THE SENSITIVITY OF CELLS TO THE LETHAL ACTION OF X-RAYS.

T H E S I S

submitted for the degree of

Doctor of Science

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by

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Scope of the Thesis.

This thesis consists of three papers which record an experimental investigation into the nature of the biological action of X-rays. An introduction has been added in order to correlate the papers with each other and to draw attention to the significance of the experiments described in the papers in relation to the records of work by other investigators.

Except for such help as is acknowledged in the papers, the investigation has been carried out independently.

Contents.

PART I. INTRODUCTION.

INTRODUCTION

This thesis describes part of an investigation of the differences which cells show in their sensitivity to the lethal action of X-rays. This sensitivity shows enormous variations. Crowther (1926) found that 80,000 r units of X-irradiation were required to kill the protozoon Colpidium colpoda, while Moppett (1929) and the author (1933) found that the cells of the allantois of the embryo chick under special conditions could be killed by doses of less than 10 r. The information concerning the sensitivity of a wide variety of plant and animal tissues has been summarised by Colwell and Russ (1924), Warren (1928), Packard (1931) and these summaries generally support the so-called Law of Bergonie and Tribondeau (1906), which states that the sensitivity of a cell is inversely proportional to its functional differentiation and directly proportional to its reproductive capacity. For instance the cells of the gonads are killed by relatively small doses of X-rays (Knox, 1932, p. 202) while muscle/

muscle cells are notoriously resistant to large doses. An unpublished experiment which the author carried out illustrates the extraordinary resistance of cardiac muscle to X-rays. Two isolated preparations of the frog's auricle were set up according to the method described by Clark and White (1930). The procedure was carried out aseptically. One auricle was exposed to 5 mg. radium element in the form of radium bromide which was contained in a glass tube, the wall of which was about 0.5 mm. thick. The distance of the radium from the auricle was 2 mm. The second auricle preparation was used as a control. In spite of the great sensitivity of this biological material to many kinds of injury, the irradiated and the control auricles both showed spontaneous contractions of approximately the same frequency and magnitude for eight days.

The Law of Bergonie and Tribondeau is therefore a convenient summary of a quantity of clinical and experimental evidence, but many important exceptions render it invalid as an accurate statement. The basal celled carcinoma which is composed of relatively differentiated and/

and slowly growing cells is radiosensitive while the melano-sarcoma which is composed of undifferentiated and rapidly growing cells is notoriously radioresistant (Ward and Smith, 1933). The experiments of Henshaw and Henshaw (1933) and those of the author (1934) show that embryonic tissues actually become more sensitive at certain stages of their development although these tissues are progressively becoming more differentiated.

Variations in sensitivity occur not only in different tissues and in the same tissue at different stages of embryological development but also in single cells. The results of Mottram (1913), Holthusen (1921), Strangeways and Hopwood (1926), Vintemberger (1928) show that the sensitivity of a cell increases during one phase of mitosis,and although there is a difference of opinion about the identity of the phase, there is general agreement that a variation in sensitivity exists.

Henshaw and Henshaw (1933) and the author (1934) have shown that embryonic tissues are much more sensitive to the lethal action of X-rays during the early stages of development than during the/

the stages immediately preceding the formation of the adult organism. This difference in sensitivity is of the same order of magnitude as that which is found between different types of tissue,but the variation in sensitivity which a cell undergoes during mitosis is a small variation. Vintemberger found that the dividing egg of the frog was most sensitive during the telophase and at this time the sensitivity is six times as great as the sensitivity of the egg at the resting phase. It is therefore concluded that the differences in sensitivity such as occur between the cells of the gonads and muscle cells cannot entirely be explained by variations in the stage of the cells in the division cycle.

Attempts have been made to alter the sensitivity of cells to a given dose of X-rays by alterations of the quality of the irradiation. The quality of the irradiation may be varied by altering the wavelength or by altering the intensity.

Moppett (1929) described experiments from which he concluded that the sensitivity to a given dose of X-rays was enormously increased

if/

if the components of the radiation were of homogeneous wavelength and further that certain bands of the X-ray spectrum were many times more active biologically than other bands. This conclusion involved the improbable implication that radiations of different wavelengths antagonise each other biologically although physically no such antagonism is known to exist. The author (1933) criticised Moppett's experiments and described experiments which showed that a homogeneous and a heterogeneous beam of X-rays produce exactly the same biological results provided that the experimental conditions are properly comparable. The experience of the author with experiments similar to those described by Moppett suggested that the biological material involved, namely the allantois of the chick is so easily damaged by a variety of agents that it is not possible to derive any certain conclusions about the differential activity of bands of the X-ray spectrum by the use of the chicken's allantois.

It was formerly believed that "in some cases, at least, the cells of a tissue are more affected by a given amount of energy of one range of wavelengths/

"lengths than they are by the same amount of energy of another range of wave-lengths" (Colwell and Russ, 1924, p. 349). Recently, however, Packard (1927 a and b; 1929) concluded from experiments on the eggs of Drosophila that a given dose of radiation produces the same biological effect even when the wavelength varies over a range as great as from 0.01 Angstrom unit to 1.7 Angstrom units. Experiments in which a more limited range of wavelength was used led Wood (1924, 1925), Holthusen (1926) and Vierheller and Saralegui (1930) to the same conclusion. There is, however, no agreement among clinical radiologists about the effect of variation of wavelength on the result of the irradiation. The accuracy of Packard's method and its simplicity suggests that his conclusion is valid because the technical difficulties of measuring the quantity of radiation which is absorbed in a tumour which is situated amongst other tissues is very great.

There is even less agreement about the effect of varying the intensity of a given dose of radiation on the biological result. Quimby and Pack (1933) have reviewed a mass of contradictory evidence/

evidence. From their own experiments these authors concluded that when the time of exposure is relatively short compared with the division cycle of the cell, then the Bunsen-Roscoe Law is valid. The results in Part IV. of this thesis are compatible with this view. Most of the evidence suggests that "the biological effect increases with increase of intensity up to a certain critical value. Beyond this point the dose of radiation required to produce a given effect remains practically constant" (Spear and Grimmett, 1933). The simplest explanation of such a state of affairs is to suppose the presence of a repair process in the cells. Such a repair process would prevent the appearance of visible cell injury if the irradiation were of a very feeble intensity. If, however, the intensity of the irradiation was very great, the repair processes would be overwhelmed so that for high intensities the Bunsen Roscoe Law would be valid. This explanation appears reasonable because Crowther (1926) showed that the protozoon Colpidium colpoda could withstand large doses of X-rays without the appearance of any signs of injury/

injury. Canti and Spear (1929) also demonstrated the capacity of cells to repair themselves after injury by X-rays. They administered sublethal doses of X-rays to tissue cultures. The division of cells of the cultures was temporarily inhibited but the cells subsequently resumed their growth which continued in an apparently normal manner.

The evidence which has been discussed suggests that the effect of a given dose of X-rays is not altered by variations of the quality of the radiation over a wide range. The evidence also suggests that differences which cells show in their sensitivity cannot be satisfactorily explained by differences in the stage of cell division. It appears to be certain, however, that embryological development eventually leads to a loss of sensitivity. Other types of modification of the cell have been produced in order to find whether the modifications would influence the sensitivity.

In Part IV. of this thesis experiments are described in which the metabolic rate and the rate of cell division were altered, but neither of these modifications produced any change in the sensitivity/

sensitivity. The cells were also injured by means of chilling and the effect of the injury so produced was compared with the effect of injury produced by X-rays. It was found that the two types of injury do not summate and that they do not potentiate each other, and it was concluded that they are not related. This conclusion suggests that the action of X-rays is not an action in which a general, non-specific injury is inflicted on the cell. It suggests that some specific mechanism of the cell is so damaged that cell death supervenes and therefore further investigation will be made into the relationship between X-ray injury and injuries produced by other means.

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The Biological Action of Homogeneous and Heterogeneous X-Rays.

BY C. M. SCOTT.

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The Biological Action of Homogeneous and Heterogeneous X-rays.

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[PLATE 16.]

The experiments described in this paper were undertaken in order to investigate the following two conclusions drawn by Moppett (1929) :—

(1) That a homogeneous beam of X-rays is enormously more active biologically than is a heterogeneous beam.

(2) That there is an antagonism between X-rays of different wave-lengths as shown by their biological effects.

Moppett based these conclusions on the effects produced by irradiating the allantois of hens' eggs. He found that the irradiation by X-rays of a portion of the allantois after removal of the overlying shell produced two types of reaction. Moderate doses of irradiation caused hyperplasia and more intense doses atrophy, and he measured the threshold doses needed to produce these effects. He used two types of irradiation, namely, a direct mixed beam of X-rays and a homogeneous beam diffracted from a crystal. Photographic measurements showed the former to be about 1000 times as intense as the latter. He found, however, that in order to produce hyperplasia or atrophy of the allantois, nearly equal durations of exposure with the different types of irradiation were needed.

He therefore concluded that the biological effect produced by a homogeneous beam per unit intensity was over 300 times greater than that produced by a heterogeneous beam. Furthermore he stated that " Direct irradiation contains all the component wave-lengths which might produce atrophy in much greater intensity than the monochromatic rays since the crystal is very inefficient. Considering the great difference between the respective threshold

doses it seems necessary to assume an active neutralisation between the components of mixed radiation in their biological effects."

The fundamental importance of these conclusions was so great that I repeated Moppett's experiments and followed his technique as closely as possible. Moppett's publications subsequent to 1929 (1930, *a*, 1930, *b*, 1930, *c*, 1931, 1932) are based on the conclusions which have been stated, and therefore no further reference to these later papers will be made.

Nature of X-rays Used.

The source of X-rays was a Muller water-cooled hot cathode tube with a tungsten anticathode. This was contained in a lead box in order to prevent interference with the experiment or measuring apparatus by scattered radiations.

Since a kilovoltage of about 70 excites the group of lines of highest frequency in the tungsten K series, a kilovoltage of 78 was used in all experiments in order to ensure the production of the characteristic K radiation of tungsten. The amperage was constant at 5 milliamps and the distance from the anticathode to the target was 77 cm. in all experiments. The apparatus was kept running for 30 minutes immediately before each experiment, after which period the output of X-rays was found to be of constant intensity.

The general method used was to expose an egg, contained in an experimental box, to a narrow beam of X-rays. Three types of irradiation were used, namely, (*a*) direct heterogeneous beam, (*b*) reflected homogeneous beam, (*c*) scattered heterogeneous beam.

(*a*) *Direct Beam.*—The general arrangement of the apparatus is shown in fig. 1. The X-ray beam reached the egg through two narrow slits in lead plates, and the rays were therefore approximately parallel. The mode of treatment of the egg and the arrangement of the experimental box will be described later.

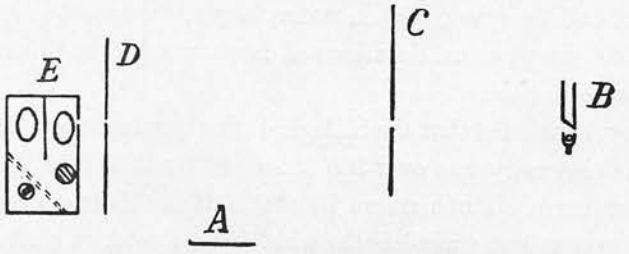


FIG. 1.—Apparatus for direct irradiation.

A, scale = 10 cm.; B, anticathode; C, lead screen with 3 mm. slit; D, lead screen with 2 mm. slit; E, experimental box.

(*b*) *Reflected Homogeneous Beam.*—In certain experiments homogeneous X-rays were used. These were obtained from a spectrometer which was simple and easy of manipulation and which was a modification of that described by de Broglie and Lindemann (1914). It consisted essentially of a sheet of mica obtained by cleavage and bent into the form of a cylinder. The advantage of this type of spectrometer lay in the curved shape of the mica which allowed a beam of X-rays comprised within a very small angle to strike the crystal in such a way as to produce a large range of possible angles of reflection.

With a slit at S, fig. 2, an extended spectrum was obtained along AB. In order to increase the intensity of the reflected rays even at a sacrifice of resolving power, a large radius of curvature, approximately 70 cm. was used. The reflected beam was received through a slit 2 mm. wide. The exciting beam was

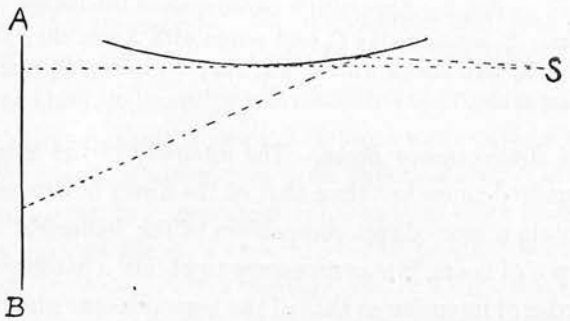


FIG. 2.—Diagram illustrating diffraction from a curved sheet of mica.

produced at a kilovoltage of 78, which ensured the production of the characteristic K radiation of tungsten which has a wave-length of 0·21 A. Since the grating constant of mica is 10 A the characteristic K radiation of tungsten is reflected from mica at a glancing angle of approximately half a degree. By experiment the beam reflected at about this angle was found to be more intense than those which were reflected at adjacent angles, and furthermore its mass absorption coefficient in aluminium $\frac{\mu}{\rho_{Al}}$ was found to be 0·24 when the absorption was about 50 per cent. These results suggest that the beam was composed of the reflected tungsten K radiation.

Mr. J. Paton of the Physics Department of this University, kindly tested the homogeneity of the beam. His method was to measure the successive absorptions in very thin sheets of aluminium by means of a tilted electroscope, and he found that the beam was absorbed exponentially in 12 successive sheets of aluminium. While it is likely that reflected radiations of other orders were

present in the beam, the exponential absorption in aluminium suggests that the intensity of these radiations was small compared with the intensity of the tungsten K radiation (Kaye, 1926, p. 104).

The general arrangement of the apparatus in these experiments is shown in fig. 3.

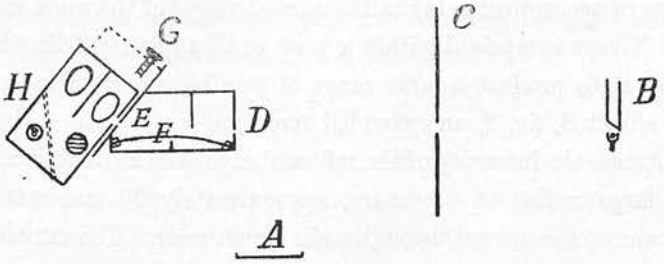


FIG. 3.—Apparatus for homogeneous irradiation.

A, scale = 10 cm.; B, anticathode; C, lead screen with 3 mm. slit; D, lead screen with 2 mm. slit; E, lead screen with 2 mm. slit; F, mica; G, screw for moving E; H, experimental box.

(c) *Scattered Heterogeneous Beam.*—The intensity of the homogeneous beam was several hundred times less than that of the direct heterogeneous beam, and in order to obtain a more direct comparison of the biological action produced by the two types of beam, it was necessary to obtain a heterogeneous radiation of the same order of intensity as that of the homogeneous radiation. A heterogeneous beam of the desired intensity was obtained from the rays scattered when the direct beam from the X-ray tube penetrated a block of hard paraffin. The arrangement of the apparatus is illustrated in fig. 4. The scattered

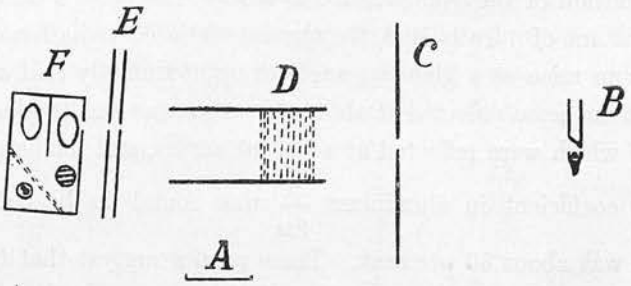


FIG. 4.—Apparatus for weak heterogeneous irradiation.

A, scale = 10 cm.; B, anticathode; C, lead screen with 3 mm. slit; D, paraffin contained in lead sleeve; E, 2 lead screens each with 2 mm. slit; F, experimental box.

radiations were allowed to pass through two vertical slits 2 mm. wide before reaching the exposed egg, so that the rays used were approximately parallel. The position of the slits and the thickness of the paraffin block were adjusted

so that the intensity of the scattered beam which reached the egg was exactly the same as the intensity of the homogeneous beam which has been described.

A scattered radiation is of the same constitution as the primary beam which excites it, and the energies associated with each wave-length are in the same proportions in both the scattered and the exciting beams (Kaye, 1926, p. 113). In these experiments therefore the scattered and the direct beams of X-rays differed only in intensity.

Measurement of Intensity.

A gold-leaf electroscope of the usual pattern was used for the measurement of intensity, and by means of this instrument the intensity of the primary beam, which was produced in all experiments at a kilovoltage of 78 and a milliamperage of 5, was found to be constant. The mass absorption coefficient for aluminium of the primary beam was measured and found to be 0·24. Owing to lack of apparatus it was not possible to measure the intensity directly in *r* units, but the absolute intensity was measured approximately by Holznecht pastilles. One skin erythema dose or 5 H units was equal to an exposure of 30 minutes at the standard distance of these experiments. The skin erythema dose at a kilovoltage of 78 is approximately equal to 450 *r* units (Knox, 1932, p. 65), and therefore one minute's exposure to the primary beam was approximately equal to 15 *r* units. For convenience of comparison the doses are recorded here as *r* units, but these doses are only approximate although the relationship between the different doses was measured accurately.

The intensity of the primary X-ray beam was enormously greater than that of the reflected homogeneous beam, and the two intensities were compared in the following way. The rate at which the reflected homogeneous beam discharged the electroscope was measured and the crystal was then removed so that the primary beam reached the electroscope directly through the same slit as was used for the homogeneous beam. Filters of aluminium (about 5 cm.) were interposed in the path of the primary beam until the rate of discharge of the electroscope was exactly that which was produced by the reflected homogeneous beam. The intensities of the filtered ray and of the primary beam therefore bore the same relationship to each other as did the intensities of the reflected ray and of the primary beam.

The electroscope was moved to a distance of about 180 cm. from the anticathode, and the unfiltered primary beam was allowed to reach it through a very small slit of known area—S_1. The rate of discharge of the electroscope was measured. The primary beam was again filtered with the same thickness

of aluminium which had previously been used. The slit was increased in size and adjusted until the filtered and unfiltered beams produced the same rate of discharge, and the area of the enlarged slit was measured—S_2. The intensities of the filtered and of the unfiltered beam bore the relationship to each other of S_1 to S_2, and this relationship was the same as that of the intensity of the homogeneous beam to that of the primary. It was thus found that the intensity of the homogeneous beam was 1/937 of the intensity of the primary beam.

Preparation of the Eggs.

