CHAPTER VII

THE SIGNIFICANCE OF BIOLOGICAL RADIATIONS IN BIOLOGY, MEDICINE AND AGRICULTURE

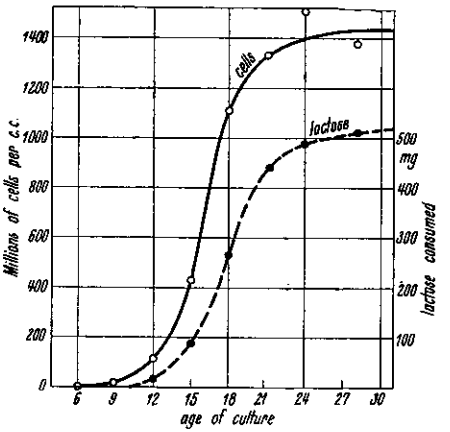
A. UNICELLULAR ORGANISMS

(1) Emission at Different Physiological Stages: It had been emphasized throughout this book that intense mitogenetic radiation has been observed almost exclusively in young, actively growing tissues or cell cultures, while fullgrown ones do not radiate at all, or only rather weakly.

According to GURWITSCH, we must distinguish between two sources of radiation, the one emitted at the moment of nuclear division, and the other resulting from general cell metabolism, such as oxidation, proteolysis, glycolysis. A culture of yeasts or bacteria should be a good sender as long as either the cell division is rapid, or the metabolism remains active. Under optimal conditions of food and temperature, both these functions should cease almost entirely within 24 hours after transfer. Fig. 43 shows the development of a culture of *Streptococcus lactis* at 21°C (RAHN, 1932, p. 401). The left-hand curves represent the multiplication of the bacteria and the gradual accumulation of lactic acid from sugar, by glycolysis. The right-hand curves show the *increases* in each 3-hour interval. These increases must be proportional to the intensity of radiation of the culture, as it is apparent that there can be no other important source of radiation. The bacteria starting with 38 000 cells per cc., can have emitted a noticeable degree of mitogenetic radiation only between 12 and 24 hours. Before that time, the radiation per cell might have been quite as strong or stronger, but the number of cells was too small; while afterwards the cells, though more than a billion per cc., have ceased to produce acid, and therefore to radiate.

With a heavier inoculation, e. g. by flooding the surface of an agar plate with a suspension of yeasts or bacteria, radiation begins sooner because the "active mass", i. e. the number of cells, is greater. At lower temperatures, the intensity is less, but the phase of active radiation is prolonged. With processes which do not result in an inhibiting product like acid, the period of active radiation may also be longer.

This *a priori* deduction is made doubtful by the secondary radiation of the medium in which the bacteria grow. The actual radiation of the bacteria themselves must follow the lines of fig. 43; the emission by the entire culture may not. Intensity of secondary radiation may not be proportional to primary intensity, and be-



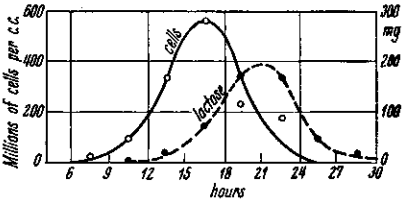


Figure 43. Development of a culture of *Streptococcus lactis* in milk. Age of culture in hours. Full line: number of cells per cc.; dotted line: mg of lactose decomposed in 100 cc.
left: total cells and total lactose fermented; right: cell increase for each successive 3-hour period, and lactose consumed during these 3 hour periods.

sides, since the nutrient medium is continuously changed by the bacteria, its property as secondary sender may change.

However, the theoretical deductions are essentially verified by the observation of WOLFF and RAS (1932) that an agar surface culture of *Staphylococcus aureus* remains actively radiating until approximately 18 hours old. When the cultures are full-grown, i. e. when there is no further appreciable increase in cells, nor in metabolic products, the culture ceases to radiate.

Whether metabolism without cell division can produce mitogenetic rays, was decided in the affirmative by a simple experiment of the authors with bakers' yeast suspended in sugar

Table 38. Mitogenetic Effects from Protozoa in Glucose Solution

	Without Sugar	After Addition of Sugar			
		immediately	15 minutes later	20 minutes later	30 mintues later
Opalina	0	—2.6	+40	+36	—
	+4.4	+2.5	+20	+24	—
Paramaecium	+2.7; +2.0	—7	+11	—	— 3.8
	—2.8; +1.0	+7	+12	—	+14.0
	—1.0; +3.0	+3.7	+27	—	—

solution. This yeast cannot grow under the experimental conditions because too many cells are present; however, even if slight growth were to occur, many hours would be required to overcome the lag. During the first hour, therefore, no radiation from cell division is possible while alcoholic fermentation starts at once.

An interesting set of data on the radiation of *Hydra fusca*, the well-known fresh-water polyp, has been given by BLACHER and SAMARAJEFF (1930). They found that a pulp of the entire organism radiates. If dissected, the hypostom and the budding zone radiate while the other parts do not. No increase in emission could be detected during regeneration.

The common infusoria do not appear to radiate under normal conditions, but produce a secondary radiation. According to Zoglina (quoted after GURWITSCH 1932, p. 64) the following mitogenetic effects were obtained:

from *Opalina* . . . . . 0; +4; +3; +6; +12
„ „ irradiated by blood . . . . . +47
„ „ „ „ frog heart . . . . +21
„ „ „ „ spectral light . . . +15; +19; +38; +50
from *Paramaecium* . . . . . +2; +1; +3; —3
„ „ , irradiated . . . . . +20; +22; +13; +40

Radiation could also be obtained from protozoa when glucose was added to the culture; it required about 15 minutes before radiation became noticeable. The mitogenetic effects observed are given in Table 38.

A very interesting claim has been made by ACS (1932). He succeeded in increasing the mitogenetic effect of muto-induction of bacterial cultures (*Bacterium typhi-murium*) by using the detector of one day, i. e. the irradiated culture, as sender for

the next experiment. His data show a very consistent increase in the mitogenetic effect through 6 to 7 such transfers.

The proof is not complete, however. It is well known that old bacterial cultures require a longer time to start growing than young ones. The rate of growth of old cultures can be increased for several successive transfers, without any irradiation. Without accurate statements concerning the number of cells in the sender and in the detector, no definite conclusions can be drawn.

(2) Reaction Upon Irradiation: Bacteria and yeasts, when transferred from an old culture to a fresh medium, do not start growing at their maximal growthrate. The old cells undergo a rejuvenation process, morphologically and physiologically (see p. 120). This results in a very slow growthrate during the first hours after transfer. The bacteriologist calls this the lag phase.

It has been observed by RAHN (1907), PENFOLD (1914) and others that bacteria after being transferred from an old culture rejuvenate more rapidly if many cells have been transferred while with a few cells in a large volume of fresh medium, rejuvenation is slow, and single-cell cultures frequently die. This observation had been very puzzling to biologists since the opposite should be expected, until it could be explained as a simple stimulating effect by mutual irradiation of the cells. The closer they are together, the stronger must be the effect, the quicker the recovery.

The best example is very likely that of HENRICI (1928, Table I) with yeast. He inoculated increasing amounts of yeast into flasks of sugar-peptone solution, so that all the decimal gradations from 2095 to 20949750 cells per cc. were present. These different inocula resulted in very different lag periods. The times required to reach the maximal growthrate were:

with an inoculum of	Lag Period	Average cell distance[1])
20 949 750 cells per cc.	2— 8 hrs.	0.036 mm.
2 094 975 ,, ,, ,,	4— 8 ,,	0.086 ,,
209 498 ,, ,, ,,	6—12 ,,	0.192 ,,
20 950 ,, ,, ,,	12—24 ,,	0.422 ,,
2 095 ,, ,, ,,	24—36 ,,	0.914 ,,

[1]) The average cell distance is computed from the equation

$$\text{distance} = \text{cell diameter} \left(\sqrt[3]{\frac{74.04}{\text{cell volume in } 100 \text{ cc.}}} - 1 \right)$$

It is absolutely correct only with spherical organisms. The volume of one yeast cell is taken as 118 μ^3, the average diameter as 6.4 μ.

Table 39. Growth Acceleration by Mutual Irradiation with Saccharomyces ellipsoideus

Inoculum	Number of Buds per 100 Cells			
	Experiment No. 1		Experiment No. 2	
	8,000 cells	80,000 cells	8,000 cells	80,000 cells
start	0	0	0	0
after 3 hours .	0	0	0	0
after 5 hours .	3	16	—	—
after 6 hours .	—	—	5	49
after 7 hours .	14	60	—	—
after 10 hours .	—	—	17	91
after 12 hours .	—	—	35	91

To these proofs that the lag phase is shortened by the mutual irradiation of cells, BARON (1930) added another. After having shown that bud formation in fresh yeast cultures begins sooner when the inoculum is larger (Table 39), he showed that this difference disappears when gelatin is added (Table 40). Gelatin

Table 40. Prevention of Growth Acceleration by Absorbing the Mitogenetic Rays by Means of Gelatin

Inoculum	Number of Buds per 100 Cells			
	Experiment A		Experiment B	
	8,000 cells	80,000 cells	8,000 cells	80,000 cells
start	1	0	0	0
after 3 hours .	2	2	0	0
after 5 hours .	18	21	12	15
after 7 hours .	30	32	—	—
after 8 hours .	—	—	35	33
after 10 hours .	41	42	48	52
after 12 hours .	—	—	62	61

absorbs mitogenetic rays very completely (see p. 59), and while all other conditions of life remained the same (gelatin contains no nutrients for yeast), muto-induction was prevented, and, as a result, the lag phase became independent of the cell concentration.

Table 41

1 cc. of a 3-day old broth culture of *Bacterium coli* irradiated for 30 minutes, then diluted in broth to various dilutions

Dilution	1 : 100		1 : 10 000		1 : 100 000	
Cell Distance	0.140 mm.		0.650 mm.		3.0 mm.	
Cells per	1 cc.		100 cc.		10 000 cc.	
	Control	Exposed	Control	Exposed	Control	Exposed
Start	530 000	460 000	495 000	505 000	570 000	540 000
2 hours	400 000	440 000	495 000	575 000	480 000	490 000
3 hours	575 000	530 000	510 000	655 000	510 000	520 000
4 hours	725 000	750 000	550 000	870 000	540 000	600 000
6 hours	7 850 000	9 950 000	2 450 000	8 350 000	1 570 000	5 130 000
8 hours	29 100 000	23 500 000	23 400 000	150 000 000	17 600 000	70 900 000

Summary of above

Cells per cc. of culture	Average Cell Distance	Cells of Controls in millions		Mitogenetic Effect	
		after 6 hrs.	after 8 hrs.	6 hrs.	8 hrs.
500 000	0.140 mm.	7850	29 100	23	—26
5 000	0.650 mm.	2450	23 400	247	541
50	3.00 mm	1570	17 600	227	303

Table 42. Offspring in 44 hours of the protozoon *Enchelys farcinem* in hay infusion drops of different sizes

Culture A			Culture B		
Drop Weight mg.	Cells after 44 hrs.	Cell Number × Drop Weight	Drop Weight mg.	Cells after 44 hrs.	Cell Number × Drop Weight
5.0	250	1250	1.4	64	90
10.0	31	310	7.4	4	30
13.6	104	1414	13.0	8	104
23.4	64	1498	23.8	6	143
34.6	33	1142	32.2	4	129

The following experiment by FERGUSON and RAHN (1933) verifies this from a different angle. 1 cc. of a culture of *Bacterium coli* was irradiated for 30 minutes, and then diluted in broth 100, 10000 and 1000000 times. The cells in all three cultures came from the same 1 cc., the only difference being their distances from one another after dilution. Table 41 presents the data comparably, i. e. it shows the development of the progeny arising from $^1/_{100}$ cc. of the old culture, when grown in different cell concentrations. The slower growth due to greater dilution is plainly evident in the controls. The more important result is the absence of a mitogenetic effect when the cells are too close together.

(3) Allelocatalysis: The lag phase of growth is not limited to fungi. Quite striking examples of lag in protozoa have been reported by ROBERTSON (1924). This investigator found that single individuals of *Enchelys farcinem* multiplied but very slowly or not at all in small drops of hay infusion while the rate was quite large when two or more individuals were in the same drop.

While this appears a duplication of the lag phase of bacteria, it differs by the fact that the size of the drops has a great influence. Table 42 gives the progeny derived in 44 hours from single cells of *Enchelys* in hay infusion drops of various sizes. In large drops, the growth is much slower, and if extrapolation is permissible, we must conclude that a single cell of culture *A* in 1.5 cc. would not grow at all; with culture *B*, the inhibiting volume is 0.15 cc. This is borne out by experience. ROBERTSON mentions that such cultures usually show no growth.

ROBERTSON assumes that a "catalyst" is produced by the cell, and that a certain concentration of this is necessary to cause cell division. This theory of "allelocatalysis" has not been accepted generally, though similar phenomena are known. WILDIER's "bios" (1901) is essentially identical with ROBERTSON's catalyst. The "bios" theory was based, among other things, upon the observation that in the same medium, a small inoculum did not reproduce while a large one did. This was verified by NAUMANN (1919) who observed that 5 yeast cells would die in a medium where 50 produced growth.

It is possible to reconcile the influence of the drop size with mitogenetic radiation. All media absorb mitogenetic rays fairly readily. The ability to grow in a small drop indicates that the cell has been able to produce sufficient radiation to induce mitosis. A considerable part of it must leave the cell, because cells can influence one another mutually. In a small drop, a good share of this emitted radiation is reflected from the air surface and finds its way back to the cell before it is absorbed. With an increase in drop size, distances become greater, absorption is greater and the probability of the rays being reflected back to the cell is smaller. In the isolation of single bacterial cells by the micro-pipette, small drops are an important factor in success (WRIGHT, 1929). However, there is no definite experimental proof that allelocatalysis is a radiation phenomenon.

