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THE ACTION OF LOW VELOCITY ELECTRONS ON MICROORGANISMS

A dissertation submitted in partial fulfillment
of the requirements of the degree of

DOCTOR OF PHILOSOPHY

to the Graduate School of the
University of Cincinnati
1929
by

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Introduction and Purpose of the Work

It has long been known that certain forms of radiant energy, such as ultraviolet light and X-Rays, are destructive to various types of microorganisms. Recently it has been shown[1] that in the ultraviolet region of the spectrum there is a rather critical wave-length below which killing of microorganisms takes place and above which there is little or no killing. This critical wave-length is approximately 2900 Angstrom units. According to the quantum theory of radiant energy, light of any wave-length has associated with it definite quanta of energy given by the equation $\epsilon = h\nu$, where ϵ is the energy per quantum, h is Planck's constant of action and ν, the frequency of the radiation. Therefore, since it is probable that the microorganisms are killed by quanta of energy greater than a definite minimum, it was thought to be of importance to investigate the action of a homogeneous beam of low velocity electrons on microorganisms and thus attempt to determine whether the energy of moving electrons produces the same general result as that of ultraviolet light or X-rays.

So far as is known, no work of this nature has previously been done. Some rough experiments on the action of high velocity cathode rays in air on microorganisms have been tried by Coolidge and . But their results give

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1 Correlated Investigations in Basic Sciences- March 1928

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no indication of the action of electrons of known energy. Again the very high voltage necessary to obtain a beam in air was of such value as to give the electrons an energy far greater than that associated with a quantum of ultraviolet light.

Experimental Procedure and Apparatus

From the relation $\mathcal{E} = h\nu = \frac{hc}{\lambda} = Ee$, it is seen that the voltage thru which an electron must fall in order to acquire the same energy as that associated with a quantum of light of $\lambda = 3{,}000$ A.U. is approximately 4.12 volts and for shorter wave-lengths the voltage must be correspondingly greater. Thus in order to irradiate microorganisms with electrons having energies of the same order of magnitude as that of the quanta of ultraviolet light, the apparatus was constructed in such a way as to give a homogeneous beam of 4 volt electrons or higher. The apparatus first used for this purpose is shown in Figure I. Electrons are accelerated, with a voltage of 90 to 135, from the platinum filament, coated with oxide, F, to the first baffle in the brass cylinder A; after which they pass thru the second opening and are retarded by the baffles of the second brass cylinder B. They then finally arrive at the platinum slide S, covered with a smear of the organism to be irradiated, and have a net energy per unit charge approximately equal to EFS. A small percentage of the electrons pass thru a hole in the center of S and then enter

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the Faraday pail. By applying a variable retarding potential, E_R, to the Faraday pail, a complete energy distribution curve of the electron beam can be obtained. From this energy distribution curve, the size of the hole in S, and the time of exposure, the total energy that falls on a square centimeter of surface can be calculated. This quantity will be referred to as P. The above apparatus was mounted in a pyrex tube with connections leading out through hard wax joints. A heavy brass disc closed the end of the tube through which the slides S were inserted or removed. The apparatus was mounted with its axle parallel to the earth's magnetic field to prevent the slowly moving electrons being deflected out of their course. It was found that the wax joints held excellently and that a very homogeneous beam of electrons of energy per unit charge as low as two volts could be obtained.

The Vacuum System

Throughout this work the vacuum was maintained by a glass mercury condensation pump backed by a Cenco Hyvac rotary oil pump. Pressures were read with a McLeod gauge. Mercury vapor was prevented from entering the apparatus by a trap interposed between the apparatus and the pumps and gauge. This trap was kept continuously in a mush of solid CO_2 and acetone. At all times great care was exercised to see that mercury vapor did not enter the apparatus. All work was done at a vacuum of between 10^{-5} and 5×10^{-6} mm of mercury. Each time throughout this work the pyrex

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containing tube was heated for some time to about 350° C before a slide was placed in the apparatus. This was done in order to reduce occluded gas and moisture on the walls of the container.

The Organism Selected and the Technique of Handling the Organism

Staphylococcus albus was chosen as the organism with which to work. This organism is one of the most common forms of bacteria, and is frequently found in the skin especially at points of inflammations. The organism is a small sphere of about 0.7 μ to 0.9 μ in diameter and most generally occurs in grape-like clusters. It will grow either in the presence or absence of free oxygen. Reasons for selecting this organism are: (1) that it is very easy to culture, (2) it grows well in definite colonies either on or under plain agar, (3) it is relatively harmless, (4) it has relatively high heat resisting power, (5) it has been used extensively in the investigation of the action of ultraviolet light on microorganisms. The technique involved in handling the organism in this work was as follows. The staphylococcus was cultured in beef broth at room temperature for twenty four hours, after which a two percent solution of this culture in clear sterile beef broth was made. A light smear of the solution was placed on the platinum slide (S). This was allowed to dry, after which the slide was placed in the apparatus and the area

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(a circle of approximately 0.6 sq. cm. in area) in front of the opening C, Fig. I was bombarded for a definite length of time with electrons of known energy. The slide was then removed from the vacuum and carefully covered with a strip of moist solidified agar (plain) about one millimeter thick. The strips were prepared in the following way. A thin layer of liquid agar was poured on a sterile glass microscope slide and allowed to solidify. A thin knife blade was then inserted under one end of the solid agar and the strip peeled from the glass. This strip, hanging from the knife, was then laid over the platinum slide in just the reverse order that it was taken from the glass. It was necessary to cover the slide in this manner in order to prevent spreading of the organisms which certainly would have taken place had liquid been poured over the slide. The slide, thus covered, was incubated at 37° C for a period of twenty four hours or more. Growth or killing of the staphylococcus was shown by the presence or absence of colonies on the irradiated area after incubation. It is important to note that each slide was automatically its own control since only a portion of the slide was exposed to the electrons. However other control slides were run in the vacuum from time to time.