Hens' eggs were incubated for 9 days, during which time the allantois had grown almost completely round the internal surface of the shell. By means of a viewing box an area of allantoic membrane, situated some distance away from the embryonic stalk, was chosen, in which the blood vessels were of medium size. Over this area a window 1 cm. long and 0·5 cm. wide was marked out on the shell with the long axis of the window in the long axis of the egg. The shell was rapidly cleaned with rectified spirit and the window sawn out. The rectangular piece of shell was carefully detached from the underlying shell membrane in one or two pieces. This shell membrane is a fibro-elastic layer of non-living tissue which lines the inner surface of the shell, is adherent to it, and separates the underlying allantois from the shell itself. Those eggs were discarded in which this fibro-elastic membrane was broken or in which any damage to the allantoic membrane was suspected. The egg so treated was placed in the experimental box with the egg window opposite the box slit so that the narrow X-ray beam crossed the centre of the shell window, fig. 5. At the end of the exposure the removed piece of shell was cleaned with rectified spirit and replaced, the whole window being sealed with adhesive plaster. The egg was returned to the incubator for 4 days, and was then fixed in " Susa " solution. Sections were made through that part of the allantois which lay adjacent to the egg window and were cut parallel to the long axis of the window.

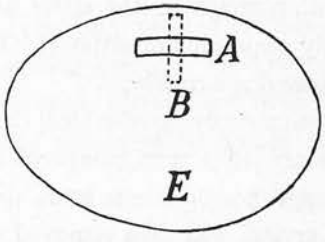


Fig. 5.—Showing position on egg of egg window and irradiated area.

E, egg; A, continuous line represents position of shell window; B, dotted line represents area over which X-rays fell.

The experiments involved the exposure for several hours of egg membranes deprived of their shell covering. It was therefore of great importance to

prevent injury by drying or cooling and also to make rigorous controls. The precautions adopted were as follows:

During their irradiation the eggs were contained in a box diagrammatically represented in fig. 6. The box was of lead with a close fitting lead cover and was heated by an electric lamp. The only opening was a vertical slit 1 cm. long and 0·5 cm. wide, through which the X-rays passed, and this was covered with gold-beaters' skin to prevent passage of air currents to and from the interior of the box. Two positions for eggs are shown in the diagram, fig. 6, separated from each other by a lead screen; that next to the window was the position for the experimental egg, and the other that of the control egg. The interior of the box was kept saturated with water vapour at a temperature of 39° C. Thus the conditions in the box were virtually the same as in an incubator.

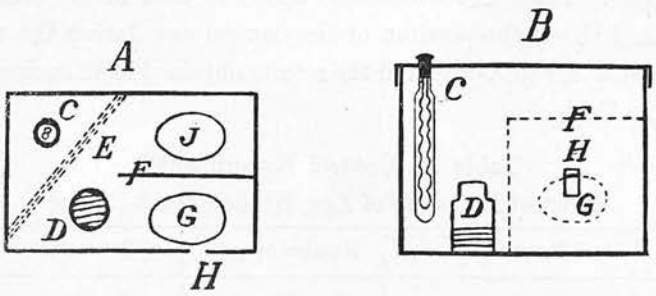


FIG. 6.—Experimental box.

A, plan of box; B, elevation; C, heating; D, water; E, asbestos screen; F, lead screen to protect control egg; G, experimental egg; H, window of box; J, control egg.

This technique in relation to eggs was employed throughout the whole course of this investigation except in those experiments in which a modified technique described later, was used.

Response of the Allantois.

The allantois in my experiments showed two types of response to stimuli:—
(1) Hyperplasia, and (2) Atrophy.

Figs. 7, 8 and 9, Plate 16, show typical photomicrographs of these two effects. They resemble exactly the results described by Moppett (1929) and by Goulston and Mottram (1932). These authors have described the conditions in detail and a repetition of their descriptions is unnecessary.

The atrophic response when present was always unmistakable. Hyperplasia when fully developed was equally unmistakable, but sometimes minor degrees of hyperplasia occurred which could not be distinguished with certainty

from the normal. For this reason chief attention was directed to the atrophic response, and I endeavoured to estimate the dose needed to produce atrophy in 50 per cent. of experiments (median atrophic dose).

Control Experiments.

Experiments were done in order to find whether the simple procedure of removing a piece of shell and leaving the shell membrane exposed for a period could produce visible changes in the allantois. Eggs treated in the way described were left in the experimental box for varying periods without any further interference. At the end of these periods the windows were closed and the eggs were reincubated. In Moppett's and in my experiments the longest times of exposure to irradiation were 180 minutes, whilst many exposures were of 120 minutes. These exposures were therefore used in the control experiments. Fig. 6 shows the position of the control egg during the exposure of the experimental egg to X-rays and the results obtained in 42 such experiments are shown in Table I.

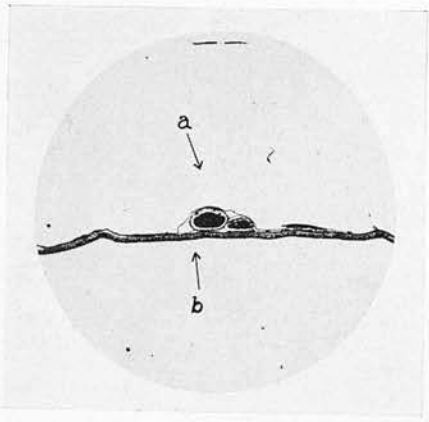
Table I.—Control Experiments.
Effect of Exposure of Egg Window for 2–3 hours.

Result.	Number of eggs.	Percentage.
No change	33	79
Hyperplasia	9	21
Atrophy	0	0

During the progress of these experiments certain criticisms of Moppett's method were published by Goulston and Mottram (1932), one of which pointed out that the control egg was not adjacent to the window through which the X-rays passed, and therefore was not subjected to currents of air which such a window might occasion. Sixteen consecutive control experiments were therefore carried out in which the eggs were in contact with the window, and hence the conditions were exactly comparable to those in irradiation experiments. The results which are recorded in Table II are identical with those obtained with the larger number of controls (Table I).

Table II.—Special Control Experiments.
Effect of Exposure of Egg Window for 2–3 hours.

Result.	Number of eggs.	Percentage.
No change	13	81
Hyperplasia	3	19
Atrophy	0	0



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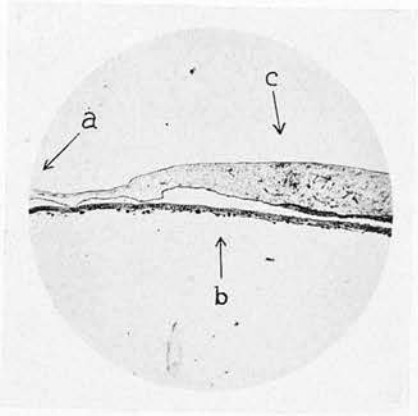

FIG. 8.

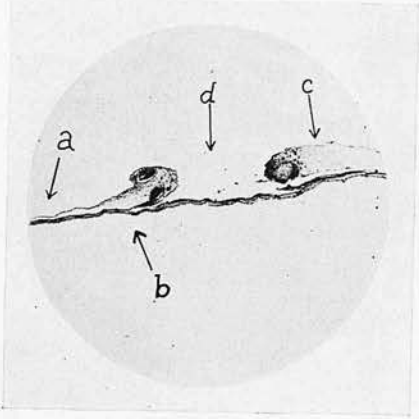

FIG. 9.

(*Facing p.* 372.)

The results show that exposure for 2–3 hours never produces atrophy but does produce hyperplasia in 10–20 per cent. of cases. Since such exposures did produce some measurable effect, the effects produced by a wide variety of durations of exposure were examined. These results are shown in Table III. The mere opening of the shell produced no effect, but exposures for periods of 6 hours or more produced atrophy in a large proportion of cases. The figures for the long exposures are scanty because in the majority of cases the long exposures caused death of the embryo within 4 days, and the local effects could therefore be determined only in a minority of cases.

Table III.—Effect of Various Exposures of the Egg Window to Air.

Duration of exposure.	No. of experiments.	No change.	Hyperplasia.	Atrophy.	Percentage of atrophy.
Less than 1 minute	6	6	0	0	0
2 to 3 hours	58	46	12	0	0
6 hours	7	0	4	3	43
9 hours	5	0	0	5	100

Table IV summarises the results obtained by Moppett (1929), by Goulston and Mottram (1932) and by me, and it will be seen that my results are intermediate between the others. Moppett thought that exposure alone produced

Table IV.—Comparison of Results of Control Experiments with less than 3 hours' exposure to Air of the Shell Window.

Author.	No. of experiments.	No change.	Hyperplasia.	Atrophy.	Percentage of atrophy.
Moppett.....	30++	30++	0	0	0
Goulston and Mottram	13	5	7	1	8
Scott	58	46	12	0	0

no effect on the allantois, whilst Goulston and Mottram found that exposure alone usually produced hyperplasia and sometimes produced atrophy. My results show that exposure for 2–3 hours does not produce atrophy but sometimes produces hyperplasia. Table III shows that more prolonged exposures can undoubtedly produce atrophy. It was also found that mechanical injury of the shell membrane easily produced atrophy.

The results of Goulston and Mottram (1932) suggest obvious doubts as to the validity of conclusions drawn from the effects produced by irradiation associated

with long exposure of the shell membrane to air. My results, however, show that exposure for periods not exceeding 3 hours does not produce atrophy and only occasionally produces hyperplasia. In my experiments, therefore, the occurrence of atrophy or the occurrence of a high percentage of hyperplasia after exposure to X-rays for periods of not more than 3 hours must be attributed to the action of X-rays and cannot be accounted for by exposure to air alone. On the other hand, there is no doubt that exposure alone injures the egg membranes, and hence very few deductions can be drawn from differences between the results produced by irradiation of intact eggs and those produced by irradiation of eggs with shell windows.

Effect of Irradiation of Intact Eggs.

Eggs were treated in the usual way except that no window was cut. They were irradiated by the direct beam from the X-ray tube over a small marked area; the arrangement of the apparatus is shown in fig. 1.

Strangeways and Fell (1928) irradiated the whole of hens' eggs with X-rays on the sixth day of incubation and found that 150 *e* units (1 *e* unit = 3–4 *r* units [Knox, 1932, p. 62]) caused the death of the embryo during the subsequent 24 hours of incubation. Eggs at the 17th day of incubation were killed by 270 *e* units.

I found that four times this dose, 3000 *r* units, produced no effect on the allantois. The lethal action of such doses was reduced as low as possible by directing the X-ray beam almost tangentially to the egg surface so that the embryo escaped the direct rays. Even with these precautions a dose of 3000 *r* units killed 30 out of 36 eggs, but the six survivors showed no change in the allantois

This negative result cannot be ascribed to absorption of the rays by the shell, for the absorption of 12 different egg shells was measured and found in all cases to be less than 20 per cent.

Direct Irradiation.

Eggs with the usual shell window were subjected to the direct rays from the X-ray tube, fig. 1. The results obtained are shown in Table V, and it will be seen that the median atrophic dose is about 700 *r* units. This dose was given in less than an hour, and therefore the atrophy observed must have been due to an effect produced by the X-rays since the controls showed no atrophy after exposure to air for 3 hours (Tables I and II). Furthermore, the atrophy

always occurred in that position of the exposed membrane which was subjected to the X-ray beam. The results recorded by Moppett (1929) appear to agree with the results shown in Table V.

Table V.—Direct Irradiation.

X-ray dosage approximate in *r* units.	Exposure in minutes.	Number of eggs.	No change.	Hyper-plasia.	Atrophy.	Percentage of atrophy.
75–300	5–20	3	3	0	0	0
450–700	30–45	5	1	3	1	20
700–900	50–60	5	0	1	4	80

Homogeneous Irradiation.

The method of the production of the homogeneous beam of X-rays has already been described, and the arrangement of apparatus is shown diagrammatically in fig. 3. The wave-length of the homogeneous beam was 0·2 A, and the intensity was 1/1000 of that of the direct beam.

Eggs with windows which were prepared in the usual way were irradiated with the homogeneous X-rays which reached the egg as a rectangular pencil of rays with the long axis of the rectangle at right angles to the shell window, in the manner which has been described in the case of the direct mixed beam, fig. 5.

The results, which are given in Table VI, show that the median atrophic dose of the homogeneous beam is about 2 *r* units. The exposure to air in this case was not more than 180 minutes, and therefore, judged by the same criteria as those applied in the case of direct irradiation, the atrophy here recorded must have been a result of X-ray injury.

Table VI.—Homogeneous Irradiation.

Approximate dose in *r* units.	Exposure in minutes.	Number of eggs.	No change.	Hyper-plasia.	Atrophy.	Percentage of atrophy.
0·5	20–35	3	3	0	0	0
0·5–1·0	35–60	5	2	3	0	0
1–2	60–100	7	2	4	1	14
2–3	100–180	7	0	2	5	71

The median atrophic dose of direct mixed irradiation, however, was about 700 *r* units, that is to say the relationship between doses of mixed and dosages of homogeneous radiations which produced the same biological action was in

the ratio of about 700 to 2 under the conditions of these particular experiments. My results therefore agree with those of Moppett in showing an apparently extraordinary difference in the biological action of the two types of X-rays. But these remarkable results do not directly prove a difference in the biological activity of homogeneous and of heterogeneous X-rays because there was an important difference between the duration of exposure of the egg window to air in the two series of experiments. The administration of 700 *r* of direct mixed irradiation took about an hour, whereas the administration of a dose of 2–3 *r* of homogeneous irradiation took 2–3 hours.

Exposure to air undoubtedly influenced the response of the allantois to X-rays, for a dose of about 3000 *r* failed to produce atrophy in unopened eggs, whereas 700 *r* produced this effect in eggs which had been opened and exposed for an hour. Exposure without irradiation for periods up to 180 minutes never produced atrophy, but occasionally produced hyperplasia. Genuine atrophy, however, was produced by exposure for 6 hours when no irradiation was given. These results suggest that exposure alone for 180 minutes produces about half the injury needed to cause atrophy and that the effects of exposure and radiation are additive.

Experiments were therefore carried out in which a weak heterogeneous beam was used and the time of exposure of the egg window to air was the same as the time of exposure in the experiments with homogeneous irradiation.

Scattered Heterogeneous Irradiation.

Eggs with the usual egg window were irradiated with a weak heterogeneous beam of X-rays which was scattered from a block of hard paraffin, fig. 4. The intensity of this beam was exactly the same as that of the homogeneous beam used in the preceding series of experiments.

The results which are recorded in Table VII show that a dose of 2–3 *r* units of weak heterogeneous irradiation, administered over a period of 100–18

Table VII.—Scattered Heterogeneous Irradiation.

Approximate dose in *r* units.	Duration of irradiation in minutes.	Number of eggs.	No change.	Hyper-plasia.	Atrophy.	Percentage of atrophy.
0·5–1	15–40	3	3	0	0	0
1–2	40–100	6	2	2	2	33
2–3	100–180	9	0	4	5	56

minutes, produced atrophy in about 50 per cent. of cases. The same result was produced by a weak homogeneous beam when 2–3 *r* units were applied over a similar period. The exposure of the egg window to air was therefore the same in both series of experiments. In the two series similar doses of X-rays of similar intensity produced identical biological effects although one beam was homogeneous and the other mixed.

This result shows that homogeneous and mixed beams act in an identical manner on the allantois of the hen's egg, and it cannot be reconciled with the hypothesis put forward by Moppett that a homogeneous beam of X-rays has an enormously greater biological action than a heterogeneous beam, and that X-rays of different wave-lengths antagonise each other in their biological effects.

There remains to be explained, however, the remarkable contrast between the biological effects produced by weak beams of X-rays, whether homogeneous or mixed, and those produced by intense beams. The median atrophic response of the allantois to weak irradiation is produced by 2–3 *r* units applied over a period of 100–180 minutes, whereas the same response to intense irradiation is produced by 700–900 *r* units applied over a period of 40–60 minutes. Now in these two types of experiment there were three factors which varied :—

(*a*) The dose of X-rays.

(*b*) The length of time which was required to administer the dose of X-rays.

(*c*) The length of time during which the egg window was exposed to air.

Experiments were therefore devised in order to determine the relative importance of these three variable factors.

Short Period of Irradiation and Long Period of Exposure of Egg Window to Air.

Since weak irradiation, both homogeneous and heterogeneous, produced atrophy in over 50 per cent. of experiments when the egg window was exposed to air for 100–180 minutes, experiments were done in which an exposure to air for this length of time was combined with direct irradiation from the X-ray tube, administered over a very short period. The dose of X-rays administered was small, namely, 1·5 to 7·5 *r*.

Eggs were incubated for 9 days and a shell window was cut. The experimental egg was exposed in the box for times varying from 100–180 minutes. Towards the end of that period the egg was irradiated over the shell window by the direct radiation from the X-ray tube for periods up to 0·5 minute. The

shell window was replaced immediately and the egg reincubated for 4 days when sections were cut. The results are recorded in Table VIII.

Table VIII.—Effect of Exposure to Air for 2–3 hours followed by Immediate Irradiation for Short Periods.

Dose in r units.	Duration of irradiation in minutes.	Number of eggs.	No change.	Hyper-plasia.	Atrophy.	Percentage of atrophy.
0·4	0·03	3	1	2	0	0
1·5	0·1	5	0	3	2	40
3·0	0·2	10	2	4	4	40
7·5	0·5	6	1	1	3	50

Similar experiments were done in which the allantois was irradiated immediately after the cutting of the shell window and the exposure to air for 100–180 minutes was consecutive. These results are recorded in Table IX.

Table IX.—Effect of Exposure to Air for 2–3 hours immediately preceded by Irradiation for Short Periods.

Dose in r units.	Duration of irradiation in minutes.	Number of eggs.	No change.	Hyper-plasia.	Atrophy.	Percentage of atrophy.
4·5	0·3	3	0	1	2	66
7·5	0·5	3	0	0	3	100

The results recorded in Tables VIII and IX show that a dose of X-rays between 1·5 and 7·5 r units administered within 1 minute in the form of an intense mixed beam can produce atrophy of the allantois in 50 per cent. of experiments provided that the shell window over the irradiated area is exposed to air for a period of about 100–180 minutes. This dose of X-rays is of the same order of magnitude as those which were used in the experiments with reflected homogeneous and scattered mixed radiations where the intensity of the X-rays was very small. Provided therefore that the egg membranes are exposed to air for a certain time (2–3 hours) the same effects are produced by the same dose of X-rays whether the radiation is intense and short (0·5 minute), or weak and prolonged (2–3 hours). The results in the latter case cannot therefore be attributed to any deviation from the Bunsen-Roscoe law. They indicate, however, that the injurious effects of irradiation and of exposure to air are additive.

Effect on the Allantois of Two Additive Stimuli applied at 24 hours' Interval.

It has been shown that irradiation of the intact egg with very large doses of X-rays produces no visible changes in the allantois. It has also been shown that exposure of the egg window to air for periods up to 180 minutes never produces atrophy. Since atrophy occurs when these two stimuli are added it appears that each stimulus produces some change in the allantois, and a few experiments were carried out to find whether these changes are reversible.

Intact eggs were irradiated over a marked area with the direct beam from the X-ray tube for periods up to 0·5 minute. The eggs were reincubated for 24 hours. A shell window was then cut over the marked area, and this window was exposed to air for about 120 minutes. The allantois was sectioned 4 days later. Three control experiments were done which differed only in the duration of the exposure of the window to air, which was in their case only 5 minutes.

