THE KILLING OF CERTAIN BACTERIA BY X-RAYS

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Plate 17

(Received for publication, May 24, 1930)

The experiments to be described are an extension to soft X-rays of those[1] previously made with cathode rays. They consist essentially of studies of the time rate of killing of single bacteria by the general radiation from a tungsten tube operated at low voltage and by the characteristic K-radiation of copper.

EXPERIMENTAL

The organisms *B. coli* and *B. aertryke* were chosen for these observations because they readily give the distribution of single cells that is needed if statistical counts are to have meaning. The bacteriological procedures were identical with those already described. As before, standard cultures were provided by Dr. L. T. Webster of this Institute. Spreads of single bacteria upon the surface of agar (ca 200 organisms per in.^{2}) were irradiated before multiplication could take place. After incubation, counts were made of the number of colonies growing out upon a stamped irradiated area and upon an equal and adjacent control area marked at the time of irradiation. From these data survival ratios have been calculated which, together with measurements of the X-ray intensity striking the agar plate, can be made to give information concerning details of the killing action of the rays. In order that the survival ratios obtained in this way should refer to enough organisms to be statistically significant, results from many plates have been averaged.

In the first group of experiments the X-rays used were the unfiltered general radiation from a Siemens Bucky tube having a tungsten target and a Lindemann glass window. The voltage across this tube was ca 12 KV peak and was provided by the unrectified output of a suitable transformer. A steady current of 8 MA was obtained by heating the

[1] Wyckoff, R. W. G., and Rivers, T. M., *J. Exp. Med.*, 1930, **51**, 921.

cathode filament with an insulated storage battery. The bacteria were distant approximately 8 cm. from the tungsten target. Because of the long exposures required to give a sufficient killing action, only about 25 plates were irradiated in a day. Since the ratios of Table I and Text-fig. 1 are each averages of the counts upon 20 to 60 plates, several days were devoted to each experiment. These results obviously fall upon straight lines when plotted on semilogarithmic paper.

TABLE I

Survival Ratios with Bucky Tube

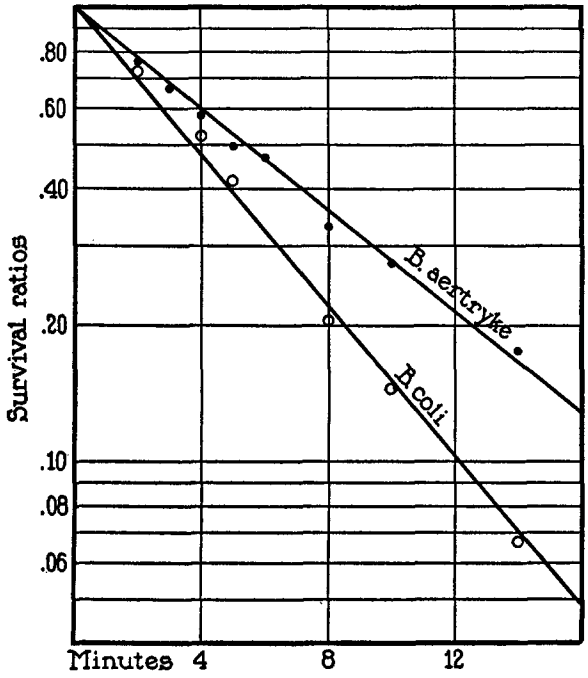
Time	Survival ratios	
	B. coli	*B. aertryke*
2 min.	0.750	0.768
3 "	—	.661
4 "	.526	.578
5 "	.416	.490
6 "	—	.466
8 "	.201	.329
10 "	.146	.274
12 "	.087	—
14 "	.064	.175

The survival ratios must therefore be expressed by an equation of the type

$$\frac{A_1}{A_0} = e^{-at}$$

Estimates of the intensity of the X-ray beam were made by measuring with a suitable chamber the ionization of air at the position of the irradiated bacteria. Subsequent experience has indicated that saturation currents were not obtained in these first experiments. No new determinations have been carried out because the general radiation of long wave length which constitutes the major output of a Bucky tube is poorly adapted to calculations of the mechanism by which cells are killed. It should be noted that the experiments of this group with *B. coli* and with *B. aertryke* were made under sufficiently different conditions so that no significance attaches to the relative killing rates found.

