CHAPTER IV

METHODS OF OBSERVING BIOLOGICAL RADIATIONS

The preceding chapters have shown that for physico-chemical reasons, we should expect ultraviolet radiations from all living organisms, as long as they have any noticeable metabolism. In this chapter, the methods used in detecting and proving such radiations will be discussed. By far the most extensive treatment is given to mitogenetic radiation because it is the most studied and the best understood. The necrobiotic rays and the injurious human radiations are, perhaps, only special manifestations of mitogenetic radiation. The Beta-radiation of living as well as dead organisms is only mentioned in passing.

The presentation in this chapter is largely historical. Occasional exceptions to this arrangement could not be avoided.

A. MITOGENETIC RADIATION

This type of ultraviolet rays was discovered by GURWITSCH in 1923. He called them mitogenetic because he observed that they stimulated cell division, or mitosis. This radiation is so weak that it was not possible for a long time to verify its existence by physical measurements. Its effect upon living organisms is very conspicuous, however. In onion roots, it increases the number of mitoses. It accelerates the growth of yeasts and bacteria, the development of eggs, and the division of certain cells in the animal body. It may cause morphological changes in yeasts and bacteria, and in the larvae of sea urchins.

a) The onion root method

The root of an onion, used by GURWITSCH and his associates extensively from 1923 to 1928, was the first detector of the rays. The detector root was placed in a narrow glass tube to permit

easy handling. The zone of meristematic growth, a few mm from the tip, was uncovered allowing it to be irradiated. In the first experiments (1923), the source of radiation was another onion root, also placed in a glass tube so that it could be directed to point exactly at the growing tissue of the first root (fig. 29). The

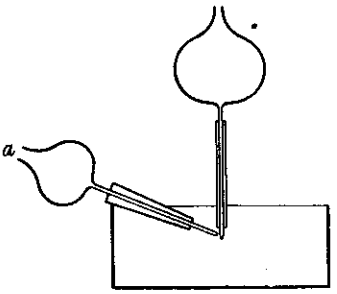

Figure 29.
The arrangement of the onion roots in the first experiments on mitogenetic rays.

roots were left in this position for one or two hours. Two to three hours after the beginning of irradiation, the detector root was fixed and stained, and, in microtome sections, the number of dividing nuclei was ascertained. GURWITSCH found that the side of the root exposed to the biological radiation showed regularly more dividing nuclei than the opposite side. For this reason, GURWITSCH coined the word "mitogenetic" rays. Table 17 gives the results of a test with the crushed base of an onion (A. and L. GURWITSCH, 1925).

Table 17. The radiation of onion base pulp

The numbers given are those of dividing nuclei in corresponding microtome sections of the exposed and the unexposed side of the detector root.

A: fresh pulp														Totals
exposed	51	59	90	82	61	60	57	53	72	62	59	37	45	788
unexposed	49	63	60	50	51	38	45	43	57	47	38	30	30	601
difference	2	−4	30	32	10	22	12	10	15	15	21	7	15	187 = +23.8%

B: same pulp heated to 60° C.																Totals
exposed	60	66	64	61	61	63	60	75	81	71	61	71	73	73	65	61	1066
unexposed	61	69	65	62	74	62	60	73	82	66	65	74	69	69	65	60	1076
difference	−1	−3	−1	−1	−13	1	0	2	−1	5	−4	−3	4	4	0	1	−10 = −0.9%

This technique was used by GURWITSCH and a number of associates to search for mitogenetic rays in the entire organic world. It would scarcely be worthwhile to compile a complete list of organisms found to radiate. The following list contains the more important earlier observations, compiled by GURWITSCH (1929):

Radiating organisms and tissues

Bacteria *Bacterium tumefaciens*, Staphylococci (MAGROU)
Bacterium murimors (ACZ) (SEWERTZOWA)
Bacillus anthracoides, Sarcina flava (BARON)
Streptococcus lactis (MAGROU)

Yeast (BARON, SIEBERT, MAGROU)

Eggs of annelids

Eggs of sea urchins before the 1st division (FRANK and SALKIND) before the 2nd and 3rd divisions (SALKIND)

Egg yolk of chicken, only during the first two days of incubation (SORIN). After establishment of circulation system, radiation ceases

Embryos of amphibia in the morula stage (ANIKIN)

Plant seedlings: root tips, cotyledons, young plumulae of *Helianthus* (FRANK and SALKIND)

Potato tubers: leptom fascicles only (KISLIAK-STATKEWITSCH)

Onion roots connected with the bulb (GURWITSCH)

Onion base pulp (A. and L. GURWITSCH, REITER and GABOR)

Turnip pulp, 24 hours old (ANNA GURWITSCH)

Young tadpole heads, pulp of tadpole heads (ANIKIN, REITER and GABOR)

Blood of frog and rat (GURWITSCH, SORIN)

Blood of man (SIEBERT, GESENIUS, POTOZKY and ZOGLINA)

Contracting muscle (SIEBERT, FRANK)

Pulp from resting muscle + lactic acid + oxygen (SIEBERT)

Corneal epithelium of starving rats, but not of normal rats (L. GURWITSCH)

Neoplasms: carcinoma, sarcoma (GURWITSCH, SIEBERT, REITER and GABOR)

Spleen of young frogs (GURWITSCH)

Bone marrow (SIEBERT)

Bone marrow and lymph glands of young rats (SUSSMANOWITSCH)

Resorbed tissue: tails, gills, intestine of amphibian larvae during metamorphosis (BLACHER, BROMLEY)

Regenerating tissue of salamander and angleworm (BLACHER, SAMARAJEFF)
Hydra: hypostom and budding zone, not other parts.

Non-radiating organisms and tissues

Tissues of adult animals except brain, blood and acting muscle (most tissues have later been found to radiate slightly)
Tadpoles over 2 cm. long
Chicken embryo after 2 days (blood radiates)
Blood serum (becomes radiant with oxyhemoglobin) (SORIN) (or with traces of H_2O_2) (ANIKIN, POTOZKY and ZOGLINA)
Blood of asphyxiated frogs
Blood of cancer patients
Blood of starving rats (becomes radiant with glucose) (ANIKIN, POTOZKY and ZOGLINA)
Active tissues with chloral hydrate
Active tissues with KCN

Further details will be given in Chapter VII.

The greatest early support to the establishment of mitogenetic rays was given through the extensive and thorough work of REITER and GABOR (1928). These two authors tested the onion root method, and verified especially the physical nature of the phenomenon; the radiant nature of this effect was thus fortified beyond doubt (see p. 59).

Considering the great claims which Gurwitsch and his associates made for their discovery, it was surprising that comparatively few biologists were sufficiently interested to repeat the experiments. Among these, some obtained negative results so consistently that they denied the existence of mitogenetic rays altogether, and considered the results of the Russian workers and of REITER and GABOR to be experimental errors. The most frequently quoted of these are SCHWARZ (1928), ROSSMANN (1928), and much later, in this country, TAYLOR and HARVEY (1932). Positive results were obtained by MAGROU (1927), WAGNER (1927), LOOS (1930), BORODIN (1930), and recently by PAUL (1933), and many Russian workers, in several different laboratories.

In order to decide whether the positive results could be considered experimental errors, SCHWEMMLE (1929) undertook a statistical investigation. He concentrated graphically all results published by GURWITSCH and the Russian school (about 200)

by plotting the percentage increase or decrease of mitoses of the exposed over the unexposed side of the root, against the total number of mitoses counted. He distinguished only between "induced" and "not induced" roots. From the "not induced" roots, he could compute the probable error of the method. The error was $\pm$ 10% when 500 mitoses were counted, and decreased, of course, as the total number increased (fig. 30). All results

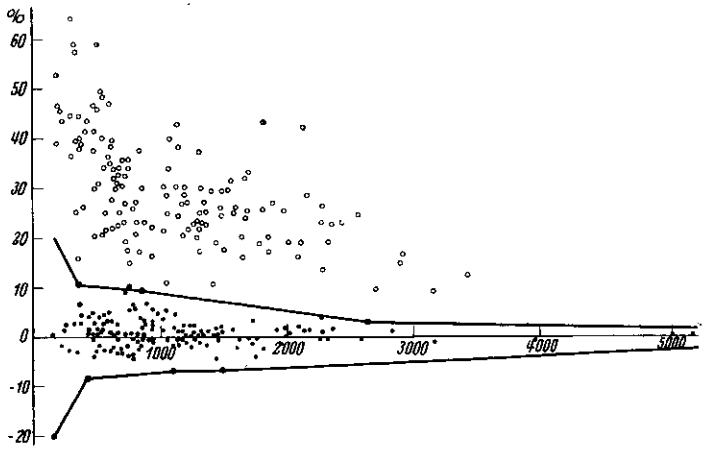

Figure 30. All results with onion root as detector by GURWITSCH and associates. Ordinate: percentage of increase over control; Abscissa: total number of mitoses counted. Circles indicate a positive induction, black dots indicate no mitogenetic effect. The line gives the limits of error.

which had been claimed to prove mitogenetic radiation were found outside the limits of error.

REITER and GABOR's experiments have a much greater error, $\pm$ 20%, and a few experiments supposed by these authors to prove induced mitogenetic effect are really within the limits of error. The error of the experiments by WAGNER (1927), SCHWARZ (1928), and ROSSMANN (1928—29) is even larger than that of REITER and GABOR's, and TAYLOR and HARVEY's few experiments (1931) indicate a similar large error. All these data gave doubtful or negative results; the mitoses of two different sides of the roots varied greatly.

SCHWEMMLE did not consider it proved that the effect is caused by mitogenetic rays, because of the possibility of other physiological factors affecting mitosis during the experiments.

The effect as such, however, must be considered established by a very large number of data, and the only question was its interpretation. That under different physiological conditions, in different countries with different onions, negative results have been obtained by some investigators is not really surprising.

GURWITSCH had always claimed that the effect of one root upon the other was caused by rays. In fact, he predicted in 1922 radiation as a factor in mitosis, and in trying to verify his prediction, found onion roots as the first reliable indicators.

The mitogenetic effect proceeds in a straight line and is reflected from glass and from a mercury surface (GURWITSCH, SIEBERT, REITER and GABOR). It will pass through thin layers of quartz and of water (GURWITSCH, MAGROU), through thin animal or vegetable membranes, thin plates of mica (REITER and GABOR), thin cellophane (STEMPELL), but not through thick layers of glass, such as glass slides, nor through gelatin even in very thin layers. It seems hardly possible to account for all of this by any agent other than ultra-violet rays.

A very vital question is that of the wave length of these rays, and it is very interesting that two distinctly different wave lengths have been claimed, both based upon apparently reliable data.

GURWITSCH could obtain the effect through quartz, and partly through very thin glass, but not through very thin gelatin, and concluded that he was dealing with an ultra-violet radiation of about 2200 Å. FRANK and GURWITSCH exposed onion roots to different wave lengths from physical sources, and obtained mitogenetic effects only from the spectrum between 1990 and 2370 Å.

Quite different were the results of REITER and GABOR (1928). They found this radiation to be transmitted through 3 mm. of Jena glass, and still noticeably through 5 mm. of common glass, and also through gelatin which indicates a wave length above 3000 Å. By means of special filters, they found the range to be between 3200 and 3500 Å. Then, by irradiating roots with known wave lengths of the spectrum, they determined the mitogenetic efficiency of this part of the spectrum. Besides a sharp maximum at 3400 Å, another smaller maximum was discovered near 2800 Å. Below this, no mitogenetic effect was observed, not even in the neighborhood of 2000 Å which was considered by GURWITSCH

and FRANK as the only efficient region. The curve resembles somewhat that for erythema (fig. 27 p. 49).

