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The Differential Action of X-Rays on Tissue, Growth and Vitality.—Part III. *The Biological Reaction to X-Radiation in Relation to the Area of Tissue Irradiated.**

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[Plate 19.]

The allantoic membrane of the embryo chick was exposed to homogeneous X-radiation obtained by crystal diffraction and a selective action was observed (Moppett, 1929). A particular wave-length 0·53 Å. produced a hypertrophic reaction with an exposure of ½ hour and an atrophic reaction in 1¼ hours and this was adopted as a standard in the investigation of problems other than selective action (Moppett, 1929 and 1930).

The Effect of Irradiating a Large or Small Area.

A variation in effect with the area irradiated was suggested because at an early stage reactions were readily produced with an imperfect crystal and it was found that the results could be imitated by placing the specimen at an oblique angle to the incident radiation. It appears that the imperfect crystal was "convex," spreading the rays out to a greater extent than usual and various aspects of the problem are illustrated by the following typical experiments.

Hypertrophy is represented by H and atrophy by A, and the transition from one to the other by placing the specimen at an oblique angle is illustrated by experiments 35 and 318 in which all other conditions are similar. In other cases the transition might be from no reaction to H, but there was always a considerable reinforcement and the general observation is well established as the results of some years' investigation. In experiment 218 atrophy was produced over an area of 1 × 4 cm. at grazing incidence and no limit to the above reinforcement has been found. In order to obtain further information,

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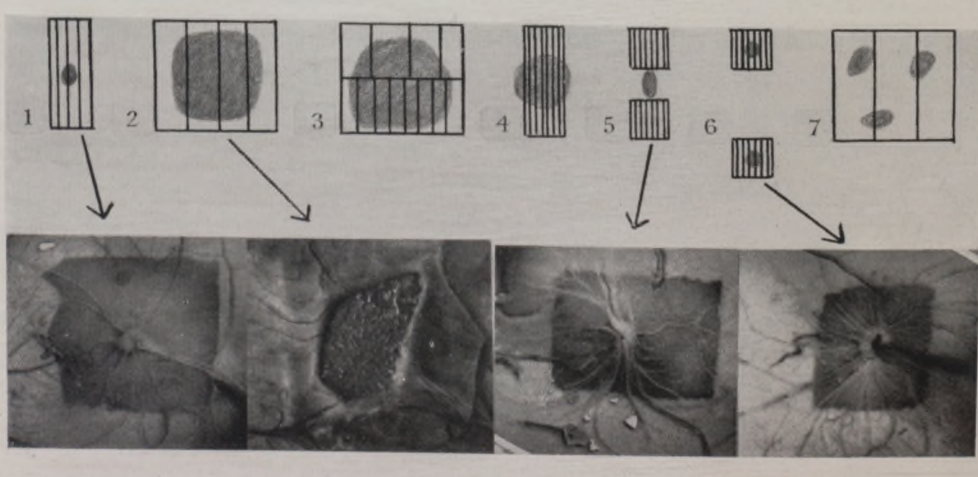
A

B

C



(*Facing p.* 303.)

experiment No. 111 was placed at an oblique angle, but the area irradiated was restricted (by a slit) and no reinforcement was observed. This result was obtained in two consistent experiments as indicated by the factor 2/2.

In experiment 96 a normal incidence was used similar to experiment 35, but the specimen was withdrawn to approximately twice the distance from the target and the reaction was A+ (R2). The area irradiated was multiplied by 4 in the above case and the general observation is well established within these limits.

In the case of 62 a large area was covered by moving the object continuously during exposure, and no reinforcement was observed in two consistent experiments, although one specimen showed two hypertrophic reactions (Plate 19, fig. 7).

The remaining experiments were carried out with a constant potential generator on the assumption that the intensity of any radiation should be proportional to the milliamperage. The threshold dose for hypertrophy at a wave-length of 0·27 Å. was accurately determined in terms of a current of 6 mA in connection with another investigation (Part II). The slit height was then reduced so that 6/10 of the original area should be irradiated, and in accordance with experiment No. 1067 the threshold dose was represented by 15 mA, the ratio being approximately inversely as the square of the area.