No commercially available electron-type X-ray tube supplies a monochromatic beam intense enough for experiments on the killing rates of bacteria. The self-rectifying gas tube developed in this laboratory and described[2] elsewhere is, however, suitable for this purpose and, equipped with a copper target, has been employed in a second series of experiments with *B. coli* and *B. aertryke.*



TEXT-FIG. 1. A plot of the survival ratios resulting from the use of soft general radiation from a tungsten target X-ray tube (Table I).

In some instances the radiation from this tube has been filtered only by the 0.001 inch aluminum forming its window; in others a thickness of 0.0009 inch metallic nickel foil was interposed between the bacteria and the target. This filter cuts down the general radiation and eliminates 99 per cent of the K-β line at the same time passing half of the K-α doublet. The tube was operated at about 4 MA and 34 KV peak (unrectified wave). During a single series of experiments the variations in the current through the tube were less than 0.1 MA and its output of X-rays was found to be steady to within 1 per cent.

[2] Wyckoff, R. W. G., and Lagsdin, J. B., *Radiology*, July, 1930, **15**, 42.

Each series required the exposure of about 100 plates, the results of which were averaged to give the survival ratios of Tables II and III. As Text-figs. 2 and 3 indicate, these ratios fall upon straight lines when plotted on semilogarithmic paper. Such a result agrees with the

TABLE II

Survival Ratios with Unfiltered Copper Radiation

Time	Survival ratios			
	B. coli (1)	*B. coli* (2)	*B. coli* (3)	*B. aertryke*
5 sec.	—	0.749	—	—
10 "	—	.617	0.645	0.793
20 "	0.358	.329	.548	.583
30 "	.233	.206	.422	.387
40 "	.167	.154	.344	.349
50 "	.112	—	.265	.270
60 "	.100	.089	.193	.234
Ionization current/sec./cm.[3]	—	—	144.2 e.s.u.	—

TABLE III

Survival Ratios with Filtered Copper Radiation

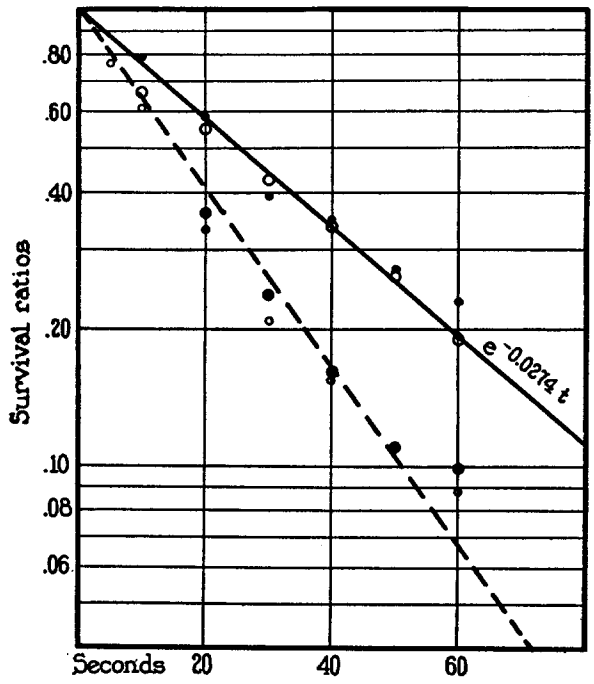
Time	Survival ratios		
	B. coli (1)	*B. coli* (2)	*B. aertryke*
20 sec.	0.667	0.711	0.804
40 "	.367	.574	.523
60 "	.345	.362	.391
90 "	.182	.291	.211
120 "	.109	.212	.189
Ionization current/sec./cm.[3]	—	67.2 e.s.u.	67.2 e.s.u.

experiments of Holweck[3] and Lacassagne[3] on *B. pyocyaneus* with silver L-radiation.

The experimental arrangement is shown in Fig. 1. During use the stamping device for the standard area A and the irradiated area B replaced the diaphragm

[3] Holweck, F., *Compt. rend.*, 1929, **188**, 197; Lacassagne, A., *Compt. rend.*, 1929, **188**, 200.