The Effect of a Very High Vacuum on Staphylococcus

It was first necessary to determine whether the organism would live in the extremely high vacuum required for this

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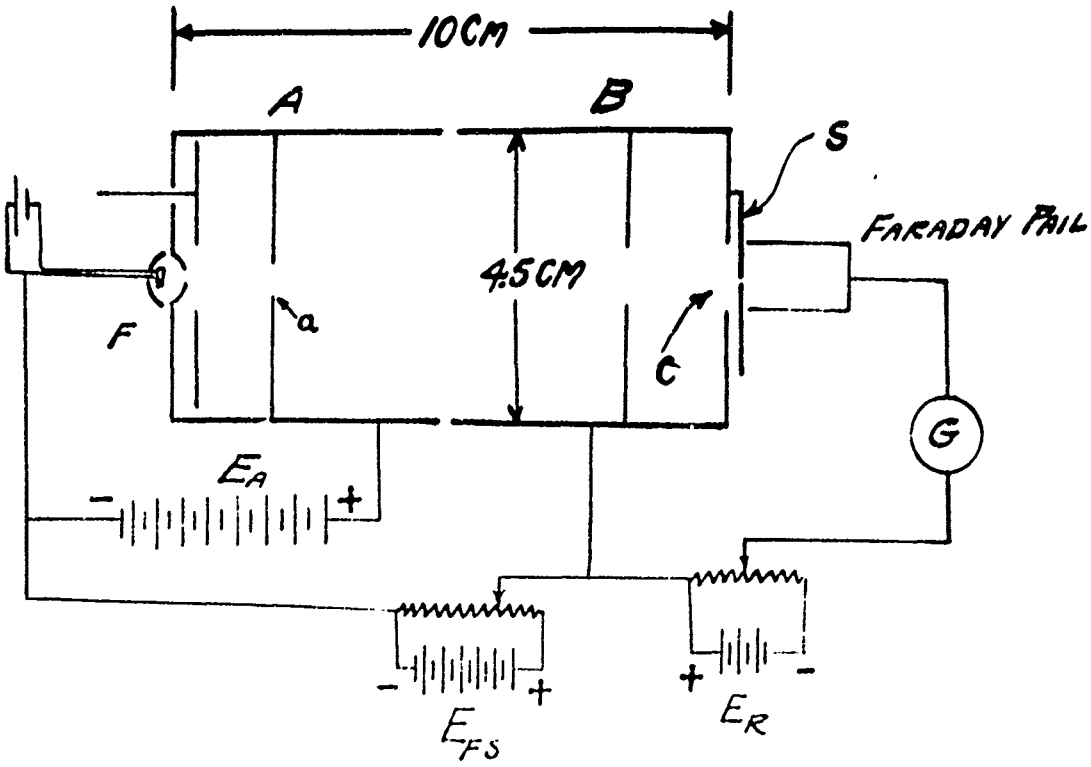
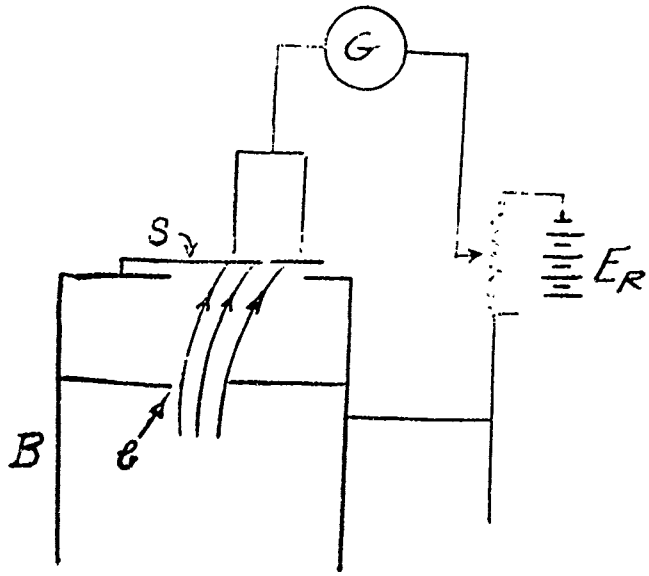


FIG. 1 FIRST ARRANGEMENT OF APPA.
(APPA. I)



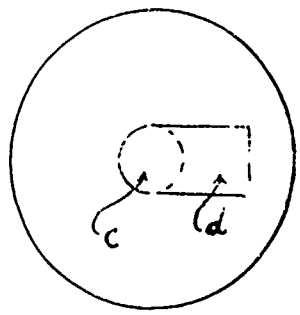


FIG. 2(a)

FIG. 2(b)

(APPA. I_a)

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work. So far as could be found no work had been done to determine the effect on microorganisms in vacuum pressures of the order of 10^{-5} or 10^{-6} mm of mercury. Staphylococcus on platinum slides was subjected to pressures of the order of 5×10^{-6} mm of mercury for as long as eight hours and after incubation, as described above, showed no signs of killing, the growth being the same as that on control slides.