The allelocatalytic effect is not limited to unicellular organisms. A very pretty exemple of mutual stimulation has been observed by FRANK and KUREPINA (1930) with the eggs of a sea urchin. With 10 to 20 eggs per drop of sea water, development went much more rapidly than if there were only a very few eggs per drop (see Table 43). It will be shown later that sea urchin eggs at this stage of development are good senders as well as good detectors. However, here too, a chemical mutual influence was not excluded.

B. HIGHER PLANTS

The first mitogenetic sender and the first detector was the onion root. It is established beyond doubt that the tip of the onion root radiates. This radiation is not entirely diffuse, but most of it points quite distinctly in the direction of growth.

Table 43. Development of Sea Urchin Eggs

		Number of Eggs per Drop		
		1—2	3—6	10—20
Experiment I		%	%	%
40 hours after fertilization	not motile	31	6	17
	slightly motile	23	34	9
	distinctly motile	46	40	28
	actively motile	0	20	46
Experiment II				
42 hours after fertilization	not motile	75	0	0
	slightly motile	25	26	4
	distinctly motile	10	40	12
	actively motile	0	34	60
	beginning gastrula	0	0	16
	perfect gastrula	0	0	8

According to GURWITSCH, some negative results obtained with onion roots are very likely due to inaccurate direction of the mitogenetic beam.

Onion roots radiate only as long as they are connected with the bulb, or at least with a part of the bulb. Radiation ceases at once when the root is severed from the bulb. (The roots of the sunflower [*Helianthus*], however, continue to radiate after being cut from the plant; they also continue to grow for a considerable time after being severed.)

This suggests that the substances which are the source of radiation are centralized in the onion bulb, and especially in its base. When the base is cut from the bulb, it radiates weakly. The pulp of the base, however, radiates strongly, and has been used as a strong source of mitogenetic rays in the early experiments. After approximately half an hour, radiation ceases. The spectrum of the pulp is purely oxidative, and its cessation is in all probability due to the completed oxidation of some unknown compound by the oxidases of the root. Heat destroys the radiating power which strongly suggests that an enzyme is the active part (Table p. 38). Heated pulp mixed with exhausted pulp starts to radiate anew, the heated pulp furnishing the compound to be oxidized and the exhausted pulp supplying

Table 44. Radiation Spectra of different parts of the onion

	Induction Effects							
	1900—1950	1950—2000	2000—2050	2050—2130	2130—2200	2200—2260	2260—2330	2330—2410
Onion base pulp	1	—5	+5	—4	+4	+7	+57	+37
Severed roots (secondary radiation) . .	44	43	4	10	37	4		
Intact roots (normal radiation, from tips)	39	32	—2	—2	39	—3	— 3	— 5

the oxidase. GURWITSCH has used the words *mitotin* and *mitotase* for these two essential factors. Considering the oxidative spectrum of the pulp, there can be scarcely any doubt that mitotase is an oxidase. The new terms might better be discontinued because they are likely to be considered as introducing a mysterious new element into life processes.

Since the base of the onion bulb as well as the tip of the root radiates, it seems likely that a relation between these two radiations might exist. Probably, the oxidase is located in the onion base, and the new oxidizable material is transported to it continuously from the leaves through the vascular system of the plant. This accounts for the radiation of the bulb, but not for the emission from the root tips. This question has been decided by spectral analysis (Table 44). The bulb spectrum is oxidative. The normal root tip radiates glycolytically, and cannot therefore be caused by the same process as that of the onion base. The "secondary radiation" of the roots (see p. 110) is also glycolytic, regardless of the wavelength of primary radiation. It seems therefore most probable to assume that the normal radiation of the intact root tip is really a secondary radiation induced by the oxidation processes in the onion base. If this part is narcotized with chloral hydrate, the tips do not radiate. If the base is cut off, the root tips do not radiate. If, however, a small part of the base remains on the root, the tip will radiate.

This conclusion is biologically very important. The conduction of mitogenetic rays through normal tissue over a distance of

several inches is bound to affect our conception of growth and of growth stimuli considerably.

Some other evidence supports this explanation. The potato radiates when cut, but only from the leptome fascicles; portions free from leptome are inactive (KISLIAK-STATKEWITSCH, 1927).

A very interesting investigation concerning the radiation of sunflower seedlings has been carried out by FRANK and SALKIND (1926). It was found that radiation can be obtained from the root tips, from the plumulae (the first young leaves) and from the cotyledons. However, only the very center of the cotyledon edge radiated, and no other part of these organs. Failure with one cotyledon which showed an abnormal curvature of the central vein led to the discovery that radiation arises from the vascular system. How mitogenetic radiation is conducted from the vascular system to the growing parts of the plant, the meristem, without great loss of energy, is unknown. It seems improbable to assume total reflection from the vascular walls. Either this or complete absorption appear the only way to explain the absence of radiation from the sides of the sunflower cotyledons.

Very little experimentation has been done with other plant tissues, and though mitogenetic radiation started with plant tissues, we know much more about radiations from animals than from plants.

The pulp of turnips radiates when 24 hours old (ANNA GURWITSCH); the pulp from *Sedum* leaves does not radiate when fresh, but after 18 hours, it begins and continues until 24 hours old. After 48 hours, radiation has disappeared (GURWITSCH, 1929). Since neither of these two experiments were carried out aseptically, and no accurate record was made of the number of bacteria and yeasts growing in the pulp, they cannot be considered as exact proofs of radiation. *A priori*, we should expect these crushed tissues to radiate because they must contain oxidase.

The radiation of wounds in plant tissues will be discussed together with the wounds of animals on p. 173. The plant tumors will be discussed on p. 178.

So far, the discussion on higher plants has been limited to the *emission* of rays. Just as important is the reaction of plants to mitogenetic rays. There are innumerable data on experiments with onion roots. The facts as well as the interpretations have been discussed in great detail in preceding chapters.

No other part of grown plants or seedlings has ever been tried as detector. We cannot as yet form any opinion about the bearing of mitogenetic radiation to total growth, to the form-controlling factors and to the reproductive mechanism of higher plants.

In the case of mold spores (see p. 80), while they have been shown to react upon mitogenetic rays, they are also senders, and muto-induction effects have been obtained.

C. EGGS AND EMBRYONIC STAGES OF HIGHER ANIMALS

(a) *Eggs as Senders.* The eggs of animals are strong senders as well as good detectors, as far as they have been investigated. FRANK and SALKIND (1927) observed that with eggs of the arctic sea urchin *Strongylocentrotus Dröbachensis*, radiation does not begin immediately after fertilization; it occurs approximately 1 hour later, and continues for about 1 hour (Table 45). At this time, the amphiaster stage is reached. The first cleavage furrow appears after 2 hours and 45 minutes. For 1 hour before and for 30 minutes after the first cell division, there is no noticeable emission of rays. Half an hour after the first division, radiation begins again.

WARBURG (1909), experimenting with the Mediterranean species *Strongylocentrotus lividus* which produces the first cleavage furrow in 40 minutes, had observed a large increase in the rate of respiration of the egg 10 minutes after fertilization. These 10 minutes would correspond to about 40 minutes in the arctic species. The increase in oxygen consumption begins about 20—30 minutes earlier than radiation. HERLANT (1918) observed a great increase in permeability 2 minutes after fertilization. GURWITSCH (1932, p. 99) assumes that a prophase of the "mitotase" diffuses from the egg plasma, becomes activated on the egg surface, and acts upon the "mitotin" which also diffuses out. The chemical reaction furnishing the radiant energy would thus take place on the egg surface, and not within the egg.

(b) *Eggs as Detectors*: Fertilized eggs not only send out rays, but also respond to radiation. MAXIA showed in 1929 that sea urchin eggs can be stimulated in their rate of development by mitogenetic rays. ZIRPOLO's data (1930) on the same subject

Table 45. Effect of Sea Urchin Eggs at Different Stages after Fertilization upon Onion Roots

Time after Fertilization	Number of Mitoses		Induction Effect
	Control	Exposed	
0 to 1 hr + 10 min . .	167	173	+ 0.3
	299	299	0
0 to 1 hr + 45 min . .	139	203	+46.0
33 min to 1 hr + 50 min . .	215	301	+40.0
1 hr + 10 min to 2 hrs + min 15 . .	67	109	+62.0
1 hr + 45 min to 3 hrs	214	226	+ 0.5
	182	186	+ 0.2
1 hr + 50 min to 3 hrs + 15 min . .	92	86	— 0.6
2 hrs + 15 min to 3 hrs + 15 min . .	268	268	0
	144	157	+ 0.9
0 to 2 hrs + 45 min . .	423	568	+34.0
	252	340	+35.0

are given in Table 27 p. 82. SALKIND, POTOZKY and ZOGLINA (1930) proved the same for the eggs of the protoannelids *Saccocirrus papillocercus* and *Protodrilus bobrezkii*. The eggs emitted mitogenetic rays during their development, and *vice versa*, their development was accelerated by the rays from isolated frog hearts, crab hearts or the hemolymph of crabs. After an exposure to these radiations for 5 to 10 minutes, a greater percentage of furrowed eggs was observed in 23 out of 26 experiments. The mutual stimulation of sea urchin eggs has already been mentioned on p. **137**, as example of allelocatalysis.

According to WOLFF and RAS (1934b), the eggs of *Drosophila melanogaster* are good detectors. They were exposed for 15 to 30 minutes to a broth culture of *Staphylococcus aureus*, 3 hours old, in quartz tubes, and the percentage of hatching eggs was ascertained in equal time intervals. Table 45a shows that at the same moment, always more irradiated eggs had hatched than unirradiated ones.

(c) Embryonic and Larval Stages as Senders: The most detailed earlier investigation of this kind is that by ANIKIN (1926) with the embryos of the axolotl (*Ambystoma tigrinum*). From very young embryos, with open medullar furrows, the medullar plates were dissected, ground to pulp and used as sender.

Table 45a. Effect of a Culture of *Staphylococcus aureus* upon the Rate of Hatching of the Eggs of Drosophila

Controls			Irradiated Eggs				Percentage Increase over Control
No. of eggs	No. hatched	Per cent hatched	No. of eggs	No. hatched	Per cent hatched	Time of irradiation	
39	25	64	51	45	88	15—20 min.	24 ± 10.6
81	15	18.6	72	30	41.7	15—20	23.1 ± 7.45
52	18	34.6	60	49	81.7	15—30	47.1 ± 8.1
304	210	69	312	300	96.1	20	27.1 ± 8.5
324	147	45.4	304	228	75	20	29.6 ± 3.7
344	255	74	327	323	98.4	20	24.4 ± 2.6
366	136	37	357	244	68	20	31 ± 3.5
118	79	67	117	98	83.5	20	16.5 ± 5.5
74	38	51.3	85	60	70.6	20	19.3 ± 7.7
1702	923	54.2 ±1.2	1685	1377	81.7 ±0.95		27.5 ± 1.54

The same was done with the rest of the embryo. As detector, onion roots were used. The results are shown in Table 46. Only the medullar plate radiated. Then, living embryos, after the removal of the surrounding mucus, were placed in a glass tube so that either only the ventral or only the dorsal side faced the detector root. Even at the morula stage, the vegetative hemisphere did not radiate. During gastrulation, the entire blastopore seems to radiate.

With slightly larger embryos, just previous to hatching, the brain could be dissected out, and it was found that the brain, but none of the other tissues, radiated. The brain pulp of the fullgrown animal did not (see however p. 158).

This agrees with the earlier statements of GURWITSCH, and of REITER and GABOR, that frog tadpoles radiate only until about 1 cm. long, and that the center of radiation appears to be in the head. Concerning the radiation of certain organs during metamorphosis, see p. 167.

Among the invertebrates, the embryos of *Saccocirrus* radiate during the entire stage of their development. SALKIND (1931) found the following induction effects:

Blastula Stage	30%	50%	48%	34% induction
Gastrula Stage	63%	30%	30%	
Swimming Larvae . .	54%	37%	49%	32%
Trochophore Stage . .	37%	28%		

Table 46. Effect of various parts of the embryos of the axolotl upon onion roots

Embryonic Stage	Number of Mitoses		Induction Effect
	Control	Exposed	%
living morula stage, animal hemisphere	750	931	+24
living morula stage, vegetative hemisphere	428	410	— 4
living embryo, before hatching; dorsal side	230	268	+16
living embryo, before hatching; ventral side	492	471	— 4
pulp of medullar plate	324	409	+26
pulp of embryo without medullar plate	482	505	+ 5
pulp of embryos' brains I	627	832	+33
pulp of embryos' brains II	865	1042	+20
pulp of embryos' brains III	920	1076	+17
pulp of entire embryo without brain I	724	728	0
pulp of entire embryo without brain II	689	696	+ 1
pulp of embryo's liver	631	635	+ 1
pulp of brain of grown animal I	617	632	+ 2
pulp of brain of grown animal II	848	847	0

Of the insects, only the larvae of *Drosophila* have been analyzed. They do not begin to radiate until shortly before pupation, and cease to radiate 90 hours after this (see also p. 169).

Quite different is the behavior of the chicken embryo. SORIN and KISLIAK-STATKEWITSCH (1928) working with entire embryos two days old and also testing the brains of older embryos could find no evidence of radiation. However, during the second and third day of incubation, positive results were obtained with the liquefied zone around the embryo.