In a second type of similar experiment the order of irradiation and exposure to air were reversed. A shell window was cut and was exposed to air for about 150 minutes. The shell was replaced and the window was sealed with thin strips of adhesive plaster around the window margin. The egg was reincubated for 24 hours and the allantois was irradiated through the closed window. The egg was reincubated for a further 4 days and the allantois sectioned.

The results, which are recorded in Table X, show that atrophy of the allantois occurs when a dose of X-rays of 4–8 *r* units is applied through the shell, followed or preceded at 24-hours interval by an exposure of the allantois to air for about 150 minutes. Tables VIII and IX showed that the same dosage of X-rays combined with the same duration of exposure of the egg window to air produces the same result when there is no interval between the time of irradiation and

Table X.—Effect of Irradiation and Exposure to Air separated from each other by an interval of 24 hours.

Type of experiment.	Approximate dose in *r* units.	Duration of irradiation in minutes.	Number of eggs.	Duration of exposure of shell window to air in minutes.	Result.
A	7·5	0·5	3	5	No change.
B	4·5–7·5	0·3–0·5	4	120–135	2 hyperplasia, 2 atrophy.
C	6	0·4	3	150	1 hyperplasia, 2 atrophy.

A.—Irradiation of intact egg with short exposure of the shell window to air 24 hours later.
B.—Irradiation of intact egg with long exposure of the shell window to air 24 hours later.
C.—Long exposure of the shell window to air with irradiation through the shell 24 hours later.

the time of exposure of the egg window. It is therefore concluded that the processes set up in the allantois by these doses of X-rays and these exposures to air are more or less irreversible in 24 hours. These results also show that irradiation of the egg through the shell does produce a change in the underlying allantois, although this change cannot be demonstrated unless the allantois is injured by exposure to air.

Discussion.

The experiments which have been described show that an extremely small dose of X-rays administered in the form of a beam of very feeble intensity can cause the complete destruction of living tissue. The significance of the experiments with reflected homogeneous and scattered heterogeneous beams of X-rays on which this conclusion is based, obviously depends on the complete reliability of control experiments. These have been fully discussed, and they have shown that while atrophy of the allantois of hens' eggs occurs after irradiation with 2–3 *r* units under certain conditions, those conditions alone without irradiation never produce atrophy.

Thus the results of Moppett's experiments with a homogeneous beam have been confirmed, but his conclusion, that there is a remarkable difference in the biological action of homogeneous and heterogeneous beams, cannot be supported. The effects of weak homogeneous and equally weak heterogeneous X-rays, Tables VI and VII, were identical, and these effects were produced under exactly similar conditions. Homogeneous and heterogeneous beams of X-rays therefore produce identical effects on the allantois of hens' eggs, and since the same doses of both types of ray produce the same result, there can in this case be no antagonism between the different components of the heterogeneous beam.

It is necessary, however, to account for the result, obtained by Moppett and confirmed by me, that a dose of 2–3 *r* units administered over a period of 2–3 hours produced the same effect as a dose of 800 *r* units administered in 1 hour. In the two types of experiment the duration of the exposure of the shell window to air was different, and the explanation may lie either in this difference or in a deviation from the Bunsen-Roscoe law. The various combinations of X-ray dosage and duration of exposure to air are summarised in Table XI. This table shows that a dose of 2–3 *r* units combined with exposure to air for 2–3 hours produces the same effect whether the radiation is given in less than 1 minute by a relatively intense irradiation or is spread over 2–3 hours in the

form of a reflected beam either mixed or homogeneous in quality. In these experiments, therefore, the Bunsen-Roscoe law holds.

Table XI.—Combinations of Exposures to Air and Irradiation by X-rays which produce Atrophy in about 50 per cent. of Eggs.

Irradiation.			Duration of exposure to air in minutes.
Quality.	Duration of irradiation in minutes.	Dose in *r* units.	
Nil	Nil	Nil	400
Feeble homogeneous } with simultaneous	120–180	2–3	120–180
Feeble heterogeneous } exposure to air			
Direct heterogeneous immediately followed by exposure to air	0·1–0·5	1·5–7·5	120–180
Direct heterogeneous immediately after exposure to air			
Direct heterogeneous with 24 hours interval between irradiation and exposure to air			
Direct heterogeneous with simultaneous exposure to air	50–60	700–900	50–60
Direct heterogeneous irradiation of the intact egg	—	>3000	Nil

It has already been shown that exposure to air alone injures the allantois, and Table XI shows that shorter exposures to air, one hour, need large doses of X-rays to produce the median atrophic response, while longer exposures, 2 hours, need only a very small dose of X-rays. The injury which is produced in the allantois is therefore the result of both X-rays and exposure to air, and the effects of these two agents appear to be in some way additive. No simple relationship, however, exists between them for 1 hour's exposure to air combined with a dose of 700–900 *r* produces a median atrophic response, but if the duration of the air exposure be doubled then a dose of only 2 *r* is required to produce the same effect. The effect of exposure of the shell window to air appears to potentiate the action of X-rays rather than to superimpose an additional injury on that produced by the rays.

This potentiating action occurs not only when the allantois is irradiated with a weak beam during the whole period of its exposure to air, but also when the irradiation is intense and is given either at the end or the beginning of the exposure. The action occurs not only when the irradiation is applied 24 hours after the exposure to air, but also when it is applied 24 hours before the exposure.

These observations offer no explanation of the nature of the potentiation, but they suggest that whatever injury is produced either by X-rays or by exposure of the allantois to air, that injury is repaired either very slowly or not at all.

The reactions of the allantois are summarized in the following way. Atrophy occurs when the allantois is exposed to air for 6 hours without irradiation. The uninjured allantois is very resistant to X-ray injury, but exposure to air for 2–3 hours alters it so that it becomes extremely sensitive to the action of X-rays.

The use of the allantois of the hen's egg for an investigation of the nature of the biological action of X-rays therefore involves the irradiation of damaged tissue. The nature of the damage is unknown, and its measurement by duration of the exposure of the allantois to air is obviously so crude that it is considered that the allantois is not an ideal preparation for the study of the action of X-rays and, least of all, for the provision of quantitative results.

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Summary.

(1) An extremely small dose of X-rays, 2–3 *r* units, can cause the death of living tissue.

(2) Homogeneous and heterogeneous X-rays have an identical biological action on the allantois of the chick.

(3) There is no antagonism between the components of different wavelengths of a heterogeneous beam of X-rays in their action on the allantois of the chick.

(4) The variation of intensity of X-rays used when administering a certain dose of radiation makes no difference to the resultant biological action, *i.e.*, the Bunsen-Roscoe law is true in these experiments.

(5) The effects of the exposure to air of a shell window in a hen's egg potentiates the action of X-rays on the underlying allantois.

(6) These effects on the allantois are irreversible in 24 hours.

(7) The allantois of the chick is not an ideal preparation for the study of the biological action of X-rays.

Note.—Since the preparation of this paper Goulston (1932) has described two experiments in which the allantois was irradiated by homogeneous rays with negative results. A technique similar to that which has been described was used, but the egg window was opened under completely aseptic conditions. The dose of homogeneous irradiation was not stated.

DESCRIPTION OF PLATE 16.

(*a*) Normal allantois ; (*b*) shell membrane ; (*c*) hyperplasic allantois ; (*d*) area of atrophy.

FIG. 7.—The normal allantois (× 13).

FIG. 8.—The hyperplasic response (× 13).

FIG. 9.—The atrophic response (× 13).

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Part III. The Action of X-rays on the Eggs of Calliphora. Proc. Roy. Soc. B. 1934. In press.

THE ACTION OF X-RAYS ON THE EGGS OF CALLIPHORA

INTRODUCTION.

The object of the experiments described in this paper was to investigate the factors which determine the differences shown by cells in regard to their sensitivity to the lethal action of X-rays when different types of cells are compared. This sensitivity is found to vary over an enormous range. For example Crowther (1926) showed that a dose of 80,000 r administered in 20 minutes was required to kill the protozoon Colpidium colpoda while, on the other hand, the author (1933) found that a dose of 2-3 r administered over a period of two hours could kill the cells of the allantois of the embryo chick under special circumstances.

The information concerning the sensitivity of cells to short wave radiations has been summarised in the so-called Law of Bergonie and Tribondeau (1906) which states that the sensitivity of a cell is proportional to its reproductive capacity and inversely/

variety of experimental conditions. Accurate measurements can only be obtained if the eggs are as uniform and as healthy as possible, and the precautions necessary to obtain this result are detailed below.

(a) Method used for breeding Calliphora erythrocephala

A constant stock of adult flies was kept in order to provide eggs. All the flies were the offspring of a single wild fly and about 20 generations of flies were used in these experiments. The flies were kept in two disused fume cupboards at a temperature of about 15°C. They were fed on loaf sugar and were provided with water. Eggs were obtained by exposing fresh uncooked liver to the flies, which laid exclusively on this medium. When a new stock was required the liver and the eggs that it bore were incubated at 23°C. In about 17 hours larvae hatched and began to feed on the liver. The larvae and the liver on which they were feeding were placed in a biscuit tin, the bottom of which was covered with two or three inches of dry silver sand. The larvae were incubated at 23°C. in the tin and after becoming sluggish they migrated into the sand on the fifth day from hatching. Here they/

pupated, and hatching occurred after a further period of 12-13 days of incubation at 23°C. Immediately before the hatch the biscuit tin was transferred to an empty fume cupboard. Fresh liver was provided as food for the newly hatched flies but as soon as they began to lay eggs, which they did after two or three days, the liver was removed and loaf sugar was substituted. Each batch of stock flies numbered about 1000. The adult flies lived for several months at 15°C., but after the first month a large proportion of the eggs which they laid were infertile. For this reason the stocks of flies were renewed every month.

(b) Method used for collecting and manipulating the eggs.

Eggs were obtained for experimental purposes by exposing a piece of fresh liver to the flies. About a thousand eggs per hour were laid and this rate was maintained for about 4 hours, provided that the flies were allowed to lay only on alternate days. Each piece of liver was exposed for half an hour. The eggs that were laid during the first hour were discarded because, at the beginning of a laying period, eggs which were in an advanced state of development, and even larvae, were often deposited.

Apart/

Apart from these exceptions, caused by the retention of fertilised eggs in the oviduct, a half-hour batch of eggs was of almost uniform development because the calliphorine egg is fertilised immediately before laying.

For experimental purposes eggs were removed from the liver by means of a platinum loop and were separated from each other in order to allow counting. They were plated on to a piece of moist blotting paper 4 cm. x 1 cm. which was stuck on to an ordinary microscope slide. The slide was contained in a Petri dish in which the air was saturated with water vapour. From a half-hour laying about 8 slides, each with about 60 eggs, were prepared and the eggs were counted. Four slides were irradiated and four were used as controls. After irradiation the experimental eggs and the unirradiated controls were incubated at a known, thermostatically-controlled temperature. When the hatching of the control eggs was complete the unhatched eggs were counted on the control and on the experimental slides.

(c) /

(c) The source of X-rays was a Muller, water-cooled, hot cathode tube with a tungsten anticathode. In all experiments the kilovoltage, milliamperage and distance from anticathode to target were constant. These were 79 K.V., 5 M.A., and 47.1 cm. A screen of 0.01 cm. Al was used in all experiments. The output of the tube was measured regularly by means of a gold leaf electroscope of the usual pattern and was kept constant by small alterations of milliamperage. The dose of X-rays delivered at 47.1 cm. was approximately 40 r per minute.

During irradiation the microscope slides on which the eggs had been plated were contained in a box which was kept at a constant temperature (Fig. 1). The box was made of lead and the atmosphere inside it was saturated with water vapour by means of wet filter paper on its walls. The slides were contained in a lead rack which accommodated three slides for irradiation and three control slides.

Fig. 1 /

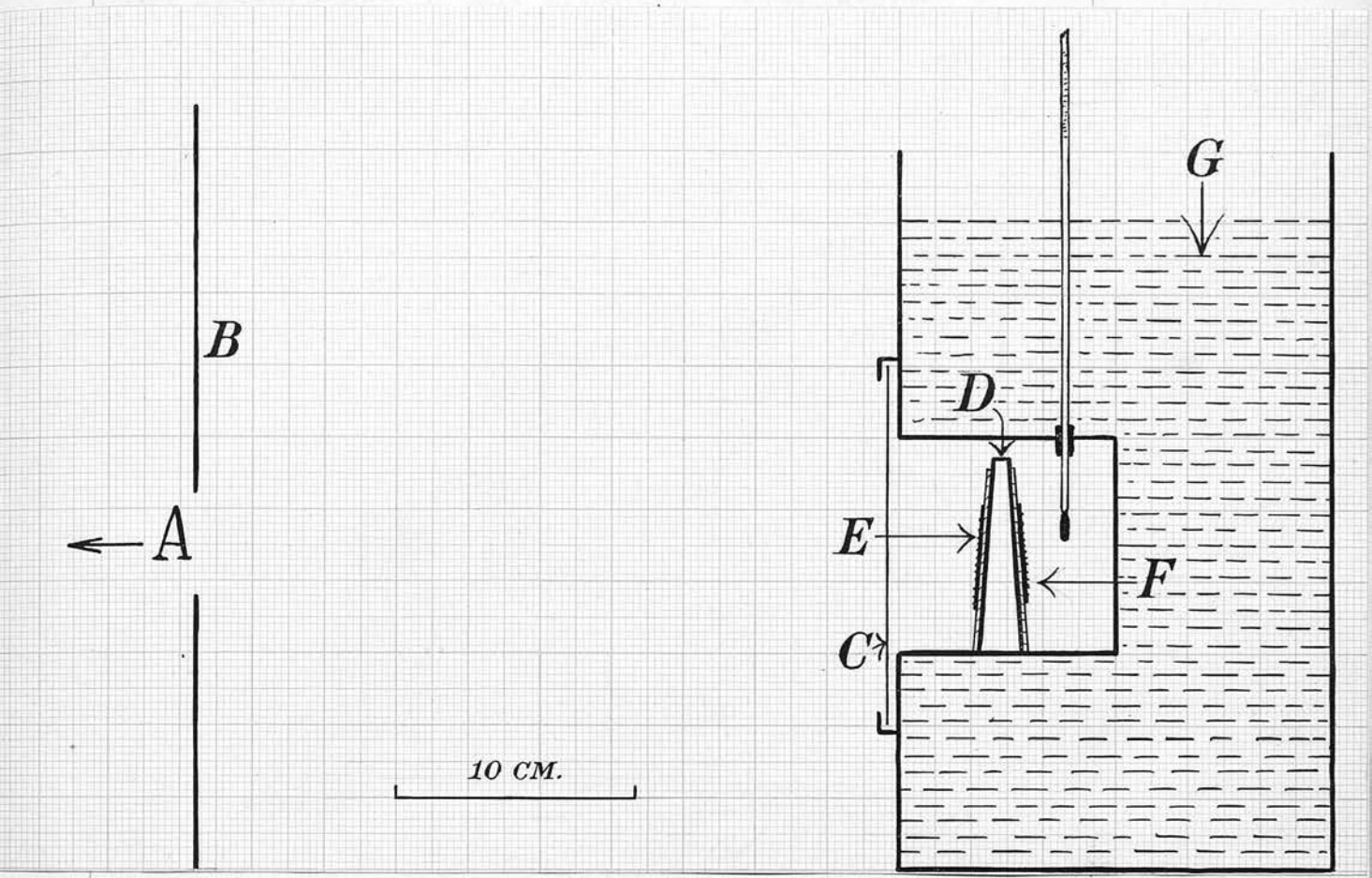

Fig. 1. Arrangement of apparatus.

A. Anticathode.
B. Lead screen.
C. Aluminium screen 0.01 cm. thick.
D. Lead rack for holding slides.
E. Irradiated eggs.
F. Control eggs.
G. Water bath.

(d) Measurement of the effect of irradiation.

The percentage of eggs which were prevented from hatching by the irradiation was the measure of the action of the irradiation. Extensive control experiments were therefore necessary in order to find the percentage of eggs which hatched normally. The control eggs were approximately equal in number to the irradiated eggs and, irradiation apart, were treated in exactly the same manner. Over 20,000 control eggs were used and 92 per cent. hatched. The percentage hatch was very uniform and experiments were discarded in which the percentage hatch of the control eggs was less than 90. Histological examination of unhatched control eggs never revealed any embryological development nor even the presence of a nucleus and it was therefore concluded that the unhatched control eggs, or at least the majority of them, had escaped fertilisation. In expressing the percentage of eggs, which had been prevented from hatching by irradiation, a correction for unfertilised eggs was necessary, and this correction was made on the basis of the corresponding control experiment.

Influence/

Influence of temperature on rate of development.

Eggs were incubated at different temperatures and the times elapsing between deposition and hatching of the eggs were measured. The following results were obtained: at 8°C. 120 hours; at 13°C. 62 hours; at 17°C. 34 hours; at 23°C. 17 hours.

These results, which are plotted in Fig. 2, show an almost exact linear relationship between the temperature and the logarithm of the time. The Q_{10} calculated from the graph is 3.64 and this appears to be constant within the limits of experimental error over the range of temperatures investigated. This temperature coefficient is similar to that found by other observers for the development of insects (Uvarov 1931).