Results with the First Apparatus

(APPARATUS I)

Upon irradiating the staphylococcus with electrons of various voltages from ten to as low as three, it was found that there was invariably killing even with the three volt electrons. (See photograph No. 1, P. 15) Slides were then tried with a retarding potential between A and B of such value that no electrons could strike the slide S and still there was complete killing over the area in front of the opening C. (See photograph No. 2, P. 15) This indicated that killing was probably due to a few positive rays coming from the space inside A, or to the extremely soft X-rays produced when electrons entering the space between A and B were caused to return to the baffle "A" by the retarding field. This is perhaps a new result. So far as is known no work has heretofore been done on the effect of positive rays, or extremely soft X-rays, on microorganisms. However, to find the action of electrons alone these two disturbing influences had to be removed. The apparatus was

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therefore modified as follows.

Modified Form of First Apparatus

The opening C (Fig. I) was elongated as shown in Fig. 2a and 2b. The small hole in S, and the Faraday pail, were shifted to the side so as not to be directly opposite the opening "B". By means of a weak magnetic field, perpendicular to the axis of the apparatus, the electron beam was deflected from its normal course and made to strike the area "D" Fig. 2b. A test run was made to find if either positive or soft X-rays were striking the area "D". A slide covered with staphylococcus showed killing over the area C, as would have been expected, (Fig. 2b) but none over the area "D". See photograph No. 3, P. 15. With this arrangement the galvanometer showned no positive reading, indicating that no positive rays were striking the area "D". It is thus seen that this arrangement excluded soft X-rays and positive rays from the region "D".

Results with Revised Apparatus (APPARATUS I a)

Eleven exposures were made with electron beams of the following voltages: 6, 9.5, 9.5, 15.5, 16, 22, 28, 28, 28.5, 29.5, 29.5. The results of these exposures are shown by photographs Nos. 1, 2, 3, 4, 5, P. 16, No. 3, P. 17, No. 6, P. 18, Nos. 1, 2, 4, 5, P. 19. (N.B. The voltages here given is the maximum voltage of any of the electrons, reaching the slide. That is to say, the voltages given represent the values at which the energy

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distribution curves reach the voltage axis. These values are given on each photograph, together with the total energy, ρ , in joules per square centimeter, near the small opening in the slide with which the organisms were irradiated. This energy density, ρ , was calculated from the total area under the energy distribution curve, the area of the hole in the slide, and the time of exposure. The areas under the distribution curves were obtained with a planimeter. It will be seen that slides irradiated with electrons from 6 to 22 volts show no appreciable signs of killing however the slides at 28 volts and higher show definite killing. It is also important to notice that the energy density at the lower voltages is in most cases as high, - in some cases much higher, - as the energy density at the higher voltages. These results therefore indicate (1) that staphylococcus albus is killed by electrons and (2) that the killing is a function of the voltage through which the electrons have fallen. After this work it was decided to redesign the apparatus in such a way as to make doubly sure of eliminating the spurious effects previously mentioned.

Second Type of Apparatus

(APPARATUS II)

Figure 3 shows the second type of apparatus together with a diagram of the connections used. The beam of low velocity electrons was obtained as before from the oxide