The very interesting observation was made that the apparently inert spectrum between the two maxima will prevent mitogenetic effects by the active wave lengths, even when the intensity of the "antagonistic" rays is only one-tenth of that of the mitogenetic rays. All wave lengths between 2900 and 3200 Å show this inhibition. The rays outside of the maxima were entirely neutral. Direct sunlight and ultra-violet arc light also inhibited mitogenetic effects.

REITER and GABOR determined further the wave length of mitogenetic rays by means of a spectrograph, letting the spectrum from roots or sarcoma tissue fall upon the length of an onion root. All three experiments gave an increase in mitoses at the place where the wave lengths between 3200 and 3500 Å had fallen on the root.

These last experiments can now be explained by an error in technique. It was not known at that time that irradiation of the older parts of a root will produce a mitogenetic effect not at the place of irradiation, but at the only reactive part, namely the meristem near the root tip. This last argument in favor of a wavelength near 3400 Å must therefore be discarded. It is considered definitely established now that mitogenetic rays range between 1800 and 2600 Å, as has already been shown in Chapters II and III. However, the deviating experiences of REITER and GABOR have never been accounted for in a really satisfactory way (see GURWITSCH, 1929).

The publication of REITER and GABOR's experiments caused the Russian workers to repeat them at once, because they contradicted all their own statements about the wavelength, and none of the German authors' results could be verified.

The first experiment was FRANK's spectral analysis (1929) of the radiation of the tetanized muscle, with a spectrograph using yeast as detector (see p. 35). In three well-agreeing experiments (fig. 31), it could be shown that no radiation above 2400 Å was emitted. Then followed the detailed study by CHARITON, FRANK and KANNEGIESSER (1930) of the effect of monochromatic light from physical sources upon yeast. The results have already been shown in fig. 28. Beyond 2600 Å, no variation of intensity produced any effect. Special efforts were made to

investigate the range around 3400 Å claimed to be so efficient by REITER and GABOR, but it yielded only consistently negative results.

Considering that REITER and GABOR used onion roots as detectors, the experiments were repeated with onion roots. Again,

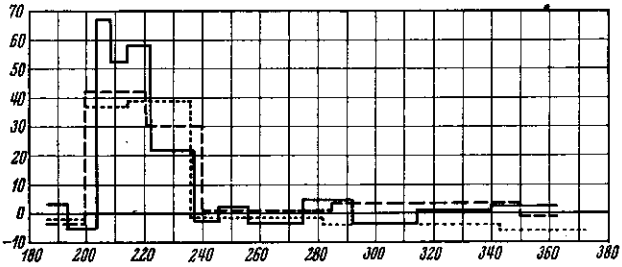

Figure 31. Results of 3 experiments on the spectrum of muscle radiation, with the technique shown in figure 22. Since the width of the agar blocks was not uniform, the results overlap partly, but show the general spectrum.

the shorter wave lengths were found to be efficient, and those in the region of 3400 Å gave no effect (see Table 18).

By these and other methods with a variety of indicators, the wave lengths of various radiations have been shown to be quite different, but all of them were below 2600 Å. No records are

Table 18. Irradiation of Onion Roots with Monochromatic Spectral Light

Efficient Wave Lengths according to GURWITSCH			Efficient Wave Lengths according to REITER and GABOR		
Wave Length Å	Relative Intensity	Induction	Wave Length Å	Relative Intensity	Induction
2190	0.02	+24	3340	0.20	−1
2350	0.02	+20	3340	0.40	+5
2350	0.10	+30	3340	2.00	−2
2350	1.00	+25	3340	80.00	0
			3340	800.00	−1
			3380	0.03	−7
			3380	4.00	+8
			3380	60.00	−2

given of wave lengths below 1900 Å. This is no proof that they do not exist, but rather, that below this point, absorption by quartz and air in the spectrograph makes accurate determinations impossible. It can hardly be doubted from the large amount of spectra analyzed by the Russian school, and confirmed by WOLFF and RAS (p. 40), that mitogenetic radiation consists essentially of the wave lengths between 1900 and 2500 Å.

Since 1928, the onion root as detector has been substituted by the time-saving yeast methods or by bacterial detectors. However, two extensive recent investigations must be mentioned which concern the question whether onion roots can be used at all as detectors. Both papers describe the technique employed very carefully, and they arrive at quite different conclusions.

MOISSEJEWA (1931, 1932) observed that roots when removed from the water show symmetrical distribution of mitoses, but after repeated removal, they do not. Pressing or rubbing of the roots will increase the number of mitoses, and after long continued pressing the opposite effect occurs. When friction and pressure were carefully avoided, no increase of mitoses was observed upon exposure to another onion root.

MOISSEJEWA denies the existence of mitogenetic effects in onion roots and explains GURWITSCH's consistent results by several assumptions: (1) One-sided pressure of curved roots in the glass tube. (2) Light applied repeatedly in centralization of roots which causes phototropic curving of the root and results in increased mitosis. (3) Selection of good roots for important experiments which results in increased mitosis through pressure, and of less uniform roots when no effect is expected, which then leads to negative results. (4) Omission by GURWITSCH of the microtome sections showing a decreased number of mitoses when they happen to come between sections showing an increased number. — With yeast and blood as senders, this author obtained also negative results.

This careful study has been considered by many critics to be the final proof against mitogenetic radiation. Most of them do not mention the still more careful work by MARGARETE PAUL (1933). Realizing the prompt reaction of roots to touching, PAUL fastened the small onions (hazelnut size) by means of gauze to perforated cork stoppers; these were held above the ground by simple stands; in a completely covered moist chamber, in a dark

room at 20° C, the roots developed in the air through the muslin in two to five days, while the leaves grew through the hole in the cork. The onions were never placed in water. When the roots were 1—2.5 cm. long and absolutely straight, one could be exposed to a root from another onion without being cut or even touched and without the need of glass tube holders. The exposed roots turn downwards, and a very careful investigation showed that the number of mitoses on the exposed part of the root was distinctly larger than on the opposite half, whether all mitotic stages were included or only the more conspicuous ones. The sender roots were taken as controls, and they showed a uniform and symmetrical distribution of mitoses. Almost always, the exposed root grew more rapidly than the other roots of the same onion.

When the sender root was substituted by a needle of stainless steel, the exposed root also turned downwards, but the microscopic analysis showed no increase in mitosis at the exposed side. However, the symmetrical distribution was disturbed.

The object of PAUL's investigation was the establishment of a good method for studying mitogenetic rays with onion roots. The number of examples given is not large enough to draw many more conclusions. The paper verifies GURWITSCH's principal experiment, however, and it will be the starting point for all future work with this type of detector.

b) The yeast bud method

BARON (1926) suggested that the rate of bud formation of yeast could be used as indicator of mitogenetic radiation. In his first experiments, he spread yeast over the surface of solidified nutrient agar containing glucose, allowed it to grow for from 9 to 15 hours at room temperature, and then exposed it to the radiating source for definite short periods, usually not over 30 minutes. The yeast was then incubated for from 1 to 2 hours to permit the radiation effect to develop. After this, the yeast was spread on glass slides, dried and stained. The measure was the percentage of yeast cells showing buds. When this percentage was higher in the irradiated culture than in unexposed controls, it was considered a proof of a mitogenetic effect. The original method has since been changed in some details by BARON (1930) and GURWITSCH (1932) (see p. 66).

Table 19. Effect of Yeast, of Sarcoma, and of Bone Marrow upon the Budding Intensity of Yeast

Sender	Percentage of Buds in Yeast					
	Yeast		Jensen Sarcoma		Bone Marrow	
	Control	Exposed	Control	Exposed	Control	Exposed
Experiment No. 1	24	33	21	32	30	38
2	22	30	20	35	27	32
3	13	25	20	27	21	39
4	14	25	20	26	28	38
5	24	33	28	35	29	39
6	23	33	30	38	20	35
7	25	34	25	35	23	37
8	27	35	25	36	22	34
9	25	36	25	35	27	36
10	26	36	21	30	26	33
Average	22.3	32.0	23.5	32.9	25.3	36.1

The most extensive early data with this method have been published by SIEBERT (1928a). Table 19 gives some of his experiments. The numbers indicate the percentages of yeast cells with buds. In all his experiments, sender and detector were separated by a quartz plate. It is now customary to record results as "induction" effect, i.e., as increase in buds of the exposed yeast over the control, expressed in percents of the control value. Thus, when the exposed yeast shows 33% buds, and the control, 24%, the increase is 9, and this is an increase of 37.5% over the control. The induction effect is 37.5%.

$$I = \frac{100\ (\text{exposed} - \text{control})}{\text{control}}$$

SIEBERT (1928b) used this method for a number of interesting studies in physiology. He observed the working, or excited muscle to radiate strongly while the resting, quiet muscle did not do this (Table 20). He attempted to produce radiation by changing chemically the pulp of resting muscle to that of working muscle. Finally, he succeeded by placing the acidified pulp in an oxygen atmosphere, since the addition of lactic acid alone would not produce radiation. Moreover, he obtained positive results in air by using a very dilute $CuSO_4$ solution as oxygen catalyst. When

Table 20. Effect of Electrically Excited and of Resting Frog Muscle upon the Budding Intensity of Yeast

Experiment No.	Percentage of buds in yeast when exposed to muscle		Experiment No.	Percentage of buds in yeast when exposed to muscle	
	Resting	Excited		Resting	Excited
1	32	45	13	23	41
2	24	32	14	22	33
3	23	36	15	23	32
4	25	36	16	21	30
5	26	41	17	22	29
6	22	30	18	22	32
7	22	31	19	22	32
8	22	28	20	23	30
9	26	35	21	25	33
10	26	34	22	25	33
11	26	35	23	24	30
12	26	31	24	24	31

he finally found that $\frac{N}{10000}$ KCN solution would prevent radiation, he concluded that the source of radiation must be chemical. Thus started the first experiments about chemical reactions as the source of radiant energy (p. 33).

SIEBERT later (1930), concentrated his attention upon blood radiation. He verified the statement of LYDIA GURWITSCH and SALKIND (1929) that blood of normal, healthy people radiated distinctly, while that of cancer patients did not. He found, further, that urine radiated, and that there was a good parallelism between blood and urine radiation. Of 35 patients with cancer, the majority showed no radiation of blood or urine. The exceptions were patients after recent treatment with X-rays and isamine blue.

Anemia, leucemia, high fever (sepsis, pneumonia, scarlatina) prevented radiation of blood, as well as of urine. With syphilis, radiation varied, but blood and urine went parallel. None of the other diseases tested caused loss of blood radiation (details see p. 153).

The experiments on the metamorphosis of amphibia and insects (p. 167), and on the healing of wounds in animals (p. 173), by BLACHER and his associates, were all carried out by the yeast

bud method. The importance of the yeast agar blocks in the establishment of biological spectra has already been mentioned on p. 35. This method is very commonly used in mitogenetic investigations at the present time.

Method: GURWITSCH gives the following directions for the yeast bud method (1932, p. 7): Beer wort agar plates are flooded with a very fine suspension of yeast in beer wort, the liquid is distributed evenly over the agar surface by careful tilting, and the surplus liquid is drawn off with a pipette. After about 5 to 6 hours, the surface is covered with a fine, delicate film of yeast, and is now sensitive, and remains so until about the twelfth hour. — Neither the temperature nor the concentration of the yeast suspension nor its age is mentioned. The temperature is probably room temperature, and it should be kept in mind here that on p. 14, GURWITSCH mentions 12° C as room temperature. The directions are probably meant primarily for the yeast *Nadsonia fulvescens* which has been used most commonly by the Russian workers, though beer and wine yeasts are occasionally mentioned.