Fig. 2 /

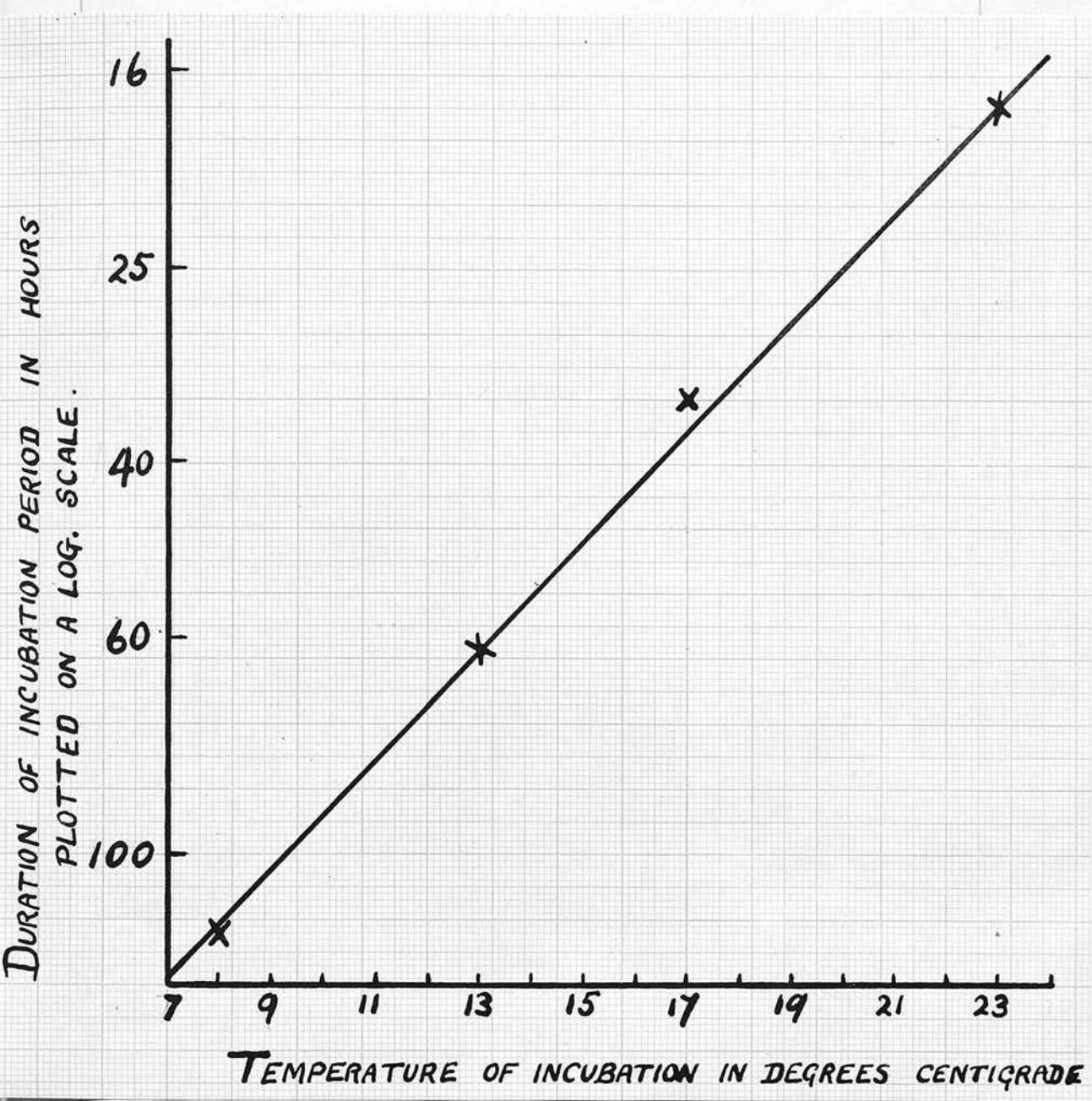

Fig. 2. Relation between the incubation period and temperature. (Q_{10} = 3.63)

The high figure for the Q_{10} made it essential to control the temperature rigidly in order to investigate the relationship between the stage of development of the egg and its sensitivity to X-rays. The uniformity of the Q_{10} between 8°C. and 23°C. made it possible to express the duration of incubation of an egg at a given temperature within the range considered in terms of the duration of incubation at some other temperature, which would result in a similar stage of development. This conclusion was confirmed later by means of the histological examination of the eggs. This method was used in certain cases as an experimental convenience to avoid prolonged incubation at low temperatures, which would often have involved work throughout the night. In this paper all the durations of incubation are expressed in terms of incubation at 14°C.

Effect/

Effect of X irradiation upon eggs at various stages of development.

The effects produced by irradiation of eggs at various ages are shown in Tables I-V and in Figs. 3-5 and are summarised in Fig. 6.

Fig. 6 shows that the changes in sensitivity are complex. There is a very great decrease in sensitivity between 4 and 7 hours incubation which is preceded and followed by a moderate increase in sensitivity. After about 16 hours the sensitivity decreases steadily.

These complex changes can be most conveniently considered in three stages, namely:

(a) the changes in the first 3.5 hours.

(b) the changes after 10 hours.

(c) the changes between 3.5 and 10 hours.

(a) Action of X-rays on eggs less than 3.5 hours old.

Experiments were made on eggs at the following ages:

1. Eggs between 0.3 and 1 hour. The results are recorded in Table I and Fig. 3, curve A.

2. /

2. Eggs between 1 and 2 hours. The results are recorded in Table II and Fig. 3, curve A.

3. Eggs between 2.5 and 3.5 hours. The results are recorded in Table III and Fig. 3, curve B.

Tables I - III. /

Table I.

Effect of X-rays on eggs incubated for 0.3-1 hour at 14°C.

Dose		No. of expts.	No. of fertile eggs calculated from control expts.	No. of eggs which hatched	Percentage of eggs which hatched	Average of the percentage hatch in individual expts.	χ^2	P_{χ^2}
In r units	In mins.							
20	0.5	1	27	26	96	96.4		
40	1.0	5	277	224	81	83.0	16.9	0.01
60	1.5	2	117	95	81	81.4	0.2	0.7-0.5
80	2.0	8	431	282	65	67.5	16.4	0.05-0.02
100	2.5	6	434	231	53	61.7	50.8	0.01
120	3.0	4	255	98	38	46.2	31.5	0.01
160	4.0	3	174	30	17	18.5	4.2	0.2-0.1
200	5.0	3	169	27	16	15.7	7.9	0.02-0.01
240	6.0	3	231	22	10	9.1	1.9	0.5-0.3
280	7.0	1	55	1	2	1.8		
320	8.0	1	74	0	0	0		

Table II.

Effect of X-rays on eggs incubated for 1–2 hours at 14°C.

Dose		No. of expts.	No. of fertile eggs calculated from control expts.	No. of eggs which hatched	Percentage of eggs which hatched	Average of the percentage hatch in individual expts.	χ^2	P_{χ^2}	t	P_t
In r units	In mins.									
20	0.5	1	40	40	100	100				
40	1.0	8	472	369	75	81.9	63.5	0.01	0.11	0.9
60	1.5	2	84	60	71	68.7			1.20	0.3–0.2
80	2.0	7	339	214	63	63.2	9.3	0.2–0.1	0.63	0.5
100	2.5	3	143	55	39	42.4			1.49	0.2–0.1
120	3.0	7	316	124	39	38.8	7.4	0.3–0.2	0.89	0.4–0.3
160	4.0	8	356	69	19	23.5	57.1	0.01	0.47	0.7–0.6
200	5.0	5	229	13	6	5.7	43.1	0.01	0·98	0.4–0.3
240	6.0	6	264	7	3	2.6	6.8	0.3–0.2	2.95	0.05–0.02
280	7.0	7	450	2	0.4	0.3				
320	8.0	1	130	0	0	0				

t and P_t compare Table II with Table I.

Table III.

Effect of X-rays on eggs incubated for 2.5–3.5 hours at 14°C.

Dose		No. of expts.	No. of fertile eggs calculated from control expts.	No. of eggs which hatched	Percentage of eggs which hatched	Average of the percentage hatch in individual expts.	χ^2	P_{χ^2}	t	P_t
In r units	In mins.									
20	0.5	1	89	71	80	79.8				
40	1.0	5	378	247	65	63.6	30.0	0.01	1.95	0.1–0.05
60	1.5	2	173	74	43	40.3			1.71	0.2–0.1
80	2.0	5	342	44	13	12.0	19.5	0.01	10.30	0.01
120	3.0	6	435	27	6	5.2	32.4	0.01	8.00	0.01
160	4.0	5	349	3	1	0.7				
200	5.0	3	220	0	0	0				
240	6.0	2	137	0	0	0				
280	7.0	2	134	0	0	0				
320	8.0	1	58	0	0	0				

t and P_t compare Table III with Table II.

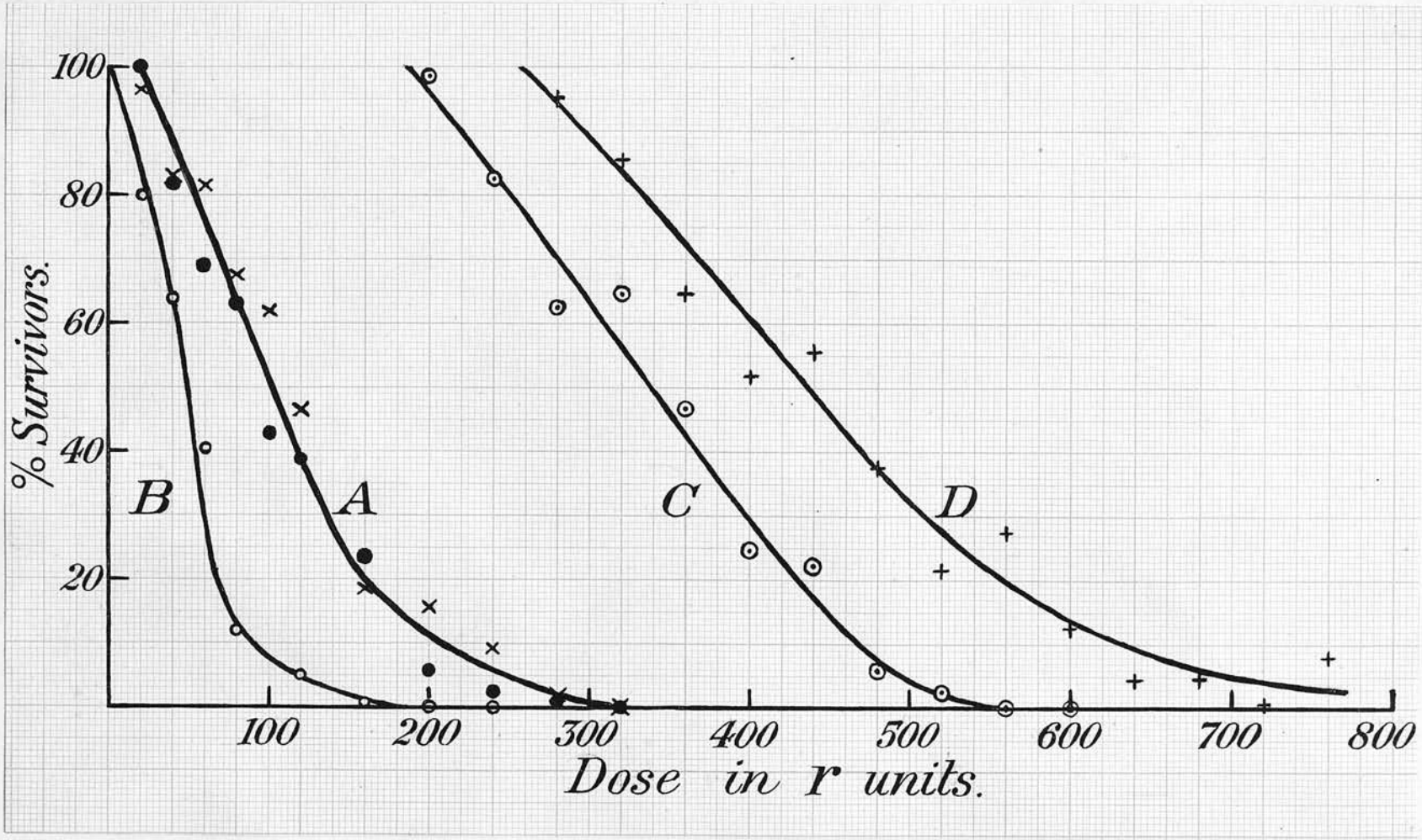

Fig. 3. Action of X-rays on eggs incubated for times equivalent at 14°C. to:-

0.3 - 1 hour	-	Curve A...×
1.0 - 2 hours	-	Curve A...●
2.5 - 3.5 "		Curve B...○
11 hours		Curve C...⊙
16 hours		Curve D...+

In the three groups of experiments eggs which had been laid during a half-hour period were plated and were incubated at 14°C. for 20, 70 and from 120-180 minutes respectively. They were irradiated and then incubated along with the corresponding control eggs at 23°C. until the hatch occurred. The percentage hatch was then measured. The total number of control eggs used in this series of experiments was 5,350 and of these 4921 or 92 per cent. hatched. The eggs were obtained from about 50 different layings and 10 different stocks of flies were used.

The results recorded in Tables I - III and Fig. 3 show that the average sensitivity of the eggs to X-rays does not vary significantly during the first two hours of incubation at 14°C. but that after 2.5 hours the eggs become more sensitive. The dose of X-rays required to kill 50 per cent. of eggs of less than 2 hours incubation is 100 r, while only 50 r produce the same effect in the case of eggs which have been incubated for 2.5-3.5 hours.

The Tables I - III and Fig. 3 show, however, averages/

averages obtained from many experiments, and, in order to decide whether the differences observed are significant, it is necessary to consider the scatter of the individual experiments. Fig. 4 shows the individual results in typical samples of experiments carried out on eggs less than 2 hours old and more than 11 hours old. The variation shown in Fig. 4 is so great that a statistical analysis is necessary. The method of analysis is described below and the summary of it is expressed in Tables I-III in columns headed χ^2 , P_{χ^2}, t and P_t.

Fig. 4. /

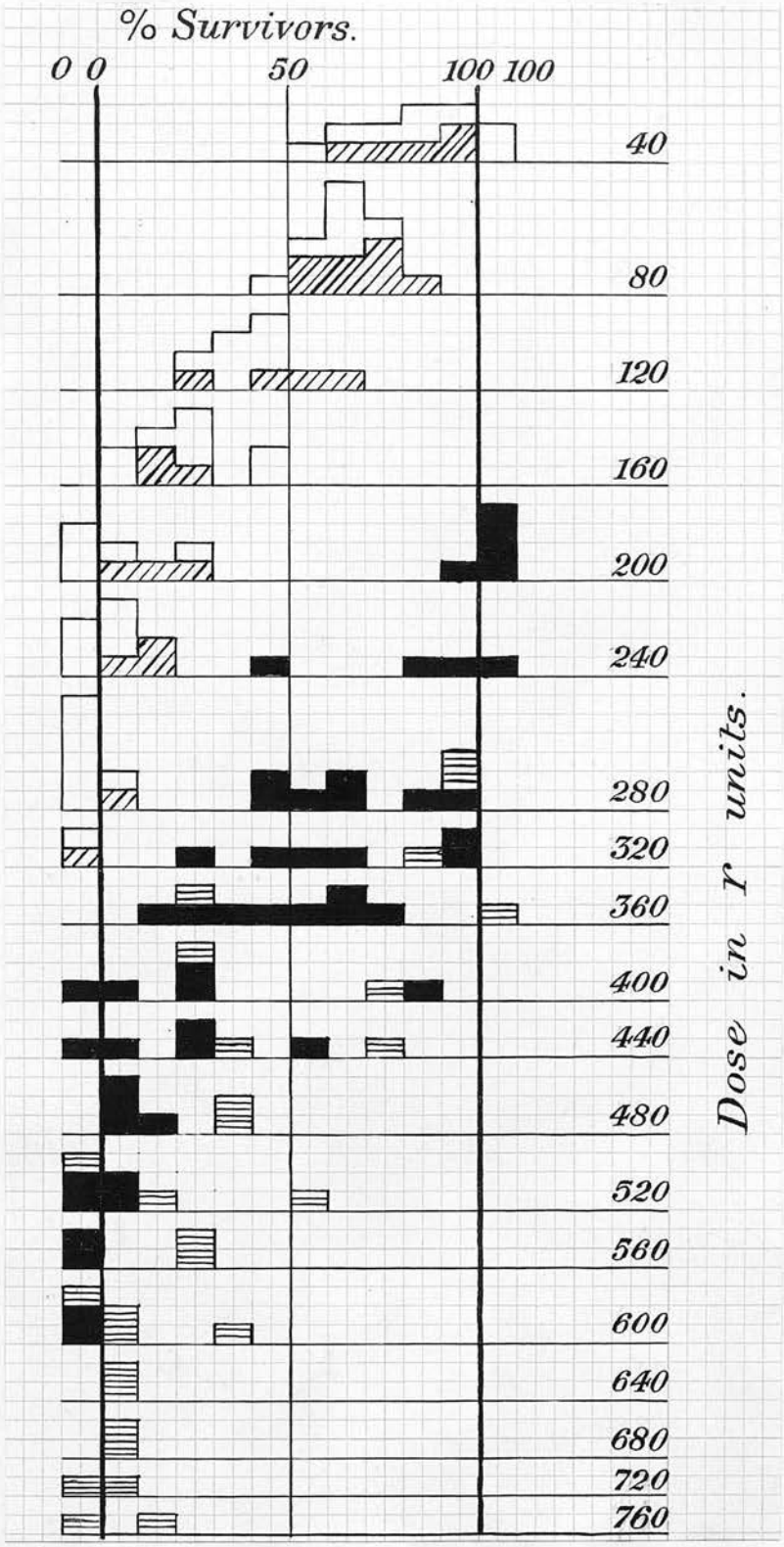

Fig. 4. Distribution graph showing the scatter of results of typical experiments. Eggs of incubation equivalent at 14°C. to:- 0.3 - 1 hour .. □
1 - 2 hours .. ▨
11 hours .. ■
16 hours .. ▤

This analysis showed that the variation in the results was not due to the random sampling of the eggs and, therefore, in expressing the mean percentage of eggs, which were killed by a given dose of X-rays, the following method was adopted. Several different batches of eggs were exposed to the same dose of X-rays and the percentage effect in each batch was measured. These percentages were averaged and this figure was considered as the mean percentage of eggs killed by a given dose of X-rays rather than the percentage derived from the total number of eggs used and the total number of eggs killed.

Method of analysis of results.

The detailed results which are summarised in Tables I-III were examined in order to find whether the variation, which they show, was due to random sampling of the eggs. The analysis was made by the use of the statistic χ^2 (Fisher, 1928, p. 69). By means of an appropriate statistical table (Fisher, 1928, p. 96) the value of P_{χ^2} reveals the probability/

probability that the variability is due to random sampling. This probability has been recorded in Tables I-III in columns headed χ^2 and P_{χ^2}. For example if $P_{\chi^2} = 0.5$ then if the variation of the results is due to a sampling error there are five chances in ten that an equal or greater value for χ^2 will be obtained. If $P_{\chi^2} = 0.01$ there is only one chance in a hundred that χ^2 will be equal to or greater than the value obtained; in other words if $P_{\chi^2} = 0.01$ the variation of the numbers is probably not due to a sampling error. Tables I-III show that the variations in my results are almost certainly not due to sampling errors and the simplest explanation of the variations is found in the rapid changes of sensitivity of the eggs, which are illustrated in Fig. 6. Averages of results were therefore calculated, not from the total number of eggs used, but from the individual percentage action obtained from each group of eggs.

The averages recorded in Tables I-III were compared with each other by means of the statistic t (Fisher, 1928, p. 107). By means of an appropriate /

appropriate table (Fisher, 1928, p. 139) the probability of the significance of the differences between averages may be estimated. This probability has been expressed in Tables I-III under the heading P_t. For example if $P_t = 0.5$ for two series of results, and if the two series are in truth parts of the same series, then the difference between the averages which occurred experimentally or a greater difference will happen five times out of ten by chance. If $P_t = 0.01$ the difference in the averages will occur only once in a hundred times by chance. In other words, the smaller the value is for P_t, the greater is the probability that there is a significant difference between the averages. The values recorded for P_t show that for a given dose of X-rays there is no significant difference between the averages recorded in Tables I and II, but that the differences between the averages of Tables II and III are significant.

(b) Action of X-rays on eggs of more than 10 hours incubation.

(i) Eggs incubated for 11 hours. The results are recorded in Table IV and Figs. 3 and 4.

Table IV./

Table IV.

Effect of X-rays on eggs incubated for a period equivalent to 11 hours at 14°C. The actual incubation was 1.0-1.5 hours at 14°C. followed by 3 hours at 23°C.

