M sodium hydrosulfite almost completely prevents the inhibition of mitosis caused by 8 r. The mitotic inhibition caused by 32 r is only partially prevented by the same pretreatment. The dose-reduction factor at 32 r is 8. The rate of recovery is the same in the cultures that received 32 r and that were also pretreated with 10^{-2} M sodium hydrosulfite as in those that were irradiated but received no pretreatment. The earlier recovery of the pretreated cultures is accounted for on the basis of less demonstrable inhibition of mitotic activity by the radiations. The primary damage responsible for mitotic inhibition at low doses, and at least partially at high doses, may be due to the oxidation of intracellular components by HO_2 or similar oxidizing radicals whose formation is dependent on the presence of oxygen during irradiation.

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