In order to give a better conception of the proper physiological condition of the detector plate, we quote from the same book of GURWITSCH's p. 317: "The most appropriate stage of the detector plate corresponds to a thickness of the yeast growth of about 25 to 30 layers of cells . . . (p. 318). We can be certain that the lowest layers of cells which are in immediate contact with the nutrient medium consist essentially of young cells in rapid multiplication . . . The cells of the middle layers are not in optimal condition, and are no more capable of developing spontaneously the maximal energy for development which was found in the lowest layers . . . It can hardly be far from the truth to deny any appreciable multiplication in the topmost layers. However, they are not real resting forms as yet."

The method of making smears to count the buds is not given in GURWITSCH's book in any detail. It consists simply in smearing the yeast cells on a glass slide, drying and staining them. Only those buds are counted which are smaller than half of the full-grown cell. Some authors limit their counts to even smaller buds. GURWITSCH recommends that the person counting the buds should not know which slide or experiment he has under the microscope; this prevents subconscious arbitrary decisions.

Attention should be called here to a leaflet published by Ing. G. TERZANO & C., Milano, manufacturers of the "hemoradiometer" of Protti's. All conditions are quite precisely standardized, and this may account for the good results of Italian investigators.

This method has been varied by other authors. TUTHILL and RAHN (1933) studied the mode of bud formation of Burgundy

yeast on raisin agar at 30° C. A typical result including all sizes of buds is shown in fig. 32. The culture immediately after being transferred contains but very few buds, and new buds are not formed at once. The old yeast cells which had ceased to multiply require some time before their reproductive mechanism is working normally. During this rejuvenation process, commonly called the lag phase, cells respond most promptly to mitogenetic rays. It is also seen that they soon reach a maximum percentage of buds. If, at this latter stage, the rate of cell division were accelerated by mitogenetic rays, it could not increase the percentage of buds (see also p. 69). The most opportune time for using such a plate as detector is evidently about an hour or two before bud formation begins. If such a detector is exposed for 30 minutes, and then incubated for one hour (so that the mitogenetic effect might manifest itself by an increase in buds) we should have bud formation beginning on the exposed plate while the control has not yet become quite ready for budding.

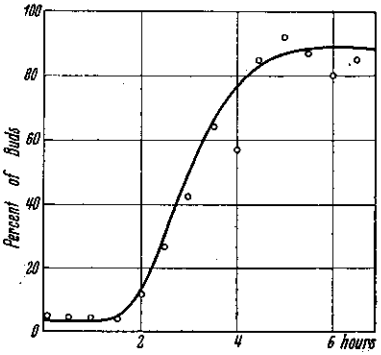

Figure 32. The development of buds in an agar surface culture of wine yeast.

The "lag period", i.e. the time interval between the seeding of the plate and the first active bud formation, depends upon the age of the culture used for the seeding. With the Burgundy yeast employed by TUTHILL and RAHN, a plate seeded with a 24 hours old culture was a good detector immediately after seeding. When a 6 days old culture was used, it was advisable to incubate the plate for about 2 hours before exposure (see Table 21), or to incubate for several hours after exposure if exposure had taken place immediately after seeding.

In liquid cultures, the old yeast cells retained their buds for many days while old cultures on agar surface lost them readily. Since a low initial percentage of buds is very desirable, inoculation with yeast from agar surface cultures is recommended.

This detector is really quite different from BARON's or GURWITSCH's described above. All yeast cells are at the same stage

Table 21. Effect of Irradiating Yeast Surface Cultures after Incubation for Different Times

Length of Exposure: 30 minutes
Incubation after Exposure: 30 minutes

Age of Parent Culture		Age of culture when exposed					
		0 hrs	0.5 hrs	1 hr	1.5 hrs	2 hrs	2.5 hrs
		Percentage of Buds					
24 hours . .	exposed area	25.0	29.2	4.6	4.2	9.8	16.4
	control area	3.6	3.5	4.4	4.6	9.5	18.4
	increase	**21.4**	**25.7**	0.2	—0.4	0.3	—2.0
	Induction effect	**595.0**	**724.0**	5.0	—1.0	4.0	—11.0
6 days . . .	exposed area	7.0	6.0	8.5	22.5	26.2	44.4
	control area	6.0	7.0	7.6	19.0	17.0	27.8
	increase	1.0	—1.0	0.9	3.2	9.2	**16.6**
	Induction effect	17.0	—17.0	12.0	17.0	54.0	**62.0**

of earliest rejuvenation when exposed, and the cells are far enough apart not to influence each other. The "mitogenetic effect" is much greater than in the other method because there are no old, inactive cells to "dilute" the counts. In fact, these are probably the largest mitogenetic effects ever recorded. It is quite permissable to count all buds because the percentage at the beginning is very low, and a limitation in size is not necessary. This should make the counting easier.

On the other hand, this type of detector is so different from the BARON type that it may react differently in certain experiments. As long as they are used merely as detectors to prove the existence of radiations, both types are good. Probably, with very weak radiations, the BARON type is more sensitive because the old cells act as "amplifiers" (see p. 127).

Method by TUTHILL and RAHN (designed for Burgundy yeast): The yeast is kept throughout the experiment at 30° C. A 24 hours old culture in raisin extract[1]) is flooded over a solidified, sterile raisin agar

[1]) Raisin extract: 1 pound of chopped, or seeded raisins is heated with 1 liter of water in steam for 45 minutes, the extract is pressed off, made up to 1 liter, 5 g. KH_2PO_4 and 5 g. yeast extract (or meat extract) are added; the resulting medium is sterilized at 100° C. The pH is about 4 to 4.5.

Raisin agar: Melted 6% water agar is mixed with an equal volume of the above raisin extract and sterilized by heating for 20 minutes at

plate; the surplus liquid is poured off, and the culture permitted to develop for 24 hours. This surface growth is washed off with 5 cc. of sterile water, the suspension is then diluted 1 : 100 with sterile water, and with this dilution, some sterile solidified plates of raisin agar are flooded, the surplus liquid is drained off at once, and the plates should be exposed within half an hour. Half of the plate should be shaded to serve as control.

The length of exposure will depend upon the intensity of the sender: 30 minutes proved a good time with young yeast cultures. One to two hours incubation, counted from the beginning of the exposure, was the most suitable time to bring out the differences.

In these plates, the yeast cells are so far apart that the buds can be counted directly on the agar surface. The organisms are killed by placing a cotton wad with tincture of iodine in the Petri dish. Soon after that, a coverglass can be placed on the agar surface, and the slightly-stained yeast is observed in situ, eliminating all possibility of breaking off buds by smearing on glass.

In all methods where growing cells in glass or quartz containers are used as detectors, radiation from these growing cells may be reflected by the glass or quartz walls, and may thus produce radiation effects in controls as well as in the exposed cultures. Protection against reflection is advisable in all such cases (see p. 80).

Liquid cultures have also been used successfully. Here, too, the rule applies, that increases in the bud percentage can be expected only during the lag phase (see fig. 32). When all cells are out of the lag period, and produce buds at a constant rate, the percentage of buds cannot be changed by a change in the growth rate. Since this has been overlooked by some experimentors, e.g. by RICHARDS and TAYLOR (1932), it may be advisable to explain this important point in more detail.

Let us, for this discussion, distinguish five equal periods in the complete cell division of the yeast, 4 with buds and one without. Fig. 33 shows a first approximation of a "cross section" through a yeast population growing at a constant rate. It requires 5 time units for each cell to complete the cycle, i.e. to produce two cells of the same developmental stage. The percentage of buds is not constant, but fluctuates between 67 and 80%. This fluctuation is due partly to the arbitrary selection of 5 stages, but mostly to an error in the cross section. In a growing culture, there must

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100° C. On account of the high acidity, the agar becomes hydrolyzed under pressure, and fails to solidify; the same is true with prolonged heating at 100°.

necessarily be more cells of the young stages than of the old. If we take all cells of the first 5 units as a more appropriate cross section, we obtain the following picture:

Yeast Culture at a Constant Growth Rate

Time units	0	1	2	3	4	5	6
	9 no buds	9 tiny	9 small	9 medium	9 large	18 no buds	18 tiny
	8 tiny	8 small	8 medium	8 large	16 no buds	16 tiny	16 small
	7 small	7 medium	7 large	14 no buds	14 tiny	14 small	14 medium
	6 medium	6 large	12 no buds	12 tiny	12 small	12 medium	12 large
	5 large	10 no buds	10 tiny	10 small	10 medium	10 large	20 no buds
with buds.	26	30	34	39	45	52	60
without...	9	10	12	14	16	18	20
Total.....	35	40	46	53	61	70	80
% buds...	74.4 %	75.0 %	73.9 %	73.7 %	73.9 %	74.4 %	75.0 %

There is a fluctuation of only about 1%.

This percentage depends only upon the variety of yeast used. Whether it grows rapidly or slowly, whether the time unit is 30 to 60 minutes (at cellar temperatures) or 10 minutes (under optimal conditions), the percentage of buds remains 74—75%. A change of the growth rate would not affect the bud percentage at all.

However, a change would become noticeable if only one certain stage of the cycle should be accelerated. If mitogenetic radiation should speed up the very first stage so much that the "tiny" buds never appeared, the number of buds at the 0 period would drop from 26 out of 35 to 18 out of 27, or from 74.4 to 66.8%, and this lower level would continue as long as radiation accelerated the one particular stage.

It is rather probable that only a certain stage of the cell cycle is affected by mitogenetic rays. Many observations suggest this, and our present conceptions of the mechanism of cell division do not contradict it. This would offer a good explanation for the "false mitogenetic depression" of GURWITSCH (1932, p. 210) and especially of SALKIND (1933) where the percentage of buds decreases while the total cell count increases under the stimulation of mitogenetic radiation.

The reliability of the BARON method has been doubted by NAKAIDZUMI and SCHREIBER (1931) who claimed to have followed BARON's method explicitly. However, they have kept their detector cultures for 9—12 hours at 25° C while BARON used room

temperature which may be as low as 13° C in Russia. That their cultures were far too old, can be seen from the fact that in most of their experiments, the percentage of buds decreased distinctly in 2.5 to 8 hours.

The claim of these authors that the error of the method has never been considered by the workers is entirely wrong.

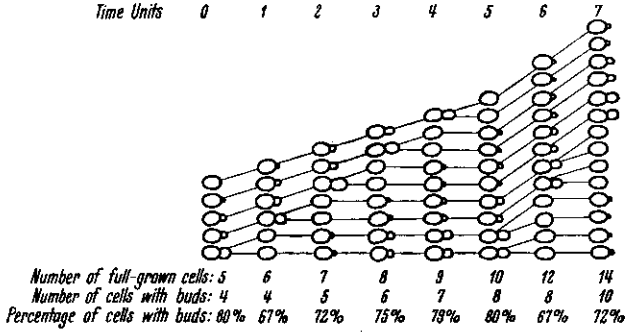

Figure 33. A schematic representation of the bud formation of a yeast culture growing at a constant growth rate.