Dose		No. of expts.	No. of fertile eggs calculated from control expts.	No. of eggs which hatched	Percentage of eggs which hatched	Average of the percentage hatch in individual expts.
In r units	In mins.					
200	5	5	275	273	99	98.6
240	6	4	189	148	78	82.7
280	7	7	287	182	63	62.6
320	8	8	318	210	66	64.5
360	9	8	406	205	51	46.8
400	10	6	328	60	18	24.6
440	11	5	312	60	19	22.3
480	12	4	235	13	6	5.8
520	13	4	238	7	3	2.7
560	15	1	85	0	0	0
600	15	1	94	0	0	0

Table V.

Effect of X-rays on eggs incubated for a period equivalent to 16 hours at 14°C. The actual incubation was 1.0-1.5 hour at 14°C. followed by 4.5 hours at 23°C.

Dose		No.of expts.	No. of fertile eggs calculated from control expts.	No.of eggs which hatched	Percentage of eggs which hatched	Average of the percentage hatch in individual expts.
In r units	In mins.					
280	7	2	126	120	95	95.1
320	8	1	80	69	86	86.3
360	9	2	131	92	70	64.5
400	10	2	129	68	53	51.8
440	11	2	121	71	59	55.5
480	12	2	168	63	38	37.6
520	13	3	174	41	24	21.4
560	14	2	128	35	27	27.3
600	15	4	270	35	13	12.5
640	16	2	121	5	4	4.1
680	17	2	132	6	5	4.4
720	18	2	178	2	1	0.9
760	19	2	78	7	9	8.1

(ii) Eggs incubated for 16 hours. The results are recorded in Table V. and Figs. 3 and 4.

(iii) Eggs incubated for 22 hours. The median lethal dose of these eggs was found to be considerably greater than 1200 r units. This dose killed about 10 per cent. in one or two experiments but in the majority of experiments all the fertile eggs hatched.

(iv) Eggs incubated for more than 22 hours. Eggs incubated from 22 hours up to hatching (which occurs at 54 hours) were so resistant that it was not possible to estimate the median lethal dose. The hatched maggot was also so resistant that no apparent effect was produced by doses up to 2500 r.

In all these types of experiment the development of the egg was accelerated for experimental convenience and the method was as follows. The eggs were laid during a half-hour period and were incubated at room temperature (14°C.) for one hour. They were then incubated at 23°C. for various times and this duration of incubation was converted into terms/

terms of incubation at 14°C. by the use of the Q_{10} (Fig. 2). This figure shows that a rise in temperature from 13°C. to 23°C. caused a 3.64 fold increase in the rate of development and that this increase was approximately uniform. A rise of temperature from 14°C. to 23°C. would therefore cause a 3.19 fold increase in the rate of development or in other words incubation for one hour at 23°C. is equivalent to incubation for 3.19 hours at 14°C.

After irradiation the eggs in all four series of experiments were incubated at 23°C. until hatching occurred.

The results of experiments on the action of X-rays on eggs of more than 10 hours incubation at 14°C. are summarised in Fig. 6. This figure shows that the sensitivity to X-rays decreases enormously between 16 and 22 hours of incubation. The sensitivity decreases slightly between 11 and 16 hours, but at 22 hours the median lethal dose is more than four times the median lethal dose at

11 hours. Eggs incubated from 22 hours up to the time of hatching, which occurs at 54 hours, are very insensitive as are also the hatched maggots.

(c) Action of X-rays on eggs incubated for periods of between 3.5 and 10 hours.

Experiments were made on eggs at the following ages in hours: 3.5–4, 4–4.5, 4.5–5, 5–6, 6.5–7, 8.5–9. The results obtained during these periods showed a much greater scatter than those obtained with eggs at other ages. The results of the individual experiments are shown in Fig. 5. It will be seen that in some cases the mortality produced by a given dose of X-rays on batches of eggs of the same age varied from 0 to 100 per cent. and a comparison of Fig. 5 with Fig. 4 shows the striking difference in variation inside and outside the limits of from 3.5 to 10 hours.

Fig. 5 /

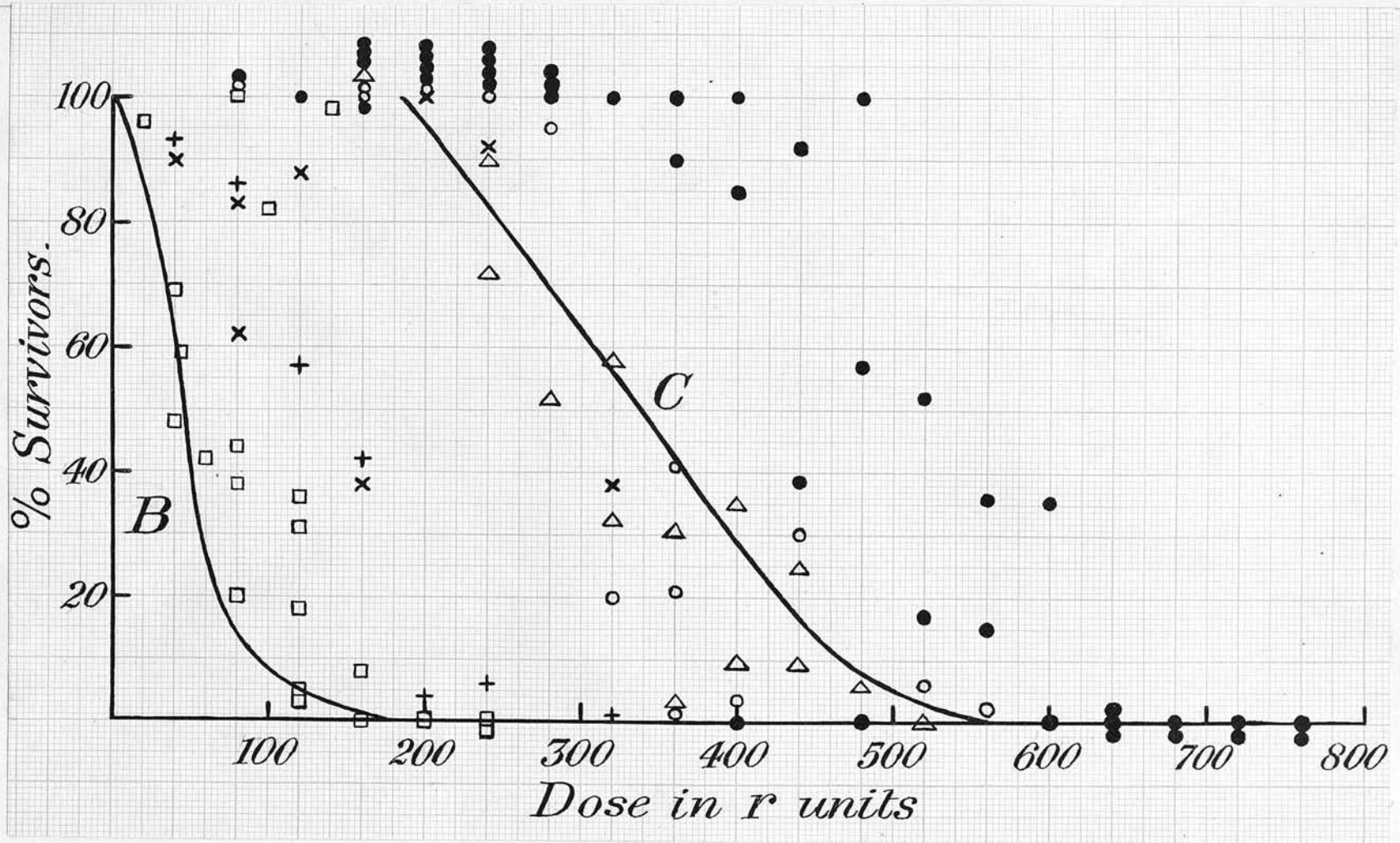

Fig. 5. Action of X-rays on eggs incubated for times equivalent at 14°C. to:-

2.5 - 3.5 hours. Curve B. (from Fig. 3)
3.5 - 4.0 hours ... □
4.0 - 4.5 hours ... +
4.5 - 5.0 hours ... ×
5.0 - 6.0 hours ... ○
6.5 - 7.0 hours ... ●
8.5 - 9.0 hours ... △
11 hours ... Curve C (from Fig. 3)

Averages obtained from the figures for eggs between 3.5 and 10 hours would have a doubtful significance, and therefore these experimental results have not been averaged in tabular form as was done with the remainder of the experiments. The points in Fig. 5 resemble the results obtained at other periods in that each point represents an experiment made on from 60–100 eggs. Fig. 5 shows that the majority of the results obtained with eggs between 3.5–4 hours old lie close to curve B. which is that obtained with eggs between 2.5–3.5 hours old, but a few of the experiments show a greatly increased resistance. The results with eggs from 4 to 5 hours old show a wide scatter but all lie between the curves B and C (Curve C shows the action of X–rays on eggs of 11 hours incubation and has been transposed from Fig. 3). The results with eggs from 5 to 6 hours old are scattered around curve C, whilst all except two of the results with eggs from 6.5 to 7 hours old lie to the right of curve C. Finally the results obtained/

obtained with eggs 8.5 to 9 hours old are scattered around curve C.

These results indicate that between 4 hours and 7 hours of incubation at 14°C. there is a very rapid decrease in the sensitivity of the eggs to irradiation and that after 7 hours the sensitivity increases slightly. The average results as shown in Fig. 6 indicate that during the period 3.5–4.5 hours the sensitivity may be reduced to less than a third in as short a time as 30 minutes. The method of egg collection only ensures that the eggs in two different batches do not differ in age by more than 30 minutes. Hence the rapid change in sensitivity fully explains the extensive scatter in the experimental results during this period. Although the results are so scattered, yet it is possible to estimate approximately the median lethal dose of irradiation at the various times and these values are shown in Fig. 6.

Fig. 6 /

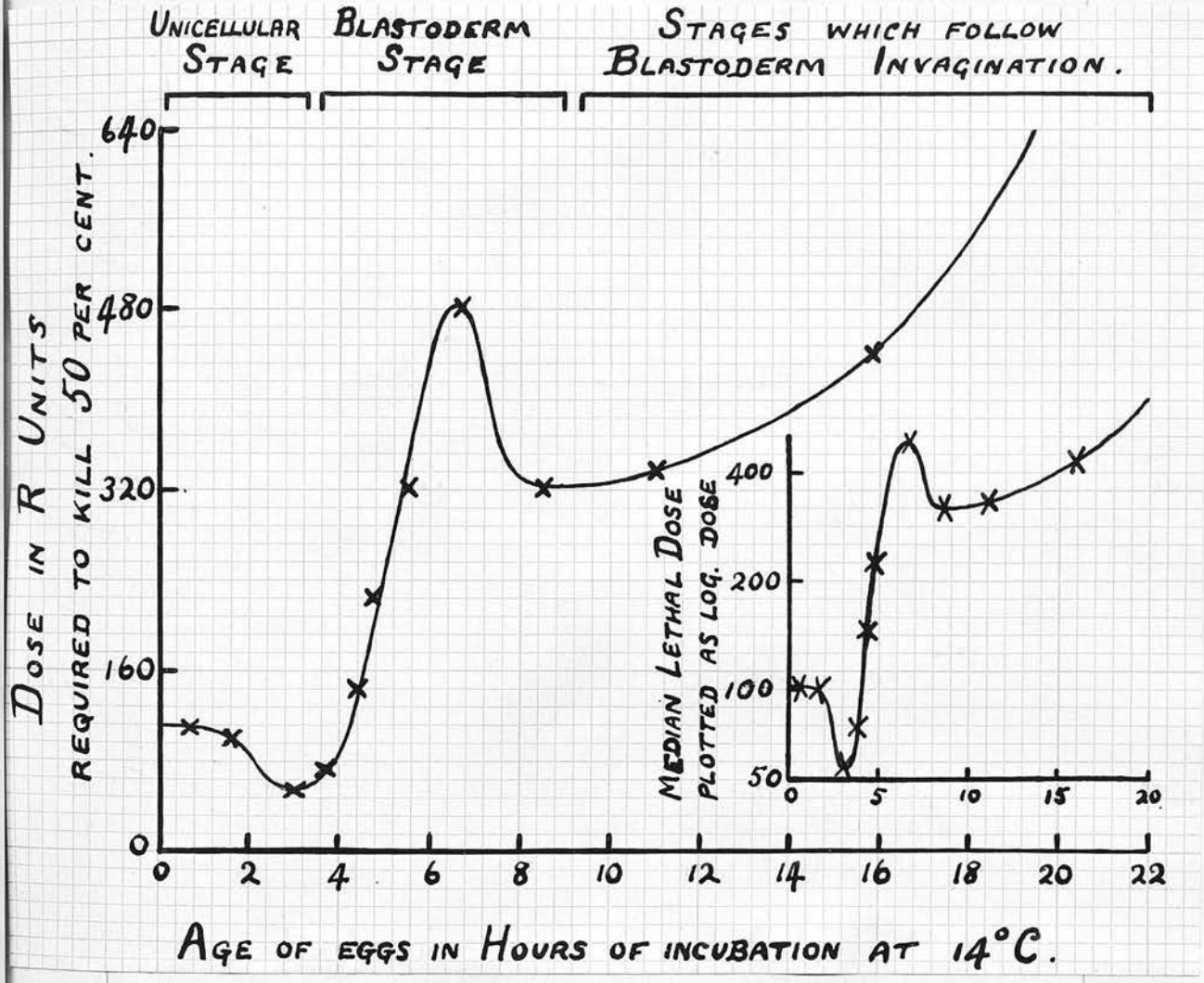

Fig. 6. Relationship between the median lethal dose of X-rays, the duration of incubation at 14°C. and the stage of morphological development of Calliphora erythrocephala.

The development of the calliphorine egg.

The eggs were examined histologically in order to correlate the changes in sensitivity with the grosser features of the development of the embryo. The technique of section cutting is of unusual difficulty and will be described in the Appendix.

The development of the egg has been described by Noack (1901), and I have confirmed many of his descriptions and have used his terminology. The calliphorine egg is fertilised as it passes down the oviduct during the actual process of oviposition. The newly laid egg (Fig. 7) consists of a mass of protoplasm in which many small yolk granules are suspended. It contains one or sometimes two nuclei which lie in a halo of condensed undifferentiated cytoplasm in which there are no yolk granules. The egg is surrounded by a vitelline membrane and an outer shell or chorion. This chorion is very tough and impermeable but it has an opening at one pole, the micropyle, through which the sperm enters.

The egg nucleus divides, but no cell membranes are/

are formed,, and the egg remains a multinucleated single cell until the 8th cleavage (Fig. 8), when there are about 500 nuclei. Prior to this stage the nuclei are scattered throughout the cell, but they now become arranged in a layer roughly parallel to the egg surface about half way between the long axis of the egg and the surface. Shortly afterwards the nuclei migrate to the surface and sections of the egg (Fig. 9) show trailing streamers of cytoplasm running from the nuclei at the surface towards the interior. The nuclei form a single-tiered layer immediately under the vitelline membrane. Cell membranes begin to form at the periphery and separate the nuclei from each other, but the cells are open to the interior of the egg and their cytoplasm is continuous with a cytoplasmic syncytium shown in Fig. 10. At this stage practically all the cytoplasm is massed towards the outside of the egg while the yolk granules occupy the inside. Finally the cell membranes are completed to form a single layer of columnar cells, the blastoderm, which surrounds the yolk (Fig. 11). A longitudinal invagination of

the/

the blastoderm along the ventral aspect of the egg produces a mesodermal tube and shortly afterwards another similar invagination of the blastoderm occurs on the dorsal aspect near the pole opposite the micropyle. (Fig. 12). Noack describes this second invagination as endodermal and from it the gut tube develops. Fig. 13 shows a still later stage of development in which the primitive tube of the gut can be clearly seen. The reduction in size of the cells does not allow of the other structures being distinguished.

Fig. 7/

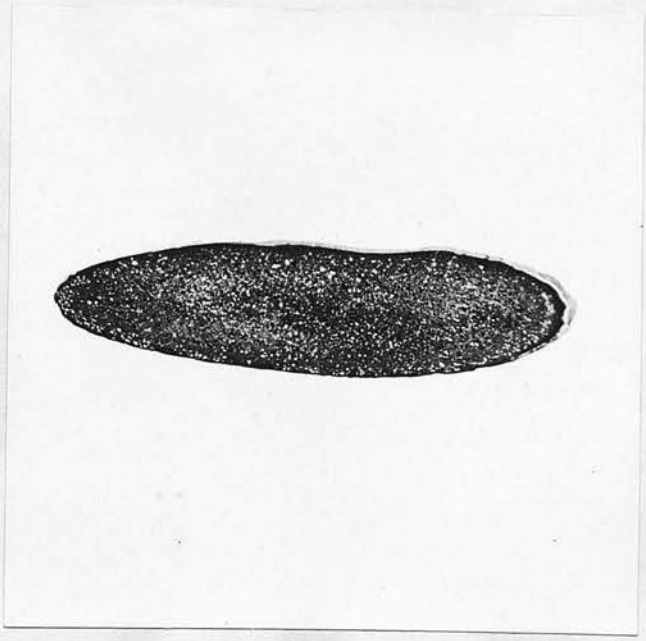

Fig. 7. A longitudinal section of a newly laid egg of Calliphora erythrocephala (x 60). The egg consists of a mass of protoplasm in which fine yolk granules are suspended. No nucleus is visible in the section.

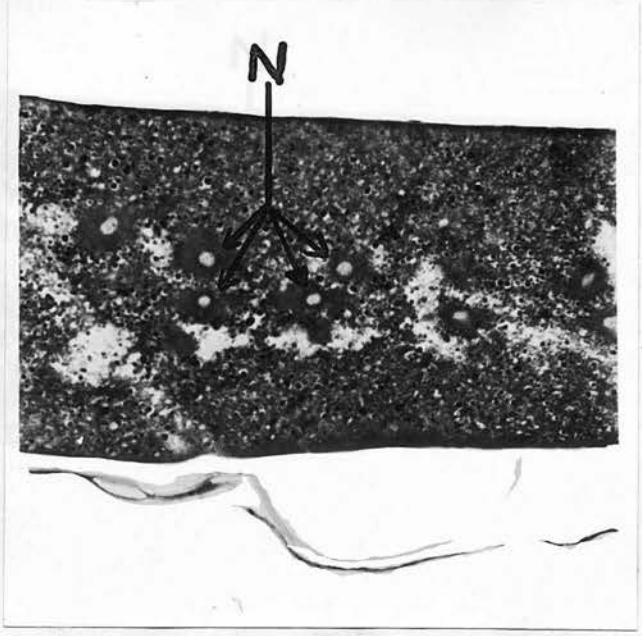

Fig. 8. Part of a longitudinal section of an egg incubated for 1.8-2.2 hours at 14°C. (x 160). N are nuclei surrounded by cytoplasm which contains no yolk granules. The nuclei are centrally situated.