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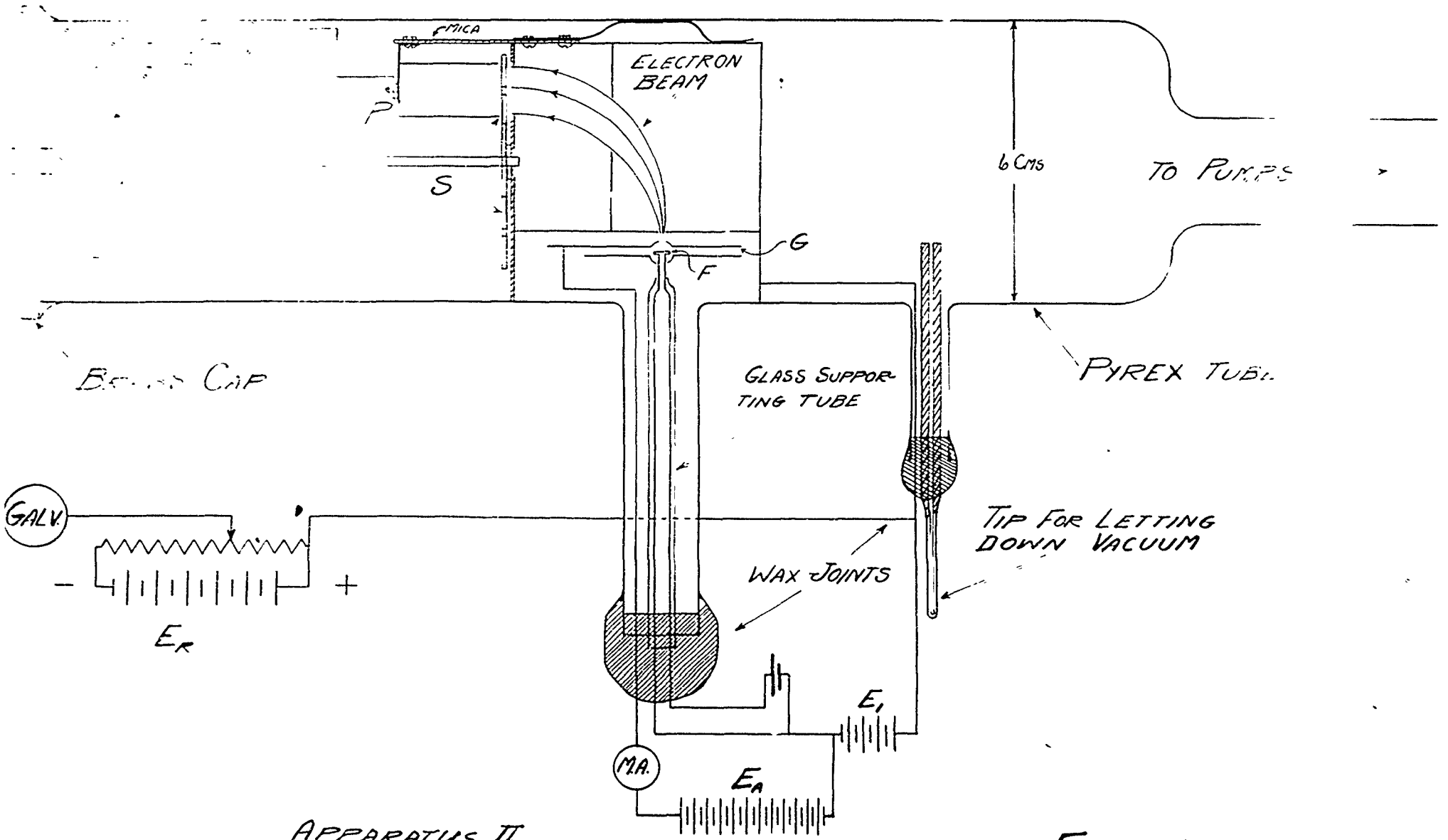

FIG. 3

coated filament F, and were accelerated to G by a potential E_a of about 90 volts. Those that pass through the opening in G are retarded in passing to the next baffle and enter C with an energy per unit charge equal to E. Thus by varying E, electrons of any desired voltage can be made to enter C. After entering C, the beam, deflected in a circular path by a magnetic field, passes through the circular openings O_1 and O_2, striking the platinum slide S. The magnetic field required to deflect the beam was produced by two large air core solenoids (See photograph of second apparatus). In order to pass the beam of electrons through O_1 and O_2 and on to the slide, the current in the solenoids was carefully adjusted while reading the galvanometer (E_R being zero). In this way, it was easy to adjust the magnetic field to its optimum value. This apparatus was also arranged so that as many as four exposures could be made, consecutively, without breaking the vacuum. This was accomplished by mounting four slides, equally spaced on the face of a disc, 5 cm in diameter. This disc was mounted on an axle and so arranged that it could be turned by the action of an electromagnet on a soft iron bar attached to the opposite end of the axle. The removable bearing supporting the end of the axle containing the iron bar is not shown in the drawing Figure 3. Each slide was pierced with a hole of area .028 sq. cm. The Faraday pail, mounted as in the first

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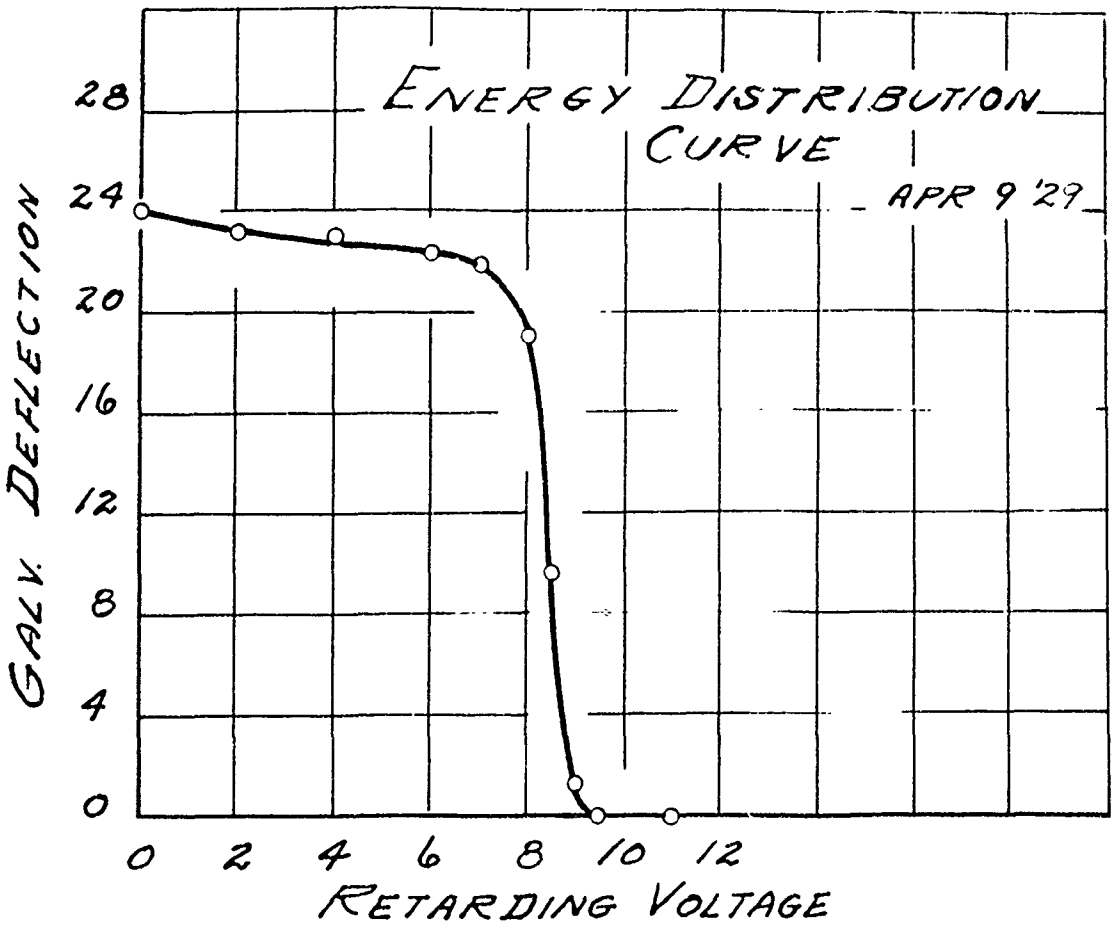

