

SEARCH FOR MITOGENETIC RADIATION BY MEANS OF THE PHOTOELECTRIC METHOD*

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According to Gurwitsch's¹ theory, based upon investigations of the distribution of mitoses in growing organisms, an oscillatory phenomenon of unknown nature must be effective in producing mitoses. This leads to the further assumption that the dividing process itself is accompanied by the emission of such radiation and, further, part of this radiation must be emitted from the biological object. His fundamental experiment is said to prove this theory: the tips of onion roots are placed opposite each other at a small distance (approximately 1 mm.). One root serves as inductor, the other as detector. It was found that the number of mitoses in the side nearest to the inductor had increased in comparison to the side furthest from it.

Numerous experiments were undertaken by Gurwitsch and others² to prove the radiation character of this agent. Reflection and refraction experiments were devised to prove this. Absorption and spectroscopic experiments indicated that this radiation consisted of ultraviolet rays. While Gurwitsch and his school concluded from their experiments that the spectral region of the mitogenetic radiation lies between 1800 and 2500 A, Reiter and Gábor² found two mitogenetic maxima at 3400 and 2800 A, the presence of which is strongly denied by Gurwitsch.

* A preliminary note on this subject was published in the *Pub. Health Rep.*, U. S. P. H. S., 1933, **48**, 1311.

¹ Gurwitsch, A., *Arch. mikr. Anat. u. Entwicklungsmechn.*, 1923, **100**, 11.

² Bibliographies are to be found in: Reiter, T., and Gábor, D., *Zellteilung und Strahlung*, *Sonderheft der wissenschaft. Veröffentlichungen aus dem Siemens-Konzern*, Berlin, Julius Springer, 1928, and Gurwitsch, A., *Die mitogenetische Strahlung*, Berlin, Julius Springer, 1932, 376.

The energy necessary for the production of such high frequency radiation is of chemical origin, as Gurwitsch assumes and tries to prove experimentally, and consists in reactions of the type of oxidation or proteolysis or glycolysis.

While the majority of the very numerous publications on mitogenetic radiation dealing with the biological side of the problem supports Gurwitsch's findings, a few³ emphatically deny the effect and criticize his experimental methods. The problem has been attacked from the physical side also. Thus, two investigators, Rajewsky⁴ and Frank and Rodionow⁵ report physical proof for the existence of this radiation, while others, Schreiber and Friedrich⁶ and Locher,⁷ with similar experimental arrangements, could not detect any trace of it at all.

The use of yeast as biological detector for mitogenetic radiation is generally accepted at present and the increase in the number of budding cells in comparison with a control is said to give the order of magnitude of the effect. For physical detector, use is made of the photoelectric effect, either by employing the photographic plate or a device that works on the principle of the photoelectric cell.

It is the purpose of this paper to show that after careful exclusion of all possible sources of error the physical experiment does not give any proof for the existence of a mitogenetic radiation.

Theoretical Considerations

Assuming that mitogenetic radiation exists, it is possible, from theoretical considerations, to make a rough estimate of the minimum intensity of the mitogenetic radiation which can conceivably be detected. Having this intensity, it is possible to make an estimate of the sensitivity of the biological and physical methods used for the detection of the mitogenetic rays.

³ Taylor, G. W., and Harvey, E. N., *Biol. Bull. Marine Biol. Lab.*, 1931, **61**, 280. Richards, O. W., and Taylor, G. W., *Biol. Bull. Marine Biol. Lab.*, 1932, **61**, 113.

⁴ Rajewsky, B., in Dessauer, F., *Zehn Jahre Forschung auf dem physikalisch-medizinischen Grenzgebiet*, Georg Thieme, Leipsic, 1931, 244.

⁵ Frank, A. S., and Rodionow, G., *Naturwissenschaften*, 1931, **2**, 659.

⁶ Schreiber, J., and Friedrich, W., *Biochem. Z.*, Berlin, 1930, **227**, 336.

⁷ Locher, G. L., *Phys. Rev.*, 1932, **42**, 540.

As the intensity of the mitogenetic radiation is extremely small, it will simplify the matter to consider the emission and absorption of the mitogenetic radiation from the point of view of the quantum theory. According to this theory the emission and absorption of radiation is a discontinuous phenomenon and, further, there exists an "atom of radiation" called quantum, the magnitude of which is given by $h \times \nu$, where h is a constant and ν the frequency of the radiation as measured; *e.g.*, with a spectrometer in this way linking the wave theory of light with the quantum theory. Now, the emission from a mitogenetic inductor consists of ultraviolet light "quanta," emitted discontinuously; these quanta fall upon the biological or physical detector where they are absorbed. In the biological material, the absorption of a single quantum or of an integral number of quanta by a single cell is said to produce a mitosis; in the photographic plate a photochemical reaction will take place and in the photoelectric device the emission of a photoelectron will be the result of the absorption if, for the present, we do not consider the efficiency of these processes.