(d) Embryos as Detectors. The only example of embryos or larvae as detectors of mitogenetic radiation is that of BLACHER and associates who could make the fore leg of a tadpole grow more rapidly by exposure to radiation (see p. 168). The morphological changes in sea urchin larvae observed by MAGROU may also be mentioned here (see p. 165).

D. TISSUES OF ADULT ANIMALS

Tissues as Senders: Until recently, most of the tissues of adult animals had been thought to be non-radiating. If we consider radiation to be produced largely by the chemical reactions in the cells, it would seem that all tissues should radiate quite strongly. GURWITSCH (1934) has given some convincing explanations for the differences between radiating and non-radiating tissues.

The very strong absorption of these short-waved rays has already been emphasized repeatedly. GURWITSCH found that extremely thin films of oil or related substances, films of practically only one molecule thickness, suffice to absorb completely a mitogenetic radiation of normal intensity. On the other hand, equally thin films of substances capable of enzymatic cleavage, such as lecithin, are also capable of secondary radiation (see p. 45), and they will pass on radiation not as a beam, but in all dimensions of the film. Thus we observe strong radiation in blood which contains no membrane, and in nerves which contain plenty of lecithin. These two will be treated separately in the next two chapters, on account of their importance.

The cornea of the eye is also a good sender, being a continually renewed tissue. WOLFF and RAS (1933*c*) consider it to be one of the strongest sources, about 10 times as strong as blood. GURWITSCH has recently favored the peptic digestion as a strong and fairly constant source. Other actively reacting digestive enzymes are likely to be good senders, but would hardly be considered as tissues. POTOZKY, SALKIND and ZOGLINA (1930) studied the tissues of two crabs, *Carcinus maenas* and a species of *Pachygrapsus*; they found the pulp from gills and from testicles inactive, while the hepatopancreas radiated distinctly. This organ is the seat of active proteolysis. Probably, autolyzing muscle would radiate.

With vertebrates, negative results had been obtained by all earlier workers with lymph glands, testicles, ovaries, skin, liver, and with the resting muscle. The working muscle, however, proved to be very active. Negative results, particularly when obtained with tissue pulp, are now considered of little significance since better methods, especially with organs *in situ*, showed distinct radiation.

On p. 65, SIEBERT's results have been given, showing that the muscle radiates only during work, and that pulp from resting muscles does not radiate, while that from active, tired muscle does. According to recent experiments by FRANK and KREPS (quoted from GURWITSCH, 1932, p. 149), this result was caused by an irritation of the muscle during grinding. By dropping the organ into liquid air and grinding it while frozen, the results were the opposite. In pulp from working muscle, the pH changed from 5.96 to 5.82 during the experiment, and radiation was absent; while with rested muscle, the pH changed from 6.33 to 5.85, and radiation was present. This refers only to the pulp, however; the muscle *in situ* radiates strongly while working, but weakly also when resting.

The spectral analysis indicates that the main source of muscle radiation is not glycolysis; partly it is an oxidation process, partly of unknown origin. It must be remembered that there is no quantitative relation between the total energy liberated by a chemical reaction, and the ultraviolet radiation emitted (see p. 41). It may well be that some proteolytic process whose energy output is negligible for the muscle work emits most of its energy in radiant form, while glycolysis, with a much larger total energy output, emits only a very small fraction of it as mitogenetic rays.

A good example of applied spectral analysis should be mentioned in this connection. GURWITSCH had been greatly puzzled in his earlier work by the observation that the very distinct radiation from the rabbit's eye disappeared during starvation, but reappeared after 8 days of continuous starving. Several years later, spectral analysis brought a simple explanation (GURWITSCH 1932, p. 67). The normal radiation is glycolytic, and ceases during starvation because of lack of sugar. After prolonged starvation, proteolysis of the tissues becomes necessary for the continuation of life processes, and this produces a proteolytic spectrum.

Tissues as Detectors: Only a few instances are known where fullgrown animals or their tissues reacted upon mitogenetic rays. The role of radiation in wounds will be treated in one of the following chapters.

With multicellular organisms, the stimulation of the growth-rate is not so readily proved. In growing organs, the cells lie so

closely together that the optimal intensity of radiation may already be furnished by the organ itself. Emanations from outside, if they are not absorbed completely before they reach the region of growth, very likely can only be harmful.

A few instances are known, however, where the rate of cell division is accelerated. The onion root is usually considered the classical example though the data have been interpreted somewhat differently by REITER and GABOR (see p. 129). Another example is the cornea (see p. 84). Further illustrations are the effects of resorbed tissue in different stages of amphibian metamorphosis (see p. 167).

These latter results together with those obtained with embryos (p. 144) suggest very strongly that the developmental mechanism controlling size and form makes use of ultraviolet radiations as well as of purely chemical means to achieve its purpose. This and the possibility that neoplasms, especially cancers arise through mitogenetic radiation will be discussed in later chapters.

The only animal tissue that has been actually used as detector is the cornea. According to GURWITSCH, it yields very good results (see p. 84).

E. BLOOD RADIATION

A comprehensive review of all work on blood radiation has been given by W. SIEBERT (1934) in the second volume of „Handbuch der allgemeinen Hämatologie".

Blood radiates quite strongly, even in adult animals and men, the only exception being extreme old age and a very few diseases which will be discussed later. The blood of various mammals, birds and amphibia, and also the hemolymph of the crabs *Carcinus* and *Pachygrabsus* and of the clam *Mytilus edulis* has been tested, and strong mitogenetic effects have always been observed. According to KANNEGIESSER and KAZWA (quoted from GURWITSCH, 1932, p. 124), dog's blood which possesses only very weak glycolysis gives very fluctuating results.

Blood radiates within the veins or arteries as could be shown by removing the tissues around the veins or arteries, and exposing a detector to the blood radiation through the inner wall of the blood vessels which is transparent to these rays (spectrum see fig. 44).

Outside the blood vessel, blood loses its radiating power within 10 to 15 minutes; this holds also for the hemolymph of crabs. In hemolyzed blood, radiation can be restored by adding glucose.

For radiation experiments, blood is mixed with an equal amount of a 4% $MgSO_4$ solution to prevent clotting, or it is hemolyzed with distilled water (POTOZKY and ZOGLINA, 1929). It is possible to dry blood on filter paper and thus preserve its radiating power for 2 to 3 days. Since this might be of practical importance, especially in diagnostic medicine, GURWITSCH's method (1932, p. 121) shall be mentioned here:

> The blood should be drawn without previous disinfection of the skin (iodine, alcohol) because traces of disinfectants appear to inhibit glycolysis. The drops are spread widely on the filter paper to prevent coagulation and thick layers. Drying must be accomplished very rapidly to preserve full radiating power; the dried sample should be kept dark. In making the test, detector and everything else must be prepared before water is put upon the dry blood. The spot is cut into very small pieces, and softened in a tiny shallow dish with 5 to 6 drops of distilled water, under continuous stirring. As soon as the water becomes dark red, it is drawn off with a pipette and used at once for irradiation. From the adding of the water to the beginning of exposure, not more than 1 to 1.5 minutes should elapse.

HEINEMANN's method for detecting blood radiation has been mentioned on p. 72.

With starving mammals, the blood does not radiate, but can be brought back by adding glucose. POTOZKY and ZOGLINA starved rats until they had lost about 30% of their body weight. The mitogenetic effects obtained with the blood of these animals were

without addition	—2.5	—5	—2	0
with 2% glucose	21	44	36	39

These strong effects could be obtained only by adding much more sugar than is normally contained in the blood.

A very important observation is the disappearance of blood radiation after continued work. BRAINESS (quoted from GURWITSCH, 1932, p. 132) investigated a number of laborers before and after the day's work. Table 47 gives a few of his data which show consistently no radiation immediately after long-continued work, but normal radiation after 2 hours of rest. This must be considered when making blood tests for diagnostic purposes.

Table 47. Mitogenetic Effects from the Blood of Factory Workers before and after Work

Person	Month	before work	at the end of the day's work	2 hours later
K	October	25	6	—
K	January	24	— 1	36
K	December	45	1	26
B	November	38	— 3	31
B	January	28	6	22
M	January	32	— 5	11
E	January	33	2	27
S	January	35	—13	10
S	January	21	12	27
D	January	26	1	30

WASSILIEFF (1934) repeated the investigation with mental work (calculation). The first results showed a decrease in radiation, but this happened before psychic analysis showed mental fatigue, and it could be demonstrated that muscular work connected with calculation caused the decrease in radiation while the mental work as such does not affect it.

The spectrum of the radiation of rabbit blood from the streaming blood in the *vena saphena* is shown in fig. 44 as measured by GOLISCHEWA (1933). It has practically all the lines characteristic for glycolysis, proteolysis, creatin hydrolysis, phosphatid hydrolysis, and oxidation, and, in addition, some new lines. Of these latter, the lines 1920—30, 1940—50, 1960—70 and 2000—2010 have also been found in nerve spectra (fig. 47).

The main source of blood radiation with mammals appears to be glycolysis. In addition to the starvation experiments just mentioned, KANNEGIESSER and KAWZA made extensive experiments with dog's blood, measuring the glycolytic power and the radiation of each sample. There was a good, though not quantitative, relation between the two. Intravenous insulin injection reduced glycolysis and radiation to zero. In some cases, addition of glucose (probably in overdose) interfered with radiation.

Rat blood treated with heparin loses its radiation by NaF, but not by KCN. This suggests glycolysis, which is affected by

NaF, but not by KCN. Hemolymph of crabs, however, loses its power promptly in the presence of 0.0001 molar KCN, and so does hemolyzed rat blood. This suggests that in normal rat blood, glycolysis is the dominant source, but in hemolyzed blood, and in the blood of crabs (which contains no hemoglobin) oxidation is the deciding reaction.

Of the various fractions of the blood, the serum radiates immediately after the blood is taken from the animal, but loses this power rapidly. It continues, however, to produce secondary

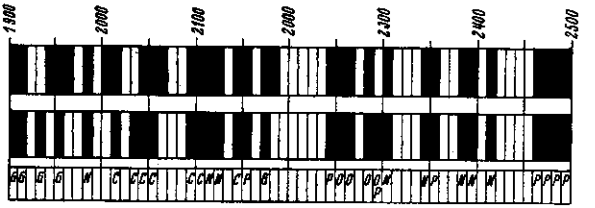


Figure 44. Above: spectrum of streaming blood in the artery of a rabbit. Below: the combined spectrum of 5 common biological processes (oxidation = *O*, glycolysis = *G*, creatinin hydrolysis = *C*, proteolysis = *N*, phosphatid hydrolysis = *P*).

radiation, even after several months (p. 43). The leucocytes, polynuclear as well as lymphocytes, radiate distinctly but not very strongly, according to KLENITZKY (1932). The spectrum indicates glycolysis, oxidation and phosphatase action, and probably proteolysis.

The intensity of blood radiation is greatly changed after wounding (see p. 174). During the metamorphosis of amphibia, it varies enormously in the different stages of development.

PROTTI (1931) observed a great decline or complete absence of radiation in blood during senility, and this result was confirmed by HEINEMANN (1932). In consequence PROTTI injected blood from young persons into old ones. Distinct clinical evidence of "rejuvenation" by this treatment has been claimed, and the blood of old people thus treated radiated again. Table 48 gives some of PROTTI's results.

A somewhat different way was used by HEINEMANN and SEYDERHELM to renew blood radiation in old persons. In his effort to find the reason why ultraviolet light was beneficial to animals, SEYDERHELM (1932) succeeded in isolating a compound

Table 48. Mitogenetic Effects Produced by the Blood of Old Persons After Injection of Blood from Young Persons

Blood Group	Mitogenetic Effects			Clinical Results
	young blood	old blood	old blood after injection	
A	70	5	30	very good
A	20	3	4	none
A	60	29	38	good
A	70	15	38	very good
A	20	10	10	little
A	80	30	53	very good
B	72	16	32	good
O	81	24	40	very good
O	62	30	37	good
O	22	17	20	little

from the blood corpuscles which he called *cytagenin*. When this substance was injected into healthy persons, it increased blood radiation (fig. 45). With patients suffering from anemia, it washed the newly-formed blood corpuscles out of the bone marrow and produced a normal blood picture (HEINEMANN, 1932). This was possible in all cases of secondary anemia, but not in pernicious anemia in which the bone marrow no longer produces blood cells.

When injected into patients 70 to 80 years old, without blood radiation, cytagenin produced either no effect or a slight depression effect during the first days, but after 1 to 2 weeks, the blood began to radiate, and repeated tests showed no decrease. How long these experiments were continued, HEINEMANN did not state.

The blood of asphyxiated animals does not radiate. It loses this power before the animal is dead, even when the process is still reversible.

Quite remarkable is the persistence of blood radiation during illness. L. GURWITSCH and SALKIND (1929) obtained radiation from the blood of tuberculous guinea pigs until almost immediately before death. Diabetes, lues, osteomyelitis and ulcer of the stomach did not decrease blood radiation. The only diseases which showed this conspicuous absence were leucemia, and severe septicemia with high fever (also poisoning with nitro-benzene),

and cancer. This was verified by SIEBERT who observed absence of radiation in severe cases of sepsis, pneumonia, and scarlatina,

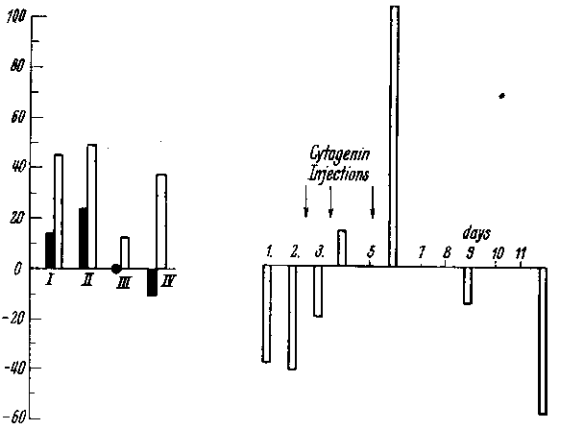


Figure 45. Increase of blood radiation by injection of cytagenin. At left: black indicates radiation before injection, white after injection. *I* and *II* are normal healthy persons, *III* is a very old person, *IV* is a carcinoma patient. — At right: the effect of repeated injections upon a carcinoma patient.

and by GESENIUS (1930). HEINEMANN (1932) added chronic tonsilitis to this non-radiating group; in some cases, radiation appeared again soon after the removal of the tonsils.