Table I.

Number of experiment.	Mean wave-length.	Slit A width.	Time of exposure.	Milli-amps.	Incidence on specimen.	Remarks.	Result.	Reliability.
	Å	mm.	hours					
35	0·53	1·5	2	4	Normal	—	H	Well-established
318	0·53	1·5	2	4	60°	—	A+	
218	0·53	2	2	4	Grazing	—	A+++	Single
111	0·53	2	2	4	60°	Slit B, 1 mm. wide	H	2/2
96	0·53	1·5	2	4	Normal	Distant 40 cm. from crystal	A+	Well-established
62	0·53	2	2	5	,,	Specimen was moved 1 mm. each ½ hour	H (double)	2/2
995	0·27	—	½	6	,,	Constant potential, full area	H	8/8
1066	0·27	—	½	12	,,	Constant potential, area 6/10	.	4/4
1067	0·27	—	½	15	,,	,,	H	4/4

Discussion.

When the allantoic membrane is placed at an angle ϕ to the incident radiation, the energy per unit area is reduced to cos ϕ times the energy at normal incidence. The allantoic membrane has an average thickness of 0·02 cm., and the total x absorption is very small so that cells in different parts will absorb nearly the same amount of energy whether the incidence is normal or oblique.

The above consideration will not account for the raising of degree of reaction or the reinforcement observed when the distance from the target is increased. In the case of an atrophic reaction one may postulate a greater interference with nutrition, since circulation proceeds from the periphery of the area under consideration.

In a discussion of the development of an atrophic reaction, it was supposed that all types of tissue are rapidly killed by the action aided by an interference with circulation due to a simultaneous injury to the blood vessels (Moppett, 1929).

The figures marked A, B and C (Plate 19) illustrate this. In A, a borderline stage between atrophy and hypertrophy is depicted as a commencing degeneration in the centre of a hypertrophied mass. In B, a central island of hypertrophied tissue remains, but the nutrition is cut off by the surrounding atrophy. Fig. C (Plate 19) is a high-power view showing a preliminary hypertrophy of endothelial cells in an area where atrophy is almost complete. An interference with nutrition will not entirely account for the state of affairs depicted in A, or in the case where a hypertrophic reaction is obtained at oblique incidence and no change at normal incidence. One must assume another factor, a summation of stimuli from adjacent parts and the relation of the threshold dose to the reciprocal of the square of the area irradiated suggests a force obeying some definite law.

General Aspects of the Area Factor.

It will be convenient to review the more general aspect of the above conclusions in terms of certain typical cases which represent the result of a very extensive observation, although it must be mentioned that the obtaining of "copper plate" specimens for reproduction is by no means easy.

1. A dose which is just above the threshold for hypertrophy produces a very small reaction, which is approximately circular and lies at what may be termed the "centre of gravity" of the irradiated area. The rectangle in

fig. 1 (Plate 19) shows the approximate size and form of a cross section of the incident beam of rays, and the dose is represented by the number of vertical lines per unit distance, while the reaction is shown in half-tone, and an actual photograph is also reproduced. In accordance with the supposition already made one may regard the reaction as due to a summation of stimuli from all parts of the irradiated area.

2 and 3. The next figure illustrates the reinforced action obtained with oblique incidence and an actual photograph is also reproduced, while fig. 3 (Plate 19) depicts the overflow to a region of inadequate dosage which was discussed in an earlier paper (Moppett, 1929).

4. If a normal incidence is used and the dose is increased, the area of membrane showing a visible reaction increases and may overflow beyond the irradiated area. This is a converse to the increased reaction which is associated with a larger area irradiated.

5. In fig. 5 (Plate 19) two discrete areas separated by a gap of 2 mm. were irradiated and the reaction was situated at the "centre of gravity" of the disturbance, although this region received no radiation apart from scattering. Three similar experiments were performed and the observation is well established by early unsuccessful attempts to produce a geometrical figure on the above lines.