These circumstances make an estimate both of the intensity of the mitogenetic radiation and of the sensitivity of the methods possible. Beginning with the biological method: yeast is usually taken as detector. The diameter of a yeast cell is approximately 6 microns. In a yeast agar culture in which the cells lie packed closely together, we obtain approximately 30,000 cells per mm.² for the top layer of cells (we need only to consider the top layer as ultraviolet radiation of a wave length of 1800 to 2500 Å (this being the wave length of the mitogenetic radiation according to Gurwitsch) will not reach beyond this layer). The number of budding cells in yeast used as control is, according to Gurwitsch, about 10 per cent of the total number; *i.e.*, in this case 3000 per mm.² Half an hour's exposure to a mitogenetic inductor placed in close proximity to the culture may give an increase in the number of budding cells of 50 per cent in the exposed area, as compared with the control. If we assume that each radiation quantum falling upon the culture is absorbed and furnishes the stimulus for the division of one cell, we have in our example 1500 quanta per mm.² per 30 minutes or approximately 80 quanta per cm.² per second as the intensity of the radiation coming from the inductor.

However, not all quanta falling upon the yeast will be absorbed; some will be reflected or scattered; not all of those absorbed will give the stimulus for a cell division since, according to their random distribution, some cells may absorb several quanta or a quantum may be absorbed by a cell already in the budding state. If an efficiency of 1/10 for this process (and this fraction is probably still too high) is assumed, as a lower limit for the intensity of the mitogenetic radiation an intensity of about 1000 quanta per cm.² per second is obtained. Here the possible influence of secondary mitogenetic radiation within the irradiated medium has not been taken into account. This must be negligible, according to the data available for yeast agar cultures.⁸ Frank and Rodionow⁹ estimate the intensity of the mitogenetic radiation to be from 100 to 1000 quanta per cm.² per second.

If this value of 1000 quanta per cm.² per second is taken as the probable intensity of the mitogenetic radiation, this radiation, according to the above example, will produce in 1 mm.² of a closely packed yeast culture (30,000 cells with 3000 budding cells) an increase of 1500 budding cells within 30 minutes. So great a number of cells cannot be counted in a single experiment. Perhaps 1/10 of this number can be counted. This would mean, out of 3000 counted cells of the example, 300 budding cells in the control and 150 additional budding cells in the irradiated sample would be counted. It is doubtful whether such a finding has any meaning at all, as long as little is known about the natural fluctuations of budding cells within a yeast culture. A positive result of a mitogenetic experiment would be obtained only if the number of budding cells in the experiment were to exceed the number of budding cells in the control by at least three times the mean error obtained by a series of counts of budding cells in a normal yeast culture. Due to the limitations of the subjective method of counting, one cannot increase the sensitivity of the biological method by counting a larger number of cells. This could be done only by the use of an objective method. Gurwitsch¹⁰ describes two such methods,

⁸ Potozky, A., *Biol. Zentr.*, 1930, **50**, 712.

⁹ Gurwitsch, A., *Die mitogenetische Strahlung*, Berlin, Julius Springer, 1932, 47.

¹⁰ Gurwitsch, A., *Die mitogenetische Strahlung*, Berlin, Julius Springer, 1932, 16-18.

a nephelometric and mycetocritic one. The data given, however, are insufficient to permit an estimate as to sensitivity and errors.

In order to make a comparison between the biological and physical methods as to sensitivity, let us assume that the above example gives a reliable positive result for an intensity of mitogenetic radiation of 1000 quanta per cm.² per second acting during 30 minutes. This interval was arbitrarily chosen as being sufficient to register any positive effect and yet insure the activity of the specimen during the period of observation.

We proceed now to a discussion of the physical methods and their sensitivity.

The simpler of the two methods is the one employing the photographic plate. A just perceptible blackening of a sensitive photographic emulsion is produced by a light energy of 2×10^8 quanta per cm.².¹¹ As the intensity of the mitogenetic radiation was assumed to be 1000 quanta per cm.² per second, 2×10^8 quanta would be obtained in a time of irradiation of 2×10^5 seconds = 55 hours. A time of exposure of a photographic plate to mitogenetic radiation of 100 hours should produce an easily perceptible blackening of a photographic plate.

The sensitivity of a photoelectric cell arrangement can be determined as follows. A cell of medium sensitivity will have an efficiency¹² of approximately 1/1000 for the wave length at its maximum sensitivity; *i.e.*, for 1000 impinging light quanta, 1 photoelectron will be liberated. With a photoelectric cell of the customary type connected into circuit with a battery and an electrometer or galvanometer, the measurement of currents of the order of magnitude of a few electrons per second is extremely difficult. Such a measurement, however, is made comparatively simple by combining the principle of the photoelectric cell with a so called Geiger counter tube. Such a counter tube consists of a fine wire axial in a metallic cylinder under a gas pressure of about 5 cm. of Hg. By applying a negative potential of approximately 1500 volts to a metallic cylinder and grounding the wire over a high resistance of the order of magnitude of 10^9 ohms,

¹¹ Geiger, H., *Handbuch der Physik*, Berlin, Julius Springer, 1926, **23**, 628.

¹² Wien, W., and Harms, F., *Handbuch der Experimentalphysik*, Leipsic, Akademische Verlagsgesellschaft, 1928, **23** (2), 1205, Table 17.

an electron, liberated from the walls of the tube by any kind of radiation, will travel toward the wire, producing on its path an ionic cloud by impact, resulting in a relatively strong current impulse through the high resistance to ground. This current impulse can be recorded by a string electrometer or by a suitable amplifier with mechanical recorder, as will be shown later. To make such a counter sensitive to light, the walls of the tube must be made of a photoelectric metal and a window provided for the impinging light. This method was first used in testing for mitogenetic radiation by Rajewsky⁴; the other authors previously mentioned used arrangements of a similar kind.