Every biologist knows the error of his methods, at least approximately. The Russian investigators have repeatedly stated the error of their method, though they have not given all the data necessary for others to check their computation. This can be done very easily, however, from the data on experiments without mitogenetic effect. SIEBERT e. g. rarely publishes less than 10 experiments to prove an yone point. The following computation is made from a study of the effect of KCN upon organic catalysts (1928b and 1929), and the differences between control and poisoned catalyst give the error which is computed here in two different ways:

	average mean deviation	average increase through radiation	standard deviation %	increase by radiation %
1928 a Table VI	1.2	9.2	3.3	35
VII	1.4	7.9	2.9	28
VIII	2.3	10.9	4.6	35
1929 Table I	1.1	13.4	2.6	67

The increases resulting from irradiation are very much larger than the error, regardless by what method it is computed.

c) Detection by increase in cell number

To the investigator not familiar with yeasts, it may seem that there should be no essential difference between the total number of cells or the percentage of buds as a measure of the growth rate. For this reason, the preceding pages were written. There is a difference so fundamental that in a number of experiments, the yeast bud method indicates a decrease in the growth rate while the total cell count shows an increase (e. g. Table 34 p. 116).

The number of cells is the best measure of the growth rate, better than any other of the methods described in this book, because it ascertains cell divisions directly while the mitoses in onion roots, the buds of yeast cells, the respiration, etc. are indirect measurements of growth, and may be influenced by secondary effects. This method is used with yeasts as well as with bacteria. While they are identical in principle, they differ in the method of measuring since yeasts are so much larger than bacteria; they shall be treated separately in this chapter on Methods.

(1) Yeasts: The number of yeast cells in a liquid may be ascertained by plate count, by hemacytometer count, by measuring the volume of all cells, or by comparing the turbidity by means of a nephelometer.

The plate count method has rarely been used. It is the same as that used for counting bacteria except that more preferable media are beer wort agar or raisin agar.

The hemacytometer has been used by the Russian workers as well as by HEINEMANN (1932) in his studies on blood radiation.

HEINEMANN's method for testing the radiation of blood consists essentially in the exposure of a 12-hour culture of beer yeast (temperature not mentioned) in liquid beer wort, to the radiation of blood diluted with an equal amount of a 4% $MgSO_4$ solution to prevent coagulation. The exposure lasts 5 minutes, and it is made discontinuous by moving some object between blood and yeast at intervals of 2 seconds (see p. 103). 0.5 cc. of the exposed yeast suspension is then mixed with an equal amount of beer wort and incubated for three hours at 24° C. The total number of yeast cells (counting each smallest bud as an individual) is determined with a hemacytometer at the start, and again after three hours' incubation, both in the control and in the exposed culture. All cultures are preserved for counting by the addition of H_2SO_4. The control is counted twice. An example of the results and of the method of calculation is given in Table 22.

Table 22. Yeast Cells Counted in Hemacytometer *before* and *after* Irradiation by Blood

	start	Control after 3 hours a	Control after 3 hours b	Irradiated	Increase of Control %	Increase of Irradiated %	„Induktion" = 100 (Irr.—Con.) / Control	actual increase in growth rate %
Healthy person .	41.5	62	63.0	84	49	102	+108	82
Carcinoma patient	46.0	68	68.5	56	48	22	—54	—42
Chronic tonsilitis.	29.0	48	48.5	49	65	65	± 0	0

HEINEMANN emphasizes that this method depends upon the physiological condition of the yeast, and that no effect can be expected when the control grows too rapidly, i. e. when the control increases to more than double during the incubation period. This means, in other words, that the yeast must be in lag phase, else it would double in less than 3 hours. To avoid errors from this source, he tested each blood sample with two different yeast strains.

The results by this method verified all former experiences with blood radiation, especially in regard to cancer patients, as will be shown in Chapter VII. He also added some important new facts regarding radiation of the blood of old people and of patients with chronic tonsilitis.

The measurement of the growth rate of yeast by cell volume has been studied in detail by LUCAS (1924). The accuracy is not greater than with plate counts, and the curves and data published by this author show so little deviation only because they are presented in logarithms instead of actual numbers. However, the volume is sufficiently accurate to prove mitogenetic radiation, and the method requires less time and less eye strain than the hemacytometer method, also giving quicker results than the plating method. BRAINESS (quoted from GURWITSCH, 1932, p. 17) has adapted the method for the small volumina available in mitogenetic work.

Volumetric Method: KALENDAROFF (1932) used beer yeast in a strong wort (18—22° Balling); only cultures 15—20 hours old (temperature not given) which are actively fermenting, are suitable as detectors. After exposure, the yeast is distributed evenly, and a definite amount of the exposed culture is measured by means of a micropipette, e. g. 0.2 cc. This is added to 1 cc. of fresh wort, and incubated for 4 hours at 28° C. The

Table 23. Height of yeast column of centrifuged yeast cultures, exposed to the various spectral regions of the radiation produced by gastric digestion of serum albumin, and of their controls

Wave Length	mm of yeast column		Induction Effect	Wave Length	mm of yeast column		Induction Effect
	control	exposed			control	exposed	
2320—	15	15	0	2360—	18	17	0
2330	15	15	0	2370	17	18	0
	15	15	0				
	14	14	0				
2330—	20	23	15	2380—	12	16	33
2350	20	24	20	2390	9	12	33
	20	25	25		9	12	33
	20	26	30				
	20	25	25				
	20	26	30	2390—	15	18	20
				2400	15	20	33
2350—	19	19	0		21	26	24
2360	19	19	0		20	24	20

yeast cells are killed by adding 0.2 cc. of 20% H_2SO_4, and are centrifuged in pipettes commonly used for measuring the volume of blood corpuscles (the illustrations of the Russian workers appear to be VAN ALLEN hematocrit tubes). The yeast column of the exposed sample is compared with that of the control.

Table 23 shows some results obtained with this method by BILLIG, KANNEGIESSER and SOLOWJEFF (1932) who used it in the determination of the spectrum of gastric digestion.

A still more rapid method for estimating the amount of growth is the measurement of the turbidity of the culture by means of a nephelometer. This method has been used occasionally by bacteriologists for several decades; the methods are reviewed and analyzed by STRAUSS (1929). The nephelometer can be used for bacteria as well as for yeasts, while the cell volume of bacteria is too small to be measured with sufficient accuracy in the earlier stages of growth. Attention may be called to the description of a simple nephelometer by RICHARDS and JAHN (1933). More complicated is the differential photoelectric nephelometer described by GURWITSCH (1932, p. 17).

Table 24. *Bacillus mesentericus* Irradiated Continuously by Yeast at 12 mm. Distance, through Quartz

Time	Cells per cubic millimeter			Mitogenetic Effect
	start	control	irradiated	
2 hrs	3168	11 440	18 290	60
2.5 „	528	7 920	8 976	13
3 „	4048	12 496	16 016	28
3.5 „	1584	10 912	13 552	24

Time	Generation Times		
	control	irradiated	increase
	min.	min.	%
2 hrs	63.7	46.8	36
2.5 „	38.4	37.7	2
3 „	110.8	90.7	22
3.5 „	75.5	67.8	11

Another way of estimating the amount of growth in yeast cultures has been suggested by BARON (1930) who compared the size of yeast colonies in hanging drops. This method has been slightly modified by BORODIN (1934) who photographed the colonies and measured their area with a planimeter.

(2) Bacteria: The stimulation of bacterial growth by mitogenetic radiation had already been observed by BARON (1926) and by SEWERTZOWA in 1929. Table 24 gives some of the results obtained by the latter. The data were verified by ACS (1931), who irradiated liquid cultures of *Bacillus murimors* with agar cultures, either of the same species, or of yeast, and found that the effect by "muto-induction", i. e., by the same species, was the greater.

BARON and ACS did not recommend the growth rate of bacteria as a universal indicator for mitogenetic radiation. This was done most successfully by WOLFF and RAS (1931). These authors worked with different species, and the number of cells was determined by the customary method of bacteriological technique, the agar plate count. Instead of making dilutions in water, they took their samples with a WRIGHT pipette which

delivers $\frac{1}{250}$ of a cc. using the slide cell method. FERGUSON and RAHN (1933) obtained good results with the $\frac{1}{100}$ cc. pipettes used in the standard (Breed) method for the microscopie count of bacteria in milk.

The plating of such minute quantities is necessary because only very small amounts of the culture (about 1 cc.) can be exposed, on account of the strong absorption of ultra-violet light by the customary bacteriological media. WOLFF and RAS (1931) showed that a layer of standard nutrient broth 0.5 mm. thick transmitted only rays above 2500 Å; if broth is diluted with 9 parts of water, a layer of 1 mm. still transmits some rays as low as 2200 Å. WOLFF and RAS irradiated their bacteria in standard broth in a layer of 0.6 mm.; even then part of the bacteria were shaded.

FERGUSON and RAHN (1933) verified this observation. 1 cc. of a standard broth culture of Bacterium coli in a quartz dish in a layer of 0.6 mm. irradiated from below showed no increase over the control, while a culture in broth diluted 1 : 10 showed a good mitogenetic effect. WOLFF and RAS pointed out that only during the lag phase, definite results could be obtained. During rapid growth, there was no effect.

A most interesting observation was the exhaustion of bacteria by continued irradiation, resulting in a decreased growth rate. The two experiments in Table 25 show a lag period of about 2 hours in the control. Irradiation decreases this period very distinctly, and with increasing intensity, or decreasing distance, the lag becomes shorter and shorter. However, continued irradiation after the lag phase retards the growth for some time, and at 5 hours, the control shows more cells per cc. than any of the cultures whose growth was distinctly stimulated. This retardation of growth is only temporary; most of the irradiated cultures almost doubled their number during the 5th hour, indicating a return to the normal growth rate (fig. 34).

Method (the most recent method by WOLFF and RAS, 1933a). A fresh suspension of staphylococci in broth, with about 20 000 cells per cc., is placed in a glass dish in a very thin layer, covered with quartz, and exposed (e. g. to milk + rennet in a quartz tube). After exposure, the dish with the bacteria is incubated at 37° C for 15 to 30 minutes; so is the control. By means of a capillary pipette, samples of the exposed culture and control are either plated on agar, or brought into "slide cells" according to WRIGHT.

Table 25. The Effect of Different Intensities of Continous Radiation through Quartz upon the Rate of Growth of *Staphylococcus aureus* Sender: Agar surface culture of *Staphylococcus aureus*, at various distances

Distance between sender and detector		Cells per cubic centimeter			
		Control	12.5 cm.	5 cm.	2 cm.
Experiment I	start	31 200	31 200	31 200	31 200
	after 1 hour	31 400	32 200	**39 700**	**50 200**
	„ 2 hours	32 100	**55 700**	54 000	48 800
	„ 3 „	45 000	57 600	51 700	46 800
	„ 4 „	133 000	128 500	117 000	51 200
	„ 5 „	262 000	237 000	135 000	79 200
Experiment II	start	14 700	14 700	14 700	14 700
	„ 1 hour	14 650	14 200	16 400	**24 500**
	„ 2 hours	15 100	16 600	**32 400**	20 250
	„ 3 „	17 100	**28 700**	32 700	22 500
	„ 4 „	50 700	80 800	46 700	21 400
	„ 5 „	123 000	108 500	79 300	44 400

The unirradiated controls never show growth in this short time while the irradiated cells do.

Wolff and Ras consider over-exposure the most common cause of failure; over-exposure either produces no effect at all, or eventually a decrease of growth rate (see p. 115).

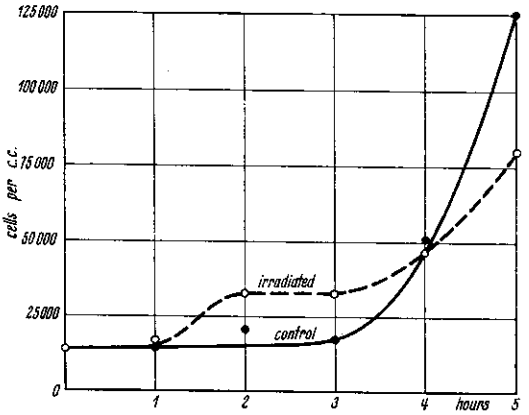

Figure 34. Developpement of two liquid staphylococcus cultures of which one was exposed continuously to the radiation from a staphylococcus agar plate.