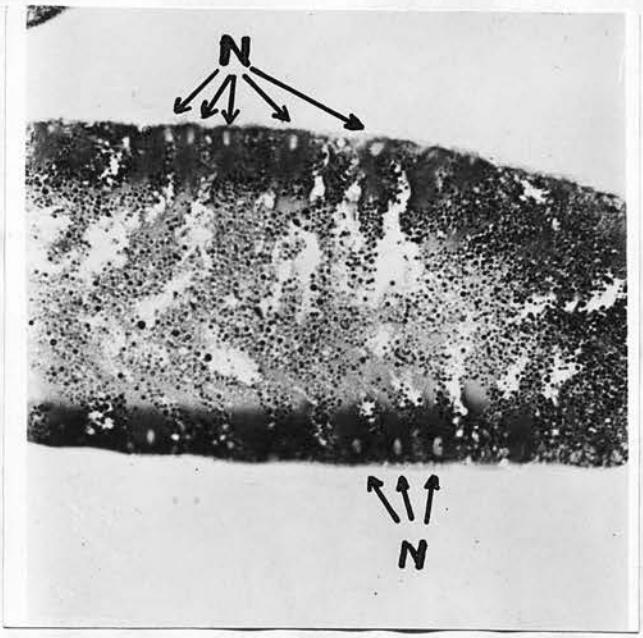

Fig. 9. Part of a longitudinal section of an egg incubated for 3-3.5 hours at 14°C. (x 60). N are nuclei which have recently migrated to the surface of the egg.

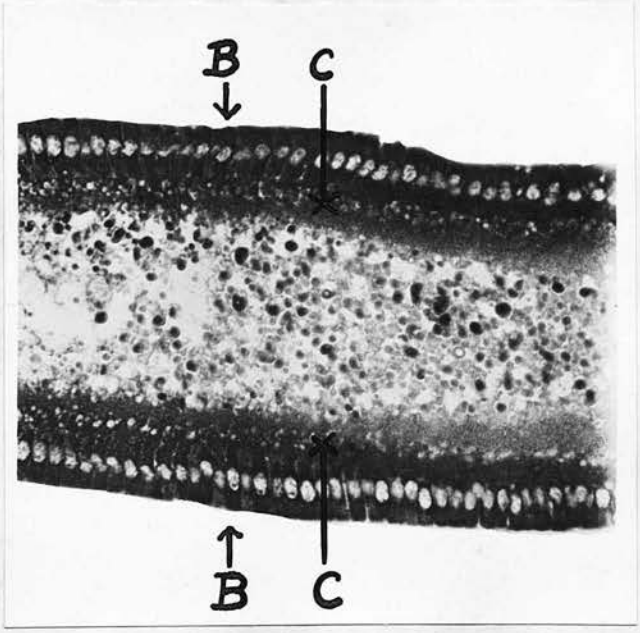

Fig. 10. Part of a longitudinal section of an egg incubated for 5-5.5 hours at 14°C. (x/60). B. is the blastoderm. Cell membranes can be seen near the margin of the section. The cells are still open to the interior of the egg and their cytoplasm is continuous with a syncytium of cytoplasm C.

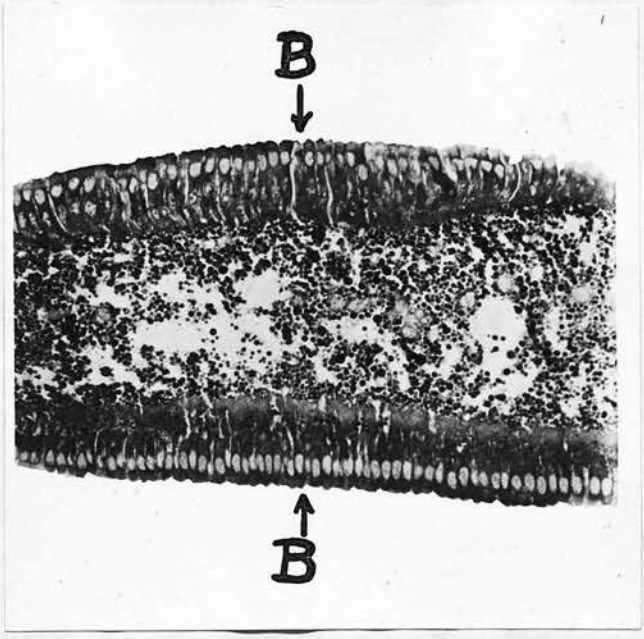

Fig. 11. Part of a longitudinal section of an egg (x 160) incubated for 7.5-8 hours at 14°C. The blastoderm B consists of a layer of complete columnar cells.

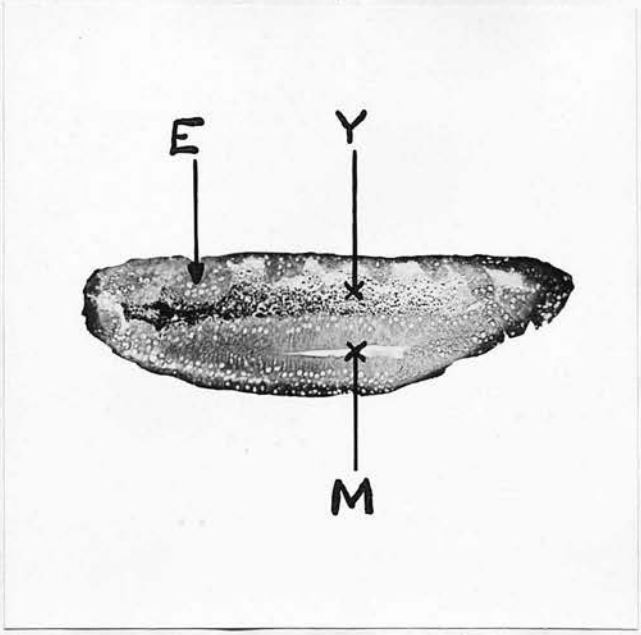

Fig. 12. Longitudinal section of an egg (x 60) incubated for 11 hours at 14°C. The mesodermal tube (M) is shown. The endoderm grows from point E. The yolk (Y) is very much reduced in quantity.

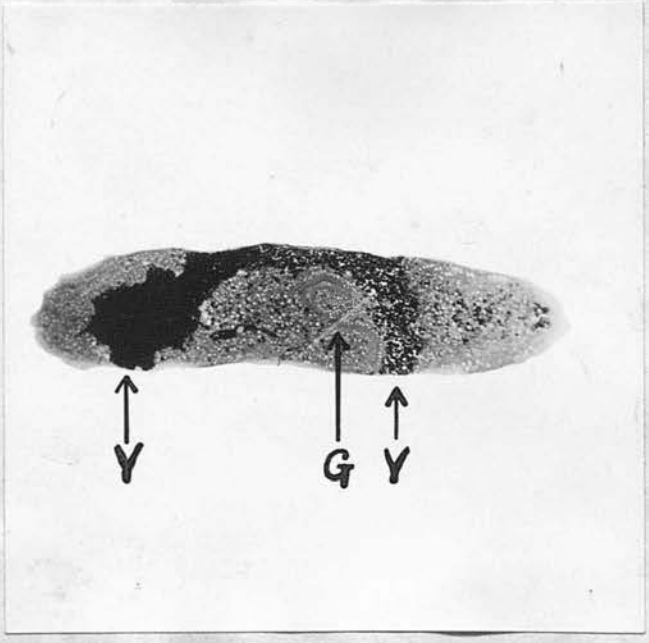

Fig. 13. Longitudinal section of an egg incubated for 16 hours at 14°C. The yolk (Y) is greatly reduced in amount. The egg consists of a cellular mass in which the gut canal G can be distinguished.

Rate of Morphological Development.

The relationship between the various stages of development and the duration of incubation is shown in Table VI and Fig. 6. The egg when incubated at 14°C. remains unicellular for 3.5 hours. During the fifth hour the blastoderm is laid down and the egg becomes multicellular. The first invagination takes place at the ninth hour and the second is complete at the sixteenth.

The rate of nuclear division was calculated in the following way. Sections of eggs which had been fixed during the fifth hour of incubation at 14°C. were examined, and in many of them the blastoderm was found to be in actual process of deposition. In 12 longitudinal sections of these eggs the nuclei were counted and the average number was 51. In 12 corresponding cross sections the average number of nuclei was 12. If it be assumed that the eggs are approximately cylindrical, then the number of nuclei in each egg is $\frac{51}{2} \times 12 = 306$. Eight nuclear divisions are required to produce this number of nuclei and therefore at 14°C. nuclear division occurs about once every half hour.

Table VI/

Table VI.

The relationship between the duration of incubation and the degree of morphological development.

Duration of incubation in hours	Duration of incubation expressed in terms of incubation at 14°C.	No. of eggs examined	Percentage in each stage of development						
			Before blastoderm formation, showing:-			Transition Stage	After blastoderm formation, showing:		
			No nucleus visible	1-7 nuclei	8 or more nuclei		No invagination	Invagination	Advanced development
0.2-0.6 at 14°C.	0.2-0.6	76	97	3	-	-	-	-	-
0.8-1.2 at 14°C.	0.8-1.2	61	39	58	8	-	-	-	-
1.8-2.2 at 14°C.	1.8-2.2	61	20	69	11	-	-	-	-
3.0-3.5 at 14°C.	3.0-3.5	79	6	-	-	94	-	-	-
4.0-4.5 at 14°C.	4.0-4.5	124	5	-	-	40	55	-	-
5.0-5.5 at 14°C.	5.0-5.5	93	8	-	-	-	92	-	-
7.5-8.0 at 14°C.	7.5-8.0	68	6	-	-	-	94	-	-
0.5-1.0 at 14°C. followed by 2.2 hrs. at 23°C.	7.5-8.0	72	8	-	-	-	92	-	-
0.5-1.0 at 14°C. followed by 2.7 hrs. at 23°C.	9.0-9.5	86	4	-	-	-	8	88	-

Table VI. contd.

Duration of incubation in hours	Duration of incubation expressed in terms of incubation at 14°C.	No. of eggs examined	Percentage in each stage of development.						
			Before blastoderm formation, showing:-			Transition Stage	After blastoderm formation, showing:-		
			No nucleus visible	1-7 nuclei	8 or more nuclei		No invagination	Invagination	Advanced development
9.0-9.5 at 14°C.	9.0-9.5	72	6	-	-	-	-	94	-
1.0-1.5 at 14°C. followed by 3.0 hrs. at 23°C.	10.5-11.0	100	7	-	-	-	-	93	-
1.0-1.5 at 14°C. followed by 4.5 hrs. at 23°C.	15.5-16.0	100	5	-	-	-	-	-	95

Development of the irradiated egg.

It has been shown that X-rays can prevent the hatching of the calliphorine egg and the following experiments were devised in order to find whether the irradiation caused an immediate stoppage of development. Eggs from a half-hour laying period were plated and were X-rayed after less than one hour of incubation at 14°C. A dose of 300 r units was given. At this stage of incubation the eggs are unicellular and contain about 8 nuclei. After irradiation the eggs were divided into two groups. One group was incubated till hatching occurred and out of 514 eggs 1.5 per cent. hatched (cf. Table I). The second group was incubated at 14°C. for 11 hours and was then fixed and sectioned for microscopic examination. It has been shown (Table VI) that unirradiated eggs incubated for 11 hours at 14°C. have reached the stage of infolding of the blastoderm. Sections of 107 irradiated eggs with the same incubation were examined and 86 or 80 per cent. of these had not formed a blastoderm. In 21 or 20 per cent. the blastoderm had been laid down but in none of the eggs was there any sign of invagination/

invagination. These results show that while at least 20 per cent. of the eggs continue to develop after irradiation with 300 r units, only 1.5 per cent. actually hatch. It is concluded therefore that the effect of irradiation is not to cause the immediate stoppage of development. The estimation of the number of nuclei in eggs before blastoderm formation is difficult but there was evidence that in many eggs nuclear division had continued after irradiation.

DISCUSSION /

DISCUSSION

The chief results of this investigation are set forth in Fig. 6 which shows the variations in the sensitivity of the developing calliphorine egg to X-rays and also the times of the various stages of its embryological development. During the process of development the sensitivity of the egg decreases greatly, but Fig. 6 shows that the process is not continuous, for there is one period (4-7 hours) of very rapid loss of sensitivity and two periods (2-4 hours and 7-10 hours) during which the sensitivity actually increases. The relative extent of the changes in sensitivity are compared in the inset graph of Fig. 6, in which the median lethal dose is plotted on a logarithmic scale. The general loss of sensitivity during development is in accord with the Law of Bergonie and Tribondeau, but this law does not explain the detailed course of the changes in sensitivity and, in particular, cannot account for the two periods during which the sensitivity increases.

The/

The relationship between the sensitivity of the calliphorine egg to X-rays and the degree of embryological development closely agrees with that found in the case of other eggs. Henshaw and Henshaw (1933) described an almost identical curve relating X-ray sensitivity to the development of the egg of Drosophila. The developmental changes in the latter egg are almost identical with those of Calliphora and the doses of X-rays used by Henshaw and Henshaw were similar to those to which I submitted the eggs of Calliphora. Glasser and Mautz (1933) obtained comparable results with Drosophila eggs although they discerned no periods of increased sensitivity. The egg of Ascaris also behaves in a fashion similar to that of Calliphora, for Schinz and Zuppinger (1928) found that the sensitivity of the Ascaris egg decreased when the egg changed from the single-celled to the blastula stage. They found that the sensitivity gradually increased between the blastula and the gastrula stages and that thereafter it gradually increased/

increased.

The results of my investigation shed some light on the nature of the action of X-rays on cells. The present conception of the mode of this action depends on three methods of biological measurement. Firstly the effect of X-rays has been measured by the degree of their lethal action, which is the method used in this investigation. Secondly the effect has been measured by the genetic mutations, which X-rays produce. In experiments of this type, the literature of which has been summarised by Hanson (1933), X-rays not only produce genetic mutations but also changes in the shape and behaviour of the chromosomes and the two effects have been correlated. The third method of biological measurement of X-rays depends on the direct observation of the behaviour of cells which have been irradiated. By this method it has been shown that a cell is most easily killed during the process of nuclear division (Mottram, 1913; Holthusen 1921; Vintemberger 1928) and also that irradiation may prevent nuclear division either temporarily or permanently (Canti and Donaldson, 1926).

The/

The latter two methods of studying X-ray action are mainly concerned with effects produced on the nucleus of the cell, and Vintemberger (1928) attempted to show by the following method that the nucleus was primarily the seat of X-ray damage He measured the dose required to kill two groups of developing eggs of the frog. In the one group an attempt was made to screen the cell nuclei and in the other to screen the cytoplasm. He concluded that the nucleus of the cell is markedly more sensitive to the lethal action of X-rays than is the cytoplasm.

The results of my investigation afford no direct evidence of any difference in sensitivity between the nucleus and the cytoplasm but they afford indirect evidence. Fig. 6 and Table VI show that the development of the calliphorine egg involves two phases of cellular activity. During the first phase the segmentation nucleus divides repeatedly but there is no cytoplasmic division and the egg is a multinucleate single cell. During the second phase nuclear division continues but is accompanied by division of the cytoplasm and the egg/

egg then consists of many cells, each of which have one nucleus. The change from the uninuclear to the multinuclear phase is abrupt and is accompanied by an enormous and sudden decrease in sensitivity to X-rays (Fig. 6). This suggests that the decrease in sensitivity is related in some way to the segmentation of the whole egg rather than to the division of the nucleus alone.

This conclusion is further supported by the fact that there was no significant difference in the sensitivity of the egg during the first two hours of development (Tables I and II and Fig. 6). During this period nuclear division occurred continuously but cytoplasmic division did not occur and there was no significant difference of the sensitivity of eggs of less than 1 hour incubation from that of eggs of between 1 and 2 hours incubation.

There is an increase in sensitivity between 2.5 and 3.5 hours and the extent of the increase is shown in the inset graph in Fig. 6. At this time the nuclei and the cytoplasm are in process of migrating to the surface of the egg and this process is preliminary to the change from the unicellular to the multicellular state. The complexity/

complexity of the change which must occur in the cells at this time is the simplest explanation of the increased vulnerability of the eggs.

This explanation also serves for the increased vulnerability which occurs between 7 and 10 hours. At this period the blastoderm is on the point of invaginating and again the complexity of the cell changes which probably precede the process of invagination is the simplest explanation of their increased sensitivity to X-rays.

My results and conclusions differ from those of other workers who have studied the action of X-rays on mitosis. There is, however, an essential difference between the material studied in the two cases. In the systems studied by other workers mitosis was followed by cell division and they measured the action of X-rays in arresting the combined process of nuclear and of cytoplasmic division. Insect eggs are peculiar in that nuclear division proceeds for several hours before cytoplasmic division commences and hence it is possible to compare the action of X-rays on the process of nuclear division and on the process of combined/

combined nuclear and cytoplasmic division. Nuclear division begins soon after the egg is laid and continues at a rate which appears to be fairly constant for the first 10 hours. In spite of this fairly constant rate of nuclear activity, the sensitivity of the cells to X-rays varies extensively and erratically. These variations cannot be explained by variations in nuclear activity, but they are correlated with obvious changes in the activity and morphological structure of the complete cell units. These facts cannot be explained by Vintemberger's finding that the action of X-rays is chiefly confined to the nuclei, but they can be explained if the action of X-rays causes greater damage to the complete cell unit when the cell is undergoing a complicated process of differentiation.

The results of this investigation also show that the lethal effect of X-rays on the calliphorine egg is not an immediate one. In spite of their having received a lethal dose of X-rays some eggs continue to develop for a short time before death occurs. A similar effect is produced in Colpidium (Crowther, 1926, Ascaris (Holthusen, 1927), the embryo chick (Strangeways /

(Strangeways and Fell, 1927) and certain tissue cultures (Spear, 1931; Cox, 1931).

The shape of the curves of action of X-rays in this paper agree closely with the results obtained in experiments with many tissues (Packard, 1931; Henshaw and Henshaw, 1933; Crowther, 1926; Canti and Spear, 1927; Cox, 1931). The significance of the shapes of the curves will be discussed later by the author, but explanations have already been put forward by Crowther (1926), Condon and Terrill (1927), Packard (1931), Clark (1933). The explanation of Packard and Clark, namely that the characteristic curve of biological action of X-rays expresses the individual variations of the biological units appears to the author to be the most reasonable explanation of the facts.

SUMMARY

1. The relationship between the sensitivity to the lethal action of X-rays of the egg of Calliphora erythrocephala and the degree of embryological development of the egg has been shown.

2. The sensitivity of the egg decreases with age but the loss of sensitivity follows an irregular course.

3. The variations in sensitivity can be explained by the accompanying changes in the embryological development of the egg.

4. The evidence suggests that X-rays exert their action on the complete cell unit rather than on the cell nucleus exclusively.

5. The lethal effect of X-rays on the calliphorine egg is not an immediate effect.

6. The shape of the characteristic curve of the lethal action of X-rays on calliphorine eggs at various stages of development has been defined.

I gratefully acknowledge the advice of Professor A.J. Clark, F.R.S. throughout the course of my work. I thank Dr R.P. Hobson of the Rothampstead Institute for his detailed advice about the breeding of Calliphora, Dr A.E. Cameron of the Department of Zoology, Edinburgh University, for his help in the interpretation of the embryology of Calliphora, and Dr W.O. Kermack of the Laboratory of the Royal College of Physicians of Edinburgh who instructed me in the methods of statistical analysis which I have used. My thanks are also due to Mr Kilgour of the Pathological Department of the Royal Infirmary of Edinburgh through whose patience and skill I obtained microscopic sections of the eggs of Calliphora.

