(*g*) To cover the syllabus required for a General Science course in the first three years of secondary education, we consider that the allotment of periods should be as follows:—

Physics	3 periods (minimum)
Chemistry	2 periods
Biology	2 periods

We further suggest that the Biology periods be equally divided between zoological and botanical science.

(*h*) These four sections of Science (Physics, itself of many sections; Chemistry, Zoology, and Botany) are surely as important as, and are worthy of as much time for study as a single language, which is now allotted six or seven periods a week, or mathematics, which is allotted seven or eight periods a week.

Ten copies of the document embodying these resolutions were sent to the Secretary of the Commission, and, subsequently, at his request, ten further copies were made available for distribution to committees.

X-RAYS AND THEIR BIOLOGICAL EFFECTS.

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(*Continued from last issue.*)

General Biological Effects.

Before proceeding to a consideration of the more precise apects of quantitative radio-biology we will note a few general results, selected from the extensive literature, which may be of some interest and serve to convey some idea of the general effects of radiations on living organisms. Some of the earliest systematic observations of the effects of X-rays upon developing forms of animal life were made and recorded by Perthes in 1908. He exposed the eggs of ascaris under certain conditions (which he described), and found that the general appearances of the irradiated eggs fell into two divisions accordingly as they were more or less severely damaged by the exposure. In the more severely damaged class are those eggs which do not proceed to the formation of a recognizable embryo, but remain as a group of cells abnormally arranged and irregularly sized. In the other class the eggs produced monstrous embryos partially resembling normal forms, but presenting irregular cell masses ; sometimes the irregular mass would occupy the position of the tail of the embryo—the head being normal, and *vice versa.* None of the eggs were killed outright, but underwent division to a greater or less degree, producing the monstrosities referred to, which were of low vitality.

In 1905 Bordier studied the effects of X-rays on ordinary silkworms. The main differences between the experimental and control animals (*i.e.*, non-irradiated animals) were that the experimental showed (1) increased restlessness, (2) smaller size, and (3) darker colour. The spinning occurred five days later in the controls, the cocoons were only half the normal size, and the moth did not emerge. On opening the cocoons four out of six chrysalides were found to be dead.

Hasebroeck irradiated caterpillars and found that, while, with the dose employed, they developed into butterflies, the power of flight was entirely absent.

In 1904 Gilman and Baetjer studied the effects of X-rays on the development of the chick. Developing eggs of the domestic fowl were exposed to various doses of radiation, and they observed deformities in the head, inhibited development of the eyes, and distorted limbs projecting from the body in various eccentric ways.

In 1911 Gaskell performed experiments of a similar nature and found that the process of cell division in the developing embryo was definitely retarded. This was a most significant observation, and modern quantitative radio-biology is essentially concerned with the effects of X-rays on the rate of cell division. Particularly is this the case in the radio-therapeutic treatment of malignant conditions (cancer) in man. Cellular division or mitosis, *i.e.*, that process by which one cell splits into two new individuals, both similar to the parent cell, is particularly active in cancerous growths, and probably the earliest effect of radiations is to inhibit cellular division. This means, of course, that the growth of the cancerous mass is inhibited.

The effects of X-rays on seeds and plants have been studied by various authors. Fabre (1911) cites the case of a flowering lily which was irradiated with a dose sufficiently strong completely to arrest development.

A considerable amount of work has been done upon the effects of X-rays on bacteria, and it is found that most of the common disease producing forms are highly resistant to the rays. On the other hand, it often happens that an ulcerated surface, the seat of bacterial infection, when given moderate doses of X-rays, shows a beneficial reaction indicating that the growth of the bacteria has been hindered, or even completely arrested.

Numerous investigators have studied the effects of X-rays on the blood. If an animal, such as a rat, rabbit, guinea-pig or dog, be irradiated *in toto*, profound changes occur, and with a sufficiently long exposure death may quickly ensue. Helber and Linsen, soon after the pioneer work of Heinike, exposed rats, rabbits and dogs with a view to detecting changes in the blood. The outstanding feature of their work was to show that the general effect of prolonged exposure to X-rays is to produce a diminution in the total number of white cells.