Taking the photoelectric efficiency as 1/1000 and assuming a 30 minute exposure to a radiation intensity of 1000 quanta per cm.² per second we obtain for a window of 1 cm.² a liberation of 1800 photoelectrons in such a tube.

Although the efficiency of the photoelectric process is much smaller than that of the biological process in the detector, the sensitivity of the photoelectric method is much higher, due to the fact that in the biological counting method use can be made of an irradiated area of a fraction of a millimeter only, while in the physical method detecting areas of 1 cm.² or even more can be employed. As will be later described, counter tubes with a window area of 6 to 7 cm.² were used which would increase the number of photoelectrons of the above calculations by a factor of 6 or 7.

To sum up: For an assumed intensity of the mitogenetic radiation of 1000 quanta per cm.² per second,—a probable value for the intensity established by the experimental data given above—the biological method, consisting in counting yeast cells, produces a perceptible effect in the detector, the photographic method should give a perceptible blackening of a photographic plate, and the photoelectric method should yield an effect far above the sensitivity threshold of that method.

EXPERIMENTS

After considering and discussing the different methods, the physical experiments—photographic as well as photoelectric—which were undertaken to study the problem of the existence of mitogenetic radiation will now be described.

The photographic experiments were carried out in the following way: Three light-tight boxes $22 \times 15 \times 22$ cm. were prepared with a sliding lid in front, to the bottom of which metal plate holders were fastened. The metal lid of these plate holders had an opening of 4×5 cm. This opening was lined with velvet. Quartz plates 6×8 cm. in size, 0.5 mm. thick, selected as to transparency, were placed upon the velvet, thus effectively sealing the photographic plate against any possible chemical influence by volatile substances from the onions or onion-base pulp used in the experiments. To the center of the quartz plate a cylinder of pyrex glass was sealed with de Khotinsky cement; the cylinder was 25 mm. in diameter and 2 cm. high in the experiments with onion pulp and 5 cm. high in the experiments with onion roots. The transparency of the quartz was tested spectroscopically down to a wave length of 1800 Å. The loss of intensity for this wave length was not higher than 20 per cent. As the distance of the onion-base pulp or onion roots from the photographic emulsion was not greater than 1 to 2 mm., practically all radiation emitted by the biological material into the lower hemisphere was necessarily absorbed by the photographic emulsion. As photographic material, Eastman Speedway plates were chosen; in some of the experiments these plates were sensitized by a thin coating of mineral oil (Nujol) to overcome the possible objection that the sensitivity of the photographic plate decreases considerably for short ultraviolet on account of absorption by the gelatine of the emulsion. Onion-base pulp was prepared from selected ordinary onions sprouting vigorously. The pulp was changed every 2 hours. In the case of the experiments with onion roots, vigorously sprouting onions were chosen with sprouts of a few centimeters in length and placed on top of the glass cylinder partly filled with a 0.1 per cent solution of KCl. Care was taken that a great number of root tips (about 10 to 20) touched the quartz plate. These onions were inspected every day and replaced every 2nd day. All operations of changing onion-base pulp or onions were carried out in complete darkness; a special device was provided insuring that the biological material was always set at the same place.

Every box was provided with an opening on top and bottom, carrying a rubber hose through which air was gently sucked by means of an aspirator.

All plates were developed in a hydroquinone solution of twice the normal strength; the sensitizing oil being removed in an ether bath before the developing process. Hydroquinone was chosen as it gives very strong contrasts.

Table I gives the experimental data of the photographic experiments.

All these plates showed a rather strong fogging which, however, was identical with that of a control plate taken from the package and developed with the same developer for 4 minutes. This fogging was due to the strong concentration of the developer used. In spite of the fog, any slight difference in density could have been detected. Not

the slightest difference in density could be found in any of the six plates which could be attributed to an effect from radiation coming from the onion pulp or roots.

There are three possible objections to the photographic method. The first one is the uncertainty as to whether all the biological material used in the experiments with onion-base pulp was equally active and whether it remained active during the total time of 2 hours for which time every single preparation was used. However, there is little doubt that the onion tips used for Experiments 2 and 4 were active.

TABLE I

No.	Biological material	Total time of exposure	Photographic material	Time of development	Remarks
		<i>hrs.</i>		<i>min.</i>	
1	Onion-base pulp	106	Eastman Speedway sensitized	7	Onions grown in dark
2	Tips of onion roots	120	" "	4	Onions grown in daylight
3	Onion-base pulp	106	" "	7	" "
4	Tips of onion roots	168	" "	8	" "
5*	Onion-base pulp	184	Eastman Speedway unsensitized	3-4	" "
6*	" "	184	" "	3-4	Onions grown in dark

* I am very much indebted to Dr. C. H. Binford for carrying out these two experiments.

The second possible objection is that the intensity of the mitogenetic radiation in reality is weaker than the estimate previously given. That this is improbable has already been pointed out. However, if it were actually only $\frac{1}{3}$ of the assumed value, it should nevertheless have been detected in the case of the onion root experiments by the photographic method.

