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EFFECTS OF X-RAYS ON ACETYLCHOLINE SOLUTIONS SHOWING THE DILUTION AND PROTECTION PHENOMENA FOUND FOR ENZYMES

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In two previous papers [Dale, 1940, 1942] the effect of X-rays on enzyme solutions has been investigated quantitatively and, without repeating the details, it has been shown that a dilute enzyme solution can be inactivated by a given dose of X-rays more completely relative to its initial concentration than can a more concentrated one, and the implications of this phenomenon have been discussed. It has also been shown that organic non-enzymatic substances dissolved in the enzyme solution inhibit the radiation effect. From this inhibition phenomenon it could be assumed that the mode of action of the radiation was not confined to enzymes but could be extended to any organic substance. A direct proof, however, which is not based on enzymatic reactions as indicators of radiation effects, is still needed, and it is the purpose of this paper to show that the effect of X-rays, found to hold for enzyme solutions, can be extended to include non-protein, non-enzymatic substances.

Acetylcholine (denoted in what follows by ACh.), has a powerful depressing effect on the contraction of the heart muscle when used in concentrations comparable to that of enzymes. It was, therefore, hoped that the effect of X-rays on ACh. solutions could be demonstrated when Clark's technique of testing ACh. solutions on the isolated ventricular strip of the frog's heart was employed. I am indebted to Dr W. Schlapp for having drawn my attention to Clark's method as an alternative to the use of the muscle of the leech which is unobtainable at present.

Clark [1926] has given a detailed account of the relation between the action of ACh. and its concentration. In the first place the activity varies 100-fold for a 10,000-fold change of concentration of ACh., i.e. the reaction of the muscle to a change of concentration is relatively small, and therefore greater doses of X-rays compared with those used for enzymatic solutions will be required to show marked inactivation effects. Furthermore, the approximately linear drop in action obtaining for medium concentrations of ACh. flattens out for decreasing concentrations, so that roughly a 60-fold drop in concentration is

required to remove the last 20% of action as compared with a 5-fold drop to lower the action from 60 to 40%. The test, therefore, is more sensitive to a change of concentration in this steeper part of the curve, i.e. towards increasing concentration. On the other hand, as shown in the author's first paper, increasing concentration requires increasing the X-ray dose to obtain a given percentage destruction. The radiosensitivity of this biological test is, therefore, lower than that of enzymatic reactions.

Methods

To show a sufficiently marked radiation effect the following layout and technique of the experiments were adopted. Two ACh. solutions, *W* and *S*, differing 10,000-fold in concentration, were exposed to an X-ray dose of 40,000 r. Solution *W* was $2{\cdot}5 \times 10^{-6}$ *M* and solution *S* was $2{\cdot}5 \times 10^{-2}$ *M* with respect to ACh. The stronger solution *S* was diluted 10,000 times after irradiation. In the tracings which follow, solution *W* after irradiation is marked *W** and solution *S* after irradiation and dilution *SW**. An unirradiated portion of solution *W* was kept as control. The ACh. content of the solutions *W*, *SW** and *W** was then tested on the ventricular strip of the heart.

The test of the activity of the ACh. solution on the heart muscle has to be carried out in Ringer's solution. During irradiation, however, the ACh. solution should not contain the Ringer constituents which must, therefore, be added after irradiation. For this purpose the NaCl, KCl and $CaCl_2$ of Ringer's solution were dissolved in 0·6 ml. of water in such a concentration that addition of 24 ml. of the irradiated solution made their concentration identical with that of Ringer's solution as used by Clark. Then 0·6 ml. of the remaining Ringer component $NaHCO_3$ was added in a likewise appropriate concentration.

This division of the Ringer components and their order of mixing is necessary to avoid precipitation of the calcium and the contact of ACh. with a too alkaline solution.

For the actual test of the ACh. solution Clark's technique was adopted without major alterations, though it should be mentioned that it was found advantageous to allow the heart muscle to settle after its preparation for about 2 hr. and then to stimulate it with regular break induction shocks for about 15 min. in Ringer's solution before the actual recording of the muscle's contraction in the experimental solutions was started. For each change of fluid the electrical stimulation was interrupted for about 8 sec. The frequency of the stimulation was usually 30 per min.

Irradiation

The ACh. solutions were irradiated with rays of half-value layer of 6 mm. Cu from a 500 kV. Metropolitan-Vickers continuously evacuated X-ray tube with a cool window. The dosage rate was approximately 370 r. per min.

The 'dilution' effect

A tracing of a typical experiment is shown in Fig. 1 A, B. In A as well as B periods of immersion in Ringer's solution without ACh. were inserted between each change of A.Ch. solution, and in both tracings the irradiated weak solution (*W**) shows the least depressing effect on the muscle's contraction, indicating a reduced ACh. content, whereas the unirradiated control solution (*W*) and the irradiated strong solution diluted after irradiation (*SW**) indicate an identical ACh. content.

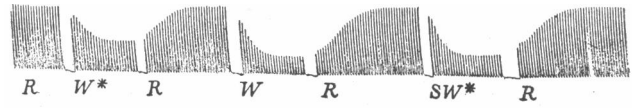

Fig. 1A.

Fig. 1B.

Fig. 1. A. The effect of irradiated and unirradiated solutions of acetylcholine on the frog's heart. *R* = Ringer's solution; *W* = acetylcholine $2{\cdot}5 \times 10^{-6}$ *M*; *W = acetylcholine $2{\cdot}5 \times 10^{-6}$ *M*, irradiated; *SW** = acetylcholine $2{\cdot}5 \times 10^{-2}$ *M* irradiated and after irradiation diluted 10^4 times. B. As A, with reversed order of immersion.**

Tracing B differs from A only in the order of immersion of the muscle into the various solutions, demonstrating that the results are independent of the order of immersion.