Fig. 46 shows the results obtained by GESENIUS (1930) who measured blood radiation by the decrease of yeast respiration (see p. 84). Since only very few easily recognized diseases prevent blood radiation, it can be used for the diagnosis of cancer (see p. 180).

A very comprehensive review of his extended research on blood radiation has been given by PROTTI (1934a). All measurements refer to apparently healthy persons, and are

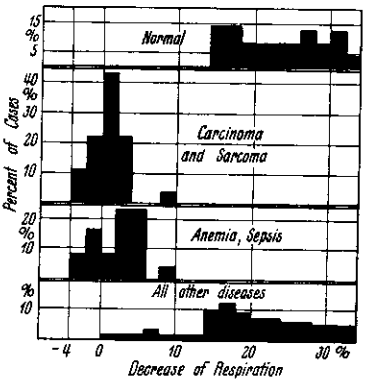


Figure 46. Decrease of respiration of yeast irradiated with the blood of healthy and sick persons.

made by the yeast bud method by means of the hemoradiometer (p. 66). In a large number of tables and graphs, he demonstrates the decrease of radiation with age, the stronger radiation of tall, slender people as compared with short individuals with a tendency for stoutness, and the slight increase of male over female blood. In the same individual, radiation increases 1 to 2 hours after each meal, and decreases with physical fatigue. During the winter months, it is slightly lower. It is increased by high altitudes, also by a trip to the sea shore, further by inhalation of oxygen, though in this latter case, the effect does not last more than one hour. Menstruation and pregnancy have characteristic curves.

PROTTI states that even among healthy individuals, "normoradiant, hypo-radiant and hyper-radiant" types can be distinguished, all reacting in a parallel manner upon the same changes.

F. NERVE RADIATION AND THE CONDUCTION OF STIMULI IN ORGANISMS

The results obtained with secondary radiation led GURWITSCH to the idea that possibly secondary radiation might be a controlling factor in the conduction of stimuli in plants and animals. Let us recapitulate briefly the facts obtained with onion roots (see p. 140).

Irradiation of the older, upper part of the severed root produced radiation from the tip. Irradiation of the tip produced radiation from the older tissue. The impulse was conducted longitudinally at the rate of approximately 30 meters per second, but there was no conduction to the diametrically opposite side of the root.

Later, FRANK observed that the same held true for muscles. The resting sartorius muscle of a frog when irradiated biologically at a definite place for 10 seconds emitted a strong mitogenetic radiation from a place 20 mm. away. In fact, at a distance of 20 mm., the effect was much stronger than at 10 mm.; the intensity had increased by conduction.

These results, together with the discovery of secondary radiation in nerve tissue, inspired in GURWITSCH the bold thought that conduction of stimuli in nerves and muscles may be ac-

complished or aided by secondary mitogenetic radiation. This statement is possibly too blunt, but it expresses in a few words the ultimate aim. GURWITSCH himself (1932a) says that this theory may appear bold, but it seems justified to approach it experimentally, on account of several suggestive facts.

One of them is the possibility of studying, by means of the mitogenetic spectrum, the metabolism of the nerve in the resting and in the excited stage. It is evident that the chemical analysis can give only a rather incomplete picture of the chemistry of nerve stimulation, because it involves destruction of the nerve, while radiation can be observed without injury. The result of the spectral analysis of nerve radiation has, as a matter of fact, revealed some chemical processes which had not as yet been discovered by chemical analysis.

The views concerning nerve radiation have undergone considerable change with the improvement of the methods. The older experiments by ANIKIN with the brain of adult salamanders (see p. 145) and those by REITER and GABOR (1928) with the sciatic nerve of the frog gave negative results. They were confirmed by WASSILIEW, FRANK and GOLDENBERG (1931) who obtained positive results only with the olfactory nerve of the pickerel. By using the yeast volume (p. 73) as detector, KALENDAROFF (1932) not only proved that the sciatic nerve of the frog radiated distinctly, but he could also study its spectrum by using intermittent exposure.

The spectrum was determined with resting as well as with irritated nerves. Irritation of the nerve was accomplished either by cutting into it (traumatisation), or by electrical tetanization with platinum electrodes 3—4 mm. apart, 8—12 shocks per minute (faradisation), or mechanically, by hitting with a light hammer, intermittently. All experiments were conducted with intermittent exposure by the rotating disk (see p. 105). This not only increased the intensity of the effect, but also eliminated the possibility of secondary radiation from the nerve induced by the yeast detector.

The minimal total exposure required to bring about mitogenetic effects was approximately 6 minutes with the resting nerve and 2—3 minutes with the excited one. The detailed spectra are shown in fig. 47. The rather surprising fact was revealed that different kinds of irritation produce slightly different spectra.

Further, it was found that radiation at the point of irritation differs from that of the same nerve a short distance away.

The spectra (of which each 10 Å strip is the result of at least three determinations) show five well-known chemical processes: oxidation, glycolysis, phosphatase action, cleavage of

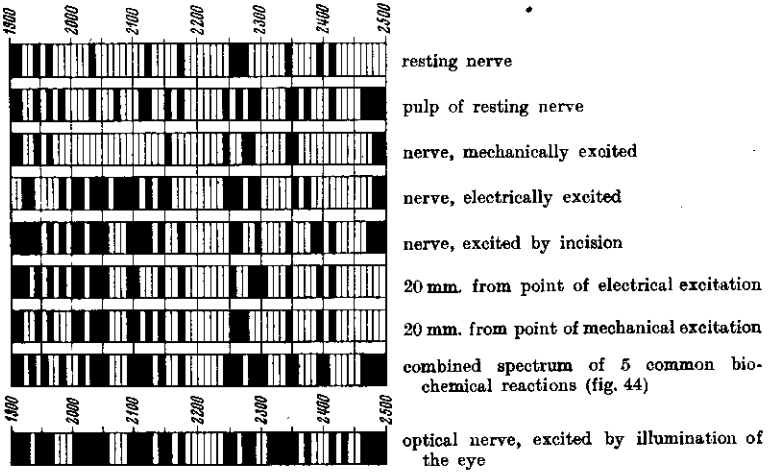


Figure 47. The spectra of the sciatic nerve of the frog under different conditions. The lowest line represents radiation from the optical nerve *in situ*.

creatinin phosphate, and de-aminisation of amino acid. A few lines were observed also which cannot at present be accounted for.[1])

These five chemical processes agree with chemical investigations on nerve metabolism. Strange is the absence of certain lines. In mechanical and electric irritation, most of those concerned with de-aminisation are missing, and in the electrically-stimulated nerve, the glycolytic regions are absent while in the spectrum of the same nerve, 20 mm. removed from the zone of

[1]) Two lines of the glycolytic spectrum, 1930—40 and 1950—60, are missing in most of the nerve spectra while they all have the neighboring lines 1940—50 and 1960—70. This may be due to a slight shift in the calibration of the instrument. Line 2410—20 might also be accounted for in this way, as a shift of the preceding line to the right, or rather an error in the calibration to the left.

However, the line 2000—10 in more than half of the spectra and 2370—80 in 3 of the spectra cannot be explained by the same error of calibration.

irritation, they are present. The creatinin lines are absent in mechanical stimulation. The spectra of nerve conduction lack entirely the phosphatase lines.

This difference in the spectra raised the question of "adequate" nerve stimulation. A good example of this has been furnished by ANNA GURWITSCH (1932) with the optic nerve of the frog. After decapitation and removal of the lower jaw, the optic nerve was laid bare by dissection from the roof of the mouth. A small window, 1.5×2 mm., is sufficient to permit access to the chiasma and tracti optici with part of the optic nerve. The radiation from this was tested in the darkroom while directing a beam of light on one of the eyes.

Increase in number of yeast cells by direct microscopic count was the method used to prove the radiation. The results were always positive while controls without illumination of the eye showed consistently no effect.

The spectrum is shown in the lowest line of Figure 47. It agrees essentially with those by KALENDAROFF. It has also some unknown lines agreeing with KALENDAROFF'S. Those corresponding to proteolysis (de-aminisation) are present only in part.

These experiments have revealed that there are differences in the metabolism of the resting and the excited nerve, and that even the type of excitation may change the spectrum. It might be well to remember here GURWITSCH'S statement that these spectra represent "minimal spectra". The established lines are doubtless correct, but there may be other lines too weak to be recorded by the detectors used.

These fine results induced ANNA GURWITSCH (1934a) to approach the more fundamental question whether this radiation of the optic nerve after illumination of the eye was only a simple secondary radiation, or represented an important functional part of nerve conduction. She laid bare the optic nerve, the optic lobes, the medulla oblongata and the spinal cord of living frogs, and kept these parts covered with the skin. For several hours after the operation, all nerves radiated strongly, but 24 hours later, lobes and hemispheres did not radiate while medulla and spinal cord usually continued to radiate weakly. As soon as light was directed onto the eyes of the frog, the lobes and hemispheres radiated strongly while the medulla and spinal cord did not change.

When the optic lobes were still in the excited stage from the operation, they did not react promptly upon illumination of the eye. This suggested an interference between traumatic radiation and normal reaction upon optic irritation. The explanation could be verified by irritating electrically the sciatic nerve. Before the circuit was closed, the lobes reacted strongly upon illumination of the eye; when the current was applied, the reaction upon light was weak and irregular; after ceasing the irritation, but continuing illumination, radiation ceased completely for a short time, and after that, the normal strong effect appeared.

It had been shown at this time (p. 45) that secondary radiation usually is a resonant radiation responding only to the wave lengths characteristic for it. ANNA GURWITSCH studied the reactions produced by various spectra applied to the chiasma of the optic system. The emission from an electrically irritated nerve made the lobes radiate weakly, while the hemispheres showed no effect. Yeast radiation, however, induced strong radiation in both. Addition of the glycolytic component (1900 —1920 Å from a monochromator) to nerve radiation produced also good radiation in lobes and hemispheres.

The spectrum of the radiation of the lobes contains always glycolytic lines even when radiation has been brought about by exposure of the chiasma to rays from the oxidation of pyrogallic acid which are very different from those of glycolysis. It is, therefore, not a mere secondary radiation, but indicates the release of an unknown independant chemical reaction in the optic lobes.

The extent of radiation in a nervous system during illumination permits thus the experimental approach to the problem of localisation of functions in the brain.

In another paper, ANNA GURWITSCH (1934 b) obtained different spectra from the hemispheres of the frog brain when the eyes were illuminated with different colors. Green light produced the lines of glycolysis, proteolysis, oxidation and phosphate cleavage. With red light, the last mentioned part was missing, and with blue light, there were no proteolytic lines. Probably, the metabolism of the nerve was not changed completely, and all lines were present, but their relative intensity was varied.

The intensity of the colored lights used and the wave lengths of the colors are not mentioned.

The effect of the intensity of light was tested by ANNA GURWITSCH (1932) by varying the distance between light and eye from 6 to 18 cm. (i. e. varying the intensity from 9 to 1) and determining the necessary exposure time. The result was as follows:

Length of Exposure . .	1	3	5	10	15	20	25	30	35 seconds
Induction Effect at 6 cm.	5	19	22.5	4	—	—	—	—	—
Induction Effect at 18 cm.	—	1	4	—	4	4	13	16	6

The weaker light (18 cm. distance) requires a longer time to produce the effect, but there is no appreciable difference in the *intensity* of the effect.

It could further be shown that continued exposure to light produces a long after-effect if the head is cut from the animal; in the living animal, however, radiation ceases when the eye is darkened (see Table 49).

Table 49. Mitogenetic Effect of the Optic Nerve after illumination and darkening of the eye

	Seconds of Exposure	Mitogenetic Effect		
		dark	light	second darkening
Isolated Head	20	2	22.0	21.8
	20	2	14.5	12.0
	20	—6	20.1	14.6
Living Animal	15	—4.5	22.5	—2.0
	10	0	15.5	—2.5

Another important advancement in the effort to correlate nerve conduction with mitogenetic radiation is the accurate measurement of the velocity of progress of secondary radiation in the sciatic nerve. LATMANISOWA (1932) found this to be 30 ± 3 meters per second (see p. 112) and this agrees, within the limits of error, with the rate of conduction of nerve stimuli.

Very suggestive, though not positive proof, are also the experiments by LATMANISOWA on the "fatigue" of the nerve by continued irradiation with strong light (see p. 111).

Later publications by LATMANISOWA (1933, 1934) have added another impressive fact to prove the role of mitogenetic

rays in nerve conduction. By exposing the sciatic nerve, with the attached muscle, in a moist chamber for about 2 hours to the radiation from protein digestion, the nerve showed all symptoms of parabiosis.

Two electrodes touched the nerve, and the place between them was irradiated. For about 2 hours, the nerve reacted normally upon electric impulses, causing a stronger muscle contraction with a stronger impulse. After this time, the nerve became at first more excitable, but soon the muscle reactions became weaker and weaker, and then, the typical parabiotic stage set in, the contraction being stronger when the impulse was weaker. Finally, the nerve ceased to react altogether, and it took two hours after the removal of the mitogenetic source before the nerve had recovered, and its reactions became normal again.