The error of the method is mentioned in 1934; four detailed series are given, the counts being 73.1 ± 3.6; 91.2 ± 3.3; 133.8 ± 4.5 and 171.1 ± 7.3. The errors are between 3.4 and 4.9% of the total count; the increase by irradiation amounted to 25% and 28%.

Method: FERGUSON and RAHN (1933) studied the best conditions for a good mitogenetic effect with *Bacterium coli*, and found that it depended primarily upon the age of the culture, and the transmission of ultraviolet by the medium. 24 hours old cultures never reacted; cultures 48 hours old or still older always responded. The best medium was 1 part standard broth plus 9 parts water. The simplest procedure is to irradiate 1 cc. of an old culture in dilute broth (either in a quartz dish of 4—5 cm. diameter, through the bottom, or in a quartz-covered glass dish, from above), dilute this 1 cc. after exposure 1 : 10 000 with dilute broth, incubate, and plate at least every 2 hours for 6 to 8 hours. The effect may not become apparent if the cell concentration is too high (over 100 000 per cc. at the start of incubation, see Table 41 p. 136). The time of exposure depends upon the intensity of the source, and no rules can be given. With a 4 hours old agar surface culture (37° C) as sender, the best results were obtained with 15 to 30 minutes of irradiation (see Table 26).

3. Computation of the Induction Effect: The induction effect in these bacterial cultures can be computed in the same manner as explained on p. 64. As a matter of fact, this has been done by SEWERTZOWA (Table 24) and ACZ. This computation implies that multiplication of bacteria is arithmetical, while in truth it is exponential. By computing the growth rates, we have a really reliable measure. The growth rate is usually substituted by the generation time, i. e. the time required for the average cell to double. It is computed from the formula

$$g = \frac{t \times \log 2}{\log b - \log a}$$

where a is the number of cells at the beginning, and b the number after the time t. Both methods have been applied in Table 26. The induction effect calculated from the numbers directly is in one case (15 minutes exposure) 183, while the effect in the same culture, computed from the generation time, is only 27. However, it must be considered that the number 183 has no real biological significance while the other indicates that during the four hours, this culture had a growth rate 27% higher than the average of the two controls.

Table 26. 3 days old culture of *Bacterium coli*, irradiated for various lengths of time by an agar surface culture of the same bacterium

(The numbers are cells per cc. after diluting the irradiated cultures 1 : 10 000 with broth)

	Control No. 1	Duration of Irradiation 60 min.	30 min.	15 min.	7.5 min.	Control No. 2
start	5 050	5 650	6 800	7 250	6 250	6 550
after 2 hours . . .	5 700	5 800	7 950	7 750	5 850	7 000
„ 3 „ . . .	—	11 000	14 350	14 400	8 300	8 500
„ 4 „ . . .	23 750	24 250	29 000	35 350	19 150	19 550
„ 6 „ . . .	188 000	223 000	434 000	457 000	139 000	137 000
Induction Effect .	—	37	168	183	—16	—
Generation Times, in minutes, for the time interval from 2—6 hours						
Generation Times	47.6	45.5	41.5	40.8	52.5	56.0
Induction Effect .	—	+14	+25	+27	—1	—

This procedure has also been used in Table 24. It could be used with yeasts as well, e. g. in Table 22. However, the error becomes very large if the increase is small. In the tables given by SCHREIBER (1933), the generation time of yeast for the first 2 hours when the mitogenetic effect is strongest is mostly more than 2 hours. This means that not all cells had divided in this time. Though SCHREIBER does not give the actual numbers of cells from which he calculated the generation times, it seems from his curves that they were computed from less than 100 cells. This makes the error very large, and a comparison of the growth rates must necessarily result in enormous percentual differences. Thus, SCHREIBER found the variations in duplicate plates of *Saccharomyces ellipsoideus* commonly to reach 40%, and occasionally more, and in one case even 163%. With *Nadsonia*, the deviation of duplicates went as high as 237%. With such a large error, there is little hope of detecting mitogenetic effects.

The "Induction Effect" as usually calculated (p. 64) has no definite meaning. It permits no comparison with the probable error of the method. It is very unfortunate, therefore, that many investigators, especially the Russian scientists, record the

obtained effects merely by giving the "Induction Effect". The critics point out very justly that such relative numbers are not convincing. It would add a great deal to the general recognition of biological radiation if the actual data obtained (numbers of cells, of mitoses, percentage of buds etc.) were given for the exposed culture, the control and also for the same culture before the beginning of the experiment.

d) Detection by cell division in larger organisms

The three detectors mentioned above are the only ones that have been commonly used to prove the existence of mitogenetic radiation, a few others have been employed occasionally, but not often.

Mention is made of the reaction of mold spores upon mitogenetic rays. The first publication of actual effects is probably that by SCHOUTEN (1933). WOLFF and RAS (1933c) mention that they react slowly and require about ten times as long an exposure as staphylococcus cultures.

FERGUSON and RAHN, in some unpublished experiments, observed that the radiation of the detector culture may be reflected, and may thus give a mitogenetic effect even in the controls which received no radiation from outside. Spores of *Aspergillus niger* were spread on an agar surface. When the dish was covered with a glass cover or a quartz plate, germination was more rapid than when the cover consisted of black paper or sterile agar. The effect varies in magnitude, and is not always present, but must be guarded against in this technique and probably in most others. This reflection may be the cause of many failures to observe mitogenetic rays. The strong effect can be explained by polarisation of the rays through reflection (see p. 45).

Exper. No.	Percentage of germinating mold spores			
	Reflecting surface		Non-reflecting surface	
	Glass	Quartz	Agar	Black paper
13	36.0	35.0	19.8	19.6
14	33.3	35.1	14.5	23.3
15	42.8	39.0	22.4	35.2
16	33.2	—	—	37.4
17	—	45.8	18.4	14.8

In the animal kingdom, the eggs of the smaller animals have been used occasionally to demonstrate the mitogenetic effect. REITER and GABOR (1928) showed that frog eggs when irradiated with the spectral line 3340 Å developped more rapidly into tadpoles than the controls. Too long an exposure retarded the development. The wave length is unusual as in all publications by REITER and GABOR (see p. 60).

In a short paper, WOLFF and RAS (1934b) showed that eggs of the fruit fly *Drosophila melanogaster* hatch more rapidly after having been exposed to the radiation from bacterial cultures (see also p. 144).

The eggs of sea urchins were found to be quite good detectors. The rate with which they divide, can be easily seen under the microscope, and the percentage of eggs in each of the different stages is a good indication of the growth rate. SALKIND, POTOZKY and ZOGLINA (1930) were the first to show that biological radiation from growing yeast or contracting muscle will increase the rate of development of the eggs.

The Italian school of mitogeneticists has also used sea urchins repeatedly. Not all species are equally well adapted as detectors, some being much more sensitive than others. Table 27 shows some data by ZIRPOLO (1930).

Table 27. Mitogenetic Effect upon the Eggs of the Sea Urchin *Paracentrotus lividus*

Sender	Percentage of Eggs					
	Control			Irradiated		
	unchan.	2 blast.	4 blast.	unchan.	2 blast.	4 blast.
Bacillus Pierantonii	82.6	13.9	3.5	10.7	87.6	1.7
„ „	74.5	25.5	0	35.0	65.0	0
„ „	70.0	30.0	0	8.4	88.4	3.2
Penicillium (in dark)	97.8	2.2	0	7.9	91.5	0.6
„ (diffuse light)	70.4	25.7	3.9			

The morphological changes of the larvae brought about by irradiation of sea urchin eggs will be discussed later (p. 164) since a different principle is involved.

Tissue cultures would appear to be an interesting subject for the study of this radiation. The first investigation was started without the knowledge of GURWITSCH's discovery.

GUILLERY (1928) observed during some experiments on the growth-promoting agents for tissue cultures, that two or three cultures in the same dish influenced one another. While the most rapidly-growing culture usually maintained its growth rate, that of the more slowly-growing ones was distinctly increased. Fig. 35 shows the relative daily increase of three cultures from the heart of chicken embryos, which were transplanted at different ages of the embryo, and therefore had different characteristic growth rates. These remained constant as long as the cultures

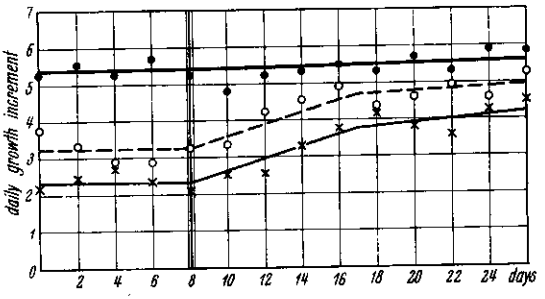

Figure 35. Daily growth increments of three cultures of chicken embryo, grown separately for 8 days, then united in the same dish.

were grown in separate dishes, but were changed when all three were continued in the same dish. Incisions in the solid medium which separated the cultures and prevented diffusion from one to the other did not prevent the mutual stimulation. Even when a strip of solid medium was completely removed between two cultures, the effect sometimes continued. When a glass slide was used to separate the cultures, the influence ceased, even if the slide did not touch the bottom, and permitted diffusion. Further experiments showed that the effect spreads rectilinearly; by placing several cultures in the same dish, and inserting small glass strips between some of them, the effect of the glass was found to be that of shading. This can be explained only as radiant energy which stimulates growth. It could also be shown that the radiation was reflected from metal mirrors.

These observations suggested to GUILLERY the possibility that the embryo extract which is necessary for the growth of tissue cultures, acts essentially as a source of radiation. He irradiated a number of cultures in a dish, from one side, with embryo extract, but the result was negative. However, with a

head of a chicken embryo, he obtained in all 4 experiments a more rapid growth in the culture nearest to the radiating substance.

The second paper on this subject was that of CHRUSTSCHOFF (1930) who observed that growing tissue cultures began to radiate some little time after transplantation from the original tissue. With a spleen culture of *Ambystoma tigrinum*, radiation began after 60 hours; with a fibroblast culture from the heart of a chicken embryo, after 12 hours. CHRUSTSCHOFF believes that radiation is due to autolysis of necrotic parts in the culture.

Next, CHRUSTSCHOFF used the tissue culture as detector. A fibroblast culture (chicken) was divided into halves, both were cultivated in separate drops on the same quartz cover glass, and one of the two was irradiated for 48 hours by a beating embryo heart, which was replaced when necessary. At this time, the irradiated culture appeared much denser than the control; after 3 days (the last day being without radiation), the exposed culture was far in advance of the control.

JAEGER (1930) observed that blood radiation retarded the growth of tissue cultures. Here, as with all other detectors, some investigators obtained negative results. LASNITZKI and KLEE-RAWIDOWICZ (1931) as well as DOLJANSKI (1932) could find no stimulation by mitogenetic radiation. DOLJANSKI used cultures which reacted promptly upon addition of embryo extract, but they did not respond at all to organic radiations, even if the distance was only $\frac{1}{2}$ mm.