C under the X-ray tube D. When set up, the tube D was carefully centered with the aid of a fluorescent screen to insure that the X-ray beam covering B should be uniform. A Petri dish carrying the desired number of bacteria spread upon its agar surface was placed on the stand E and raised by the remote control rod F until the knife edges of A and B cut through the agar. On opening the metal shutter G, the surface marked by B could then be given an exposure of desired length. After lowering E, it and the dish were removed with the help of F. This remote control was needed because the X-ray tube itself operated continuously

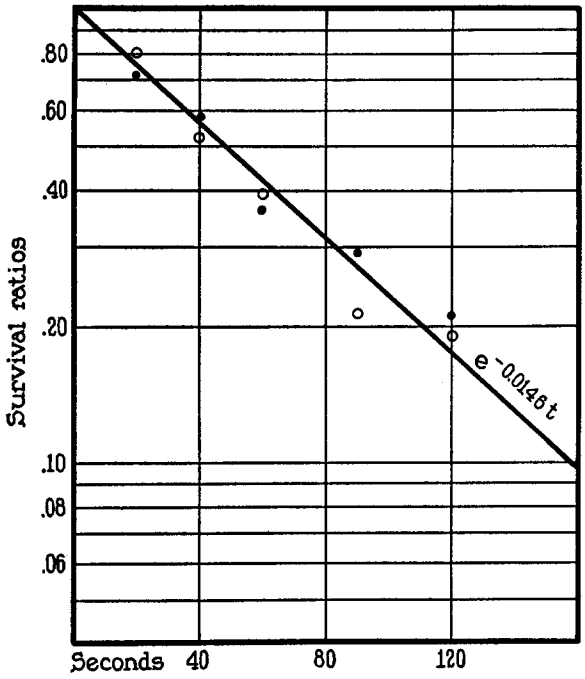


TEXT-FIG. 2. A plot of the data obtained by irradiating *B. coli* and *B. aertryke* with filtered copper rays. The small open, large black, large open and small black circles refer to experiments (1), (2), (3) and (4) of Table II.

throughout the entire experiment. When in use, the tube was covered by a lead housing such as that shown at H and during exposures the door K was kept closed. These precautions are essential to the safety of the experimenter.

The intensity of the X-rays striking the bacteria spread on the agar surface was obtained from measurements of the amount of ionization in air produced by these rays. Because of their absorbability, it was

considered inaccurate to employ for this purpose a small enclosed type ionization chamber. Accordingly, an open air chamber of standard design but of small size was made. Its over-all length was 7.0 cm. The actual length of the amber-insulated collecting electrode was 1.31 cm., its effective length 1.39 cm. The grounded electrodes on either side were each 2.54 cm. long. All three electrodes were 3.8 cm. wide. The charged shield surrounding and facing these plates was 3.2 cm.



TEXT-FIG. 3. A plot of data obtained by irradiating *B. coli* and *B. aertryke* with the same dose of unfiltered copper rays. The small black and large open circles refer to experiments (2) and (3) of Table III.

distant. On account of the extremely great ionizing power of the X-rays used, saturation in a chamber of this type can be obtained only if beams of small cross-section are measured. The currents produced in this chamber were measured by the usual balance methods[4] employing

[4] See for instance, Makower, W., and Geiger, H., Practical Measurements in Radio-activity, London, 1912, 15.

a variable voltage and a standard condenser of known capacity. This capacity was obtained by comparison against a small standard condenser and checked by calculation from its accurately determined dimensions.

The intensity of the X-rays striking a small area of the agar surface was found by replacing the stamping device AB of Fig. 1 with a small lead stop, C, having an opening 0.117 cm. in diameter. The air chamber was brought as near to the stop as possible (ca 3 mm.) and the ionization produced by the X-ray beam passing through it was ascertained. Knowing the length of the measured column of ionized air and its absorption coefficient under the conditions of temperature and pressure of the experiment and for copper rays, it is obviously possible to calculate the approximate ionizing power of the beam at the irradiated surface. A typical calculation will make clear the procedure followed and the approximations introduced.

Area of stop in position of bacterium = 0.0107 cm.^{2}
Height of air column the ionization of which is measured = 1.39 cm.
Corrected density of air = 0.001165
Temperature = 26°C., Barometer = 747 mm. Hg.
Absorption coefficient μ/ρ of air for copper radiation = 8.43
Balancing voltage on potentiometer = 18.8 V in 20 sec.
1 e.s.u. potential = 0.33×10^{-2} volts
Measured capacity of standard condenser = 321.4 cm.