This experiment has been repeated more than 40 times with the same success. Controls with gastric juice without protein were not affected. The nerve outside of the irradiated zone reacted normally at the same time when the exposed part of the same nerve showed parabiosis. The author quotes a paper by LAPITZKI who obtained the same effect by rays from a mercury vapor lamp in a few minutes.

The nerve at the parabiotic stage has not ceased to radiate; on the contrary, the emission seems to be much stronger than that of the normal nerve.

It has not been possible as yet to produce muscle contraction, or a corresponding effect, by irradiating a nerve. This may be due to the absence of an "adequate" stimulation. Perhaps, a combination of definite wave lengths is necessary to produce such effects. The one example of true adequate stimulation are the above-mentioned experiments by ANNA GURWITSCH with the optic nerve.

It seems too early to speculate on the relation between the mitogenetic radiation of nerves and the action current. Though a number of physiologists oppose this idea, it does not seem impossible that the two observed facts may be some time be combined to produce a more comprehensive explanation of the nerve mechanism.

G. MORPHOLOGICAL EFFECTS

While the classical example of morphological effects produced by biological radiation is that of sea urchin larvae, they shall be preceded, for the sake of logical arrangement, by a short note on unicellular organisms.

1. Yeasts and Bacteria: CHRISTIANSEN (1928) observed striking morphological changes in yeast cells as well as bacteria under the influence of radiations emanating from menstrual blood. It seems that most of these experiments were carried out without exclusion of chemical effects from volatile substances of the blood, but the same effects could be obtained when a quartz coverglass protected the test organisms perfectly against vapors from the blood or from outside.

At times, the blood was strong enough to kill microorganisms; more commonly, it affected their cell forms. With *Bacterium coli*, the non-motile cells immediately above the drop of blood were 3 to 5 times as long as the farther removed cells; *Bacterium vulgare* lost its pellicle formation; *Lactobacillus bulgaricus* grew to long threads without cell division; *Oidium* formed no oidia. *Streptococcus lactis* and *cremoris* did not appear to be changed morphologically.

Just as striking were the changes in yeasts. Frequently, retardation of growth was accompanied by an enormous expansion of vacuoles, leaving the protoplasm only as a very thin layer around the cell membrane. In other cases, there was a decided tendency to grow into hyphae. Still other cultures showed formation of giant cells.

Similar morphological changes could be produced by exposure of yeast to saliva of apparently normal persons (FERGUSON, 1932). Particularly the large spherical cells with tremendously extended vacuoles and with complete loss of granulation are considered typical "saliva cells". It has not been possible, however, to produce these same forms by interposing a quartz coverglass between saliva and yeast. Thus, the physical nature of this phenomenon is not proved. On the other hand, it is evidently not a purely chemical effect of some saliva constituent because the yeasts grew quite normally in mixtures of equal volumes of saliva and raisin extract; these cultures must have obtained more saliva constituents than could possibly distil over from the

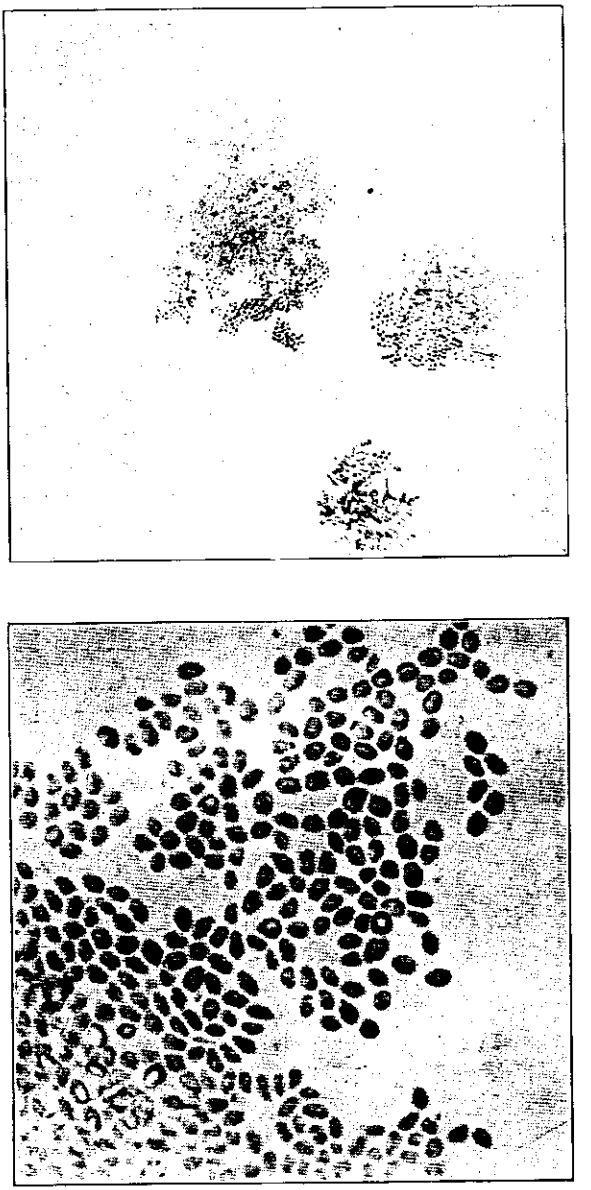


Figure 48 a. Normal growth of *Saccharomyces Mycoderma punctisporus*. Above: 100 times; below: 500 times.

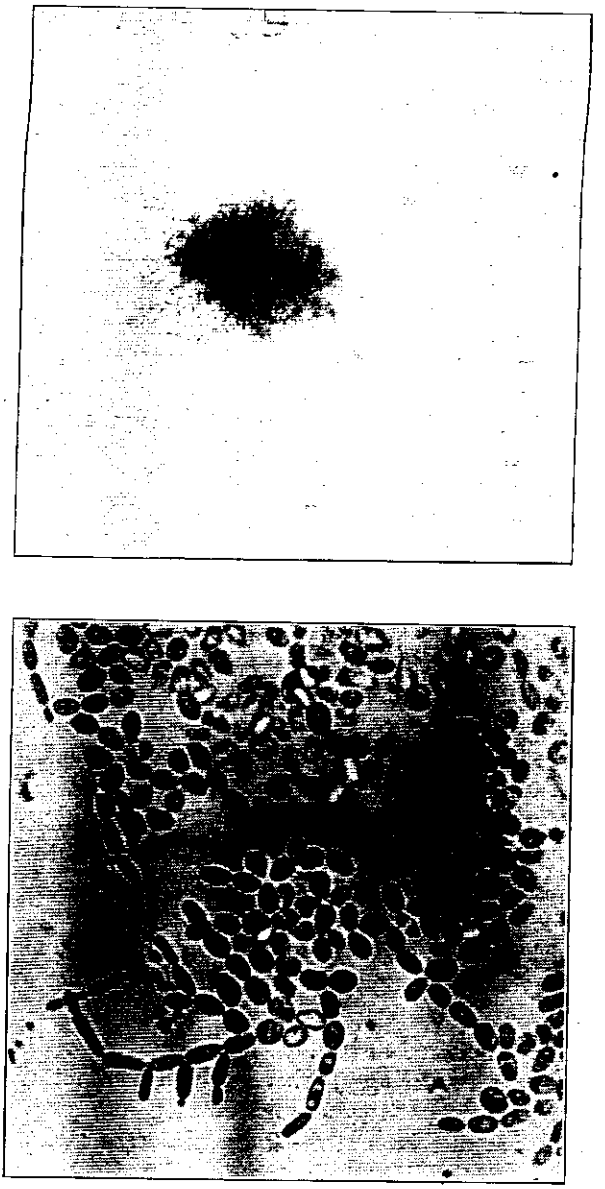


Figure 48b. Growth of irradiated *Saccharomyces Mycoderma punctisporus*.
Above: 100 times; below: 500 times.

saliva drop to the yeast culture. These experiments, as well as those by CHRISTIANSEN, were made in hanging droplets on a coverglass which was sealed to a moist chamber slide with vaseline or lanolin; the "sender" was at the bottom of the cavity.

By the same method, the effect of plants upon the morphology of yeasts was studied. It was found that most parts of the various plants stimulated yeast growth considerably, and also produced great morphological changes. The most sensitive yeast was *Saccharomyces Mycoderma punctisporus* GUILLIERMOND which was frequently greatly elongated so that it appeared under the low power like a mold colony. However, true branching was never observed, and the growth resembled more that of a *Monilia* Fig. 48 shows the change produced by exposure to a young agar culture of the same species. Some of the wine yeasts also showed changes of cell form and size.

Seed embryos, pollen, and roots produced the strongest effects; leaves, the weakest effects.

These results could not usually be repeated with the interposition of a quartz coverglass between yeast and sender. Only the one or the other symptom appeared occasionally (see fig. 48), but scarcely ever the complete set of morphological changes. However, the steam distillates of carrots or potatoes, when added to the culture medium in large amounts did not affect the morphology; nor did the juice of crushed carrots produce any change. When the sender was poisoned chemically, killing all cell activity, no effects were observed.

Perhaps we are dealing here with a combined chemical and physical effect, such as STEMPELL (1932) assumed to be rather common in nature.

In the author's laboratory, Mrs. BARNES has isolated a bacillus which through a quartz coverglass, will change morphologically the yeast cells, giving them the appearance of "saliva types".

2. Sea Urchin Larvae. It has already been discussed on p. 86 that sea urchin eggs, when exposed continously to mitogenetic rays from various sources, develop into very abnormal larvae. It has also been stated there that recently, it has been suggested that this is not due to a real radiation, but to an electric effect. Microphotographs of these forms are shown in fig. 49.

J. and M. Magrou (1931) have studied the abnormal larvae histologically, and have come to the conclusion that they are primarily due to overproduction of the mesenchyme, while the ectoderm and endoderm appear normal (fig. 50). This gives a simple explanation of the morphological changes by the mito-

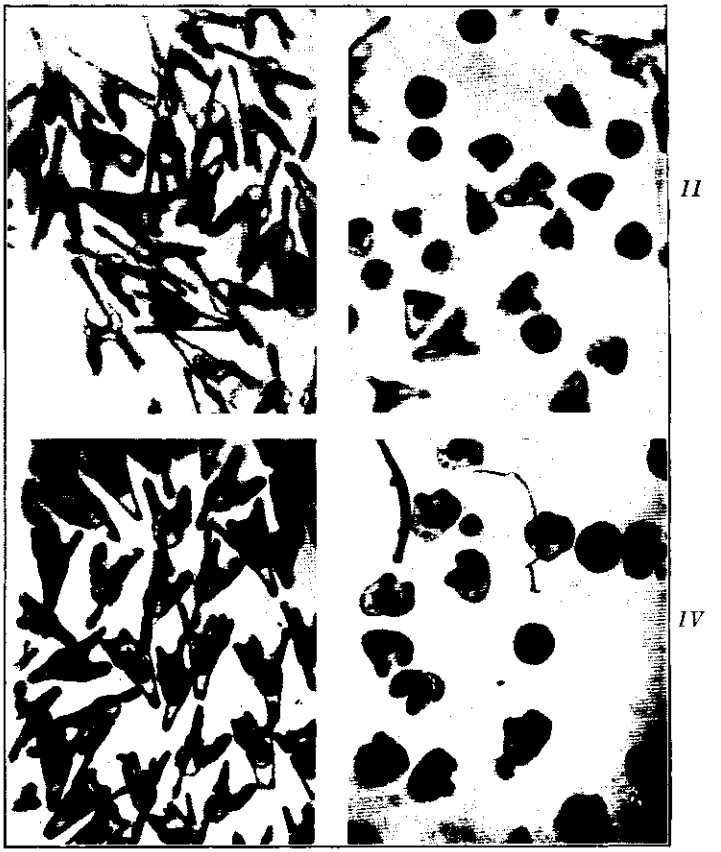


Figure 49. Larvae of the sea urchin, *Paracentrotus lividus*.

I. Control, untreated.

II. Same origin and age as *I*, but exposed continuously through quartz to an acid solution of phenosaffranine reduced by $KHSO_3$.

III. Control, fertilized with normal sperm.

IV. Eggs of the same origin as *III*, but fertilized with sperm exposed for 45 minutes to the same solution as *II*.

genetic effect. However, there is considerable difference in the reaction of ectoderm and endoderm cells on one side, and mesenchymatic cells on the other. The former are not essentially affected (there may be some retardation eventually), while the mesenchyme grows entirely out of bounds though it is really protected against radiation by the outer cell layers.

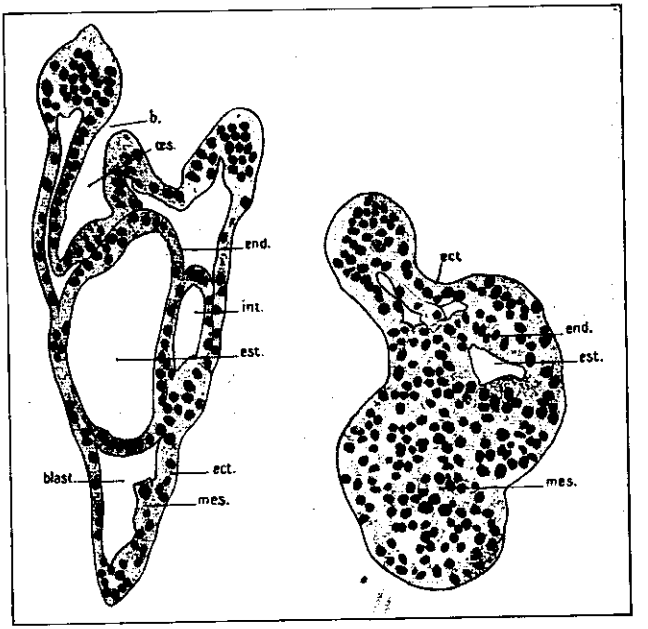


Figure 50. Cross section through sea urchin larvae.
Left: the normal larva. — Right: larva exposed to the radiation of *Bact. tumefaciens*.
b = mouth; *blast* = blastophore; *ect* = ectoderm; *end* = endoderm; *est* = stomach; *int* = intestine; *mes* = mesenchyme; *oes* = oesophagus.