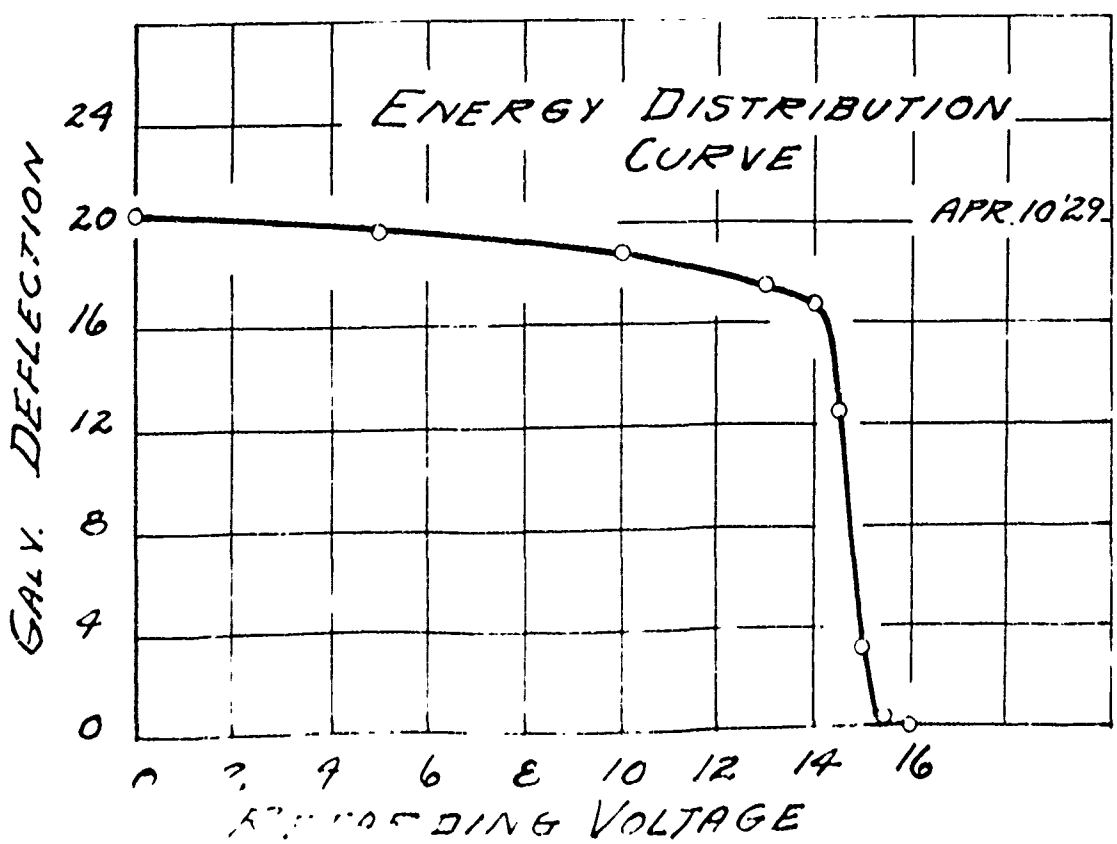

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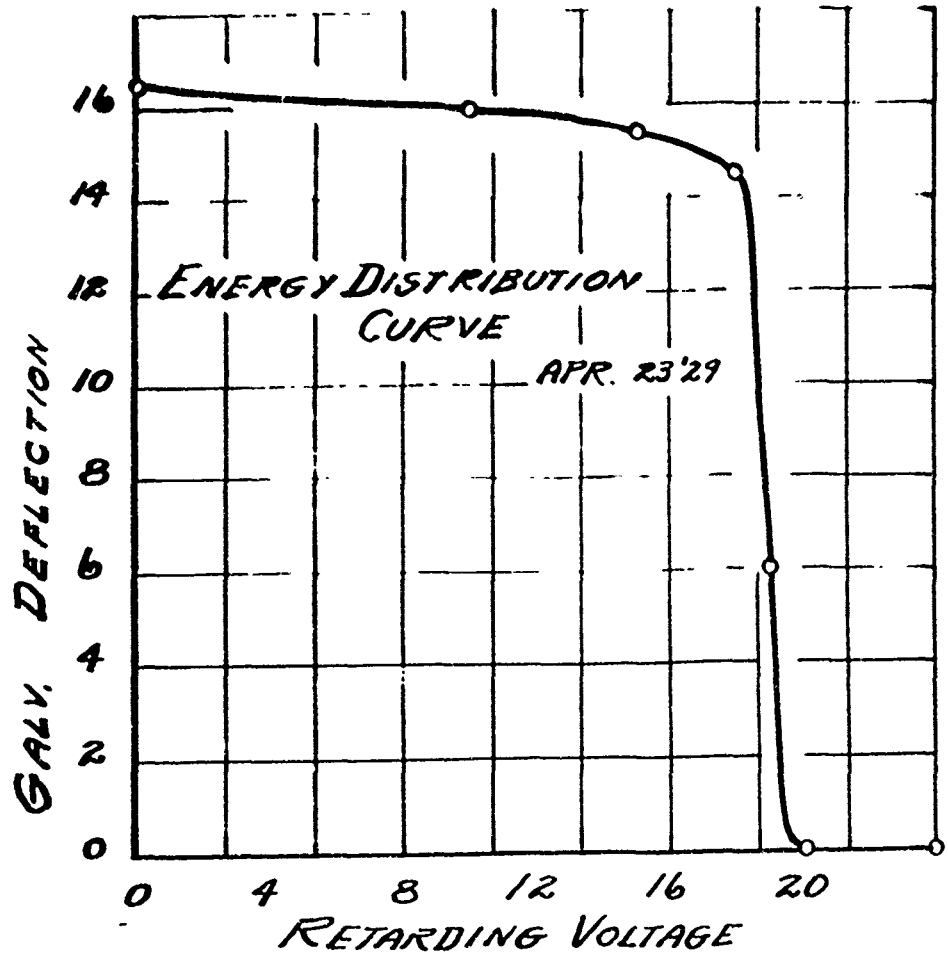

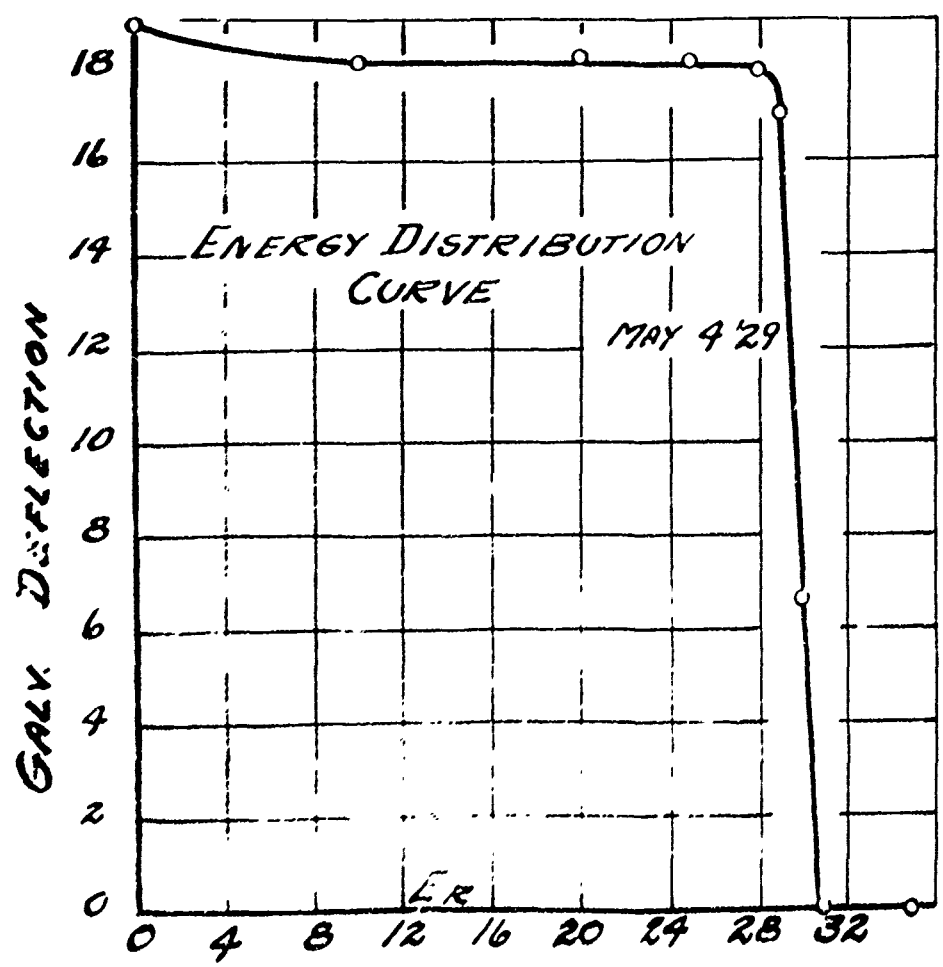

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apparatus, served to determine the energy distribution of the beam. Typical energy distribution curves for this apparatus, as well as for the previous arrangements, are shown in Figures 4 and 5. The diameter of the circular opening, O_2, is one centimeter. Therefore a spot of 1 cm. diameter is irradiated with electrons. This is larger than the area that could be irradiated with the first apparatus.