Very recently, JULIUS (1935) obtained definite growth stimulation of chick fibroblast cultures, but only on poor media. By placing one half of a culture on glass, the other half on quartz, and exposing both to radiation from staphylococcus culture, those on quartz grew more rapidly as measured by means of a planimeter. The induction effect was recorded as the ratio of increase in the quartz culture over that in the glass culture. In 56 such pairs, the average effect was 1.92 ± 0.15. Another set of 48 pairs, but without irradiation, gave the ratio 1.01 ± 0.08, proving no chemical effect from glass or quartz. The actual stimulation by radiation from bacteria was therefore 0.91 ± 0.17, the effect being 5.3 times the probable error.

It may be that a certain stage of development is necessary to make the cells sensitive to the mitogenetic stimulus, as was shown for yeast and bacterial cultures (pp. 61) and 120).

Table 28. Mitogenetic Effects produced in the Corneal Epithelium of vertebrates by 3—4 minutes exposure of the left eye to the spectral line 2030 Å

Number of Mitoses								
Triton			Frog			Rat		
left eye exposed	right eye control	% incr.	left eye exposed	right eye control	% incr.	left eye exposed	right eye control	% incr.
54	27	100	277	108	158	3086	1944	78
134	66	100	3200	2070	50	3312	2050	60
130	51	150	615	333	85	1644	1187	40
50	30	68	695	205	240	440[1])	196	123[1])
53	38	40	885	221	300	2593[1])	1578	70[1])

A very good detector is the corneal epithelium of vertebrates, according to LYDIA GURWITSCH and ANIKIN (1928). It is the only easily accessible tissue of the grown animal showing frequent mitosis. The number of mitoses varies with the physiological state; it increases rapidly with good nourishment, dropping in rats during starvation from approximately 2000 to about 50. Fortunately, the two corneae of the same animal always show very nearly the same number of mitoses, so that one eye can be irradiated, and the other used as control.

Method: For physical light sources, an exposure of 3—4 minutes was sufficient, the animal's head being held in the hands of the experimentor. With biological sources, exposure had to be continued for 20 minutes, which necessitated the tying down of the head into an immovable position. Ordinarily, after irradiation, 3—4 hours time was given for the manifestation of the effect. The cornea was fixed for 40 minutes in 70% alcohol + 5% acetic acid, stained with hämalaun, and clarified in glycerol.

The results in Table 28 show very strong mitogenetic effects.

e) Detection by changes in yeast metabolism

GESENIUS (1930a) concluded that such decisive changes as the acceleration of the growth rate of yeast must be accompanied, or perhaps preceded by changes in metabolism. He studied, therefore, the influence of irradiation upon the rate of respiration and of fermentation of yeast. The technique employed was

[1]) The source of radiation was a yeast culture.

essentially that of WARBURG (1923), or of RUNNSTRÖM (1928) for measuring respiration of tissues or tissue pulps. The organism used as detector was a wine yeast, which was exposed in quartz-bottomed dishes to yeast radiation for 4 hours before being tested. The result was a stimulation of fermentation, but a retardation of the oxygen uptake.

The results can be briefly summarized in the following way:

Fermentation in N_2—CO_2 atmosphere	102 experiments	68 increase 4 decrease 30 within the limits of error
Respiration (oxygen-uptake) in O_2 atmosphere, without sugar	54 experiments	40 decrease 1 increase 13 within the limits of error

The number of yeast cells in these tests was very large, 7 to 10 billion cells per cc. This is 10 to 100 times the maximal population which can develop in the medium used. The mutual irradiation of the cells must play an important role in these experiments, and probably accounts for the depression of respiration.

GESENIUS tried further the influence of radiation upon macerated yeast, free from cells, i. e. upon zymase. While yeast radiation produced no effect, blood radiation decreased the fermentation in 28 out of 30 experiments, the average depression being 14%. The same retardation of respiration also could be obtained with sea urchin eggs during the early stages of cell division.

GESENIUS (1930b) applied this test, after the improvement of the technique, to blood radiation and found that normal blood always radiated[1]). He observed further that from patients with most diseases, the blood radiated, and that the consistent exceptions were only with cases of pernicious anemia, leucemia, carcinoma and severe sepsis (see fig. 46 p. 153). His results agree very well with those of L. GURWITSCH and SALKIND and of SIEBERT. The role in cancer diagnosis of this loss of radiation will be discussed in Chapter VII.

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[1]) "Healthy blood never fails. If a failure occurs, it is time to test either the yeast or the apparatus." (GESENIUS, 1932.)

f) Morphological changes by biological radiation

Quite different from the previously described manifestations is a decided morphological change in the irradiated organisms. The first observations of this kind were those by J. and M. MAGROU (1928) who exposed the eggs of the sea urchin *Paracentrotus lividus* to radiation from bacteria, yeasts or other organisms, and even from chemical reactions. They obtained quite abnormal, more or less spherical larvae while the normal larvae possess a very characteristic conical form (see fig. 49 p. 165). Upon various criticisms, the greatest care was taken in later experiments (1931) to prevent any chemical influences. In each case, the effect was transmitted through quartz, but not through glass. Table 29 shows a summary of the results of these experiments, and the purely physical nature of the effect cannot possibly be doubted.

While the MAGROUS originally explained this morphological change through mitogenetic rays, later experiments, together with REISS (1931), offered another possible explanation. They found that though a layer of glass prevents the effect, two layers of glass with a coat of paraffine between them permit the effect to pass. From this and similar experiments, these authors conclude that they are dealing with an electric effect. The larvae become abnormal if the source of radiation is separated from the medium of the sea urchin eggs by a very good electric insulator, and if further the difference in oxidation-reduction potential between the two liquids is very great. When the electric insulation was prevented by a metallic connection, the larvae remain normal. This explanation is tentative. In their recent publications, the MAGROUS do not give it preference to the ultraviolet radiation theory.

In 1929, CHRISTIANSEN observed very strange morphological changes in yeasts and in bacteria brought about by menstrual blood. The effect passed through quartz coverslips, and must therefore be considered as the result of biological radiation. The yeast cells either became large and spherical, with enormously distended vacuoles; or, they elongated and produced hyphae; or, they did not grow at all, but died.

During the last two years, the author and his associates have regularly observed similar morphological changes.

Table 29

MAGROU's Results with Biological Irradiation of Sea Urchin Larvae

No. of Experiments	Source of Radiation	Larvae Separated from Source of Radiation by	% of Experiments Showing Abnormal Larvae
76	none	glass	0
4	dead bacteria	glass	0
76	*Pseudomonas tumiefaciens*	quartz	75
7	same	glass	43
36	none	glass	0
25	*Staphylococcus aureus*	quartz	75
3	same, agglutinated	quartz	0
16	none	glass	0
12	*Streptococcus lactis*	quartz	93
2	same	glass	0
4	bacteria-free serum of Streptococcus culture	quartz	25
7	none	glass	0
5	Saccharomyces and Debaryomyces	quartz	100
30	none	glass	0
18	BERTHELOT'S culture medium	quartz	100
6	same	glass	0
56	none	glass	0
36	glucose oxidized by ferricyanide, permanganate or bichromate	quartz	78
14	same	glass	0

Summary

221	none		0
118	microorganisms	glass	33
9	microorganisms	quartz	78
54	chemical reactions	quartz	85
20	chemical reactions	glass	0

The effects in beer and wine yeasts produced by saliva were essentially identical with those observed by CHRISTIANSEN. When irradiated by plants, however, the tendency was not a shortening but rather a lengthening of the cells; this was so pronounced with some Mycodermas that their growth strongly resembled that of mold mycelium. However, we could never observe the true branching of cells which CHRISTIANSEN has described. Of the various parts of plants, the roots, young seeds, seedlings and pollen were the most effective, while leaves had little or no effect (see fig. 48 p. 162, and Chapter VII).

g) Physico-chemical detectors

LIESEGANG Rings: It was recognized by STEMPELL that a physico-chemical detector would carry much more weight than biological ones for the proof of mitogenetic radiation. He observed that the LIESEGANG rings are disturbed by biological radiation. These rings appear when, on a gelatin gel containing certain salts, a drop of another solution is placed causing a precipitate with the salts in the gelatin. The precipitant diffuses gradually into the gelatin, and the precipitate is deposited in concentric rings.

Method: 2 cc. of chromate gelatin (12 g. gelatin, 160 cc. water, 0,4 g. ammonium bichromate, being mixed, immediately before use, with 1 cc. water + 1 drop of 3% aqueous pyrogallic acid) are poured hot upon a clean glass plate 3.5×4 inches. After solidification, 2 drops of a 20% $AgNO_3$ solution are placed on the center of the plate by means of a fine pipette. Silver chromate is gradually precipitated in concentric rings wich spread over the entire plate in about 24 hours. The uniformity of these rings is disturbed by radiating material placed in quartz tubes as closely as possible over the gelatin surface.

STEMPELL's first experiments (1929) with onions were not accepted since it could be shown that the allyl-mustard oil of the onion caused a chemical disturbance of the LIESEGANG rings. In later publications, however, STEMPELL could prove disturbance of the rings when chemical influences were completely eliminated.

The situation is rather complicated. Strong ultraviolet light from a quartz mercury vapor lamp thrown through a narrow slit upon the gelatin intensifies the ring formation at the exposed places, while weak light, at the edge of the slit, decreases it. Onion oil acts in the opposite way; a large dose of onion oil gas lessens the ring formation while small doses intensify it.

STEMPELL (1932, p. 46) states that the disturbance of LIESEGANG rings is usually brought about by a combined action of radiation and chemical effect of a "gas", i. e. a volatile substance produced by the sender. He considers this chemical effect to be very important, biologically, and states that the LIESEGANG rings at present are the only detector for this substance, since none of the other detectors for mitogenetic radiation react to it.

Since this book is meant to be limited to biological radiation, the interesting speculations of STEMPELL (1932, p. 46) regarding

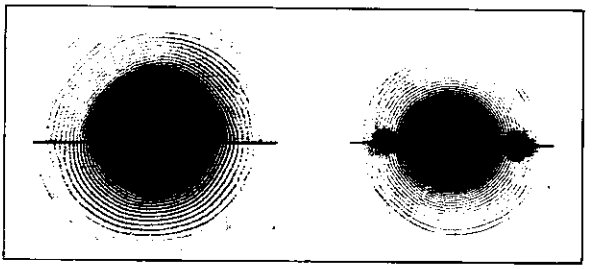

Figure 36. The effect upon LIESEGANG rings of onion base pulp in metal tubes with a slit whose position is indicated by the black line
left: effect through cellophane; right: effect through 0.5 mm of quartz.

the possible biological meaning of the chemical emanations will be omitted.

Decomposition of Hydrogen Peroxide: Another, quite different detector, has been found by STEMPELL (1932, p. 55). It is based on the deterioration of H_2O_2 into $H_2O + O$, under the influence of ultraviolet radiation. One onion root, or a bundle of several roots, is fixed so as to touch the underside of a thin quartz plate. On the opposite side of the quartz plate is placed a drop of H_2O_2, just over the root tips. After long exposure in a moist chamber, the peroxide concentration in this drop is less than that of the control.

Flocculation of Colloidal Solutions: A promising method has been worked out by HEINEMANN (1934, 1935) who observed that inorganic sols flocculate more readily when exposed to mitogenetic rays. Gold sol was found to be more satisfactory than iron hydroxides. The gold sol was prepared in the following way: To 1000 cc. of a slightly alkaline solution of 0.8% glucose,

150 cc. of a neutralized solution of 0.1 g. $AuCl_3$ are added. Upon heating, the gold salt is reduced by the glucose to a bright-red, clear gold sol. A small amount of NaCl solution is added just before the beginning of the experiment to make the gold sol unstable. This is done in complete darkness.