We will conclude this qualitative discussion by stating certain general propositions which have been gleaned from the multiplicity of investigations similar to those considered :

(1) All living cells, normal or pathological, are sensitive to the action of X-rays.

(2) This sensitivity for the same amount of absorption varies from one type of cell to another ; and also, to some extent, for the same type of cell from one animal to another.

(3) According to the amount of energy absorbed the function of cellular division (mitosis) may be inhibited or permanently destroyed.

(4) The sensitivity of a cell is a function of its age.

It is possible by the use of X-rays to destroy certain types of cells as though by a surgical operation of surpassing delicacy ; we can reach within the cell and effect changes, especially in the nucleus, and it seems that we can change the genes and also inheritance.

We will now pass on to a consideration of some quantitative aspects of radio-biology and will commence with a brief study of the living cell.

The Cell.

The cell is the fundamental unit of living matter, and the mysteries of life lie hidden within these units. A group of cells constitutes a tissue, a combination of tissues constitutes an organ. The function of any organ is the sum of the activities of its constituent cells, and the metabolism of a complex organism is the sum of the changes in the cells that comprise it. It is logical, therefore, to study the functions and activities of the structural unit in order that one may apply the knowledge so gained to the more intricate problems of the body as a whole, in health and in disease.

The living cell in size may vary from structures only discernible through high-power lenses, to large eggs distended with yolk ; in shape it may be spherical, oval, polygonal, or assume thread-like or other shapes ; within these cells there is a substance of a distinctive nature called protoplasm, which is found nowhere else. Protoplasm is not a definite chemical compound, but rather is it a varying mixture of chemical compounds. The cell is by no means a simple structure, for within it we find an organ called the nucleus, one or more nucleoli, *et cetera.*

A diagrammatic illustration, showing the essential features of a cell very greatly magnified, is given in Fig. IV.

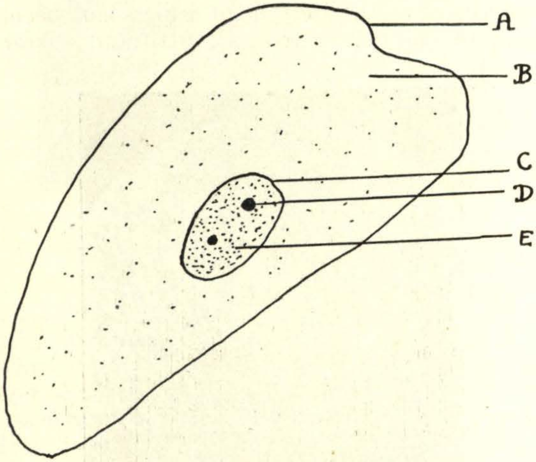

Figure IV.
A, cell membrane; B, cytoplasm; C, nuclear membrane; D, nucleolus; E, nucleus.

Viewed from the physico-chemical viewpoint the living cell is a peculiarly constructed energy machine or transformer through which a continual flux of energy ceaselessly goes on, and the entire life of the cell is an expression of the variations and alterations in the rates of flow of energy and changes in equilibrium between the various types of energy. In other words, the most striking characteristic of living organisms is the perpetual state of change which they exhibit. There is a building up of matter and a breaking down of matter. If the building up process predominates over the breaking down process the organism grows; if the two processes just balance, the organism exists, and if the breaking down process predominates the organism tends to disintegrate.

This brief introduction to quantitative radio-biology will serve to indicate the nature of the system with which we are dealing, and it will be conceded that in the organic world the conditions are more complex than those with which the pure physicist has been used to deal.