Finally, the exponent in Schwarzschild's law ($s = i \times t^p$, where s = density, i = intensity, and t = time of exposure) which lies between 0.9 and 1.1 for different photographic emulsions may have been much smaller than 1 for the photographic emulsion used in these experiments. There is nothing known about the value of this ex-

ponent for short ultraviolet; however, it is probable because of several considerations that it differs but little from 1. Assuming a value of 0.9 for the exponent, the effective time of an experiment lasting say 160 hours would be reduced to $160^{0.9} = 96$ hours, an interval which still should be sufficient to produce a perceptible blackening.

Because of negative results, further photographic experiments were abandoned, especially as the photoelectric method permits an experimental arrangement the sensitivity of which is such that it can detect radiation intensities far below the estimated intensity of the mitogenic radiation. Although by themselves the results of the photographic experiments are not conclusive, they serve to corroborate the more clear-cut results obtained with the photoelectric method.

The principle of the photoelectric counter tube has already been explained. After testing different kinds of counter tubes, a tube consisting entirely of quartz was finally adopted. For counter tubes used in cosmic ray work, for instance, any metal tube with ends sealed by means of rubber stoppers and cement will do, as the sensitivity of the tube is independent of the surface properties of the metal and of the filling gas. However, in tubes to be used either for ultraviolet or visible radiation work, great care has to be taken that the surface conditions of the photoelectric metal remain unaltered, as time goes on, by chemical changes such as oxidation. Otherwise, considerable changes in sensitivity will occur. For this reason, the counter tubes used in this work consisted of thin walled quartz tubes (wall thickness approximately 1 mm., length 10 cm., diameter 2 cm.) of high transparency for ultraviolet light. The transparency of the tubes was tested with a quartz spectrograph and it was found that absorption in the quartz was negligible down to 2200 Å, the limit of the spectrograph. An area of 6 to 7 cm.² was flattened out to serve as window. The wire of the tube consisted of tantalum, 0.02 cm. diameter, and was connected to two thick copper wires held in place by 2 quartz capillaries at the end of the tubes. These copper leads were sealed vacuum tight into the capillaries by silver chloride cement. Three side tubes were provided, one for exhausting purposes, one for distilling in the metal, and a third for carrying a wire cemented in by silver chloride and making contact with the photoelectric layer. The tube was exhausted by a mercury diffusion pump with liquid air trap for 8 to 10 hours, heated several times with a blow torch to yellow heat; the wire was degassed for 3 hours by heating it with a battery. Spectroscopically pure cadmium was then distilled into the tube, the wire and window were heated to remove any cadmium deposit, and pure argon was filled in to a pressure of 4 to 6 cm. of Hg. Counter tubes prepared this way do not show any change in sensitivity with time.

Although cadmium is not a very sensitive photoelectric metal, it was nevertheless chosen, because its sensitivity increases rapidly for wave lengths shorter than

3100 Å, where, according to Gurwitsch, the mitogenetic spectrum lies. A further advantage is that visible stray light of longer wave lengths will not affect the counter tube, since the threshold sensitivity of a cadmium counter tube was found to lie between 3400 and 3500 Å. Moreover, shielding for very small amounts of stray visible light would have been very difficult inasmuch as the work could not be carried out in complete darkness. Counter tubes with zinc as photoelectric metal were also made. They were similar to the cadmium tubes, both in sensitivity and threshold wave length. The counter tubes were connected into circuit both with an amplifier operating a mechanical counter, and with a string electrometer provided with a photographic recorder. Fig. 1 gives the experimental arrangements.

The negative pole of a dry cell battery¹³ of 1500 volts with means for changing the tube potential in steps of 1.5 volts and a voltmeter consisting of a microammeter in series with twenty resistors of 10^6 ohms each was connected to the cadmium deposit. The axial wire grounded over a resistance of 2×10^9 ohms¹⁴ was connected to the fiber of a string electrometer in which the potential difference of the plates was 100 volts. In this connection it may be stated that the deflection of the string is approximately proportional to the voltage. Coupling to the amplifier was effected by means of a variable condenser. The amplifier had two resistance-condenser coupled stages. The tube of the last stage was a thyratron, the plate current of which, limited by means of a resistance to 80 milliamperes, was large enough to operate a mechanical counter of the type used for counting telephone messages. The movable arm of this counter carried a small pin, breaking a contact switch in the thyratron circuit at the highest position of the arm. This arrangement is necessary since the grid of the thyratron becomes ineffective the moment it has "triggered" the gaseous discharge between cathode and anode.

The photographic recording device used for recording the movements of the string of the electrometer and studying the character of the discharge consisted of an electric motor driving a photographic recording paper through a train of gears having a variable ratio so as to obtain different recording speeds. Most of the experiments with biological material as a radiator were counted with the mechanical counter as well as photographically recorded. The number of counts obtained by the two methods were identical within a few tenths of 1 per cent.

Since the counter tubes just described are sensitive not only to ultraviolet radiation but also to radiation coming from radioactive substances in the ground, air, and walls of the building, as well as to cosmic radiation, every counter tube will give a residual effect; *i.e.*, the apparatus will record a certain number of counts per minute due to the electrons liberated by these radiations, the number of which depends, other things being equal, upon the cross-sectional area of the tube and its sensitivity. In every experiment with another source of radiation, this back-

¹³ Burgess P. L. batteries.

