CHAPTER VI

ANALYSIS OF THE MITOGENETIC EFFECT

At the present time, the mechanism by which short ultraviolet rays affect living cells is not understood. This chapter does not offer one theory, but presents a number of attempts to account for the various phenomena observed.

These rays were not discovered by chance. From a certain rhythm observed in the division of the sperm cells of amphibia, and in plant roots after special treatment, GURWITSCH (1922) predicted a factor which controlled cell division. From the mode of action, he concluded that this factor could not be chemical, but must be of a physical nature, and in 1923, he succeeded in proving it with onion roots.

Mitogenetic radiation was considered at first merely from the cytological viewpoint, as an emanation produced somehow in the very complicated process of cell division. Only after 1928, when SIEBERT proved this radiation to be emitted also from purely chemical oxidations, the physico-chemical viewpoint entered into consideration. The theories which were developed during the "biological stage" of the discovery have never been fitted completely into the physico-chemical facts observed later. Hence, we lack a clear conception of the working mechanism of these rays.

GURWITSCH has always distinguished between the "*Ureffekt*", the primary effect which is the increase in the number of mitoses, and all other effects of secondary importance. All of GURWITSCH's speculations and explanations start with the results obtained with onion roots; these were the first detectors, and since he made a large number of experiments with them, they are to him probably the most familiar of all detectors.

However, they have the great disadvantage of not offering perfect controls. The number of mitoses in different roots even from the same bulb varies greatly. The customary method is to

use the shaded, unexposed side of the same root as control; but we cannot be at all certain that irradiation of one side does not influence the cells on the opposite side as well. In fact, REITER and GABOR claim that they are affected.

The authors of this book who have made no experiments with onion roots, but are familiar with yeasts and bacteria, prefer to start with these simple, unicellular forms as the first objects for an attempt to interpret the primary mitogenetic effect. The best method for this purpose is the yeast bud method by TUTHILL and RAHN (p. 68) where all cells are of the same age; no secondary radiation from older cells complicates the analysis, and the percentage of buds is a true measure of the rate of cell division.

Any interpretation of the mitogenetic effect should account at least for the most remarkable facts observed. The following have been selected as the most important:

(1) The necessity of a particular physiological stage of the cell.
(2) The relation between the intensities of radiation and of effect.
(3) The minimal intensity required for an effect.
(4) The harmful effect of over-exposure.

A. THE NECESSITY OF A PARTICULAR PHYSIOLOGICAL STAGE

This necessity will not appear improbable to a cytologist. The cells of growing tissues are morphologically and chemically quite different from the old, resting cells of the same tissue. This holds not only for the larger plants and animals, but also for cultures of yeasts and bacteria (see e. g. HENRICI, 1928).

When any cell changes from the stage of active cell division to the resting stage, this is caused by some external or internal factors. These factors must be removed, or changed, before old cells can divide again. With unicellular organisms, rejuvenation is brought about by transferring the old cells to a fresh medium. The old cells need from one to several hours before they are "rejuvenated", i. e. able to multiply at the normal rate. This period of adjustment is called the lag phase (see p. 67). In multicellular organisms, old cells can be induced to cell division by wounding, or by unknown outside stimuli as in the case of gall formation in plants, or neoplasma in animals.

It would not appear probable that a resting cell can be induced to a new cell division by a short, weak irradiation. Though we do not really understand physico-chemically the ageing process of a cell, it does not seem likely that the factors which induce ageing could be removed by irradiation. This is borne out by experiment. Mitogenetic effects are not, as a rule, observed with old cells left in an old environment.

The stage of active cell division, does not appear very favorable either for mitogenetic effects. WOLFF and RAS (1932) make the unrestricted statement that mitogenetic effects can be obtained only during the lag phase, but not later, i. e. not during the phase of constant growth rate. Their experiments support this claim. They explain it by the assumption that the rapidly multiplying cells irradiate one another, and being so close together, their own radiation is stronger than that from any external source, which is necessarily weakened by distance and absorption.

If this explanation were correct, such cultures should react to outside irradiation at low temperatures where the rate of metabolism, and consequently the intensity of radiation, is weak; they should also react to an external source when they are widely dispersed so that the cells are far apart. The latter was tried without success by FERGUSON and RAHN (1933). Cultures of *Bacterium coli*, 24 hours old, never reacted upon irradiation, whether exposed as such or diluted 1 : 10000, while older cultures gave very pronounced effects. The fact that actively dividing cells do not respond readily to mitogenetic rays is thus verified, but the explanation by WOLFF and RAS is doubtful.