The most remarkable fact is perhaps the observation that the irradiation of the sperm alone or of the unfertilized ovum alone will suffice to bring about the same morphological changes. Thus, the radiation received by the single egg or sperm cell affects the entire future development of the larva, and the stimulus received by such a cell affects only one part of the offspring, i. e. the mesenchymatic cells.

3. Metamorphosis of Amphibia. The role of mitogenetic radiation in the metamorphosis of amphibia has been studied extensively by BLACHER and his associates. The most detailed investigation concerned the development of tadpoles.

The following developmental stages are distinguished:

Stage I : hind legs differentiated
II : hind legs scarcely motile
IIIa: hind legs actively motile, belly rounded, forelegs not visible through the skin.
IIIb: belly becomes lean, elbows of forelegs distend the skin.
IV : forelegs are out; tail has full length
Va: about one-fourth of the tail is resorbed
Vb: about one-half of the tail is resorbed
Vc: about three-fourths of the tail is resorbed
VI : the entire tail is resorbed; metamorphosis completed.

Radiation arises from the resorbed tissues. The newly-formed fore or hind legs do not radiate. The intensity of radiation was estimated by the amount of the mitogenetic effect (yeast bud method) which is not a particularly good measure. The results are shown in fig. 51. All organs radiate only during their resorption. The gills initiate the process, followed closely by the intestine which is shortened considerably, the main shortening occurring at the time the forelegs develop. When the gills are almost completely resorbed, the tail begins. Other parts of the tadpole were tried, e. g. the back of the skin, but they did not radiate.

In a later paper, BLACHER and LIOSNER (1932) estimated the intensity of blood radiation of *Rana ridibunda* during metamorphosis. They ascertained the minimal time necessary to bring about a distinct mitogenetic effect. The results were

at Stage II :	5 minutes; relative intensity:	normal
IIIa:	30 minutes	one-sixth normal
IIIb:	15 seconds	20 times normal
IV :	5 minutes	normal
Vb:	30 seconds	10 times normal
VI :	5 minutes	normal

The strongest blood radiation coincides with the stage where gills, intestine and tail are all near the maximum of radiation. Otherwise, there is no parallelism. The second pronounced

maximum of blood radiation occurs after gills and intestine have completed their metamorphosis.

BLACHER and associates (VII) further showed that there was a relation between the resorbed tissue and the developing limbs, and that transplantation of gills increased the growth rate of the corresponding foreleg. Removal of the gill, or the freeing of the foreleg from the gill cavity where resorption of the gills occurs, retarded growth. Finally, it could be shown that a tadpole lying in a quartz-bottomed vessel will show a more rapid growth of

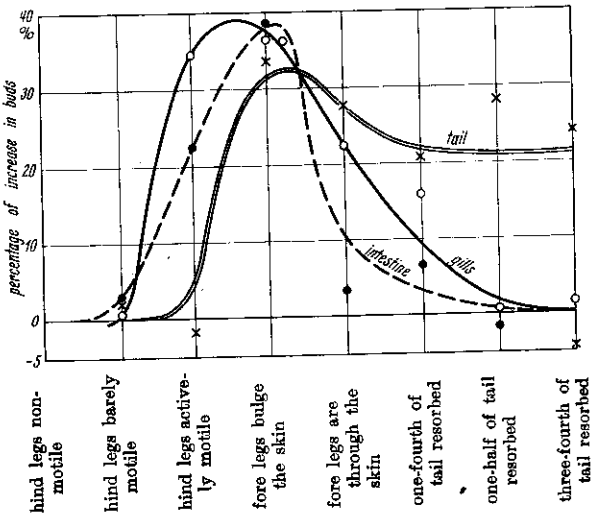


Figure 51. Intensity of radiation of the resorbed organs of frog tadpoles, during successive stages of metamorphosis.

the legs if the bottom is placed over pulp of tissues in the process of resorption. The foreleg to be irradiated was freed from its cavity by a cut in the covering skin, while the other leg which served as control remained covered, and therefore shaded against radiation. The result of the operation as such was that in the vessels with glass bottoms, the freed leg grew a little more slowly than its mate in the cavity: $6.5\% \pm 0.8$ less in one series of 22 tadpoles, and $4.5\% \pm 1.3$ less in a second series with 44 tadpoles. In the quartz-bottomed vessels, however, the freed legs grew more rapidly; $4.1\% \pm 1.1$ more in one series of 33 tadpoles and $7.4\% \pm 1.2$ in the other series with 51 tadpoles. The actual

gain by irradiation was therefore $10.6\% \pm 1.4$ in the one set and $12.1\% \pm 1.8$ in the other set of experiments. The gain is 7 times that of the average error.

The triton *Pleurodeles Waltii* shows similar radiations (BLACHER etc., III), though less intense; and the axolotl *Ambystoma tigrinum* which retains gills as well as tail throughout its life radiates still less.

Experiments were made also with *Drosophila* larvae (BLACHER etc., V). They do not radiate until about ready to pupate. The maximum intensity is reached 24 hours after pupation, and radiation nearly ceases after 30 hours.

There is no direct proof that in these cases, the radiation of the resorbed parts had any distinct form-giving effect. Positively established is only the radiation of the resorbed tissue, and an accelerating effect of this radiation upon the *rate* of development of the organs concerned.

4. Change of Chromosome Number. It has been repeatedly observed that polyploidy occurs fairly frequently in some individual branches of tomato plants infected with *Bacterium tumefaciens*. KOSTOFF and KENDALL (1932) conceived the idea that polyploidy might be somehow linked with the strong mitogenetic radiation which is known to be emitted by this bacterium.

To test this, 120 tomato plants were inoculated with *Bact. tumefaciens*. After the development of the tumor, the plants were cut off 3 to 5 cm. above the tumor in order to induce the development of new shoots. Seven shoots arose directly in the tumor and of these, 5 could be rooted. One of them proved to be tetraploid.

While these data are, of course, too scant to permit the drawing of conclusions, they are suggestive of a new cause for polyploidy.

5. Parthenogenesis by Radiation. REITER and GABOR after having verified GURWITSCH's statement that there is always radiation around a wound, refer to the theory of BATAILLON that the developmental mechanism of an egg is released by the wound produced through the entrance of the spermatozoon. He had succeeded in causing cell division in unfertilized frogs' eggs by very fine needle pricks; only a very few percent of the eggs thus treated initiated development, but in some cases, BATAILLON obtained tadpoles and even frogs by this method.

REITER and GABOR speculated that this effect was in all probability due to the mitogenetic radiation of the wound (see wound radiation p. 173), and they reasoned that in this event, radiation alone, without wounding, should produce the same result. They exposed unfertilized eggs of salamanders and frogs, obtained with great precautions from the females, to various sources of ultraviolet. Of all the wave lengths tried, only those of 3340 Å proved efficient (Attention may be called again to the circumstance that REITER and GABOR have always claimed that only the rays around 3340 Å are mitogenetic, see p. 60). Even with this wave length, only 2 unfertilized eggs of the salamander could be induced to develop, the first division being completed 3 hours after the 5-minute period of irradiation. A repetition caused only one such egg to develop. In the controls, as well as in all experiments with longer or shorter irradiation, or with different wave lengths, the eggs died in a short time. The number of eggs exposed is not given, except that it was stated that each group contained about 5 eggs. Of these "induced eggs", one developed to 32 cells, one to the 8-cell stage.

In a later experiment with "a few hundred" unfertilized frogs' eggs, 3 could be induced to start cell division by irradiation with monochromatic light of 3340 Å.

H. SYMBIOSIS, PARASITISM AND ANTAGONISM

It seems very likely that biological radiations play an important role in the mutual relationship not only of cells, but also of cell groups and multicellular organisms. This field of mitogenetic radiation has as yet scarcely been entered. A few isolated facts stand out plainly.

From the material mentioned in the preceding chapters, biological radiations appear to be largely stimulating, or beneficial. Possibly, this conception is wrong. It may be that only for reasons of technique, we have been able to observe for the most part this one type.

It has been shown that as a rule, unicellular organisms further each other's growth. The radiation from yeast stimulates the cell division of many bacteria, and the rate of germination of mold spores. Similar effects with multicellular organisms are imagineable, but not proved. The radiation from an onion root will stimulate cell division in another root under laboratory

conditions, but that can hardly occur under normal conditions of plant growth, with the soil absorbing all radiation.

The role of radiation in parasitism is strongly suggested, but not proved in the case of plant tumors caused by *Bact. tumefaciens*. MAGROU (1927*c*) observed that cell division in the tumor was not limited to the immediate presence of the bacterial cells. However, it was not attempted to prove that a tumor would be formed even if chemical effects by bacteria were excluded from action through a wall of quartz.

The abnormal proliferations on the leaves and stems of plants due to insect eggs (commonly called galls) are usually attributed to chemical irritation by the larva, but it does not seem impossible that the young larvae radiate strongly during their early period of growth, and cause abnormal proliferation of tissue by irritation through ultraviolet rays.

Parasitic existence is limited to a very few species of plants or animals. It is customary to assume that for chemical reasons, all other plants and animals are prevented from living as parasites. The assumption that specific radiations also play a part offers itself naturally. However, this assumption would imply very specific radiations: either beneficial, which favor one species, but none of its nearest relatives, or, more probably, antagonistic radiations which prevent growth, or cell division, of all species except the parasite; for some reason, the parasite is not affected by the harmful radiation. The assumption of specific radiations has very little experimental evidence for its support. While we are still far from knowing all sources of mitogenetic rays, all well-known radiations arise from entirely unspecific biochemical processes such as proteolysis, glycolysis, oxidation, etc., processes which are common to most plants and animals. The pathogenicity of the typhoid bacterium to man, but not to rats could not be explained by different radiations from the two hosts, at our present state of knowledge. But it must be admitted that we have no chemical explanation either.

On the other hand, there has never been observed any specific action of definite wave lengths upon definite organisms. All experiments so far point to the conclusion that any radiation between 1900 and 2600 Å can stimulate the division of any cell, plant or animal, unicellular or part of a tissue, providing that the physiological conditions permit (see e. g. fig. 28 p. 49).

It is known, of course, that an overdose of rays will not stimulate, and might even retard growth, but there is no evidence that parasites are more tolerant to radiations of this kind than related, non-parasitic species. While the assumption of specific radiations would be a very convenient explanation for some problems in parasitism, it has as yet no experimental foundation.

Antagonistic Radiations: The only good case of specific antagonistic radiations is the investigation of Acs (1933) who experimented with microorganisms known to antagonize each other when grown simultaneously in the same culture, such as *Bacillus anthracis* and *Pseudomonas pyocyanea*, or yeast with staphylococci or streptococci.

Acs used for his experiments cultures which were 6—8 hours old, i. e. at the stage of rapid growth, and exposed the one pure culture to the radiation from the other. In this way, he obtained distinct retardation of growth. Irradiation of *B. anthracis* for 1 to 2 hours by *Ps. pyocyanea* gave growth retardations of 22 to 136%, in 14 experiments. In two experiments, he reversed the arrangement, using *B. anthracis* as sender, and found *Ps. pyocyanea* 42 and 48% retarded. The same organism was used simultaneously to irradiate *Bacillus ratimors*, which was not retarded, but stimulated 42 and 56%. Yeast radiation was found to retard staphylococci very distinctly, in 5 experiments, and also streptococci in the one experiment made.

Since such selectivity of antagonistic radiation cannot be explained by our present knowledge, a more detailed spectroscopic investigation of such types might add greatly to our understanding of the significance of biological radiations.

Different from the specific harmful radiation which injures one species, but stimulates other species, are the generally harmful human radiations observed by CHRISTIANSEN and by BARNES and RAHN (see p. 184) which are linked with certain pathological conditions. The radiation may be truly specific; only one species of yeast, *Saccharomyces Mycoderma punctisporus*, could be killed, while other yeast species were retarded, or not at all affected.

The example of the sea urchin larvae (p. 86 and 164) is quite characteristic of a harmful effect produced by a primarily beneficial radiation. In this case, the harm is probably done by an overdose and not by any specific wave lengths since such widely different sources as chemical oxidation, glycolysis by

yeasts or streptococci, and proteolysis by staphylococci gave the same results. The main cause of the harmful effect in this case is the difference in sensitivity or reactivity, the mesenchyme cells being the only ones which were stimulated out of proportion.

I. THE HEALING OF WOUNDS

When wounds begin to heal, this is usually accomplished by mitosis of the cells nearest to the wound. In most cases, as with grown animals or plants, this would mean that cells which have already come to a resting stage, revert to multiplying cells. This resembles somewhat the situation of old yeast or bacterial cells transferred to a fresh medium. Something like a rejuvenation process of the old resting cells is necessary before they can divide again. It was precisely at this stage that we found mitogenetic radiation to be most effective upon bacteria or yeasts.