¹⁴ Manufactured by the S. S. White Dental Co., New York.

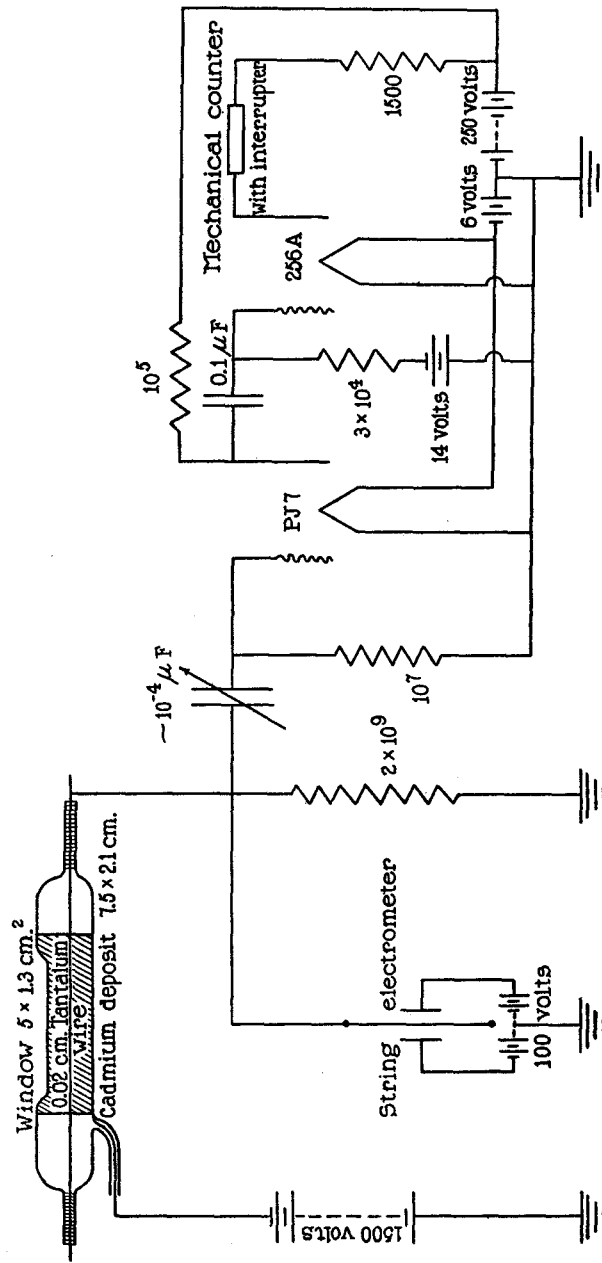


FIG. 1

ground radiation has to be taken into account and its relative intensity can only be found from the difference between the number of counts produced by the source plus the background radiation and the number of counts of the background radiation within the same interval of time. As the time during which a biological object acting as a radiator remains alive is relatively short, it can readily be seen that a strong background radiation can mask the effect of a weak additional radiation. On account of its random distribution it renders the number of counts produced by it in a given time subject to the statistical error, the magnitude of which is given by the square root of the total number of counts. Therefore, the trustworthiness of a measurement of the intensity of an additional weak radiation depends upon the magnitude of this additional effect. This will be discussed later. Consequently, it is necessary to cut down as much as possible the effect of the background radiation without, at the same time, impairing the sensitivity of the counter tube. For this reason the counter tube was enclosed in a lead box with walls of sufficient thickness to surround this tube on all sides with 10 cm. of lead. This lead was, of course, selected as to its freedom from radioactive substances. Although a lead shield 10 cm. thick cuts out only the softer components of the background radiation, nevertheless the shield effected a reduction of approximately 50 per cent in the number of counts from this source.

Experiments with biological material as a possible source of radiation were carried out for the most part as follows: First the effect of the background radiation was measured by counting the number of counts during a certain time, usually 30 minutes. Then the biological object was placed upon the window; usually a few drops of tap water or distilled water or potassium nitrate solution or an inorganic serum solution¹⁵ were added to prevent drying out as an air current had to be passed through the lead box to prevent the formation of a water film on the quartz of the counter tube which would have acted as a short circuit to ground. Several tests were made of the biological material used in these experiments as to viability before and after the 30 minutes of the experiments. In all the tests the tissue was viable after the experiments.¹⁶ Finally the test for the background radiation was repeated after removing the biological material.

The number of counts produced by the counter tubes was, on an average, approximately 20 per minute; *i.e.*, in half an hour about 600 counts were recorded. As already stated, this number is subject to a statistical error = $\sqrt{600} = \pm 24.5$ counts. The number of counts obtained from another source of radiation added to the number of counts of the background radiation is likewise subject to a statistical error. To obtain an estimate of the weakest possible intensity of a source of radiation that we still can measure without involving a statistical error large enough to invalidate the result, we shall arbitrarily assume that in the presence of a radiator, an increase in the number of counts which is twice the statistical error of the number of counts produced by the background radiation in the same time

¹⁵ Shear, M. J., and Fogg, L. C., *Pub. Health Rep., U. S. P. H. S.*, 1934, **49**, 229.