With yeasts as well as with bacteria, the stage of rejuvenation, the lag phase, is one of strong response. Very striking are the results of TUTHILL and RAHN (Table 21, p. 68) where the yeast produced buds very rapidly when exposed within half an hour after being transferred to the fresh nutrient medium, but failed to respond an hour later, though the control had not as yet started to produce buds. There seems to be one stage during rejuvenation when the cells are most susceptible.

The explanation may be cytological, chemical or physical. It may be that but one mitotic stage can take advantage of the energy introduced into the cell by this radiation. Perhaps, a certain chemical process in the rejuvenating cell is greatly stimulated; e. g. the ultraviolet, by means of a chain reaction, might

set up the reduction potential necessary for normal cell functions (light the candle which then keeps on burning as long as the cell feeds normally). Or, possibly, the cell wall becomes transparent to these rays only at one certain stage of development. Whatever be the explanation, it must be kept in mind that so far, the later stage of active cell division does not seem to be greatly influenced by these rays. It appears that the difference between rejuvenation and active cell division might give us the clue for the mitogenetic effect.

There seems to be another stage where cells respond, namely immediately before entering the resting stage. The description of the physiological condition of BARON's yeast plate (p. 66) suggests this strongly. The volumetric method as described by KALENDAROFF (p. 73) appears to make use of this stage, and so does HEINEMANN's hemacytometer method (p. 72). Apparently, the cells, at the point of going to rest, are stimulated to at least one more cell division by irradiation. This may also be the cause of the mitogenetic effect in onion roots (see p. 129). This need not necessarily involve a mechanism different from that assumed in the rejuvenation process. It may well be that in the ageing cell, the additional, properly dosed energy from the sender prevents a certain phase of the ageing process, for a short time, sufficiently long to permit one more cell division. This may be the same mechanism which, under the more favorable conditions of rejuvenation, is stimulated so greatly.

B. RELATION BETWEEN THE INTENSITIES OF RADIATION AND OF EFFECT

It has been one of the most annoying puzzles of mitogenetic experiments that there seemed to be no proportionality between cause and effect even when polarisation is excluded. We could not expect this with detectors involving secondary radiation by old cells, such as the onion root, BARON's yeast plate, or the volumetric yeast method. But even with the yeast plate of TUTHILL and RAHN, where mutual cell influences are practically excluded, the percentage of buds was not at all proportional to the length of irradiation time. When freshly prepared detector plates were exposed for different lengths of time, the following percentages of buds were found (after 2 hours of incubation):

exposed for . .	minutes 0	10	20	40
	%	%	%	%
through quartz .	21.0	25	36.5	24.5
direct	22.5	23	37.5	27.5

The sender was a 6 hours old yeast surface culture. The exposure of 20 minutes produced a strong effect, either directly or through quartz, 15% more than the control. If there were proportionality, the 10-minute exposure should have produced an increase of approximately 7%, and the 40-minute exposure a 30% increase. Neither of these other times showed any great effect, however.

This may be explained by the recent discovery of WOLFF and RAS (p. 43) that nutrient media produce secondary radiation when they have been in contact with microorganisms. The entire detector plate begins to emit radiation as soon as it is exposed to a sender. The intensity of secondary radiation does not depend so much upon that of the primary source as upon the chemical composition of the medium. All hope for proportionality must be given up in this case. Only with a medium which does not produce secondary radiation, does a biological measurement of intensity seem at all possible.

C. THE MINIMAL INTENSITY REQUIRED FOR AN EFFECT

All measurements of the intensity of mitogenetic rays are very inaccurate, but the order of magnitude of the strongest senders appears to be about 100 to 1000 quanta per cm^2 per second. The detector plate by BARON is completely covered with cells, but that of TUTHILL and RAHN has single cells. The probability that a yeast cell of $6 \times 7\ \mu$ is hit in one second, assuming 1000 quanta/cm^2/sec, is

$$P = 0.000\,000\,42 \times 1000$$
$$= 0.000\,42$$

The probability of being hit in one minute is 0.0252. It will require 40 minutes of continuous, uniformly dispersed radiation before each of the yeast cells is likely to be hit by one quantum of ultraviolet light. Since we find the strongest effect under this

arrangement at 20 minutes (see above), it would seem that one quantum per cell is sufficient to produce the mitogenetic effect. There has been a good deal of speculation as to the mechanism by which one single quantum could affect the cell so greatly.