The first discussion concerning the possible rôle of biological radiation in the healing of wounds occurred in 1929. HABERLAND had found that full-grown, but young leaves of *Sedum spectabile* and *Escheveria secunda* can be torn without rupturing the cells of the mesophyll. They separate, and leave a dry surface. Such a surface will not "heal", i. e. the cells show no signs of division if the torn leaf is kept in a moist chamber. They will begin to divide, however, if smeared with juice of crushed leaves. They will also reproduce if the leaf is cut instead of carefully torn, leaving the wound surface wet with cell debris.

GURWITSCH (1929) expressed his opinion that such new division of old cells could not take place without a mitogenetic stimulus, and that very probably it came from the cell pulp. HABERLAND (1929) attempted to discover whether radiation by sedum leaf pulp would cause a healing or renewed mitosis of a dry, torn leaf. He found that leaf pulp did *not* induce any division of cells when held at short distances from the injured leaf and concluded that the wound hormones act chemically and not physically.

GURWITSCH (1929) found, using yeast as detector, that pulp of sedum leaves did not radiate when fresh, but would do so after 18 to 24 hours, losing this power again after 48 hours. He states that in his opinion, radiation alone will not cause the healing process in this case; that there are chemical requirements

besides the physical ones; but, that radiation is *one* of the necessary factors.

While the argument concerning the sedum leaf wounds has never been settled experimentally, BROMLEY (1930) has proved that the tail of the salamander *Ambystoma tigrinum* radiated strongly after being amputated. It is not the new tissue which radiates, but the old cells immediately under the newly-formed tissue. Exposing yeast for 18 minutes to the ground tissue, the following "induction effects" were obtained:

1	2	3	5	6	12	24	hours after amputation
6.2%	2.1%	29.6%	41.1%	39.9%	48.4%	56.4%	induction

It requires 3 hours for radiation to be established. The radiation of the injured tissue reaches a maximum after 1—2 days, decreases decidely and reaches a second maximum after 5—6 days. This agrees with the two maxima in pH of healing wounds as observed by OKUNEFF (1928).

The same double maximum could be observed with wounded tadpoles of the frog *Pelobates fuscus*, by BLACHER, IRICHIMOWITSCH, LIOSNER and WORONZOWA (1932b). They cut 10 mm. from the tip of the tail, and after different times, removed the old tissue which produced the regeneration, ground it up and used the pulp to irradiate yeast plates. The threshold time necessary to produce distinct mitogenetic effects was the measure of intensity. In Table 50, two distinct maxima are seen at 12 and 36 hours after wounding, and a minimum at 24 hours, and a great drop in intensity after 4 days.

Very interesting was the observation by the same authors that the blood of the tadpoles changed its radiation decidedly upon wounding of the tail. The blood of normal, uninjured tadpoles produced a marked effect in 5 minutes. 12 hours after injury, 1 minute of exposure to blood sufficed for a similar effect; 24 hours after injury, 2 minutes were required, and on the fourth day, an exposure of 15 minutes became necessary. This means that at this stage, the blood radiated less than half as strongly as that of uninjured tadpoles, and it stayed at this low level during the entire 18 days of the experiment.

By this long-continued effect of the wound upon blood radiation, the entire body is affected and brought into play.

Table 50. Induction Effects Produced in Yeast by Irradiation with Old Tissue Bordering the Regeneration of Wounded Tadpole Tails (*Pelobates fuscus*)

Time after Wounding	Irradiated for							
	10 sec.	15 sec.	30 sec.	1 min.	2 min.	5 min.	10 min.	15 min.
6 hours	—	—	0	+28	—	—	—	—
12 „	+2	+18	+29	+ 7	—28	—29	+ 7	—
24 „	—	—	—	— 5	+18	—	—	—
36 „	—	+ 4	+36	—	—	—	—	—
2 days	—	+ 2	+26	+12	—16	— 9	—23	—
3 „	—	—	+ 3	+24	—	—	—	—
4 „	—	—	—	—	—	—	— 8	+36
5 „	—	—	—	—	—	—	+ 1	+52
6 „	—	—	—	—	—	—	+ 2	+28
8 „	—	—	—	—	—	—	+ 2	+21
11 „	—	—	—	—	—	—	+25	—
12 „	—	—	—	—	—	—10	+20	—
18 „	—	—	—	—	—	+ 2	+31	—

SAMARAJEFF (1932) repeated the tadpole and salamander experiments with earthworms. He cut them through, leaving them in earth, and found that the first mitogenetic effect could not be detected until 20 hours after inflicting the wound. Radiation was weak throughout, but was noticeable even after 6 days.

Considering that regeneration in wounds is accompanied by a radiation of the injured tissue, it seemed probable that this radiation was essential in bringing about healing. BLACHER,

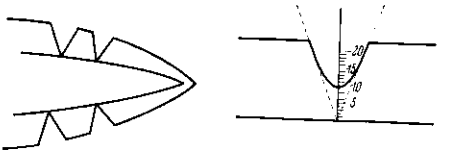


Figure 52.
At left, method of wounding the tadpole tails for irradiation from below; at right, method of measuring the amount of regeneration.

IRICHIMOWITSCH, LIOSNER and WORONZEWA (1932a) succeeded in proving this by producing a more rapid regeneration of wounds by biological irradiation. More than 500 tadpoles were used for this proof. Into their tails were cut triangular wounds, as in fig. 52. The rate of healing was measured as shown in the same figure, under a low power lens, and it is here recorded in μ of new

Table 51. Amount of Regeneration in the Wounds of Tadpole Tails, Irradiated from Below

Irradiated during	Irradiated				Controls			
	No. of tadpoles	new growth, in μ under side	upper side	Induction Effect	No. of tadpoles	new growth, in μ under side	upper side	Induction Effect
1st 24 hours	18	582	513	+13	16	444	480	— 7
after	10	324	210	+54	17	246	243	+ 1
wounding	16	645	555	+16	12	477	528	—10
2nd 24 hours	20	777	678	+14	20	744	753	— 1
after	17	576	459	+26	16	489	498	— 2
wounding	16	666	528	+26	14	657	705	— 7
3rd 24 hours	14	849	765	+11	14	786	762	+ 3
after	16	585	486	+20	18	561	558	+ 1
wounding	19	1014	813	+25	16	702	645	+ 9

growth from the deepest part of the wound. The tadpoles were placed in dishes with quartz bottoms which rested on a pulp of tadpole tails and intestine. This pulp is known to radiate strongly, and the wounds on the under side of the tail were thus exposed through quartz, while the wounds on the upper side were not. The controls were in similar dishes with glass bottoms. These experiments, of which a few are reproduced in Table 51, showed that in the controls, there was no difference in the rate of regeneration between the upper and the under side of the tail. Irradiation produced in all series of experiments a distinctly more rapid healing of the irradiated wounds.

However, this statement needs some modification as a careful checking of the individual wounds showed. When all the upper (non-irradiated) wounds of the exposed tadpoles were compared with those of the controls, there was no difference when the tadpoles were exposed for 24 hours immediately after wounding. There was also no difference when the tadpoles were left without treatment for 48 hours and then exposed for 24 hours. If, however, the tadpoles were left untreated for 24 hours and then exposed for 24 hours, the upper wounds had healed 26% less than the controls. A comparison of the lower wounds showed an increase in healing of 33% when irradiated at once, but no difference when irradiation began after 24 hours.

This signifies that irradiation immediately after injury affects only the irradiated wounds, stimulating them; irradiation 24 hours after wounding does not stimulate these, but retards the others, manifesting an effect which is only apparently beneficial. Irradiation 48 hours after wounding again produces a true stimulation. There is a suggestion of the same double maximum which we have already encountered. No explanation has been attempted.

The proof that the rate of healing of wounds can be accelerated by mitogenetic rays, may be of importance for the future of wound treatment. Two treatments might be, partly at least, explained by mitogenetic rays. One is the beneficial influence of the presence of Lactobacilli. NORTH (1909) and GILTNER and HIMMELBERGER (1912) applied cultures of *Lactobacillus bulgaricus* to inflamed mucous membranes (gonorrhoea, hay fever, conjunctivitis, utero-vaginal affection of cows after abortion) and to suppurating wounds with very good healing results. The good success is ascribed to the lactic acid. The other method is the rapid healing of wounds infested with maggots of flies. While doubtless the present explanation is correct, that the continuous removal of pus and dead tissue cells by the maggots induces more rapid healing, it may well be that in addition, there is a mitogenetic effect upon regeneration by the larvae.

J. THE CANCER PROBLEM

The word *cancer* is not very accurately defined. It is meant to indicate the most common form of malignant tumor. From the physician's point of view all tumors are neoplasms. Since the authors do not feel competent to pass judgment upon medical definitions, they have adopted the following of FELDMAN'S (1932):

"With certain reservations, a neoplasm may be defined as an autonomous proliferation of cells, non-inflammatory, which grow continuously and without restraint, the cells resembling those of the parent cell from which they derived, yet serving no useful function, and lacking orderly structural arrangement."

"Carcinoma is preferable to the older term cancer as designating tumors of epithelial origin that are malignant."

"Sarcoma refers to a tumor consisting of immature connective tissue elements, that is clinically or histologically malignant."

It has already been mentioned repeatedly in previous chapters that contrary to the very weak radiations of the normal tissues of grown animals, the malignant tumors radiate strongly, while the benign do not.

The first study on the relation between tumors and mitogenetic rays, however, was the investigation by the MAGROUS (1927a and b) of plant tumors caused by the crown gall organism, *Bacterium tumefaciens*. They proved, with onion roots as detectors, that cultures of the bacterium radiated; they also showed (1927c) that in the tumors, cell division had taken place quite removed from the location of the bacteria. This made a physical effect probable, but did not exclude chemical effects. They did not produce experimentally plant tumors by radiation.

Soon afterwards, the carcinoma problem in man and animals was attacked from various angles; (1) the radiation of cancerous tissues; (2) the radiation of blood of cancer patients, as a means of diagnosis; (3) the origin of cancer.

Radiation of Cancerous Tissues: The strong radiation of cancerous tissue as contrasted with the non-radiating normal tissue has been established in a number of cases by various investigators, e. g. GURWITSCH, REITER and GABOR, SIEBERT, STEMPELL, GESENIUS, and by various methods. It is one of the most definite facts of mitogenetic radiation. It is also an established fact that only malignant tumors radiate.

A. and L. GURWITSCH (1929) and KISLIAK-STATKEWITSCH (1929) had found that there were two kinds of carcinoma radiations; one requires glucose in order to emit rays, while the other occurs primarily in the necrotic parts of cancerous tissue which are not capable of glycolysis. By comparing the spectra of these two different types with the known spectra, it could be shown that the one was plainly a glycolytic radiation while the lines emitted by the necrotic parts of the tumors agreed with those of proteolysis (see Table 52). An analysis with strips of 50 to 60 Å was sufficient to prove the difference.

The nucleic acid spectrum is also found in carcinoma itself if the exposure is sufficiently long; the proper time for the production of a glycolytic spectrum is too short for that of nucleic

Table 52. Analysis of the Two Spectra of Carcinoma (the numbers indicate induction effects)

Carcinoma Type	Wave Length in Å	in situ	in RINGER's solution + glucose	Glycolysis of Normal Blood
Intact Metastases	1900—1950	50	44	25
	1950—2010	55	33	32
	2010—2070	—3	—9	5
	2070—2150	1.3	3	—1
	2150—2220	50	66	30
	2220—2340	0	0	2

		necrotic carcinoma	serum albumin + pepsin
Necrotic Parts	1900—1960	—1.5	—3
	1960—2020	38	50
	2020—2080	53	80
	2080—2140	45	70
	2140—2210	8	1
	2210—2290	2.5	11.6
	2290—2390	44	50
	2390—2430	42	36

acid. This difference in intensity of various reactions occurring simultaneously adds greatly to the difficulties of spectral analysis.

Absence of Blood Radiation: The second outstanding and thoroughly established fact is the absence of blood radiation in cancer patients (see fig. 46, p. 153). GESENIUS (1932), in summarizing 3 years of clinical experience, states that patients with teratomes (monstrosities) and probably with mixed tumors never show blood radiation; while normal radiation is observed in all cases of sarcoma[1]), hypernephroma (benign kidney tumor), glioma (benign tumor of the brain, retina or auditory nerve), and myoma (benign muscle tumor), even in severe anemia caused by bleeding of the myoma.

BRAUNSTEIN and HEYFETZ (1933) determined the time when blood radiation decreases. They inoculated rats with tumor

[1]) This seems strange since the sarcoma tissue shows the same strong radiation as carcinoma tissue.

Table 53. Glycolysis and Radiation in Blood from Rats Inoculated with Tumor Cells

	No. of Rats	Decrease of Sugar in Blood, in mg, after 1.5 hrs	3 hrs	Mitogenetic Induction
Normal Animals	14	50.1	67.6	+33.4
5 days after implantation of cancer	3	38	69	+26
6 days	6	40	66	+13
8 days	7	62	78	+ 7
10 days	7	43	70	+ 7
13 days	6	46	69	— 4
16 days	3	52	77	+ 5

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Table 53. Glycolysis and Radiation in Blood from Rats Inoculated with Tumor Cells

	No. of Rats	Decrease of Sugar in Blood, in mg, after 1.5 hrs	3 hrs	Mitogenetic Induction
Normal Animals	14	50.1	67.6	+33.4
5 days after implantation of cancer	3	38	69	+26
6 days	6	40	66	+13
8 days	7	62	78	+ 7
10 days	7	43	70	+ 7
13 days	6	46	69	— 4
16 days	3	52	77	+ 5

cells through an incision of the skin of the back; after 6 days, a tumor the size of a barleycorn had developed. In short intervals, they determined the decrease in blood sugar, and the mitogenetic radiation (Table 53). While the latter decreased distinctly from the fifth day, glycolysis and sugar content were not affected. There is really much more to this negative induction effect of cancer blood than merely the absence of radiation. HEINEMANN (1932) very definitely found growth retardation when he used the actual rate of cell increase as a measure (Table 22, p. 73 and fig. 45, p. 153). As early as 1929, LYDIA GURWITSCH and SALKIND observed that the blood of cancer patients suppressed the radiation of normal blood upon mixing the two. Rats, injected with cell-free tumor extract, showed no blood radiation after 2, 3, and 4 days, but were normal again after 9 days. Injection of cytagenin (see fig. 45) caused a temporary increase of blood radiation. When cytagenin is discontinued, emission of rays drops to its negative level in 1 to 3 days.