Therefore the observed ionization current is

$$i' = \frac{18.8 \times 0.33 \times 10^{-2} \times 321.4}{20 \text{ sec.}} = 0.997 \text{ e.s.u.}$$

Since $I/I_0 = e^{-\mu l}$ where l is the length in centimeters of the absorbing column and $\mu = 8.43 \times 0.001165$, a column of air 1 cm. long will absorb 0.721 as much as a similar column of length 1.39 cm. Hence the ionization produced per second in 1 cm. depth of air by the rays striking 1 cm.^{2} of irradiated bacterial surface is taken as

$$i = \frac{i' \times 0.721}{0.0107} = 67.2 \text{ e.s.u. per cm.}^3 \text{ per sec.}$$

The intensities of the X-rays used in each of the three final standardized experiments when expressed in the foregoing units are recorded in Tables II and III.

Analysis

Existing knowledge of the properties of X-rays and of the mechanism of their absorption in inorganic matter makes it seem inevitable

that their absorption in bacteria is a quantized process. Thus the X-rays incident upon a cell will either pass through without altering it or else they will give up one or more quanta whose energy content is connected with the wave length λ of the rays through the familiar relation

$$E = h\nu = h\frac{\lambda}{c}$$

where h is Planck's constant, ν is the frequency of the rays and c is the velocity of light. It is known that a high velocity electron is liberated as a result of such an absorption. This electron gives rise to a chain of ions in the matter through which it passes and to X-rays which, in their turn, liberate more ions of less and less energy. The volume within which this cluster of ions resulting from a single quantum absorption is freed increases rapidly with the magnitude of the original quantum but, in matter having the density of a bacterium, it is at greatest only a very small fraction of a cubic millimeter. The changes X-rays produce in protoplasm are naturally identified with the physico-chemical changes induced by this ionic shower.

The physical consequences of the absorption of an X-ray quantum so closely resemble those attending the direct absorption of a high velocity electron that it seems permissible to treat the killing action of X-rays by the same statistical analysis which has previously been used in studies of the killing of bacteria by cathode rays.

On this basis, the straight line obtained by plotting survival ratios upon semilogarithmic paper means that, on the average, the death of a bacterium is the result of the absorption of a single X-ray quantum. As the earlier analysis[5] has shown, survival ratios under these conditions can be calculated from the expression

$$\frac{A_1}{A_0} = e^{-\alpha t}$$

where t is the time and α is the expected, or average, number of quantum absorptions in unit time.

A more intimate picture of the killing process is to be had from a study of this expected number of hits. Under conditions of satura-

[5] Wyckoff, R. W. G., and Rivers, T. M., *op. cit.*

tion, the air ionization chamber measures the total number of ions produced by the quanta absorbed in a known volume of air. If the number of pairs of ions liberated by a single quantum can be judged, then the number of quanta absorbed in this volume of air is known. Furthermore, if the ratio of the absorption coefficients of air and of a bacterium can be estimated, the *average* number of quanta absorbed by a single bacterium in unit time is readily computed from the size and shape of the organism. This has been done for experiments with filtered and unfiltered copper rays.

The following typical calculations based upon the standardized experiment of Table III using *B. aertryke* will show how the average number of hits has been obtained and will make clear the approximations that have been introduced in the process.

$$67.2 \text{ e.s.u.} \doteq 67.2 \times 0.21 \times 10^{10} \text{ ion pairs/sec.} = 1.411 \times 10^{11} \text{ ion pairs/sec./cm.}^3$$

A small amount of radiation is absorbed in the air between the diaphragm C and the collecting electrode of the air chamber. This absorption is readily calculated from the dimensions of the apparatus and the absorption coefficient ($\mu/\rho = 8.43$) of copper radiation. With the resulting correction it is found that 1.46×10^{11} ion pairs will be produced per second per cm.^{3} at the position of the irradiated bacteria. In the absence of direct experimental knowledge of the absorption coefficient of protoplasm, the mass coefficients of air and of living cells are customarily assumed equal. If this assumption, which cannot depart far from the truth, is made, the amount of absorption in 1 cm. thickness of air is about 27.9 times that of one bacterium. Both the *B. coli* and the *B. aertryke* have been taken as rods 0.5μ in diameter and 2μ long. Computation shows that to a first approximation one bacillus may be considered equivalent in absorption to a rectangular block of protoplasm 1×10^{-8} cm. in area, 0.42μ thick and of unit density. From these quantities the average number of ion pairs absorbed per bacterium per second becomes

$$\frac{1.46 \times 10^{11} \times 1 \times 10^{-8}}{27.9} = 52.3 \text{ ion pairs/sec./bacterium.}$$