However, since certain solutions such as blood serum, or broth in which bacteria have lived or are living, will produce secondary radiation, the assumption of a single quantum acting upon the cell has become unnecessary, even improbable. The raisin agar upon which the yeast is spread will gradually become transformed into a secondary sender by the very presence of the yeast. Irradiation will then set the entire mass of agar radiating, and the number of quanta thus produced, or absorbed by the cells, cannot be estimated.

It is known that yeast cells, or onion root cells, or pulp of tissues, yield secondary radiation; it seems safe to assume that living protoplasm generally will respond in this way. Then, if the cell absorbs one or a number of quanta of ultraviolet, the entire cell begins to radiate, not visibly, but measurably. This induces a state of excitement, and it is quite probable that a more rapid cell division may be brought about, provided that the cell is at the proper cytological stage. Possibly, the synthetic powers of the cell work to a certain morphological and physiological culmination which can be released only by a very accurately measured impulse, i. e. the absorption of one quantum of ultraviolet of fairly definite wave length. Considering the systematic arrangement of all molecules in the cell, it can be well imagined that such a release will start many wheels turning, many processes going on automatically and exothermically, until cell division is completed.

This is, in slightly different terms, GURWITSCH's original conception of the mechanism of the mitogenetic effect. He claimed, and seems to assume even now that no cell division is possible without this external, ultraviolet stimulus. It would appear that single-cell cultures of bacteria and yeasts were a proof against this assumption, but they may be explained in some other way.

One fact, however, makes the above explanation too simple. Every fermenting yeast cell liberates, within the cell, energy of definite mitogenetic wave lengths, namely of 1900, 1910, 1930, 1950 and 2170 Å (p. 37). If all yeast cells produce this wave length, how can cells be stimulated by the same wave lengths from

an external source? We may return to the first of our fundamental facts that only at a certain cytological stage, cells will react to mitogenetic radiation. No reaction has been observed at the stage of most active multiplication which is also that of most active metabolism. A very definite and strong response was obtained at the first stage of the rejuvenation process. If we could make the assumption that during the period of sensitivity, the cells show no metabolism, or at least emit no ultraviolet, then the mitogenetic effect could be easily explained. But the assumption is not justified. RAHN (1928) and RAHN and BARNES (1932) found that old yeast cells, compressed baker's yeast as well as beer yeast stored for several weeks at low temperature, fermented strongly within 10 minutes after being placed in sugar solution.

Whatever the explanation, it is certain that the mitogenetic effect does not occur merely through the increase in energy content of the cell.

D. THE HARMFUL EFFECT OF OVER-EXPOSURE

The harmful effect of over-exposure is more easily understood by the secondary radiation of the cell and of the medium. Before this was found, the stimulating effect which the first quantum had produced, appeared to be counteracted by the absorption of a second quantum. Now we realize that the first as well as the second quantum are probably multiplied manyfold within and outside the cell.

It has been shown (p. 43) that too long exposure destroys the ability of a solution to produce secondary radiation. After a day or two of rest, this property returns. Nothing is known about the chemistry involved, but the assumption of an equilibrium, disturbed by irradiation and slowly re-established after discontinuance, fits best into our present conceptions of life functions.

Something similar to these effects may happen in the cell. We have already seen that very likely they are all capable of secondary radiation. They also will become exhausted upon long-continued exposure (p. 110). Moreover, it has been shown that recovery is slow. If we assume that all cells are brought to a state of radiation, or excitation, but that only those cells which

are at the proper cytological stage can respond to this stimulus by dividing more rapidly, the cells of other stages will soon become exhausted. This would mean at first a normal rate of cell division, and eventually a temporary interruption of mitosis, on account of exhaustion of certain chemicals in the cells, by the prolonged secondary radiation.

Since exhaustion of solutions lasts for a day or two (p. 43) and exhaustion of cells for hours (p. 110), it would be difficult to explain the periodical alternation of stimulation and depression observed with long-continued irradiation by SALKIND (p. 116). The data of WOLFF and RAS (Table 25, p. 77) also seem to indicate recovery from depression though irradiation is continued.