Fig. 2 shows the same reduced ACh. content in *W**, the difference of procedure being that the change from one fluid to the other was done directly

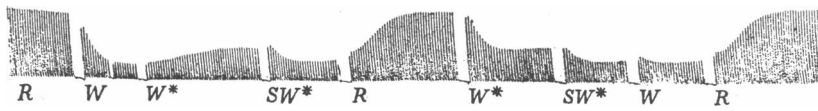

Fig. 2. Immersion without intervening Ringer periods; in the second half of the tracing order of immersion changed.

without intervening Ringer periods. The gradual increase of the muscle contraction for *W** shown in the first half of the tracing to its proper final value demonstrates that a change from a more concentrated solution to a weaker one has taken place. In the second part of the tracing after one Ringer period, the order of immersion was changed again. These tracings prove that the weak solution is inactivated more completely relatively to its initial concentration than the strong one.

The 'protection' effect

In a similar way, it can be shown that the presence of another organic substance in the ACh. solution during irradiation inhibits the radiation effect. Glucose, being innocuous to the working of the muscle, was chosen as an inhibitor.

For this experiment the following solutions were required:

For irradiation
- (*X*) ACh. 10^{-6} *M*.
- (*Y*) ACh. 10^{-6} *M* containing glucose of the concentration of $1{\cdot}1 \times 10^{-2}$ *M*.
- (*Z*) ACh. 10^{-6} *M* as an unirradiated control.

Solutions *X* (after irradiation) and *Z* as well as the Ringer's solution are made up to the same glucose content as solution *Y*.

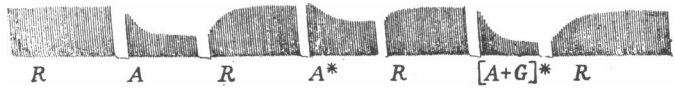

Fig. 3. The effect of irradiated and unirradiated solutions of acetylcholine on the frog's heart in the presence and absence of glucose during irradiation, with intervening Ringer periods. *R*=Ringer's solution; *A*=acetylcholine 10^{-6} *M*; *A**=acetylcholine 10^{-6} *M* irradiated; $[A+G]^*$=acetylcholine 10^{-6} *M* irradiated in the presence of $1{\cdot}1 \times 10^{-2}$ *M* glucose.

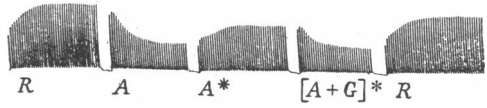

Fig. 4. The same as Fig. 3 without intervening Ringer periods.

The experiments have been carried out again with (Fig. 3) and without (Fig. 4) intervening Ringer periods. In both tracings the presence of glucose in the ACh. solution ($[A+G]^*$) protected against the irradiation effect, whereas the ACh. solution without glucose (*A**) was reduced in activity.

Discussion

The results of the present paper cannot be seen in their right perspective without briefly recalling relevant points of the two previous papers on the enzymes carboxypeptidase, polyphenoloxidase and *d*-amino-acid oxidase. There the dilution and the protection phenomena in their relation to the inactivation of these enzymes have been shown. The results excluded the possibility of a direct action of radiation on these enzymes and could be understood only if the X-rays form an intermediate product from the water which in turn reacts with the enzymes. Since many organic, non-enzymatic, non-protein substances protected the enzymes against inactivation by X-rays, it was assumed that the mode of action of radiation could be extended to these substances. A fair

justification for this assumption was provided by the inactivation of the organic prosthetic group of the amino-acid oxidase—alloxazinadenine dinucleotide—itself non-enzymatic and non-protein, though still related to an enzyme.

The present ACh. experiments are not based on enzymatic reaction at all, and therefore show clearly that both the dilution and the protection phenomena hold for a representative of biologically active, organic, non-protein substances, and that such substances obey the same laws of inactivation by X-rays as do enzymes.

Another point of importance only deduced in the discussions in the two previous papers now appears to be demonstrated experimentally. There it was stated that radiosensitivity, in the biological sense, is not a fixed entity but a variable, the value of which depends on the concentration of the material subjected to radiation, on the presence of 'protecting' substances and, by deduction, on its particular function within the living cell. The response produced by acetylcholine varies only narrowly for large variations of its concentration. Any absolute radiation effect therefore appears to be expressed on a diminished scale in terms of this response. This instance of radiation effect shows that in living material a reaction of this kind must be far less important from the point of view of radiosensitivity than that found for enzymes, for which radiation effects are expressed on a greatly enlarged scale in terms of the response measured, owing to the catalytic nature of their action.

Summary

1. Solutions of acetylcholine as a representative of organic, non-protein, biological substances, entirely unrelated to enzymes, have been exposed to X-radiation and then tested for their biological activity on the isolated ventricular strip of the frog's heart stimulated by regular break induction shocks.

2. It is shown that a dilute solution is inactivated by a given dose of X-rays more completely relatively to its initial concentration than a more concentrated one, and that glucose present in the acetylcholine solution during irradiation inhibits the radiation effect.

3. This proves that the mode of action of X-rays on acetylcholine is the same as that previously found to hold for enzymatic solutions.

4. The significance of these results and the relative radiosensitivity of acetylcholine as compared with enzymatic reactions are discussed.

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