A great advance in our knowledge of quantitative radio-biology coincided with the development of the "tissue culture" technique. The earliest attempt to grow animal cells and tissues in a glass vessel appears to have been made by Leo Loeb. He noticed that certain cells would grow upon clotted blood, and in 1902 succeeded in growing epithelium on the surface of a blood clot contained in a glass vessel at the bottom of which had been placed a fragment of kidney. Burrows (1910) and Carrel (1911)

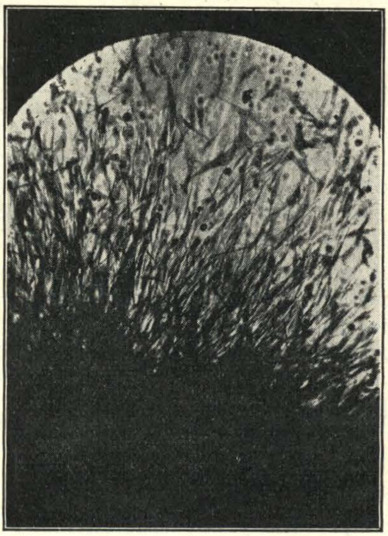

Figure V ($\times$ 150 approx.).

improved upon this method, and finally succeeded in maintaining growth and cell division for an indefinite period.

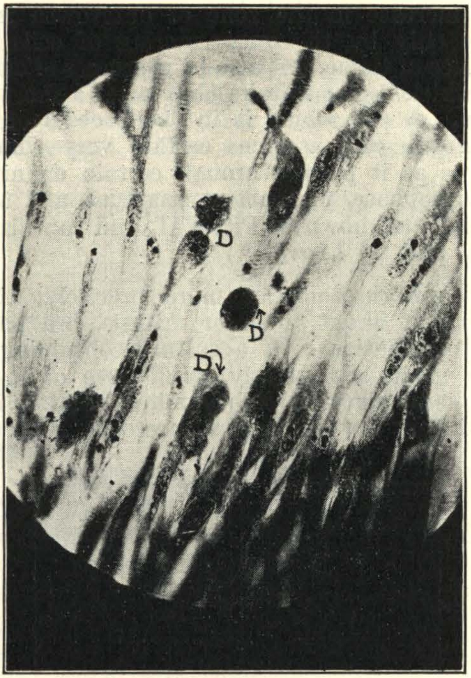

Figure VI.
D = Dividing or Mitotic Cells (× 1,000 approx.).

In the " hanging drop " technique a fragment of tissue is planted in a drop of nutritive medium upon a cover glass which is then inverted over a hollow-ground glass slide and sealed down with melted paraffin wax. The whole procedure is carried out under strictly aseptic conditions, and the preparations are incubated at the normal temperature of the animal from which the tissue was taken. After some time the first signs of growth appear and a thin film of cells gradually forms around the original fragment. It is possible by this means to preserve a tissue in a state of life and growth over very long periods. The cells in the zone of outgrowth (*i.e.*, the thin film of cells surrounding the implant), can be examined under the microscope with the greatest ease, and we thus have at our disposal a method of observing the direct effects of radiations on living cells. Photographs of living cells growing *in vitro* (*i.e.*, on glass slides) and magnified some hundreds of times are shown in Figs. V and VI.

Now increase in the zone of outgrowth is brought about not only by the outwandering of cells from the original piece of tissue, but also by a process of cell division (mitosis) which begins to take place after a short period of incubation. A cell which is in the process of dividing[1] (*i.e.*, a mitotic cell) assumes certain very characteristic appearances as it passes through certain definite phases, known as prophase, metaphase, anaphase and telophase; these phases are shown in Fig. VII, and can also be seen in Figs. V and VI above.

These mitotic cells, or more precisely, those cells which are just about to enter mitosis, are particularly sensitive to radiations. If a culture containing plentiful mitoses is given a relatively small dose of X-rays and examined some sixty to eighty minutes later, it will be found that very few mitoses are present.

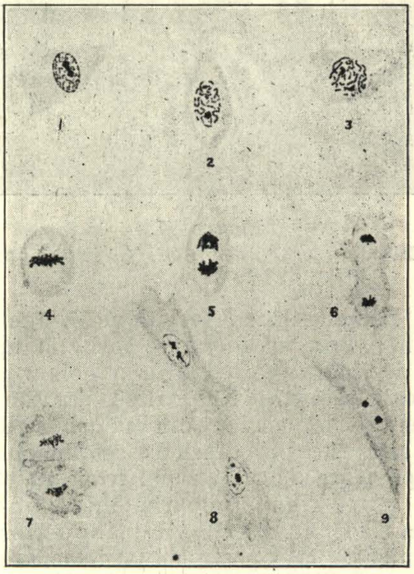

Figure VII.