The time during which mitogenetic effects can be observed seems to vary with the detector. The sharpest limitations observed are those by WOLFF and RAS (Table 12, p. 44): strong positive effect with 5 minutes exposure, none whatever with 4, 6, 7 or 8 minutes, etc. This is doubtless caused by the uniform age of all cells in this kind of detector while BARON's yeast plate with cells of many different physiological stages, has a more gradual range of response and tolerance.

E. STORAGE OF MITOGENETIC CHARGES

FERGUSON and RAHN (1933) observed that bacterial cells could be kept in their old environment for 2 hours after exposure to mitogenetic rays, and still show stimulation of growth when transferred to a fresh medium (Table 36).

Table 36. 3-day old culture of *Bacterium coli*, irradiated by an agar culture of *Bacterium coli* for 30 minutes

	transplanted immediately after irradiation		transplanted 2 hours after irradiation	
	Control	Exposed	Control	Exposed
start	4 950	5 050	4 350	3 950
after 2 hours .	4 950	5 750	3 700	5 900
„ 3 „ .	5 100	6 500	—	—
„ 4 „ .	5 500	8 700	3 650	5 200
„ 6 „ .	24 500	83 500	10 500	44 000
„ 8 „ .	234 200	1 500 000	—	—

The storage of energy as such appears out of the question. A continued internal secondary radiation is also impossible. We can only assume that the cells were changed chemically, that the unknown process of rejuvenation was released, but could not materialize under unfavorable environmental conditions; as soon as this situation was altered, rejuvenation set in at once. This observation may eventually help to locate the exact process released by the mitogenetic impact.

F. MECHANISM OF THE BARON YEAST DETECTOR

In his monograph, GURWITSCH devotes 50 pages to the analysis of mitogenetic effects in the BARON yeast plates. The limited space of this book does not permit detailed quotation, especially since the complexity of this detector leaves too many possible explanations. However, since it is a good parallel to multicellular detectors, we shall at least give a brief summary here.

The complex structure of the BARON yeast plate, with old cells, beyond the stage of cell division, on the surface, and with normally-dividing cells at the bottom, has been described in detail on p. 66. The old surface cells react upon irradiation by producing secondary radiation. They can emit only a definite (though unknown) amount of radiation. After that, they are exhausted and remain inactive, though absorbing ultraviolet, for the duration of the experiment. The number of these secondary senders decreases therefore gradually during exposure.

The absorption of one quantum is sufficient to induce secondary radiation in a surface cell provided that the cell is not too old. Secondary radiation consists in the emission of a number of quanta. Since the emission takes place in all directions, the intensity decreases very rapidly with the distance from this cell. However, since in this detector, cells are lying closely side by side, a quantum emitted through secondary radiation may be absorbed by another reactive cell which then, on its part, emits new secondary quanta. On p. 110, it has been shown that in these yeast plates, mitogenetic effects may be observed 10 mm. removed from the exposed cells. Some of these secondary quanta will penetrate into younger cells, and stimulate them to bud formation.

GURWITSCH considers the "mitogenetic field" similar to an electro-magnetic field. He believes that a uniform stream of quanta striking a cell from all sides will not produce a mitogenetic effect. The mitogenetic stimulus consists in the one-sided discharge (release) of a neighboring secondary sender. GURWITSCH assumes that if two or more quanta strike the surface of the detector at the same moment, the peripheries of the streams of secondary quanta may be partly superposed and thus by interference, produce no effect; though the total energy is increased, the potential necessary to initiate cell division is lacking. Such "equalization" will occur more commonly with physical sources of ultraviolet because the light is more uniform, while in biological sources, radiation comes from many cells unevenly distributed. Consequently, there is less equalization, and therefore a relatively stronger mitogenetic effect must be expected from biological senders.

By irradiating a liquid bacterial culture in quartz from above and below at the same time, Miss FERGUSON, in an unpublished experiment, obtained a strong mitogenetic effect. The two radiations did not cancel. Such interference seems rather improbable if we look at the mitogenetic effect as a photochemical one. We would not expect the reaction between hydrogen and chlorine (p. 46) to be suspended if the gas mixture were irradiated from two opposite sides. Such interference is imagineable in a one-dimensional system, e. g. a nerve fiber, but hardly in three-dimensional media.