With this arrangement the action of soft X-rays produced in the vicinity of the filament should be completely eliminated, since the area to be bombarded is out of direct line with the filament and the baffles in front of the filament. Also the action of positive rays should be eliminated since any rays produced between G and the next baffles would move forward with a slight deflection opposite to that of the electrons. The result of a test run of three hours with 31 volt electrons entering C, but not deflected to S, is shown in photograph No. 5, P. 15. The slide shows no killing whatever.

Results with Second Type of Apparatus

(APPARATUS II)

Eighteen exposures under these conditions were made with electrons ranging in voltage from 19.5 to 31.5, and one exposure at 48 volts. Photographs of the slides after incubation are shown on PP. 16, 17, 18, 19, 20. For convenience in comparing corresponding voltages all slides are arranged in order of ascending voltage. Exposures made with the first modified form of apparatus are marked "APPA. I a"; those made

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with the final apparatus are marked "APPA. II". It will be seen that exposures made with electrons of 19.5, 20, 22 and 24 volts show no appreciable killing, while exposures at 25 volts and higher show definite killing. It is important to note that the exposures at the lower voltages were made with as great, and in some cases greater, total energy per square centimeter than at the higher voltages, yet they show no killing. These results confirm those obtained with the first apparatus (APPA I $_a$), namely, that this type of microorganism is killed by electrons, and that the killing is a function of the energy of the individual electron.

To show definitely whether the killing was a function of the total energy with which the organisms were irradiated, a series of three slides were exposed, at a constant voltage of 31.5, to total energies in the ratio of 1:2:4. A control slide was kept in the apparatus at the same time. These four slides are shown in photographs Nos. 3, 4, 5, 6, P. 20. The corresponding total energy per sq. cm. of each exposure is given below the photographs. It is seen that there is a definite increase in the percent killing with increase in total energy of exposure which indicates that the percent killing is a function of the total energy of bombardment.

As to whether the organism would ever be killed (under the conditions of this experiment) with electrons of less than 25 volts, by increasing the total energy of irradiation, is a question which can only be settled by more data. The

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answer depends largely on the mechanism by which the electron does the killing.

General Considerations and Conclusions

In considering the results of this work it is well to note that the destruction of microorganisms by electrons may be either a primary or secondary effect. The abiotic action may be due to the direct impact of the electrons or to the radiant energy emitted when the electrons strike. Likewise the destruction of microorganisms by ultraviolet light may be either a primary or secondary action; for it has not been shown whether the killing is due directly to the ultraviolet radiation or to the photoelectrons ejected by the radiation. Furthermore an answer to this problem would not tell how the killing takes place since that ultimately leads to the question of what is involved in life itself.

Although no attempt has been made to deduce quantitative relations, the results indicate that, under the conditions of the experiment, there is a rather rapid rise in the percentage of killing within the range of 25 to 30 volts with total energy constant. On the other hand, with ultraviolet light, there is a rapid rise in killing in the vicinity of from 3000 to 2600 A.U, equivalent to 4.12 to 4.75 volts. Again, according to Coblenz and Fulton (A Radiometric Investigation of the Germicidal Action of Ultraviolet Radiation, By, W. W. Coblenz and H. R. Fulton, U. S. Bureau of Standards, Scientific Papers No. 19, 1923) the minimum energy to kill a bac-

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terium of B. Coli with ultraviolet light in the region of 1700 to 2700 A. U. is about 2×10^{-4} ergs; and according to Ellis and Wells (The Chemical Action of Ultraviolet Rays by C. Ellis and A. A. Wells, The Chemical Catalogue Co. 1925) the time required to kill staphylococcus is somewhat less than that required to kill B. Coli at the same intensity. Thus the energy to kill a staphylococcus bacterium with ultraviolet light is probably somewhat less than 2×10^{-4} ergs. Now in order to get complete killing of staphylococcus with electrons, the energy density required in the region of 25 to 30 volts was about 13×10^{5} ergs per square millimeter and since the average exposed area of a staphylococcus bacterium is about 5×10^{-7} sq. mm. the average energy required to kill a single bacterium is approximately 0.65 ergs which is about 3000 times the energy necessary with ultraviolet radiation. It would thus seem that the action of electrons on staphylococcus is quite different from that of ultraviolet radiation. However, it must be remembered that even though the results appear to be so different, the conditions of the two experiments are also quite different. All the work on the action of ultraviolet has been done with the organisms in a rather moist state, (The organisms could probably be considered as being in a solution.) in the open air, whereas this work was done in a very high vacuum where the organisms are of necessity free from moisture. In order to make a direct comparison of the two actions it will be necessary therefore to carry out the experiments with ultraviolet under the same

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h'gh vacuum conditions.

Summary of Results

The results of this work indicate:

(1) That staphylococcus albus can be subjected to a vacuum of the order of 5×10^{-6} mm. of mercury for as long as eight hours without showing signs of killing.

(2) That staphylococcus albus is killed when exposed to a beam of low velocity electrons.

(3) That under the conditions of this experiment there is a rapid rise in killing in the vicinity of 25 to 30 volts, with constant total energy, and therefore that the lethal action is a function of the energy of individual electrons.

(4) That the percent killed at a definite constant electronic energy is a function of the total energy of exposure.