The solution is then distributed between two beakers, and each is covered with a quartz dish. They are placed into a very sensitive photoelectric differential nephelometer, and the supposedly radiant substance is placed into one of the quartz dishes. The difference in turbidity between the two beakers is read every minute, by means of a galvanometer. Radiation causes a more rapid flocculation, and therefore a change of the galvanometer reading while in the absence of radiation, the readings remain fairly uniform. The following readings were obtained in 15 consecutive minutes, the arrow indicating the moment when the radiating material was applied to the quartz dish:

control:	0	0	0	0	0	0	1	1.5	1.5	1	2	2	2	2	2
blood:	0↑	2	3	5	7	7	8	10	11	16	20	19	19	25	26
control:	0	0	0	0	0	0	1	1.5	1.5	2	2	2	2.5	3	3
NaCl, dissolving:	0	2	0	1	1	0↑	5	10	10	11	11	15	21		

h) Measurement by physical instruments

It may be surprising that radiation by organisms has not been recognized and proved conclusively long before this. The reason must be sought in its very weak intensity. The intensity is so slight that the most sensitive photographic plates and the most elaborate physical instruments have in most cases failed to record this radiation. The best detector is still the living organism. This is unsatisfactory since we must allow for considerable individual variation of the detector organisms, and as a rule, the measurements are not as accurate as with physical experiments.

The only photographic records are the ones reproduced in fig. 15 of REITER and GABOR's monograph, and those by BRUNETTI and MAXIA (1930) and by PROTTI (1930). None of them are absolutely convincing, and GURWITSCH has refused them all as proofs of the physical nature of mitogenetic radiation. TAYLOR and HARVEY (1932) could obtain no effect by exposing plates to frequently renewed fermenting yeast for ninety days.

Table 30. Measurements of Mitogenetic Radiation by Means of the GEIGER Counter

Radiator	Time interval	Impacts per interval	
		Control	Exposed
Experiments of RAJEWSKY			
Onion root	5 minutes	42.0±1.0	51.0±7.0
Onion base pulp . .	10 minutes	42.2±1.2	50.0±1.5
Onion base pulp . .	10 minutes	39.8±0.3	49.3±2.2
Carcinoma of mouse	10 minutes	23.4±0.6	30.3±0.5
Onion root	9 minutes	33.3±1.4	36.7±0.5
Experiments by FRANK and RODINOW			
Frog muscle . . .	6 minutes	12±1.3	40±2.6
Frog muscle . . .	8 minutes	12±1.3	20±1.7
Frog heart	11 minutes	19±1.1	23±1.2
Frog heart	5 minutes	34±2.4	46±2.8
Muscle pulp	4 minutes	13±1.8	20±2.2

In 1929, RAJEWSKY succeeded in obtaining direct physical proof of this radiation by means of a photo-electric counter (see p. 28). His data with onion roots and carcinoma are shown in Table 30. These experiments were repeated successfully by FRANK and RODIONOW (1930) with working muscle (see Table 30 and Fig. 21, p. 29). Later experiments of this nature are those by BARTH (1934) and SIEBERT and SEFFERT (1934).

However, these results have not been accepted generally, at least not by physicists. LORENTZ (1933, 1934) could show that bringing the radiating material near the counter, or opening a shutter between the counter and source, may definitely change the counting rate even if there is no radiation present. To enumerate: (1) If the biological material is not in a closed quartz container, the water vapor from the material, even though slight, will condense upon the quartz of the counter, changing its resistance and thus altering the counting rate. Some experimenters have even placed their moist material directly upon the quartz window which will certainly change the counting rate. (2) If the water vapor is carefully kept away from the counter, the charges which are inevitably present on the outside of the quartz tube containing the biological material are almost sure to change the counting rate. (3) In one case, at least, muscle was tetanized by means of an induction coil directly in front of the window.

Table 30a. Photo-electric yields obtained for ultraviolet light

Authors	Photoelectric material	Yield in quanta per electron	wavelength Å
Rajewsky, 1934 . .	Cd	about 1×10^3	2650
Schreiber, 1930 . .	K	„ 2×10^4	2540
Grey and Ouellet, 1933	Pt	„ 6×10^3	2540
Frank and Rodionow, 1932 . . .	Cd, Al	„ 2×10^3	2540
Lorenz 1933 . . .	Cd	„ 3×10^3	2540
Kreuchen, 1934 .	Cd, Al, Zn	„ $2—5\times10^4$	2540

To separate the effect of extremely low intensity from these other effects is very difficult even when their existence is realized. In none of the physical measurements of mitogenetic rays is it absolutely certain that these errors have been excluded, and it seems well to view with caution the positive results claimed for the physical detection experiments so far carried out.

A more careful description of the method of exposure, and a number of experiments with water blanks or non-radiant organic materials will be necessary to produce evidence which is physically irreproachable. The experiments of Siebert and Seffert (1934) who obtained increased counts with several hundred normal blood samples, but no increase with blood from carcinoma patients, are a step in that direction. Most convincing is the experiment that a counter gave a definite increase when exposed to normal blood, but showed no increase when this was removed and replaced by blood + KCN. Many more extended experiments of this general nature will be necessary to establish finally the radiant nature of the biological effects.

In a recent paper, Kreuchen and Bateman (1934) reviewed the field of physical detection and present their results in a table (see Table 30a) which gives the photoelectric yield (see p. 29) of the surfaces used by the various investigators. In all cases except the first, more than 2000 quanta are required to eject one electron. Though some early workers indicated higher sensitivities, it seems from later work that this value has never been surpassed if, in fact, it ever was reached. In his latest paper, Kreuchen (1935) obtained yields of from 10^5 to 10^4 quanta per electron from hydrogen-activated zinc and cadmium surfaces.

It would be of great advantage if some surface having a higher efficiency for the ultraviolet could be obtained. Preliminary experiments by one of the authors using magnesium surfaces sensitized by oxygen seem to offer encouraging results.

RAJEWSKY estimated from his experiments the intensity of this radiation, and found it for onion roots and for carcinoma tissue to be of the magnitude of 10^{-10} to 10^{-9} erg/cm^2/sec. (10 to 100 quanta/cm^2/sec. for the wave length 2300 Å). FRANK and RODINOW observed higher values; they obtained with pulp from muscle, with the working frog muscle and heart, values up to 2000 quanta/cm^2/sec.

For the reproduction of the various mitogenetic phenomena with physical sources of light, much larger intensities are required (about 6.6×10^5 quanta, according to STEMPELL, 1932).

i) Unaccounted failures in proving radiation

It must be stated with perfect frankness that biological detectors sometimes fail for unknown reasons. Probably all investigators working with biological detectors have been worried by such failures. Some of them have published short remarks. GOLISHEWA (1933) mentions that out of 373 experiments with blood radiation, in GURWITSCH's laboratory, 54 failed on account of a poor quality of the yeast culture which was used as detector. This cause became evident through the fact that all other associates using the same culture on the same day obtained negative results. No reason for the abnormality of the yeast culture is mentioned.

WOLFF and RAS (1933c) working with Staphylococci had a similar experience. Twice it happened that all the experiments of one day proved to be negative though the culture had reacted promptly on the previous day. It was found that the sensitivity of the culture had changed, and that a longer exposure was necessary. These authors believe that a change in the opposite direction may also take place. ACS (1932) claims to have increased sensitivity by selection.

Most of the sudden failures of cultures to react have not been published, but by discussing this point with the various investigators in this field, practically all seem to have had the same experience. Professor GURWITSCH has told the author that in his experience such a condition usually remained for several days, or even for a number of weeks, and it was impossible to produce even

the simplest mitogenetic effect. Eventually the culture reacted normally again. Doctor HEINEMANN, after a very successful diagnosis of cancer by the absence of blood radiation (see p. 181) in Frankfurt and in London, with yeast as detector, suddenly experienced a complete lack of reaction, and none of the various attempts to obtain normal reactions proved successful, not even the testing of a large number of different yeast cultures. This failure induced him to look for physico-chemical methods of detection (see p. 89). Professor WERNER SIEBERT's many succesful experiments with a yeast detector have been mentioned in practically every chapter of this book. But with him, too, the yeast suddenly ceased to react, and he resorted to the GEIGER electron counter as a more dependable detector (see p. 92). The author himself has also had long periods of negative results in his laboratory, and they come and go at irregular intervals.

As a rule, the investigators do not discuss these periods of failure because there is still considerable doubt among physicists and some biologists concerning the existence of the mitogenetic phenomena, and the emphasis of such periodical failures might increase this doubt.

While this can not be denied, it does not seem wise to belittle this experience. On the contrary, by calling attention to it, it may help to explain the cause of these failures, and thereby may bring about a better understanding of mitogenetic effects. Whether it is due to disturbance by short radio waves (suggestion by GURWITSCH), to a change of sensitivity of the detector culture (WOLFF and RAS), to a retarding effect by human radiation (RAHN), to climatic changes or some other cause, is not known. The cause of this disturbance might be more readily traced and overcome by the cooperation of various laboratories. At the present, we do not know whether the culture, the experimenter or the environmental laboratory conditions have changed, in fact, it is only an assumption that the change has influenced the detector. If the cause should prove to be of such general nature as e. g., weather, cosmic rays, terrestrial magnetism, sunspots, it might be that the senders do not function under the prevailing condition.

These occasional failures have nothing to do with the error of the method. When mitogenetic effects are observed, they are outside the limits of error. The failures might be compared to

the experience of expert florists that sometimes, certain plants refuse to bloom in the greenhouse. This was not caused by poor seed nor wrong soil, but remained unexplained for a long time until it was found that the number of hours of light per day decided this.

The consistent observation of this disturbance by most (if not all) investigators who have obtained large series of positive results, points out a common error in some criticisms. It has been claimed that many simultaneous parallel experiments prove more than similar experiments spread over a longer period of time. E. g. KREUCHEN and BATEMANN (1934) state that one series of theirs is equivalent to 140 single experiments by GURWITSCH. That is a mistake. As long as it is not known why the occasional failures occur, no great stress can be laid upon the results obtained on any one day. It might be a day where the detector fails, and the multiplication of experiments on such days would only reveal the error of the method as such, but would not increase the proof or disproof of biological radiation.

B. INJURIOUS HUMAN RADIATION

It is an old "superstition" that a harmful emanation comes from the body or the hands of menstruating women. It is believed that bread dough kneaded by them will not rise, that food preserved by them will not keep, that flowers in their hands wilt readily. Experiments to prove this have been successful with some investigators (SCHICK, 1920; MACHT and LUBIN, 1927; BÖHMER, 1927) and gave no results with others (SÄNGER, 1921; FRANK, 1921; POLANO and DIETL, 1924). Still, the belief in this effect seems to have been rather prevalent among medical men, and the term "menotoxin" for the hypothetical compound causing it is in general use.

CHRISTIANSEN, as bacteriologist of a dairy laboratory in Germany, observed that the pure cultures used for dairy starters occasionally developed poorly and abnormally. After eliminating all other causes, it was ultimately found that this abnormality occurred during the menstrual period of the woman technician in charge of the cultures.

A detailed investigation by CHRISTIANSEN (1929) led to the discovery that the effect from menstrual blood passed through

quartz, and must, therefore, be considered a radiation. Aside from this proof, CHRISTIANSEN did not go into the nature of the effect. He worked with menstrual blood and saliva, but not with radiations from the body.