1, 2, 3, prophase; 4, metaphase; 5, anaphase; 6, 7, telophase; 8, daughter cells; 9, mature daughter cells.

[1] We are not concerned in this article with divisions other than mitotic.

The mitotic cell thus provides an ideal indicator of quantitative biological reaction to a given X-ray treatment ; the quantitative effect is usually expressed as a percentage survival, which is the percentage ratio between the number of mitotic cells present after treatment, and the number originally present. The notion "percentage survival"

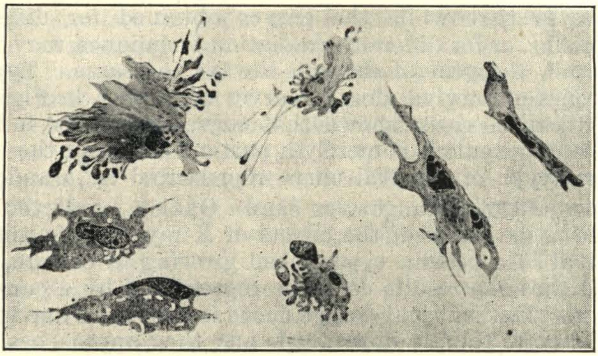

Figure VIII.

is of fundamental importance in quantitative radio-biology, and its use is, of course, not restricted to problems relating to mitotic cells in tissue cultures. For example, let us consider the irradiation of a large number of very small eggs, such as those of *ascaris* or *Drosophila*. The percentage survival in this case is the percentage ratio between the number of eggs which hatch out after irradiation and the number originally present. In this simple definition it is assumed that all the eggs originally present would have hatched out had they not been irradiated. Again we may take as our criterion of biological effect the number of cells which have been completely disintegrated by a given dose of X-rays ; in this case the percentage survival simply measures the percentage number of cells that have not suffered complete disintegration. These completely disintegrated cells are known as "breaking down" cells, and are always associated with very large X-ray doses. Typical breaking down cells are shown in Fig. VIII, and it will be seen that they form a striking contrast with the normal mature cell, 9, in Fig. VII.

The Survival-Dosage Curve.

A survival-dosage curve is simply a curve showing the relation between percentage cellular survival and the dose of radiation administered for a series of dosages. Such curves have been obtained for a large number of cells, tissues, organisms, *et cetera*, and the study of these curves has made a material contribution to the elucidation of the fundamental problems relating to the X-ray treatment of malignant disease (cancer) in man.

The first significant feature in regard to these survival curves is that while the curves obtained for different organisms, or for different irradiation techniques, may vary in detail, the general shape is always the same. Typical examples of survival dosage curves are shown in Fig. IX, and it can be easily shown that curve *a* which is, in fact, simple exponential, is merely a particular case of the more general type of survival curve represented by *b* and *c*.

In 1923 Strangeways and Oakley undertook a quantitative study of the effects of X-rays on the mitotic survival of a certain type of cell growing in culture, and found that the results could be represented by a curve of type *a*. This survival dosage curve is seen to be one which first plunges rapidly downwards and then approaches zero very slowly indeed for very large doses. This means that the rate at which cells are passing into mitosis in these irradiated tissue cultures is decreasing exponentially with the dosage ; or, in other words, the action of X-rays on the cell which produces incapacity for entering mitosis follows a law of probability, and the probability can be shown to be identical with the probability that a certain structure or organ within the cell would actually be affected

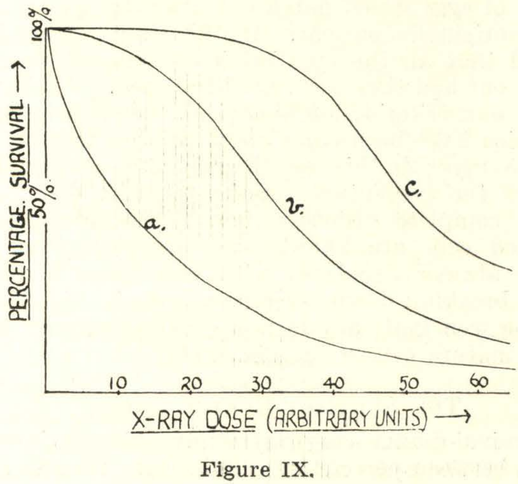

Figure IX.