Cancer Diagnosis: Blood radiation is so conspicuous even with patients suffering from many kinds of severe illnesses that its absence in cancer has been considered since the earliest observations as a diagnostic means. GESENIUS reports (1932) that in pernicious anemia, radiation begins again after liver diet, while in carcinoma, even the complete removal of the tumor and radium treatment will not restore normal blood radiation.

Recently, KLENITZKY (1934) observed the return of blood radiation when every last trace of cancerous tissue had been removed.

Since practically all diseases producing decreased mitogenetic radiation in blood are readily recognized, cancer diagnosis by this method has been studied systematically. The best results obtained are very likely those recorded by HEINEMANN from one of the hospitals in Frankfurt, Germany. "On the one side, further observations with carcinoma suspects justified the conclusion that a positive mitogenetic effect excluded the possibility of a tumor with certainty. On the other hand, a negative effect changing promptly to positive after cytagenin injection, caused us repeatedly to search for a tumor and to prove it beyond doubt, though the preceding, most careful clinical investigation had given no reason to suspect tumors (e. g. a small carcinoma in the upper rectum which caused no direct pain)."

Very encouraging is further the summary by GESENIUS (1932) of 3 years experience in the Berlin University clinics. The method consisted in the decrease of yeast respiration by blood radiation (see p. 84). During the last year, only such cases in which diagnosis was uncertain were investigated.

Occasional exceptions have been observed; blood radiation has been found infrequently connected with severe carcinoma, and has rarely been absent in patients where autopsy revealed absence of carcinoma. Nevertheless, blood radiation is a valuable diagnostic means when used as a part of the clinical examination. Loss of radiation is independent of the size and location of the tumor. It remains absent after removal of the tumor, and cannot be used as a test for the success of treatment.

Origin of Cancer: It has been stated repeatedly that normal adult tissues radiate very weakly while normal blood radiates strongly; in cancer, the opposite takes place, the new growth radiating strongly while the blood ceases to do so. These facts plainly suggest that the growth-stimulating source of radiation is removed from the blood and concentrates in the neoplasm. However, this simple assumption does not agree with our explanation of these radiations as originating from biochemical reactions. It has just been shown that sugar content and the rate of glycolysis in the blood of cancer patients are not greatly altered. For this

reason, this explanation of the origin of cancer is not usually stressed.

Somewhat different is PROTTI's conception (1934*b*) who assumes that a cellular disorder may arise when blood radiation becomes very low, eventually resulting in a neoplasm. He proved his point by injecting a cell suspension of adeno-carcinoma into two lots of 12 mice each; one lot was being fed normally, while the other was on a starving ration. For the first 15 days, the tumors grew more rapidly in the starved mice whose blood radiation had decreased, on the day of injection, from about 50 to about 10. It must be remembered that cancer is most frequent in old age when blood radiation has a tendency to become very low.

PROTTI observed further that a mixture of neoplasmic cells and yeast cells *in vitro* resulted in a destruction of the neoplasmic cells while cells from normal tissues were not influenced by yeast. The same happened when the neoplasmic cells were separated from the yeast cells by a quartz plate. PROTTI calls this "cytophotolysis".

Repeated injections of yeast suspensions into the Galliera-Sarcoma of rats caused a liquefaction of the tumor, without pus formation, leaving finally a cavity with thin fibrous walls. Intravenous injections produced no effect upon the tumors. With the adeno-carcinoma of mice, injections of small amounts of yeast into the tumor stimulated its growth while large amounts retarded it. The same was the case with intravenous injections. Yeast heated to 60° produced no effect which suggests, together with the abovementioned "cytophotolysis", that the results are not due to enzymes, but to radiation.

Cancers have been produced by frequent application of certain chemicals to the skin. Since the compounds found so far are not normal or pathological products of the human body, as far as is known, the discovery of their effect does not really explain the formation of cancers, but may give valuable suggestions towards the solution of the problem.

More important is perhaps the discovery that cholesterol metabolism is in some way connected with cancer. It has been claimed by SHAW, MACKENZIE, MORAVEK and others that cholesterol stimulates the growth of malignant tumors. ROFFO (1933) found the cholesterol content of the skin near cancerous or pre-

cancerous lesions to be much higher than the normal skin of the same patient. He found the frequency of skin cancer to be distributed in the following way, as average of 5000 cases:

skin of the face	95.51%
skin of the back of the hand	3.07%
scalp	1.02%
skin of the foot	0.50%

KAWAGUCHI (1930) had shown that the cholesterol content of the skin increases when it is exposed to sun light. According to MALCYNSKI (1930), ultraviolet irradiation of healthy persons increases the blood cholesterol while with cancer patients, there is a decrease of 25 to 40%.

ROFFO (1934a) studied the "heliotropism" of cholesterol very thoroughly. He proved it to take place only in living organisms. With white rats, sunlight as well as ultraviolet light increased the cholesterol content of the exposed parts of the skin. The wavelength of the ultraviolet was above 2300 Å. X-rays or radium rays produced the opposite effect.

Then, ROFFO (1934b) studied the tendency for cancer development in white rats. 700 rats were kept in sunlight daily for not more than 6 hours, avoiding the hottest sun. After 10 to 11 months, 52% of all rats had developped cancer, which was exclusively on the naked parts of the body (ears and eyes mainly, also twice on the hind feet and once on the nose). 150 rats were exposed to ultraviolet light, of an intensity of 14 erythemal units, beginning with 5 minutes per day and gradually increasing the exposure to 6 hours. Within 4 months, every one of these rats showed tumors, and many of them had several tumors. Of the control rats receiving light from a tungsten filament lamp, not a single animal had developped a tumor. This agrees very well with the above-mentioned frequency of skin cancer on different parts of the human body.

The mode of development and the histology of the rat cancers corresponded exactly with that of human cancers.

The author has not been able to ascertain whether ROFFO has tested the mitogenetic range of rays by itself, i. e. the range between 1800 and 2600 Å. It would be a splendid agreement if this range which is known to stimulate cell division could also be shown to produce abnormal cell division of the epithelial cells. The fact that sunlight can do this speaks very much against

it since sunlight contains no wavelengths shorter than 2700 Å. However, very small amounts, such as have been shown to produce mitogenetic effects, might possibly be present (at least in Buenos Aires where these experiments were made). The much stronger effect of artificial ultraviolet containing also the shorter waves suggests that the shorter waves are more efficient in bringing about abnormal cell division.

Attention may be called here to the effect of radiation of oxy-cholesterol upon yeast cells. It is impossible to say whether any relation might exist between oxy-cholesterol and the cancer problem.

K. RADIATIONS OTHER THAN MITOGENETIC

In Chapter IV, three types of radiation from organisms have been mentioned which are not identical with mitogenetic radiation. One was entirely different, and does not really belong in this group, namely the *Beta*-radiation of the potassium in the cells. Then, there was *necrobiotic* radiation. This type, though apparently stronger than mitogenetic rays, is emitted by dying cells, and does not have any definite biological purpose or effect as far as has been ascertained. It is an emission of energy typical for the chemical changes connected with death, but it is absorbed by the surrounding medium, and should it produce biological effects, it would be purely accidental.

The third group are the harmful human radiations, and they produce distinct biological effects. Quite commonly known are the reactions brought about by menstruating women: flowers wilt readily in their hands; menstruating women are excluded from collecting flowers for the perfume factories of France; mushroom beds are said to be utterly ruined by their mere presence; pure cultures of yeasts and bacteria become abnormal when handled by them (see p. 95), and the common belief that bread dough will not rise normally, and that pickles and sauerkraut packed by them will not keep, does not sound improbable, since CHRISTIANSEN proved that wine fermentation was distinctly affected (see p. 96).

The facts as such can be considered fairly definitely established. Details may be found in the paper by MACHT and LUBIN (1923—1924). But it cannot be considered proved that the effect

is of physical origin. The more common conception among medical men is that it is chemical, produced by a compound called menotoxin.

The intensity of this effect varies greatly with the individual. We have but rarely been able to obtain good results with wilting flowers. CHRISTIANSEN found the effect to be much stronger in summer than in winter.

Attention should be called here to the observation by HILL (1933) that certain persons kill bacteria on agar plates when placing their fingers right upon the seeded plate. In this case, it has not been decided as yet whether the effect is physical or chemical.

Probably the same type of harmful radiation has been observed in a very few cases of illness (p. 97); at least, the yeast was killed by emanation from the finger tips, and even from the face, through a thick quartz plate excluding chemical influences.

The Source of Harmful Human Radiations: MACHT and LUBIN (1923—1924) came to the conclusion that the "menotoxin", i. e. the compound in the blood during menstruation responsible for the various phenomena just mentioned, must be either oxycholesterol or a closely related compound. This induced BARNES and RAHN (1933) to test whether this compound radiated. The results were quite definitely positive. Out of 5 batches of oxycholesterol made at different times, 4 killed Mycoderma during the first day when exposed continuously through quartz, but not on the second day. With Batch No. III which had no effect upon the yeast, an error was made in the separation of the oxycholesterol from the other reagents; on account of this, the exposure could not be started until one day later, and by this time, this product usually has lost its power. The colorimetric tests mentioned for oxycholesterol can be obtained long after radiation has become too weak to be detected biologically.

Since it is not probable that a compound as such radiates, it appears more likely that the radiation comes from some reaction of the cholesterol or oxycholesterol, and we would think above all of oxidations. In the above experiments the oxycholesterol was emulsified with a little water to make oxidation possible.

Why this radiation is harmful instead of stimulating, cannot be stated at present. The simplest assumption would be that the death of the Mycoderma cells is due to over-exposure because in

Table 53a. Effect of Oxy-cholesterol upon Mycoderma through fused Quartz

time after manufacture of oxy-cholesterol	Batch I	Batch II	Batch III	Batch IV	Batch V
1 day	dead	dead	normal (stimulated)	mostly dead	dead
2 days	—	—	—	normal	retarded
3 days	—	—	—	stimulated	—
4 days	greatly retarded	greatly retarded	—	—	—
5 days	slightly retarded	—	—	—	normal
6 days	normal	—	—	—	—
7 days	normal	—	normal	—	—
8 days	normal	—	—	—	—
14 days	—	—	normal	—	—

all experiments the yeast was exposed continuously to this radiation. However, complete killing of all cells is not usually observed even in over-exposure, and only rarely was stimulation found. The other alternative that we are dealing with different wave lengths does not seem very probable either. All wave lengths between about 1800 and 2600 Å give the same mitogenetic effect (see fig. p. 49), and no exceptions are known; the oxycholesterol radiation passes through quartz but not through glass, and can therefore be hardly anything but ultraviolet. An analysis of the spectrum may give the explanation.

Oxycholesterol in the Body: A study of the distribution of cholesterol and oxycholesterol in the fats of the human skin, by UNNA and GOLODETZ (1909) shows that oxycholesterol is not present in the normal cellular fats but only in secreted fats (Table 54). An exception is the fat in the finger nails and toe nails. This agrees quite well with our observation that radiation was obtained from hands (and feet) and from the face where sebacious glands exist, but not from the chest where they are very rare.

While a good deal of attention has been paid in recent years to cholesterol metabolism, very little is known about oxycholesterol in the body. Even its chemical composition is not certain. The only more recent investigation on oxycholesterol in the body is that by PFEIFFER (1931). According to his analyses, the largest

Table 54. The Cholesterol and Oxycholesterol Content of Human Skin Fat

		melting point °	Unsaponifiable fraction %	In % of the unsaponifiable fraction: free Cholesterol	Cholesterol Esters	Oxycholesterol
secreted fats	Comedon fat (blackhead)	53.0	30.50	9.16	—	very much
	hand sweat	46.5	28.40	5.40	0.79	very little
	foot sweat	36.5	22.30	18.70	1.60	fairly much
cellular fats	surface skin fat	48.0	32.20	50.00	2.06	0
	horny skin fat	51.0	36.30	54.00	8.91	0
	nail fat	38.0	41.60	43.70	—	fairly much
	vernix caseosa	38.0	36.00	45.00	8.38	0
	subcutis	liquid	1.15	15.80	0.04	0

amount of oxycholesterol, on the basis of total solids, is found in the brain (0.44%), next are bone marrow, adrenals and bile (about 0.29%), followed by the liver (0.09%). Lowest are the erythrocytes with 0.003%. While previous authors, e. g. UNNA and GOLODETZ, estimated the oxycholesterol colorimetrically, PFEIFFER determined it by the difference in melting points between cholesterol and oxycholesterol. The results may therefore not be comparable, on account of the uncertain chemical composition. It has been stated already that oxycholesterol radiation ceased when the colorimetric tests were still very strong. The relation between this substance in the tissues and radiation is therefore not definitely established.

In the preceding chapter, attention has been called to the close relation between cholesterol and cancer. The above results suggest that an investigation about the role of oxy-cholesterol in connection with the cholesterol metabolism in cancer might yield interesting results.