G. MITOGENETIC EFFECTS IN MULTICELLULAR ORGANISMS

There is one essential difference between the cells of unicellular and multicellular detectors which must be kept in mind to prevent misleading generalizations. Bacteria and yeasts in the detectors mentioned have a very large amount of food at their immediate command, whereas in tissues, e. g. in onion roots or in the cornea, the food supply is limited. Thus, in the latter case, there may not be enough food readily available to permit rapid cell division, even after adequate stimulation; or after a premature mitosis, the food may be insufficient for continuing at a subsequent normal rate of cell division.

The one multicellular detector that has been studied cytologically is the onion root. The interpretations of the onion root effect by GURWITSCH (p. 55) and by REITER and GABOR do not agree. The latter have based their intrepretation upon a cytological analysis of the onion root which deserves attention because it suggests a close analogy of the root with the BARON yeast plate. The root can be divided into transverse cross sections which may be designated by the number of successive cells from each section to the tip. This number is fairly uniform whether

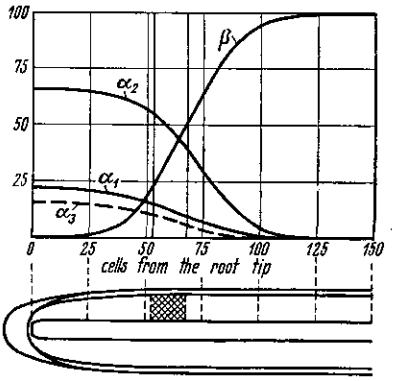

Figure 42.
Distribution of various cells in the onion root.
Below: cross section through the root, with its vascular bundle; the shaded zone indicates the sensitive part of the root.
Above: distribution for the four cell types in different parts of the root; the abcissa represents the distance from the tip, measured by the number of cells.
β = elongated, resting cells; α = short dividing cells. α_1 = newly-born cells, α_2 = ripe nuclei, α_3 = mitotic stages.

the successive cells are counted in the center of the root or along the sides. The most sensitive region in respect to mitogenetic rays is approximately 50 to 70 cells from the tip.

REITER and GABOR studied the distribution of cell types along the root. They distinguished the α-type, short actively-growing cells, and the β-type, elongated, resting cells. The first type could be subdivided into 3 nuclear stages: α_1 = newly-born nuclei; α_2 = ripe nuclei; α_3 = various mitotic stages. The distribution of these types is shown in Table 37 and fig. 42. In the root tip, as far as about 25 cell layers upwards, no cells of the β-type, i. e. no resting cells are found. All cells are in active division. In the region which is 125 or more cells distant from the tip, practically all cells are resting; dividing stages are very rare. The zone of mitogenetic sensitivity contains cells of all types, the resting cells amounting to less than half of the dividing types.

To analyze the effect, the authors raised two questions: How much does the exposed side of the root differ from the

Table 37. Distribution of the Nuclear Stages

	α_1 newly-born nuclei	α_2 ripe nuclei	α_3 dividing nuclei	β resting nuclei
at the root tip	21	64	15	0
50 cells upwards from the tip	16	56	11	17
80 „ „ „ „ „	5	21	3	71

opposite, shaded side? and How much does the exposed side differ from the normal? The latter question could be answered only by analogy.

The conclusion was that "under the influence of mitogenetic irradiation, all cells born during the experiment remain in the actively-dividing stage, and produce again ripe nuclei while normally, without irradiation, a certain percentage would go into the resting stage. On the opposite side of the root, more cells go into the resting stage than would normally do so".

GURWITSCH (1932) does not agree with this interpretation. He doubts the possibility of accurately distinguishing between nuclei of resting and dividing cells. He criticizes the method of counting "ripe nuclei" only, and points out that a decrease of mitoses in the opposite side of the root has been observed occasionally, but only in about half of all his (GURWITSCH's) and also of ROSSMANN's experiments, and this can be accounted for by the method of sectioning and counting.

There is a certain similarity between the onion root thus described, and the BARON yeast plate. Both consist of many closely-packed cells; both have the oldest, non-dividing cells on top, and very young, dividing ones at the bottom. It will be seen later that the onion root is irradiated continuously from above, by the bulb, and this stimulus is transmitted by secondary radiation of the old cells to the young, growing cells in the root tip.