In order to obtain the number of X-ray quanta absorbed per second it is necessary to know how many ions are liberated when one quantum is absorbed in air. The best available measurements[6] indicate that for X-rays of the quality used in these experiments about 35 volts are required to produce each electron pair. The voltage equivalent of the K-α lines of copper as computed from the familiar quantum relation

[6] Kulenkampff, H., *Ann. d. Physik*, 1926, **79**, 97.

Voltage (in KV) = 12.34/λ(in A) = 12.34/1.537

is 8.029 KV. From this the number of ion pairs arising through the absorption of one quantum of Cu K-α radiation is

$$8029/35 = 229.$$

The average number, α, of quantum absorptions per second then is

$$52.3/229 = 0.228.$$

The observed survival ratios in Table III lead to a straight line having the equation $A_1/A_0 = e^{-0.0146t}$ (Text-fig. 2). It will be noted that this experimental $\alpha' = 0.0146$ is much smaller than the α calculated from ionization measurements.

DISCUSSION

Accepting the foregoing type of analysis as an essentially correct, if rough, description of what happens when X-rays strike bacteria, the smallness of the ratio α'/α when taken in connection with the linearly exponential character of the experimental results shows that though on the average the absorption of one quantum of these radiations is sufficient to kill a bacterium of either *B. coli* or *B. aertryke*, relatively few of the absorbed quanta are lethal. In the experiment just calculated 0.0146/0.228 or about one in 15.6 kills. In the standardized experiment of Table II using *B. coli* one in 17.8 is deadly ($\alpha = 0.489$, $\alpha' = 0.0274$). This agreement is satisfactory since it is scarcely to be expected that the results with filtered and unfiltered rays should be identical.

The fact that so many quanta can be absorbed by a bacterium without causing death apparently means that the vital elements within the cell which can be destroyed by a direct quantum hit are much smaller than the cell itself. If the volume through which the quantum acts were negligible compared to that of the vital element of the cell and if there were only one such element in a bacterium, then the "sensitive volume" that would have to be hit in order to bring about death would be α'/α. In the two standardized experiments with filtered rays this ratio is 0.064; in the series using unfiltered radiation it is 0.056. The spheres of action of these quanta cannot, however, be disregarded and the quantities α'/α are to an important degree measures of these regions of quantum action. Furthermore, if, as seems natural, the

vital parts of the cell are identified with its chromatin material, more than one "sensitive volume" exists within it. It can nevertheless be concluded that the volume of the "vital elements" contained within one of the bacteria studied scarcely exceeds 0.05 of its entire volume. This estimate seems to be in serious conflict with the earlier conclusions of Holweck and Lacassagne with *B. pyocyaneus*. The published data are, however, too few to allow of a satisfactory comparison.

Within the limits of experimental error there is no difference between the sensitiveness of *B. aertryke* and *B. coli* to copper K-radiation (Text-fig. 2). A similar result was found when studying the killing action of cathode rays upon them.

Additional experiments are being carried out to ascertain in what way differences in X-ray wave length affect the killing of these organisms.

Valuable help in the carrying out of these experiments has been given by Charles G. Porskieves.

CONCLUSIONS

Both copper K X-rays and the soft general radiation from a tungsten tube operated at 12 KV kill *B. coli* and *B. aertryke* in a linearly exponential fashion. Within the experimental limits, the two organisms appear to be equally sensitive to these radiations.

By making use of the fact that X-ray energy is absorbed in quanta, an approximate picture can be formed of the mechanism of this destructive action. If the average numbers of quanta (α) absorbed per bacterium per second are calculated from measurements of air ionization using the quantities outlined in the text, survival ratios for these bacilli can be approximately represented by the equations

$$\frac{A_1}{A_0} = e^{-0.064\,\alpha\,t}$$

for filtered copper rays and

$$\frac{A_1}{A_0} = e^{-0.056\,\alpha\,t}$$

for unfiltered copper rays (peak voltage = 34 KV).