Appendix.

Method of preparing microscopic sections of the eggs of Calliphora erythrocephala

by

D. Kilgour

(Department of Pathology, Royal Infirmary, Edinburgh)

No satisfactory method of preparing sections of the eggs of Calliphora has yet been described, but the following method succeeds in two-thirds of cases. The difficulty of the technique is due to the nature of the chorion of the egg, the hardness and impermeability of which make fixation and section cutting very difficult. The following method gave the best results.

About 200 eggs are immersed in Carnoy fixative for at least 2 hours. They are washed in industrial spirit and after successive dilutions of the spirit, in water. The chorion is dissolved by immersing the eggs in sodium hypochlorite solution (about 0.02 g. chlorine per 100 c.c.) for about 2 minutes. This part of the procedure/

procedure requires much practice. If the eggs are immersed in the hypochlorite solution for an insufficient period the chorion remains too hard to cut and if the immersion period is too prolonged the underlying structures are seriously damaged.

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Part IV. The Influence of Temperature on the Sensitivity of Calliphorine Eggs to X-rays. (This paper is about to be submitted to the Royal Society for publication)

THE INFLUENCE OF TEMPERATURE ON THE SENSITIVITY OF CALLIPHORINE EGGS TO X-RAYS.

In a previous paper (Scott, 1934) a method was described by which the lethal action of X-rays on the eggs of Calliphora erythrocephala (common bluebottle) can be measured. The results in that paper show that the sensitivity of the eggs to X-rays, as measured by the median lethal dose, varies enormously as the eggs develop; the median lethal dose of eggs incubated at 14°C. for 3 hours is 50 r but after 22 hours incubation it rises to more than 2,500 r. The results also show that the loss of sensitivity does not follow a uniform course and that changes in sensitivity are associated with well marked developmental changes in the eggs such as the laying down of the blastoderm and the invagination of the blastoderm. In all the previous experiments the eggs were kept at a temperature of 14°C. during the irradiation and after irradiation they were incubated at 23°C. until hatching took place.

The rate of development depended on the temperature at which the eggs were incubated and the Q_{10} of the rate of development was found to be/

be 3.6 between 8°C. and 23°C. At 14°C., which was taken as the standard temperature, the nuclei divided about once every half hour. It was found that the sensitivity to irradiation depended on the stage of development reached at the time of irradiation and was not affected by changes in the temperature of incubation. For example eggs incubated at 14°C. for 11 hours reached the same stage of development as eggs incubated for one hour at 14°C. followed by 3.1 hours at 23°C. and the two groups of eggs at these times showed the same sensitivity to irradiation.

The objects of the experiments which will be described in this paper were:-

1. To find whether the effect of a given dose of X-rays is altered by changes in the intensity of the irradiation. This part of the investigation was not exhaustive and was carried out rather as a test of the validity of the method than as a complete test of the Bunsen-Roscoe Law.

2. To find how the sensitivity to X-rays is affected by changes in the rate of development at the actual time of irradiation.

3. To find how the sensitivity to X-rays is affected by changes in the rate of development after irradiation.

4. To find how the damage which is produced by irradiation is related to the damage which is produced by very low temperatures.

In general the method of interpreting the new results was to compare them with the standard curves of action which were described in my previous paper and which represent the action of X-rays on calliphorine eggs at various stages of development under standard conditions.

METHOD.

Essentially the same method as that described in my previous paper (1934) was used. Eggs were irradiated at three stages of development:

(a) Eggs incubated at 14°C. for 0.5-1.0 hour.

(b) Eggs incubated at 14°C. for 2.8-3.3 hours.

(c) Eggs incubated at 14°C. for 10.8-11.3 hours.

At the first two stages the eggs are unicellular and/

and multinucleated. At the third stage the eggs are composed of a large number of uninucleated cells. The second stage is the stage of maximum sensitivity to X-rays.

During irradiation the eggs were contained in a lead box with an aluminium front which has already been described. The inside of the box was kept at a known and constant temperature by means of a water bath. The eggs were placed in the box for about half to one hour before irradiation in those experiments in which a temperature other than room temperature (14°C.) was required. Those other temperatures were always considerably less than 14°C. and therefore any development of the eggs which might occur during the period of temperature adjustment was insignificant.

After irradiation the eggs were incubated at known temperatures and the percentage which hatched was measured. Control experiments in which the number of eggs used was approximately equal to the number of experimental eggs were carried out in the manner described in my previous paper and from these control experiments the percentage of fertile eggs/

eggs was calculated. The average percentage hatch in control experiments was 92 per cent. and experiments were discarded in which the control hatch fell below 90 per cent. The discarded experiments were few and all others are recorded.

The source of the X-rays was a water-cooled Muller tube with a tungsten anticathode. The kilovoltage was 79 and the milliamperage 5. In all experiments an aluminium screen 0.01 cm. thick was used. During individual experiments the output of the tube was constant. Day to day variations in the output were corrected by small alterations of the milliamperage. In most experiments the dose delivered was 40 r per minute at 47.1 cm. In some experiments the dose was 30 r per minute at 47.1 cm. The intensity was measured by means of a gold-leaf electroscope of the usual pattern.

The physical measurements of dosage were biologically confirmed by means of repeated standard experiments. Fig. 1 shows the averages of the results of these confirmatory experiments. They were carried out with eggs between 0.5 and 1.0 hour old; the eggs were at 14°C. during irradiation and were reincubated at 23°C. until hatching. In each/

each experiment about 200 eggs were used and 35 experiments were done. The various average percentages of eggs surviving fall closely along a curve, derived from exactly similar experiments, which has been transposed from my previous paper. The results of the confirmatory experiments, which were carried out periodically during the investigation, not only confirm the results recorded in my previous paper, but also confirm the relative accuracy of the physical measurements.

RESULTS /

distances from the anticathode, 47.1 cm., which was the distance used in the standard experiments, 29.8 cm., and 81.6 cm. The intensity varies inversely as the square of the distance and therefore the ratio of the intensities at 29.8 cm., 47.1 cm., and 81.6 cm. was 7.5:3:1.

About 30 experiments were done at 29.8 cm. and the same number at 81.6 cm. In each series about 2,000 eggs were irradiated. Approximately the same number of eggs was used in the control experiments and between 91 and 92 per cent. hatched.

The averages of the results are recorded in Fig. 1. In this figure the curve is reproduced from my previous paper and represents the action of X-rays at 47.1 cm. The various points all lie approximately along the curve and it is therefore concluded that the Bunsen-Roscoe Law is valid for calliphorine eggs within the range of intensities from 100 r to 13 r per minute, which is a 7.5 to 1 range.

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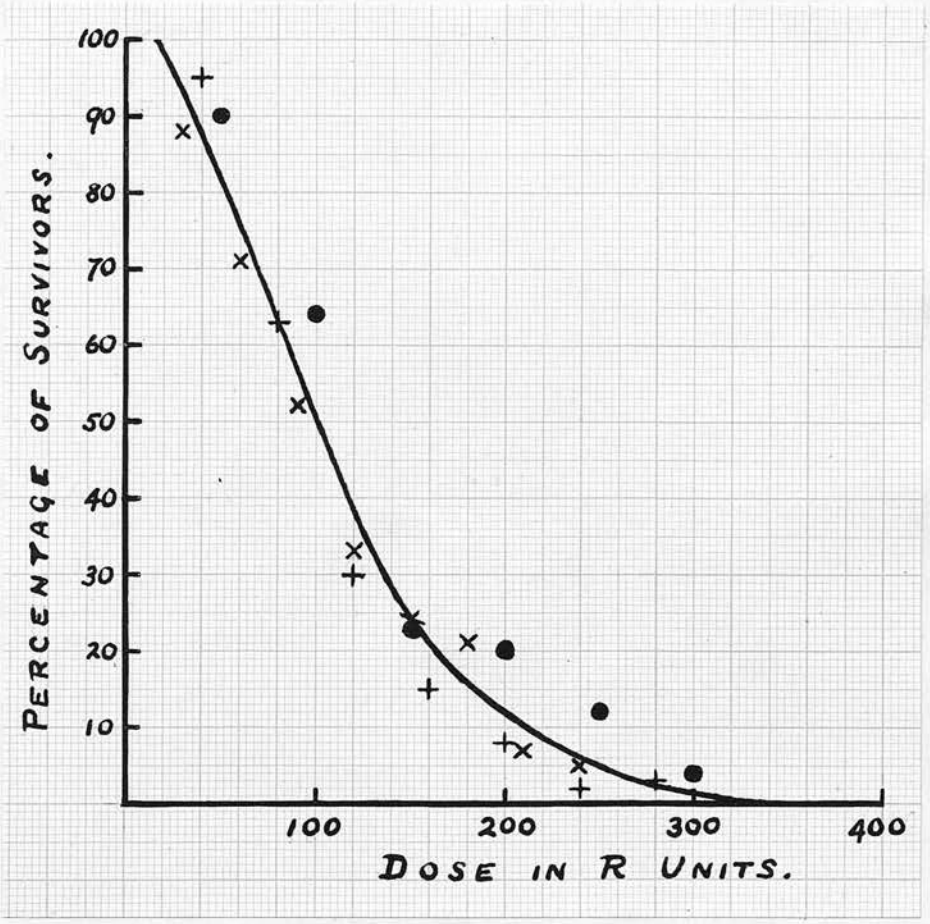

Fig. 1. Effect of varying the intensity on the lethal action of X-rays.

The curve is the standard curve reproduced from my previous paper (1934).

× Distance from anticathode to target = 47.1 cm. (standard distance)

● Distance from anticathode to target = 29.8 cm.

\+ Distance from anticathode to target = 81.6 cm.

The effect of different rates of development during irradiation.

Eggs at two stages of development were used, namely eggs which had been incubated for 0.5-1 hour at 14°C. and eggs which had been incubated for 11 hours at 14°C. The former group are composed of unicellular eggs and the latter of multicellular eggs.

In experiments with both groups the eggs were placed in the experimental box along with control eggs and were chilled to a low temperature. In order to ensure chilling to the temperature of the box the eggs lay in it for half an hour and were X-rayed in the last few minutes of that period. After irradiation they were reincubated at 23°C. till hatching occurred.

The group which had been incubated for 0.5-1 hour were chilled to 8°C. and at this temperature all the fertile control eggs hatched. This temperature was chosen because chilling to 6°C. for half an hour caused the death of a few fertile control eggs . The group which had been incubated for 11 hours/

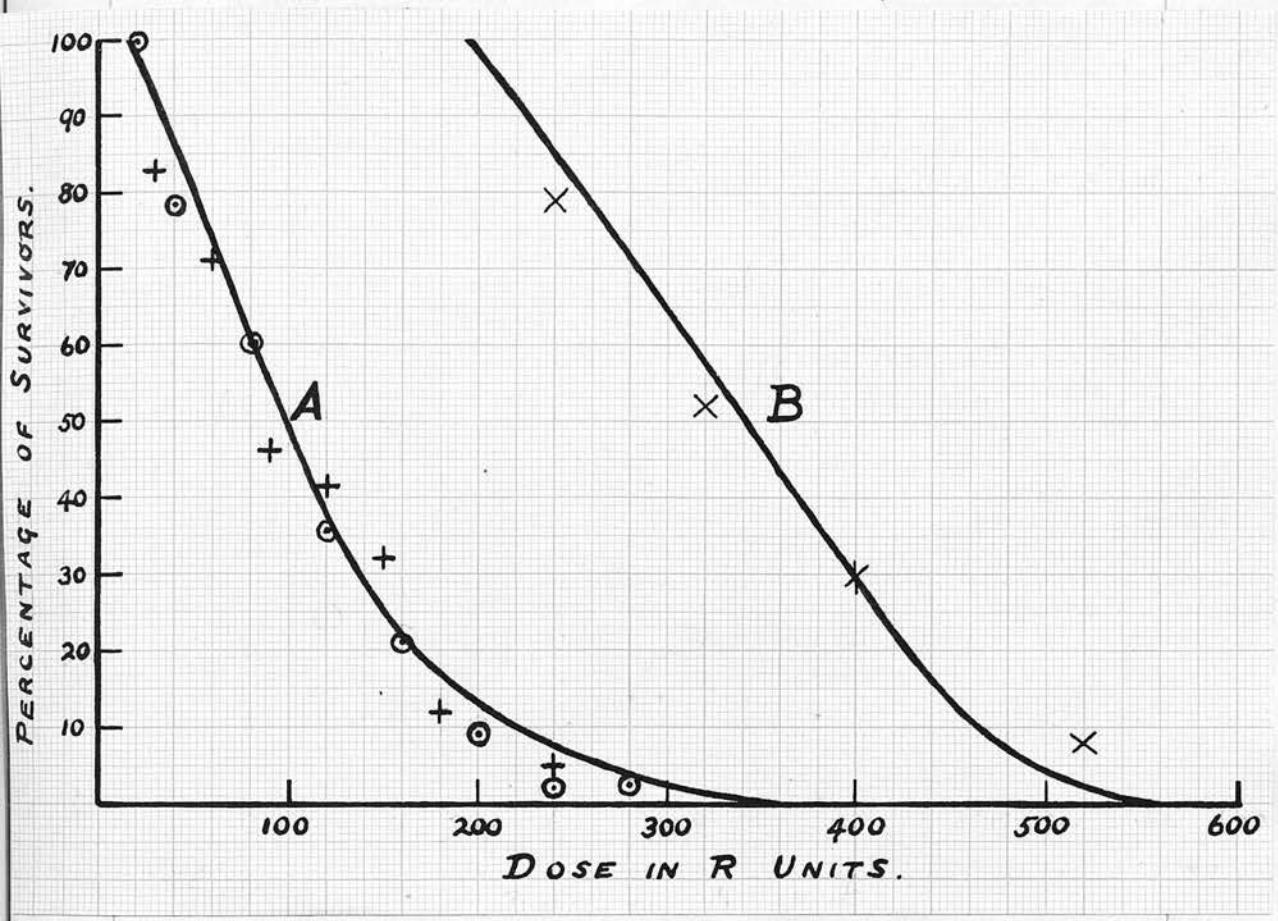

Fig. 2. Effect of varying the temperature during irradiation and subsequent to irradiation on the lethal action of X-rays.

Curves A and B are standard curves reproduced from my previous paper (1934). Curve A = Action on eggs incubated for 1 hour at 14°C., irradiated at 14°C. and incubated at 23°C. subsequent to irradiation. Curve B = Action on eggs incubated for 11 hours at 14°C., irradiated at 14°C. and incubated at 23°C. subsequent to irradiation.

⊙ Action on eggs incubated at 14°C. for 1 hour, irradiated at 14°C., and reincubated after irradiation at 8°C.

\+ Action on eggs incubated at 14°C. for 1 hour, irradiated at 8°C., and reincubated after irradiation at 23°C.

× Action on eggs incubated at 14°C. for 11 hours, irradiated at -5°C., and reincubated after irradiation at 23°C.

was 14°C. The results of the experiments with chilled eggs conform very closely to these curves.

These results show that the retardation of the rate of development which is produced by a change of temperature from 14°C. to 8°C. and from 14°C. to -5°C. does not influence the sensitivity of the eggs to X-rays.

The effect of the rate of development after irradiation on the sensitivity to X-rays.

Eggs which had been incubated for 0.5-1.0 hour at 14°C. were irradiated at a temperature of 14°C. After irradiation they were reincubated until they hatched in a cold cellar where the temperature varied from 7°C. to 9°C. Hatching took place after 120 hours. About 2,000 eggs were irradiated and 28 experiments were done. The control eggs were about 1,500 and 91 per cent. of these hatched. The averages of the results are shown in Fig. 2 as crosses around Curve A, where they are compared with the standard curve of action. The results lie closely along this standard/

standard curve which was obtained from experiments in which the temperature of incubation after irradiation was 23°C. Apart from the difference in the temperature of incubation after irradiation, the experiments from which the standard curve is derived and the experiments which have just been described, were carried out in an identical manner.

The eggs incubated at 23°C. hatched in 17 hours whereas those incubated at 7°C. to 9°C. hatched in 120 hours. The difference in the rate of development therefore was a sevenfold difference and the Q_{10} of the development rate was 3.6.

Fig. 2 shows clearly that this great variation in the rate of development subsequent to irradiation makes no difference to the lethal effects produced by the irradiation.

The lethal effect of cold on calliphorine eggs at various stages of development.

Eggs were chilled for a period to various low temperatures and were reincubated at 23°C. The percentage of fertile eggs which hatched was measured and the results are recorded in the Table.

This table shows that exposure to cold produces injury and the lower the temperature the greater the injury. The table also shows that the injury is approximately the same whether the eggs are chilled for half an hour or for one hour. As the eggs become more developed the resistance to cold increases and although resistance to X-ray injury also increases, the table shows that the increase in resistance to cold does not follow the same course as the increase in resistance to X-rays. Eggs which have been incubated for 3 hours are more sensitive to X-rays than eggs incubated for 0.5-1 hour, while they are more resistant to the effect of cold. The eggs of other types of insects respond in a similar way to the action of cold but there is a wide variation in their response (Uvarov, 1931).

The effect of superimposing the injury produced by X-rays on the injury produced by cold.

Experimental and control eggs were chilled for periods of half and one hour to temperatures which produced damage in the control eggs. During the last few minutes of the chilling period the eggs/

eggs were irradiated and the percentage of fertile eggs which hatched was estimated for both the irradiated and the unirradiated eggs.

Eggs at two stages of development at the time of chilling were used:

1. Eggs incubated at 14°C. for 0.5-1.0 hour. These eggs were chilled to 3°C. in one series of experiments and to -5°C. in another series. In the 3°C. series over 5,000 eggs were irradiated and in the -5°C. series over 3,000 eggs. A comparable number of control eggs were chilled.

2. Eggs incubated at 14°C. for 2.8-3.3 hours. These eggs were chilled for 1 hour to -5°C. About 3,000 eggs were irradiated and about 2,000 control eggs were chilled. The percentage hatch of the control and irradiated eggs was measured.

The averages of the results are shown in Figs. 3, 4 and 5.