¹⁶ I am very much indebted to Dr. L. C. Fogg who carried out these tests.

is an indication of the presence of additional radiation and we shall call this the "minimum effect." Even then only a series of observations, all of which show an effect of the same order of magnitude, will furnish definite proof of the existence of an additional effect. 600 counts per 30 minutes were, on an average, observed as the effect of the background radiation; twice the statistical error is 49 counts. The minimum effect would be observed if the background radiation and the additional radiation together produce 649 counts per 30 minutes. The statistical error of the difference of 49 counts would be $\sqrt{600 + 649} = 35.6$ counts = ± 72 per cent.

These considerations show the importance of cutting down the background radiation and of extending the time of duration of an experiment as both factors will increase the sensitivity of the arrangement.

From the minimum effect of approximately 50 additional counts in 30 minutes; it is possible to calculate the theoretical number of light quanta which one should

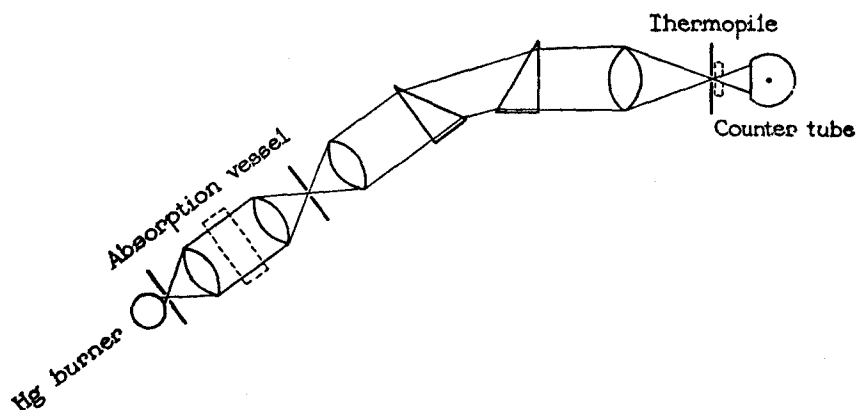


FIG. 2

be able to detect. 50 counts in 30 minutes correspond to 0.03 counts per second. The photoelectric efficiency being of the order of magnitude of 1:1000, 0.03 counts per second will be produced by 30 quanta per second that have passed through the window. The area of the window being approximately 6 cm.², a theoretical number of 5 light quanta per cm.² per second is obtained, which should be detected in a series of experiments. This is far below the theoretical minimum intensity of the mitogenetic radiation.

The experimental calibration of the counter tubes was carried out with an arrangement shown in Fig. 2. The monochromator used was manufactured by Bausch & Lomb; according to the manufacturer, the amount of stray radiation reaching the exit slit being of the order of magnitude of a few tenths of 1 per cent for a slit width of 0.05 mm. As sources of light a d.c. mercury arc lamp, an a.c. mercury arc lamp, and an a.c. cadmium arc lamp were used. The final measurements were carried out with the d.c. mercury arc lamp. A small slit of 2 × 8 mm. was placed directly in front of the arc lamp to obtain as source of light an area of

the same luminous intensity per unit area. An image of this slit was thrown upon the entrance slit of the monochromator by the two condenser lenses. A vessel with an absorbing material of known density could be placed between these two lenses to decrease the intensity. Directly behind the exit slit a vacuum Coblentz thermopile was placed, mounted in a carrier which could be moved up and down so that the thermopile or the counter tube could alternately be exposed. The diverging beam emerging from the exit slit was of almost uniform intensity and well defined cross-section. The counter tube was placed at such distance that the cross-section of the beam corresponded to the dimensions of the window of the tube.

The calibration of the counter tube in quanta per cm^2 per second was carried out in the following way. The intensity of the Hg line 2536 A was measured with the thermopile which was calibrated in absolute units against a standard lamp. Then the intensity of the line was decreased to 1.9×10^9 of its value by putting between the two condenser lenses or between exit slit and counter tube an absorption vessel containing a solution of $\text{K}_2\text{Cr}_2\text{O}_7$ of known concentration. The extinction coefficient of $\text{K}_2\text{Cr}_2\text{O}_7$ was carefully determined with the thermopile by using a series of more dilute solutions of known concentration. In addition, checks of the validity of Beer's law were made, although it could be assumed that Beer's law was valid for the concentration of 8 gm. in 10 liters of distilled water used to produce the reduction in intensity given above (1.9×10^9). The law was found to be valid within the experimental error. After removing the thermopile, this weak radiation fell upon the counter tube. As the intensity of this beam is known in quanta per cm^2 per second, the number of counts (difference of background counts and background plus radiation counts) now given by the counter tube in a certain time corresponds to the number of quanta passing through the window in this time. From this value the minimum effect could be calculated. For the Hg line 2536 A, 50 additional counts in 30 minutes are produced by an intensity of 10 to 15 quanta per cm^2 per second falling upon the window of the counter tube. As the steeply rising branch of the sensitivity-wave length curve for cadmium extends to still much shorter wave lengths up to the wave length at which the absorption in quartz begins to become considerable, it is obvious that the counter tubes will approach the calculated minimum effect in the region of the wave length of the mitogenetic radiation. For cadmium, the measured sensitivity for the wave length 2300 A is 1.7 times that for the wave length 2536 A.¹⁷ For these measurements as well as for the biological measurements to be reported later, the voltage on the counter was raised as high as possible, to obtain highest sensitivity; *i.e.*, near to the point at which erratic operation of the counter tube begins, due not to the incident radiation but to spontaneous discharges within the tube. However, at the applied voltage the counter tubes work normally for any length of time if certain precautions are taken.