by the X-radiation. An analogy may help to make the matter clearer. Suppose that we are firing at a swarm of midges with a machine gun. The number we should hit per second would be proportional to the number in the swarm. At first we should claim a large number of victims, but as the swarm gradually melted under our fire the chance of hitting a midge would become smaller and smaller. To hit the last two or three would entail the expenditure of much ammunition and considerable patience. The survivors, however, would not owe their prolonged existence to any biological factor or to any immunity, either inherent or acquired, but simply and solely to their good luck. The number of survivors would, in fact, decrease exponentially as the number of bullets fired into the swarm increased.

We have already considered the analogy between a beam of X-rays and a fusillade of bullets and, in addition, have learnt that the initial effect of a beam of X-rays on matter is the expulsion from some of the atoms of a high speed electron. Now the X-ray dosage per minute that was administered to the cultures in the experiments of Strangeways and Oakley was such that an individual atom would be ionised about once in a million years, and to explain the results it becomes necessary to assume that each cell contains some particular sensitive zone or organ of mass m (*i.e.*, a group of atoms), and that the cell will be unable to pass into mitosis if an ion is formed within this structure. The probability that an ion will be formed within a given particle increases with the size of the particle, but yet may be quite small even for particles sufficiently large to be visible under the microscope.

A simple mathematical calculation shows that for such a structure the mass m would be about $3 \cdot 5 \times 10^{-14}$ grms., and that the diameter of the structure would be about $^1/_{2500}$ millimetres. The diameter of the cell employed in these experiments was about $^1/_{100}$ millimetre. Dr. Strangeways suggested that the particular structure indicated by calculation could be identified with the centrosome, an organ within the cell which is known to play an important part in the phenomenon of mitosis.

The establishment of this result gave a great impetus to the study of quantitative radio-biology, and in the hands of such investigators as Holwerk, Lacassagne and Zuppinger the subject progressed rapidly.

It was soon found, however, that the simple exponential relation was rather exceptional, the more general relation between percentage survival and dosage being represented by curves of types *b* and *c*. This type of curve indicates that practically no effect is produced until the dosage reaches a certain value, after which the number of cells affected increases rapidly (*i.e.*, the percentage survival decreases rapidly), and a few cells always survive much larger doses than the average.

In 1926 Professor Crowther made the very logical and fruitful suggestion that more than one hit may be necessary to put the sensitive organ of the cell out of action; and working on the theory of probability he deduced a mathematical expression for the number of particles which have been hit less than n times, and thus calculated the number of survivors if n hits are required to put the particles out of action. The expression takes the form

$$S=N\left\{1+\frac{\lambda q}{\underline{|1}}+\frac{(\lambda q)^2}{\underline{|2}}+\ldots\ldots\frac{(\lambda q)^{n-1}}{\underline{|n-1}}\right\}e^{-\lambda q}$$

and connects the number of survivors S with the total number of irradiated cells N, the chance of one hit λ, the number n of hits required to put the particle out of action, and q the dose of X-rays.

Experiments were made on a pure culture of the protozoan *Colpidium colpoda*, and it was found that this organism requires a succession of forty-nine hits on some particular structure before it is killed by X-rays.

It will now be seen that the above relation reduces to the simple exponential

$$S=Ne^{-\lambda q}$$

when only one hit is required to put the organ out of action. It is clearly a special case of the more general equation.

The significance of survival curves has been considered by other authors, particularly by Packard, and the view that the living cell contains a sensitive zone is not universally accepted. It seems, then, an alternative interpretation may be possible, but whatever the ultimate result may be, it is certain that the survival curve is represented by the general equation given above, and this equation has formed the starting point of many important researches and provides the basis of modern quantitative radio-biology.

A LEVEL SURFACE.

PORTION of a question in the last (1933) Intermediate Certificate Examination in Physics (N.S.W.) called for the definition of "a level surface", and apparently some Science teachers in schools have