These figures in the first place show the experimental results (crosses); secondly, they show the standard curves (A) for the lethal action of X-rays on unchilled eggs at a similar stage of/

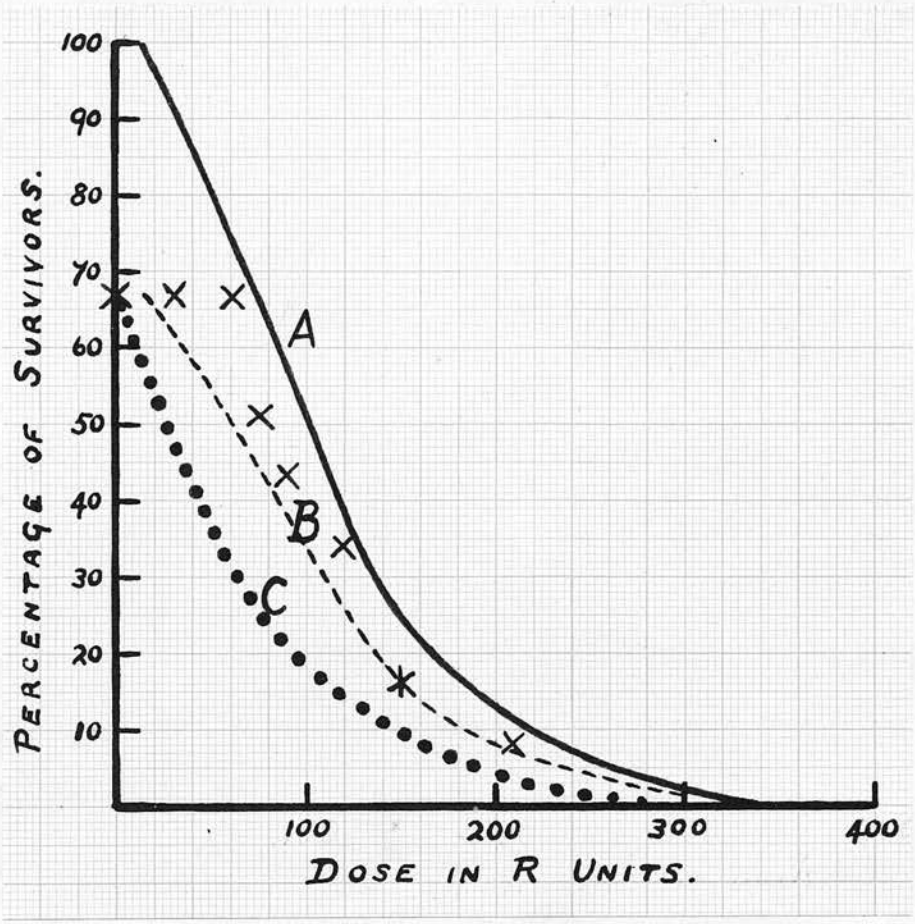

Fig. 3. Effect of superimposing injury by X-rays on injury by cold on eggs incubated for 0.8 hour at 14°C.

× Action on eggs chilled to 3°C. and X-rayed while chilled.

Curve A is the standard curve from my previous paper (1934).

Curves B and C are theoretical curves which are described in the text.

of development; thirdly, the figures show two theoretical curves (B and C). Curve B is a theoretical curve which expresses the number of eggs which would hatch after various doses of X-rays if the eggs which survived the cold showed the same variation in response to X-ray injury as the unchilled eggs. For instance Fig. 3 shows that 67 per cent. of the eggs survived the action of cold. The median lethal dose of the unchilled eggs is 100 r and if the response of the chilled eggs were the same, then 50 per cent. of the survivors would be killed by 100 r, and hence the total mortality would be $33 \times 67 \times \frac{1}{2} = 66.5$ per cent. and the survivors would therefore amount to 33.5 per cent.

Curve C is also a theoretical curve and expresses the percentage of eggs which would hatch if the injury produced by X-rays were added to the injury produced by cold. For instance Fig. 3 shows that 67 per cent. of the unirradiated chilled eggs survive. Fig. 3 also shows that 67 per cent. of unchilled eggs survive a dose of 73 r. Curve C in this figure has therefore been drawn on the assumption/

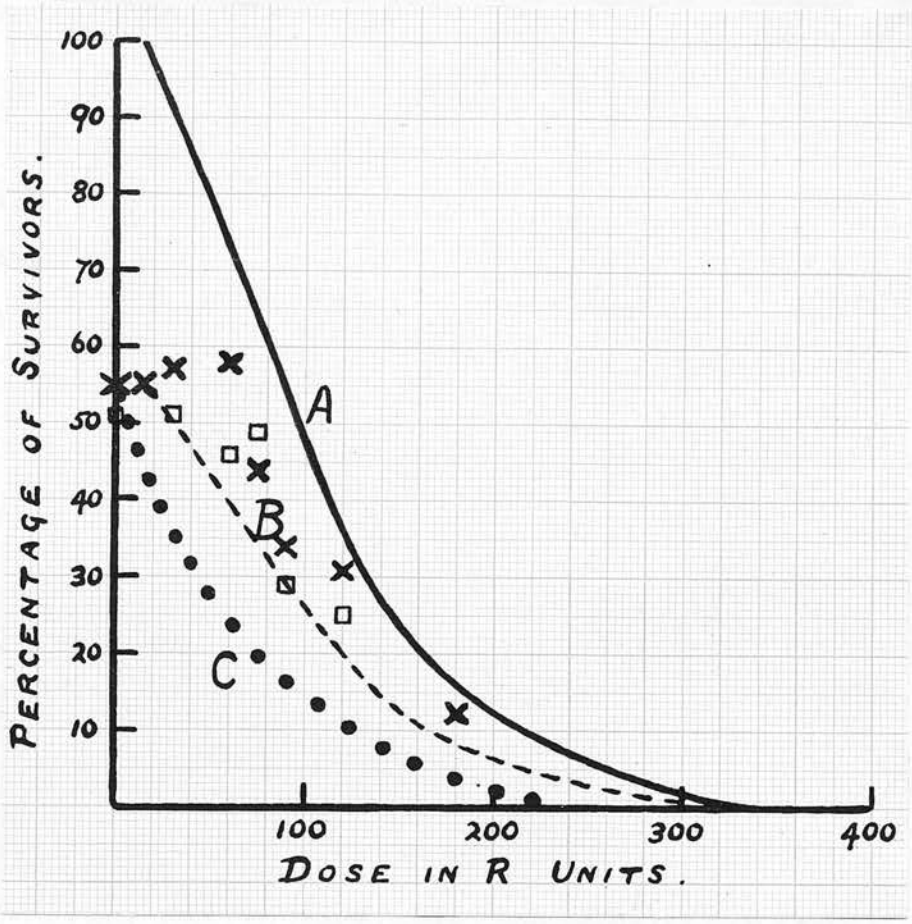

Fig. 4. Effect of injury by X-rays and injury by cold on eggs incubated 0.8 hour at 14°C.

× Action on eggs chilled to -5°C. and X-rayed while chilled.

□ Action on eggs which had been chilled to -5°C. and X-rayed at 14°C.

Curve A is the standard curve from my previous paper (1934)

Curves B and C are theoretical curves which are described in the text.

assumption that the effect of chilling was the same as the effect of a dose of 73 r. Hence the effect of a dose of 100 r to the chilled eggs is calculated as equal to a dose of 73 + 100 r to the unchilled eggs.

There is a general similarity in the position of the results of the experiments which have been plotted in Figs. 3, 4 and 5. In Figs. 3 and 4 they lie between Curves A and B, but in Fig. 5 they lie along Curve A. The results in Fig. 5, however, are derived from experiments on eggs incubated at 14°C. for 3 hours and my previous paper showed that at this stage of development the sensitivity of the eggs is changing very rapidly. This rapid change renders the results less reliable when eggs at this stage of development are used. Therefore while Fig. 5 shows general agreement with Figs. 3 and 4, the results recorded in Figs. 3 and 4 are probably more reliable.

In Figs. 3 and 4 all the points plotted fall between Curves A and B with one exception which falls on Curve B. This distribution at once eliminates/

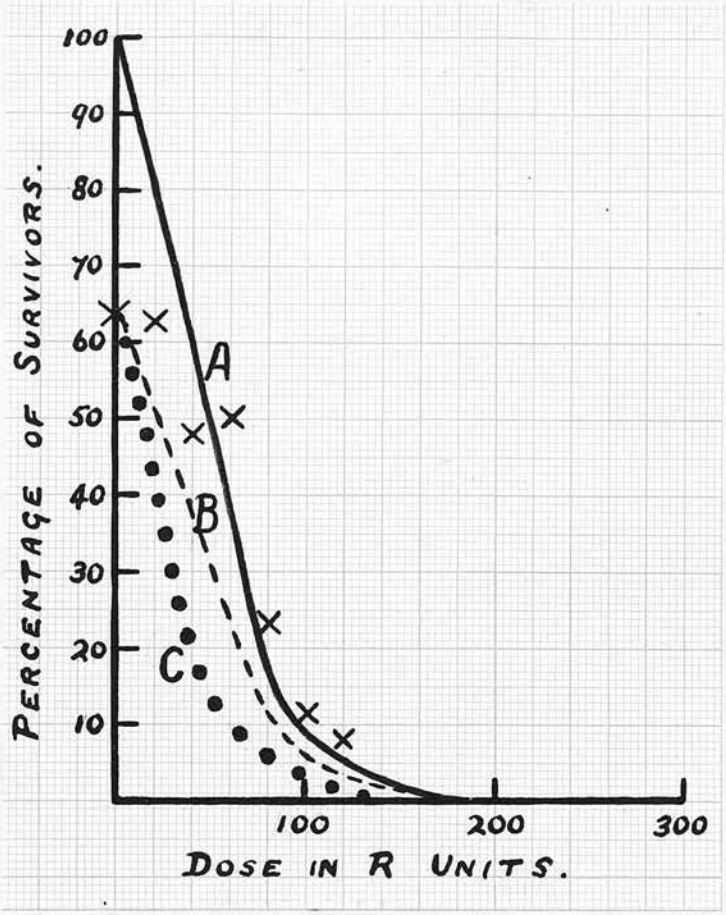

Fig. 5. Effect of injury by X-rays and injury by cold on eggs incubated for 3 hours at 14°C.

× Action on eggs chilled to -5°C. and X-rayed while chilled.

Curve A is the standard curve from my previous paper (1934).

Curves B and C are theoretical curves which are described in the text.

eliminates curve C as a possible interpretation of the results, but it is necessary to consider whether the results can be interpreted as falling along either curve A or curve B. The results were therefore analysed statistically by a method which will be described later, and the following conclusions emerged.

In the case of both Fig. 3 and Fig. 4 all the points except those plotted for a dose of 60 r are insignificantly different from points at a corresponding dosage in either curve A or curve B. The exceptional points, those plotted for a dose of 60 r in both figures, are significantly different from the corresponding points in curve B, but insignificantly different from the corresponding points in curve A.

The method of statistical analysis was essentially the same as that described in my previous paper. The averages of the various percentages of survivors to the doses of X-rays in the experiments with chilled eggs were compared with similar averages which occurred in the standard experiments where the eggs were unchilled.

The/

Table I.

Effect of cold on calliphorine eggs.

Stage of development of eggs at the time of chilling	Median lethal dose of X-rays at that stage (Scott 1934)	Temp. to which the eggs were chilled	Duration of chilling in hrs.	No. of eggs used	Percentage of fertile eggs which hatched
Eggs incubated		8°C	120	1541	100
at 14°C.		6°C	1	346	93
for		3°C	0.5	4711	69
0.5-1.0	100 r	3°C	1	977	64
hour		-5°C	0.5	1351	55
		-5°C	1	520	54
		-9°C	1	286	4
Eggs incubated at 14°C. for 2.8-3.3 hours	50 r	-5°C	1	1829	64
Eggs incubated at 14°C. for 11 hours.	340 r	-5°C.	1	2391	100

The statistic t (Fisher, 1932) was used in the manner I have previously described. If P_t was less than 0.01 the difference in the averages was assumed to be a significant difference and if greater than 0.01 the difference was assumed to be insignificant.

The theoretical curves were calculated from the standard curves and therefore the statistical data for these curves could be obtained from the individual results of standard experiments. For instance the individual percentages of survival after doses of 60 r in standard experiments were converted to terms of curve B by multiplying by 0.67 since 67 per cent. was the percentage of chilled eggs which survived.

Effect of X-rays on eggs which have survived injury by cold.

Eggs of 1 hour incubation at 14°C. were chilled to -5°C. for a period of one hour. Their temperature was then raised to 14°C. and after 5 or 10 minutes the eggs were irradiated. The results are recorded in Fig. 4 as squares. About

1200/

1200 eggs were chilled and subsequently X-rayed and about the same number were used as chilled control eggs. The results are not significantly different from the results of experiments in which the eggs were chilled to -5°C. and irradiated at that temperature nor do they differ significantly from corresponding points in Curve B. Fig. 4, however, shows that the results of chilling the eggs to -5°C. and subsequent irradiation at 14°C. are probably the same as the results of chilling to -5°C. followed by irradiation at that temperature.

DISCUSSION /

DISCUSSION

The results recorded in Fig. 1 show that the Bunsen-Roscoe Law holds for intensities over a 7.5 to 1 range. The literature concerning investigations of the validity of the law in relation to X-rays is very great and has been summarised by Quimby and Pack (1932). These authors reviewed a mass of contradictory evidence and eventually concluded that a greater biological effect was produced by high intensities. My results over so small a range of intensity as 7.5 to 1 are not incompatible with such a view. The conclusion which can be drawn from my results is that the method of measurement of irradiation which was used, namely the measurement of the time of exposure to a uniform intensity, was not influenced by a deviation from the Bunsen-Roscoe law in the experiments under discussion.

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The results recorded in Fig. 2 show the effect of varying the temperature of an embryonic tissue during its irradiation. The variation in temperature was from 14°C. to 8°C. in experiments recorded around Curve A, and from 14°C. to -5°C. in experiments recorded around Curve B. Such variations in temperature produce very great alterations in the metabolic rate (Uvarov, 1931) and therefore Fig. 2 shows that changes in the metabolic rate do not influence the sensitivity of cells to X-rays.

The results recorded in Fig. 2 also show that alteration of the rate of development of an embryonic tissue by variation of temperature actually at the time of irradiation does not influence the sensitivity of the tissue to the lethal action of X-rays. This result agrees with the conclusions of Ancel and Vintemberger(1927)who used the eggs/

eggs of frogs and of hens as their biological material and of Ancel (1927) who irradiated germinating seeds at various temperatures. Other investigators found that an increased rate of development at the time of irradiation caused an increased lethal action. This was the conclusion of Holthusen (1921), Dognon (1926) and Schinz and Zuppinger (1928) who worked with Ascaris eggs, and of Politzer (1925) who used a tissue culture of the salamander's cornea. Packard (1930) used the developing eggs of Drosophila which is a tissue similar to that which I used. Packard found that the median lethal dose of newly laid eggs was increased 1.46 fold if the temperature of the eggs at the time of irradiation was reduced from 28°C. to 13°C. This result of Packard's is open to criticism because he incubated newly laid eggs at 28°C. and at 13°C. for half an hour before irradiation and this would cause the eggs/

eggs of the two groups to reach different stages of development at the time of irradiation. The results of Henshaw and Henshaw (1933) suggest that the difference in sensitivity which Packard described was due to differences in the degree of development at the time of irradiation and was not due to the differences in the rate of development as Packard concluded.

Redfield and Bright (1919) found that the sensitivity of unfertilised Nereis eggs to X-rays was unaffected by changes in temperature, but Dognon and Piffault (1931) found the opposite result in the case of a culture of Paramoecium, a tissue which resembles unfertilised Nereis eggs in that it is not in a state of rapid development. The results of Dognon and Piffault, however, can be criticised because the damage which their tissue showed might have been ascribed, at least in part, to the action of heat.

There is common agreement that cells are most vulnerable to X-rays during miotic division (Mottram, 1913; Holthusen, 1921; Strangeways and Hopwood, 1926; Vintemberger, 1928). It might therefore/

therefore be argued that rapidly dividing cells would be more sensitive to X-rays because they would pass more frequently through the period of increased sensitivity. Such an argument is fallacious because with a large colony of cells each of which divides at approximately the same rate the percentage of cells at each stage of division will be constant for any one moment regardless of their rate of division. In the second place the cells would not be likely to pass through more than one process of mitosis during the irradiation because it has been shown that cells are inhibited from dividing by X-rays although those cells actually in process of division when the irradiation begins complete the process (Canti and Donaldson, 1926; Regaud, Lacassagne and Jovin, 1925; Juul and Kemp, 1933). My results therefore are not incompatible with the view that cells are more sensitive to irradiation during mitosis.

My results also show that the rate of development after irradiation does not affect the sensitivity of calliphorine eggs. This result does not confirm the finding of Packard (1930) who concluded that slow development subsequent to irradiation/

irradiation protected the irradiated eggs of Drosophila from injury. Strangeways and Fell (1926) found a similar effect in the case of the hen's egg. Clark (1933) has suggested that the biological effect of X-rays is the result of a chain of more or less complicated processes, namely a conversion of the physical energy of the rays into a chemical effect, a succession of chemical changes which result in a biological action. Such a sequence would be accelerated by an increase in temperature but there is no reason why the eventual conclusion should be altered. Fischer (1931) for instance found that the effect of varying the incubation temperature of a tissue culture after irradiation was to prolong the latent period in the case of low temperatures and to accelerate it in the case of high temperatures but not to alter the amount of the final injury. Packard and Strangeways and Fell explained their results by suggesting that repair processes occurred in the tissues and these processes were favoured by low temperatures.

Fig. 4 shows the results of experiments in which eggs which had survived injury by cold were irradiated/

irradiated. (The results are recorded as squares). These results lie between curves A and B. Curve B represents the rate of destruction which would occur if the survivors from cold were to have exactly the same sensitivity to X-rays as have normal unchilled eggs. Fig. 4 shows that the actual results fall to the right of curve B, and although there is not a significant difference between any of these plotted results and corresponding points on curve B, the fact that no results occur to the left of curve B suggests that the eggs which survive the action of cold are slightly more resistant to X-rays than are normal unchilled eggs. The difference is, however, small and there appears to be some correlation between the sensitivity of the eggs to the injury produced by cold and the sensitivity to X-rays. The table supports this view for it shows that as the eggs grow older there is an increase in resistance both to cold and to X-rays. The correlation is, however, not exact for after 3 hours incubation at 14°C. the eggs are slightly more resistant to cold than are newly laid eggs while their resistance to X-rays is actually at its minimum.

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The results recorded as crosses in Figs. 3, 4 and 5 show the effect of superimposing an injury produced by X-rays on an injury produced by cold. Curve C in these figures represents the theoretical mode of action if the two types of injury were to be added. The results obviously do not fall along this curve, and therefore it is concluded that the injury produced by X-rays is not added to the injury produced by cold. The results are still more conclusive in showing that the chilling does not in any way potentiate the action of X-rays.

Inspection of Figs. 3 and 4 shows that all the results lie between curves A and B and Fig. 4 shows that they are not significantly different from the results of experiments in which the eggs which had survived the effect of cold were irradiated at 14°C. It is concluded therefore that when the injury produced by X-rays is superimposed on the injury produced by cold the result is the same as when the injuries are produced consecutively and that sublethal damage produced by cold does not render the eggs more sensitive to X-rays.

SUMMARY.

1. The sensitivity of cells to the lethal action of X-rays is not related to the metabolic rate of the cells, neither to the rate of cell division during the actual irradiation.

2. Neither the metabolic rate nor the rate of development of an embryonic tissue, calliphorine eggs, after the irradiation, influence the sensitivity to the lethal action of X-rays.

3. The injury of calliphorine eggs produced by X-rays is not added to the injury produced by cold, nor do the two injuries potentiate each other.

4. The two types of injury, that produced by cold and that produced by X-rays, are distributed in a somewhat similar manner, but they are entirely unrelated to each other.

5. Over a 7.5:1 range of intensities the Bunsen-Roscoe Law is valid for the action of X-rays on calliphorine eggs.

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