¹⁷ International Critical Tables, New York, McGraw-Hill Book Co., Inc., 1929, 6, 68, Table 3.

The electrostatic field within the counter tubes, due to the arrangement of a thin wire in the axis of a conducting cylinder, is logarithmic, which means that almost the entire potential difference between cylindrical electrode and wire lies within a narrow region around the axial wire. In this region the ionic cloud is produced by the photoelectron released from the wall, and the consequently formed secondary electrons which charge the wire and produce the impulse which is recorded. The high resistance of 10^9 ohms prevents the immediate removal of this charge, resulting in diminishing the potential difference of the electrodes to a value for which the potential difference is insufficient to produce additional ions by impact. Thus the discharge stops; the wire discharges, which brings the potential difference between the electrodes once more to its original value. The counter tube is then ready for the next discharge. It is evident that the window will have an influence on this logarithmic field, especially as it consists of quartz, the insulating properties of which are very high. During the operation of a counter tube provided with a window, stray ions will go to the window and produce a change in the field around the wire, thus influencing the sensitivity (counting rate) of the tube until an equilibrium is reached. The greater the sensitivity of the counter tube, the more noticeable is this effect.

During the first series of experiments in which the counter tube was used in the study of mitogenetic radiation, somewhat erratic results were obtained which, nevertheless, seemed to point toward the existence of this radiation. In the endeavor to exclude any spurious effect, experiments were made with substances which could not possibly emit any radiation, *e.g.* water, and effects consisting in an increased counting rate were obtained. It was finally found that mere touching of the window produced an effect. After what has been said about the influence of the window upon the operation of the counter, it seemed obvious that all the effects just noted were produced by disturbing the field in the counter tube. When counting rates were taken from minute to minute, it was found that the effect gradually died off until the equilibrium was again reached. In the case of biological material this might be falsely interpreted as due to gradual loss in viability.

A series of experiments was undertaken to show the magnitude of this spurious effect.

Table II gives data on some of these experiments.

Especially interesting are the experiments with tubes of glass and quartz, respectively, closed at one end and partly filled with water. Since these tubes were in contact with only a small part of the window,

one would expect a comparatively small effect. Both tubes were cleaned before placing them on the window by rubbing them gently with cheese-cloth. Although both tubes were of the same size, the quartz tube produced a much larger effect while that of the glass tube is somewhat larger than the statistical error. Upon wiping off the tubes with moist tissue paper, in the case of the quartz tube the effect was decreased, while in the case of the glass tube it disappeared. This shows that the larger effect with the quartz tube was due to charges on the quartz. Due to the high insulating power of quartz, an electric charge is easily obtained and can be removed only with difficulty. This is true for glass also, but the charge is much smaller in this case, as the insulating properties of glass are inferior to those of

TABLE II

Counting rate per min. for uncovered window	Material put on window	Counting rate per min. for covered window
15.6 ±0.9	Piece of lead foil, not grounded	20.9 ±1.4
25.6 ±1.6	Piece of lead foil, grounded	47.8 ±2.2
21.5 ±1.0	Water, not grounded	28.2 ±1.7
24.7 ±1.0	Quartz tube slightly rubbed	38.5 ±2.0
23.0 ±1.1	Glass tube slightly rubbed	29.0 ±1.7
23.5 ±1.5	Glass tube wiped off with moist paper	26.6 ±1.6
22.9 ±1.5	Quartz tube wiped off with moist paper	32.6 ±1.8

quartz. It can be shown by means of an electroscope that quartz, once well rubbed, retains its charge for several hours when kept in a dry room, while that of glass will disappear in a few minutes. Since, in experiments for demonstrating the presence of mitogenetic radiation by putting the biological material in both glass and quartz tubes in order to show its ultraviolet nature, tubes will be rubbed clean to avoid possible absorption, the effect shown above gives a possible explanation of the positive results that have been reported in such experiments.

The experiments with metal foil or water on the window may explain why it is that some of the investigators who employed photoelectric methods for the demonstration of mitogenetic radiation found this radiation to be present while others did not. In Schreiber and

Friedrich's, as well as in Locher's experiments, the biological material was not placed directly on the window; there was an air space between. Rajewsky, on the other hand, placed his material on the window. Frank and Rodionow, so far as can be learned from their brief publication, apparently tetanized a frog's muscle by means of an induction coil in front of their window, thus creating violent electric disturbances which must have influenced the static field of their cell.

The data of an experiment with onion roots may be given to show how large this spurious effect was in some cases:

September 4, 1931. Onion Root Experiment

Counter voltage: 1600 volts.