In terms of the foregoing interpretation this means that when death results, it is caused by the absorption of a single X-ray quantum of energy. Since only about one in twenty of the absorbed quanta kills, the sensitive cell constituents whose destruction leads to cell death must have a volume which is less than 0.06 of the bacterium itself.

EXPLANATION OF PLATE 17

FIG. 1. A photograph of the experimental arrangement for irradiating bacteria.

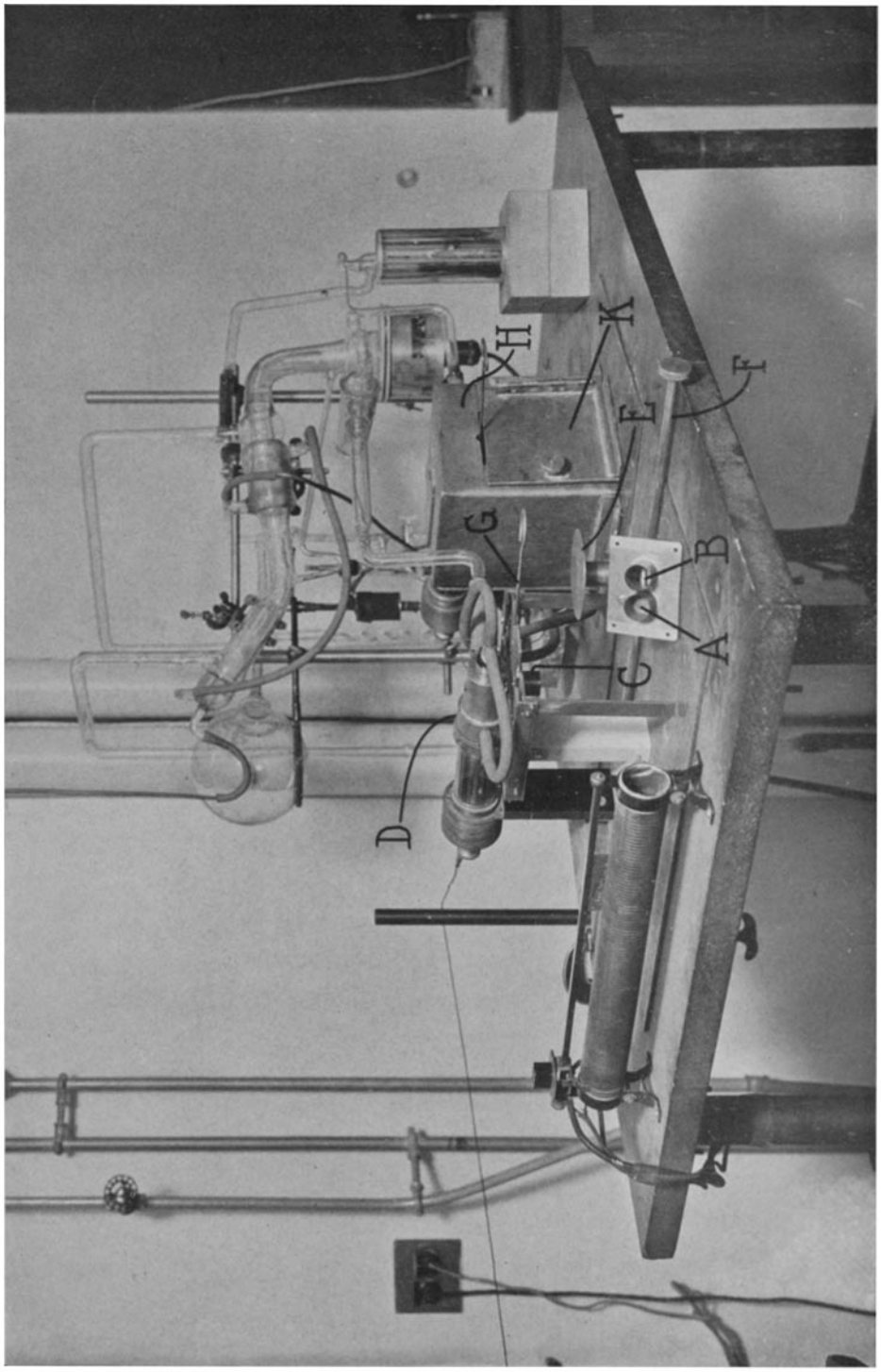


FIG. 1