The blood produced either abnormal morphological changes in yeasts and bacteria, or killed them. The effect was much stronger in summer than in winter, and since one woman who had been treated with ultraviolet light in winter, continued to show

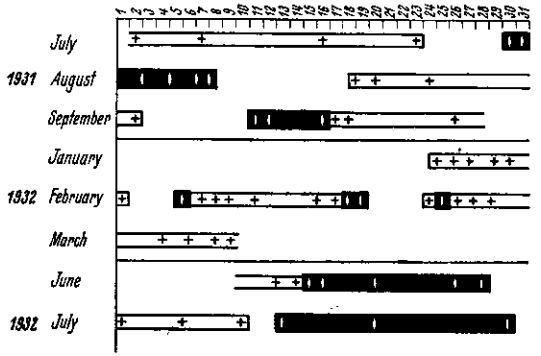

Figure 37. Alternation of growth and no growth of yeast in droplet cultures transferred by the same person.
+ signs indicate growth, o signs in black bars indicate no growth.

strong radiation, he thought it very probable that the seasonal difference was brought about by the variation in solar irradiation. He called attention to the fact that the above-mentioned investigators obtaining positive results had made their experiments in summer, while the negative ones had been obtained in winter. Further, he showed that wine made with a pure culture yeast by a menstruating woman fermented feebly, while the control showed a vigorous normal fermentation.

FERGUSON (1932) during an investigation of morphological changes in yeast by plant radiation, observed occasionally that for short periods, coverglass cultures of one yeast did not grow when made by one certain woman student. The periods of no growth alternated with those of growth through summer and fall, while in winter, the controls nearly always grew normally (fig. 37). The droplet culture on the coverglass requires that the coverglass be held in the fingers while the droplets are made with a pen dipped into the yeast suspension.

The above-mentioned experiments of CHRISTIANSEN made it probable that this failure was due to human radiation, and some experiments proved that an emanation from the fingertips of this person killed yeast in 5 minutes while others making the same test with the same culture produced no marked effects. In this experiment, the fingertip was held closely over the yeast by the support of a glass ring (fig. 38). In another experiment, a quartz plate of 2 mm. thickness was placed between finger and yeast (fig. 38); by this method, it required 15 minutes to kill the yeast. This student menstruated rarely and irregularly, and the case suggests an abnormal parallel to CHRISTIANSEN's observations.

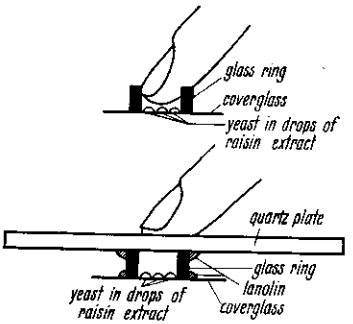

Figure 38.
Methods of testing finger radiation.

This test has been applied to a number of people, and it was found that this radiation occurs only very rarely. It was most pronounced with a man who had recently recovered from *herpes zoster* of the face; for about 6 months after recovery, he frequently killed yeast through quartz in 15 minutes, but this was not always the case. During this time, he did not feel quite well. After a summer vacation, he felt perfectly normal, and his power of radiation was gone. With this person, radiation also occurred from the tip of the nose and from the region of the eye.[1])

A third case was a hypo-thyroid patient tested only once.

A fourth case was one of the authors, during 3 successive days of sinus infection. Never before or afterwards did this person show any effect upon the yeast.

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[1]) This has been interpreted by imaginative but uncritical newspaper reporters as a scientific proof of the "Evil Eye", regardless of the fact that this radiation does not reach further than a few inches, and that only one especially sensitive species of yeast could be killed in this way; the authors are in no way responsible for this kind of publicity, but have not been able to prevent it.

The experiments were always made with droplet cultures through quartz, as shown in fig. 38. In all more recent work, the droplets were prepared by the same person who had been found over a 2-year period never to radiate.

The only organism which reacted upon this radiation was *Saccharomyces mycoderma punctisporus* GUILLIERMOND, isolated from the scum of a fruit juice. This yeast does not cause fermentation. Other yeasts showed slight retardation, but never was complete killing observed.

This radiation is quite likely due to a skin excretion, as will be shown later (p. 185).

Another killing effect was frequently observed with saliva. The saliva of many persons changes the oval or elliptical, granulated cells of beer and wine yeasts to spherical cells of increased size, with greatly distended vacuoles and homogeneous cell contents. Often, the organisms either do not grow at all, or cease to do so after a few cell divisions.

It is not certain, however, that the effect is one of radiation. It was observed in droplet cultures which were mounted immediately above the saliva; this did not exclude a chemical effect. When saliva was mixed with the raisin extract in which the yeast was cultivated, it produced no abnormal cells, not even with half saliva and half raisin extract. This seems to exclude any chemical effect. On the other hand, the typical saliva reaction could not be produced through quartz; with yeast on one side and saliva on the other, only partial effects could be obtained, such as absence of granulation, or tendency to become spherical. The pictures were never convincing, and it must be left to a later investigation to solve this problem.

This harmful effect is not typical for all saliva. It is typical for the individual, and pratically independent of the diet. An investigation of the saliva reactions of several members of a family showed the above-mentioned injurious effect with male members of the family, and one female member. Two other females stimulated yeast growth, produced elongated forms, and, with *Saccharomyces mycoderma punctisporus*, a manner of growth strikingly resembling mycelium.

C. NECROBIOTIC RAYS

LEPESCHKIN had observed (1932a) that the stability of living matter (plant cells, erythrocytes) is increased by irradiation with weak ultraviolet light, while strong intensities made cells less stable. The two effects are independent of each other. He concludes (1932b) that weak intensities of ultraviolet must help in the synthesis of cell constituents, and that *vice versa*, when cell constituents break down, the energy absorbed during synthesis must be released again, emitted, partly at least, as ultraviolet radiation.

The experimental proof is given in considerable detail in a later paper (1933). The test organism was nearly always yeast, though parallel experiments were made also with leaves of *Elodea*, with petals of flowers, e. g. of *Papaver*, and with suspensions of *Bacillus subtilis*. When silver nitrate was added to a living yeast suspension in the dark room, the yeast died within 12 minutes, and the suspension was gray. When the yeast had been killed by ether or by heat before the silver salt was added, the suspension remained white, but turned gray upon exposure to light. The gray color of the silverprotein precipitate with living yeast was supposed to be brought about by ultraviolet rays emitted by the dying cells. A similar difference could be observed when yeast was suspended in a mixture of solutions of KBr and $AgNO_3$. Living yeast with ether caused a dark discoloration, but when the yeast had been killed by ether before the AgBr mixture was added, the mixture remained light-colored.

Since these experiments did not exclude chemical effects, AgBr suspensions in small quartz tubes were inserted into tubes with dead yeast, and also into those with living yeast plus ether. After exposure with continuous shaking in the dark room, the AgBr suspensions were removed and mixed with a photographic developer. In all experiments, the suspension exposed to dying cells proved to have received some radiation.

Then, very sensitive photographic plates (EASTMAN SPEEDWAY) cut in small strips, were submerged directly into the yeast suspension. Part of the plate was covered with filter paper which excluded physical, but not chemical effects by soluble substances. After 10 to 25 minutes exposure to yeast previously killed by ether, the plates upon development remained light. With living

yeast, they were also light. However, when ether was added to the living yeast, the dying cells affected the plates so that during developing they turned dark, except for the little strip shaded by the filter paper. This proved to LEPESCHKIN's satisfaction that the effect was physical and not chemical.

From the absorption of these rays by glass and by gelatin, LEPESCHKIN estimates their wave length to be largely between 1800 and 2300 Å, with a very weak emission of greater wave lengths. This agrees fairly well with the range of mitogenetic rays. From the above experiments, it seems as if the necrobiotic rays were stronger than those from actively fermenting yeast cells. LEPESCHKIN could obtain an indication of an effect upon AgBr suspensions if he used living beer yeast instead of baker's yeast, and added 10% sugar. However, though there was a slight effect from the fermentation upon the AgBr, the effect from the same mixture with ether i. e. with dying cells was much stronger.

LEPESCHKIN then ventures further to state that many of the mitogenetic phenomena are in reality due to necrobiotic rays. He emphasizes the radiation of necrobiotic processes e. g. autolysis and of wounds as proof for his contention. Evidently, LEPESCHKIN was not familiar with the latest literature on this subject. In the case of wounds, it is not the injured cells which radiate, but the uninjured cells next to the wound. The radiation spectra of the various chemical processes are ample proof that dying cells are not necessary for the production of mitogenetic rays. LEPESCHKIN apparently feels this; he believes it possible that "necrobiotic rays" might also be emitted from a decomposition of vital compounds in living cells which might be imagineable during very rapid physiological processes. He mentions respiration as a possibility. However, it would be necessary to consider almost all exothermic reactions as necrobiotic processes in order to combine the two types of radiation.

SUCHOW and SUCHOWA (1934) have perhaps found the link between LEPESCHKIN's and GURWITSCH's explanation. They conceived the idea that the "necrobiotic rays" were emitted from the coagulation of proteins, and they tested it by coagulating egg white by alcohol in quartz or glass vessels which were placed over AgBr-suspensions as in LEPESCHKIN's experiments. The experiment was carried out in the dark, and after exposure, the two

suspensions were brought into light. In 25 experiments, the suspension which stood under the quartz vessel uniformly darkened sooner than the other.

D. INFRA-RED RADIATION

It might appear rather probable that some organisms would emit infra-red rays since some are capable of producing visible and ultraviolet rays. The only case of near infra-red emanation known to the authors is, however, a series of observations by STEMPELL (1931) that sprouting peas will increase distinctly the rate of spontaneous decomposition of a saturated solution of H_2O_2. The effect passed through glass, and was not visible, it must therefore be of an infra-red nature. The temperature of the peas rose 0.5° by their own respiration, but an artificial increase of 5.5° was necessary to bring the rate of peroxide decomposition to that produced by peas.

Equally rare are observations of an effect of infra-red rays upon living organisms. The only one known to the authors is an experiment by NELSON and BROOKS (1933). They exposed the unfertilized eggs of 2 sea urchin species and of one worm to infra-red rays of 8000 to 12 000 Å, obtained from a Mazda lamp by means of a monochromator. After 15 to 45 minutes exposure, the eggs were fertilized by the usual method. The irradiated eggs showed, in each of the 9 experiments a distinct decrease in the percentage fertilization. The decrease varied between 18.5% and 87.1%. The temperature difference between control and exposed eggs was not more than 0.06° C. The authors believe therefore that the reduced fertilization is caused by a photochemical effect.

E. BETA-RADIATION

An entirely different type of radiation should be mentioned only in passing, namely the beta-radiation of potassium. Though it has been proved experimentally, and is of importance to life processes, it will not be discussed extensively here because this type of radiation is utterly unlike the mitogenetic and related rays. It originates from the radioactive fraction of potassium, and

Nevertheless, this radiation is biologically important, and may be the chief reason for the indispensibility of potassium in living organisms. ZWAARDEMAKER (1921) was the first to test experimentally the physiological importance of potassium radiation. He succeeded (1926) in keeping isolated frog hearts beating by substituting the potassium of RINGER's solution by radioactive equivalents, rubidium, uranium, thorium, radium or ionium. In 34 experiments, he could show that frog hearts which had ceased to beat in RINGER's solution minus potassium, started again in the same solution after about half an hour's irradiation by mesothorium (in glass) or radium (through mica). After removal of the radioactive substance, heart beat soon ceased again and could frequently be brought back a third time by new exposure to beta rays.

SCOTT (1931) determined the total energy from potassium of the average human heart to be 9.64×10^{-6} ergs per second. He states that "one may readily conceive that the free energy of the beta particles can be cumulative and, reaching a maximum, transform the potential energy of the heart muscle in response to node and bundle impulses into the enormously greater manifestation of kinetic energy, the systolic contraction".