1. Background radiation	903 counts in 40'	22.6/min. ± 0.7
2. Onion roots on window	2187 counts in 50'	43.7/min. ± 0.9
3. Chloral hydrate (1 per cent) dropped on onion roots	2962 counts in 50'	59.2/min. ± 1.1
4. Background radiation	904 counts in 40'	22.6/min. ± 0.7

These effects disappeared after proper shielding of the window was effected.

The shielding consisted in surrounding the tube with a grounded metallic wall, with an opening for the window. In order to keep the window at the same potential during the measurement of the background radiation as well as during measurements with biological material, the window was covered with any one of the several liquids previously mentioned, which were used in some cases to keep the biological material moist, and a wire connected the liquid to ground. Inasmuch as tap water or the other liquids are conducting to some degree the surface of the window was kept continuously at the same; *i.e.*, ground potential. For the experiments with biological material the water was removed and enough material put on the window to cover it completely.

Another means of preventing spurious effects would be the placing of the biological material at some distance from the window, say 1 to 2 cm. But this would necessarily result in loss of intensity which, if possible, has to be avoided.

With this arrangement numerous tests for the presence of mitogenetic radiation were made.

The biological material tested consisted mainly of onion-base pulp and tips of onion roots. Mouse sarcoma 180, mouse embryo tissue, and tetanized frog muscle, all alleged to be excellent radiators, were likewise tested. The results of some of the experiments are given in Table III.

TABLE III
Results of Some of the Tests

Biological material	Time <i>min.</i>	No. of counts		
		Control	With biological object	Control
Onion-base pulp.....	30	529 ±23.0	523 ±23.0	534 ±23.0
“ “ “.....	30	562 ±23.6	562 ±23.6	516 ±22.7
“ “ “.....	30	552 ±23.5	534 ±23.0	497 ±22.3
Mouse embryo.....	30	543 ±23.3	552 ±23.5	515 ±22.7
“ “.....	30	646 ±25.4	611 ±24.7	617 ±24.8
Onion-base pulp.....	30	535 ±23.1	521 ±22.8	527 ±22.9
Mouse embryo.....	30	507 ±22.5	517 ±22.7	543 ±23.3
Onion root.....	40	643 ±25.3	643 ±25.3	641 ±25.3
“ “.....	40	981 ±31.3	977 ±31.2	898 ±29.9
“ “.....	40	1112 ±33.3	1092 ±33.0	1159 ±34.0
Frog muscle (tetanized).....	30	656 ±25.6	673 ±25.9	669 ±25.8
Mouse sarcoma.....	40	504 ±22.4	521 ±22.8	624 ±24.9
“ “.....	40	597 ±24.4	582 ±24.1	625 ±25.0

DISCUSSION

The data given show that no mitogenetic radiation could be detected. If mitogenetic radiation exists at all, its intensity must be smaller than the minimum effect as established for the counter tube; *i.e.*, its intensity must be smaller than 10 to 15 quanta per cm.² per second. The estimate of intensity, as given at the beginning, gives as the smallest—though highly improbable—value approximately 100 quanta per cm.² per second. The counter tube would have detected such an intensity. Therefore we must conclude that there is no physical proof for the existence of mitogenetic radiation.

Consideration of the energy content of the chemical reactions which, according to Gurwitsch,¹⁸ are responsible for the emission of the mito-

¹⁸ Gurwitsch, A., *Die mitogenetische Strahlung*, Berlin, Julius Springer, 1932, 47-68.

genetic radiation and which may be reactions of either oxidation or proteolytic or glycolytic character shows that their heat of reaction is insufficient for the production of ultraviolet radiation of a wave length between 1800 and 2500 A. The energy necessary to produce quanta of a wave length of 2000 A is 142.2 kg. cal. So far as known there is no biological reaction which will produce in a single step a heat of reaction greater than approximately 70 kg. cal. It must either be assumed that there exist unknown reactions of the above type which produce sufficient energy, but in this case the atoms or radicals would have to take part in the reaction, or that in the case of reactions with insufficient energy, rare single processes may occur which result in the production of an ultraviolet quantum corresponding to the wave length of mitogenetic radiation. There is no physical or physico-chemical evidence that either of these cases is possible.

SUMMARY

The intensity of mitogenetic radiation was estimated from data given by Gurwitsch.

The sensitivity of the biological method and of the physical methods were compared.

With onion-base pulp and onion roots as mitogenetic inductors, the photographic method gave no perceptible blackening for exposures up to 184 hours.

A photoelectric counter tube was described with cadmium as photoelectric metal. Its sensitivity was such that a radiation intensity of 10 to 15 quanta per cm.² per second of the Hg line 2536 A was detectable.

Spurious effects produced by the counter tube were described and means for their avoidance given.

A number of different biological materials, all supposed to be excellent mitogenetic radiators, were investigated by means of the counter tube. No mitogenetic radiation could be detected.

Addendum.—After the manuscript was written, a paper was published by Gray, J., and Ouellet, C., *Proc. Roy. Soc. London, Series B*, 1933, 114, 1, describing experiments on mitogenetic radiation with a photoelectric Geiger counter tube of a sensitivity of 50 quanta per cm.² per second at 2500 A which

gave no indication of a radiation from fertilized eggs of sea urchins, cultures of active spermatozoa, or of growing yeast. Spurious effects due to condensation of water vapor or other volatile substances on the counter tube were observed and means for their prevention given.