6. If a sufficiently large gap is provided, discrete reactions can be obtained, and a photograph is given of an intermediate stage showing a dumb-bell shaped reaction. When small circular apertures were used, discrete reactions could not be obtained, but the threshold dose was found to vary inversely as the square of the distance between centres for the following values 3·5, 4·5, 5·5 and 6·5 mm.

The above facts preclude the production of geometrical figures on the scale of magnitude available.

Fig. 7 shows a patchy reaction which is occasionally observed when a comparatively large area receives a dose near the threshold value. There are presumably small variations in intensity or sensitivity over the area covered, and the double reaction recorded in Table I may be explained on this basis.

Experiments with Mixed Radiation on Mammalian Tissues.

The above laws were tested by means of mixed radiation on the assumption that it was unbalanced radiation (Moppett, 1930), having a component of force similar to that of homogeneous radiation. The skin covering the back of a rat was immobilised in a wooden frame by means of four stitches, and the hair was removed by sodium sulphide. A lead mask was used with four square apertures of various sizes, and the various doses tabulated below were given by means of a constant potential apparatus, the results being observed after an interval of 7 days.

Table II.

Dose.	Apertures.			
	$0 \cdot 25^2$.	$0 \cdot 5^2$.	1^2.	2^2.
Milliamp. minutes				
200	—	—	—	E slight
250	—	—	E slight	E
300	—	—	E	E
450	—	E	E	Covered
600	E	E	E	,,
450, 600	Killed when large aperture included.			

It will be seen that the erythema dose increased as the aperture diminished slowly at first and then more rapidly, and no doubt an inverse square law could be demonstrated if fixation was sufficient to justify the use of very small apertures.

To test the phenomenon of fusion a mask was prepared with 5 pairs of circular apertures, 0·25 cm. diameter and 3·5, 4·5, 5·5, 6·5 and 7·5 cm. apart. The discrete erythematous areas were readily identified and a connecting link of reddened skin was observed as indicated by the letter F in the following table.

Table III.

Dose.	Apertures.					(Fusion distance)^2.
	3·5.	4·5.	5·5.	6·5.	7·5.	
Milliamp. minutes						
675	F	—	—	—	—	1·4
1080	F	F	—	—	—	1·3
1600	F	F	F	—	—	1·3

Conclusions.

There is ample evidence that the threshold dose for a biological reaction varies as the area irradiated both when the allantoic membrane is exposed to homogeneous radiation and the skin of the rat to mixed radiation. This result, together with the phenomenon of fusion, may be explained in part by a summation of stimuli from distant parts in such a manner that an inverse square law is manifested when the area irradiated becomes small. It is to be observed that distant regions may be affected even if an area under consideration shows no visible change. The mechanism of remote action is therefore not concerned with the production of visible changes, such as hypertrophy and atrophy, but with an intermediate stage which follows the absorption of quanta and presumably the production of ionised atoms. The present discussion strengthens the supposition made in connection with prior action that the biological change is due to the production of ionised atoms (Moppett, 1930). It is reasonable to suppose that the threshold dose would become enormously great if a very small area involving very few atoms was affected and the law may presumably be extended to three dimensions.

Prof. V. A. Bailey has kindly pointed out the following consequence of the supposition that in the production of a particular biological change the following law holds, $IA^2 =$ constant, when I is the intensity of the radiation and A the area.

If N represents the number of ions (of one sign) produced in the area A, N is proportional to IA and the above equation becomes $NA =$ constant.

In a similar manner, if there are N_1 other factors (ions of opposite sign, cells, etc.) involved in the area then N_1 is proportional to A and so $NN_1 =$ constant. The inverse square law may, therefore, indicate that the reaction requires the co-operation of two different factors (ions, etc.) to produce a definite number of transactions each involving one factor of each kind.

One must recognise a purely biological overflow in the general rounding off of inflammatory processes, but in the present instances it appears best to regard gross biological changes such as hypertrophy and atrophy as distinct phenomena which somewhat complicate the interpretation of effects depending on the atom.

REFERENCES.

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