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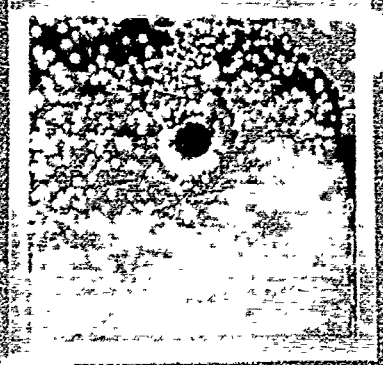
E = 3 VOLTS
DIRECT BEAM
APPARATUS I

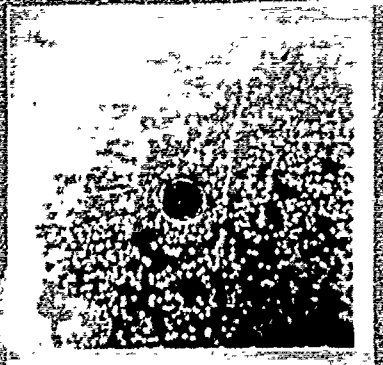
ZERO CURRENT
TO SLIDE
APPARATUS I

TEST RUN WITH
APPARATUS II



CONTROL SLIDE
KEPT IN VACUUM
DURING EXPOSURE
OF SLIDE No. I

TEST RUN WITH
APPARATUS II
E = 1 CURRENT



CONTROL KEPT IN
VACUUM DURING
EXPOSURE OF

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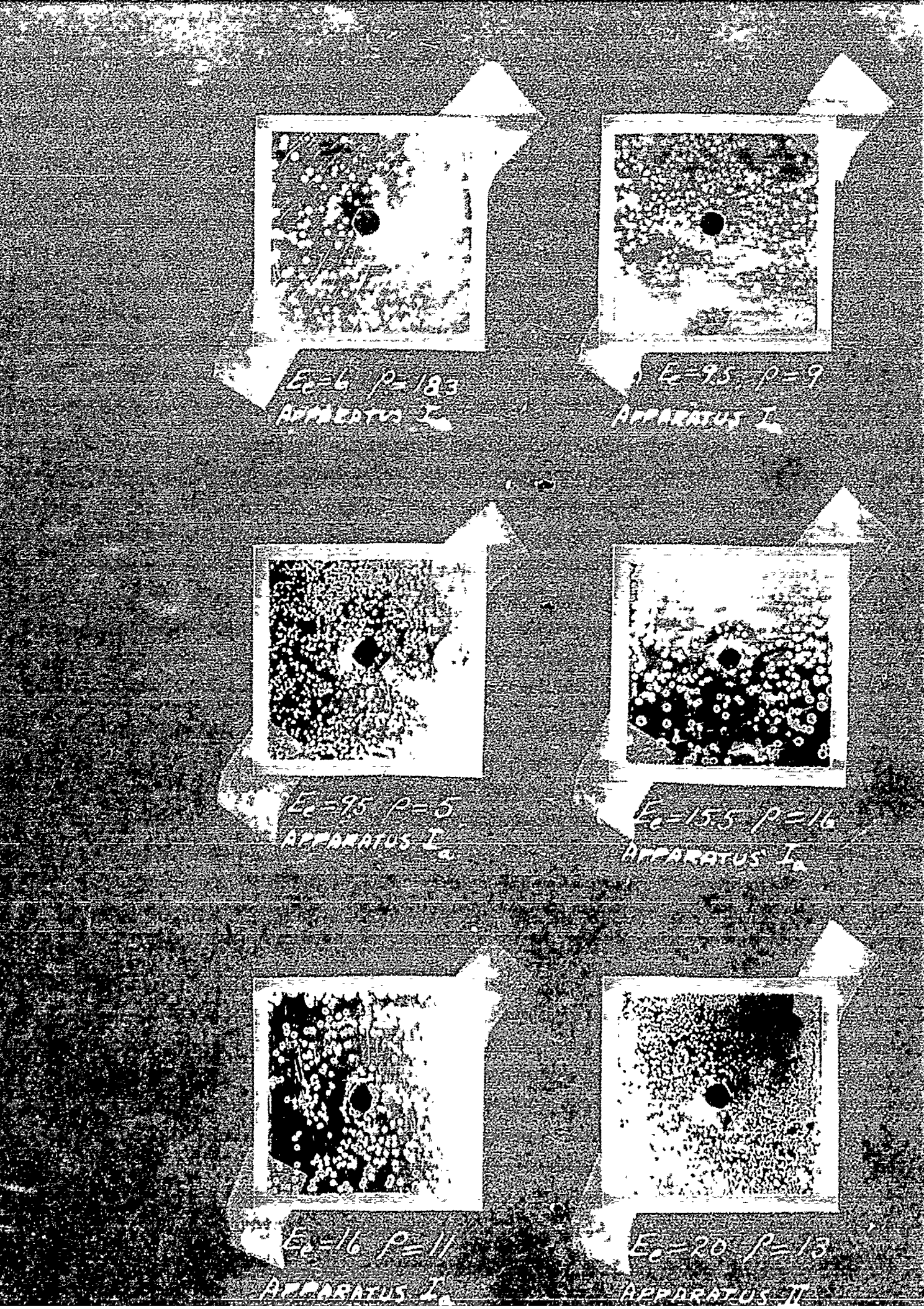

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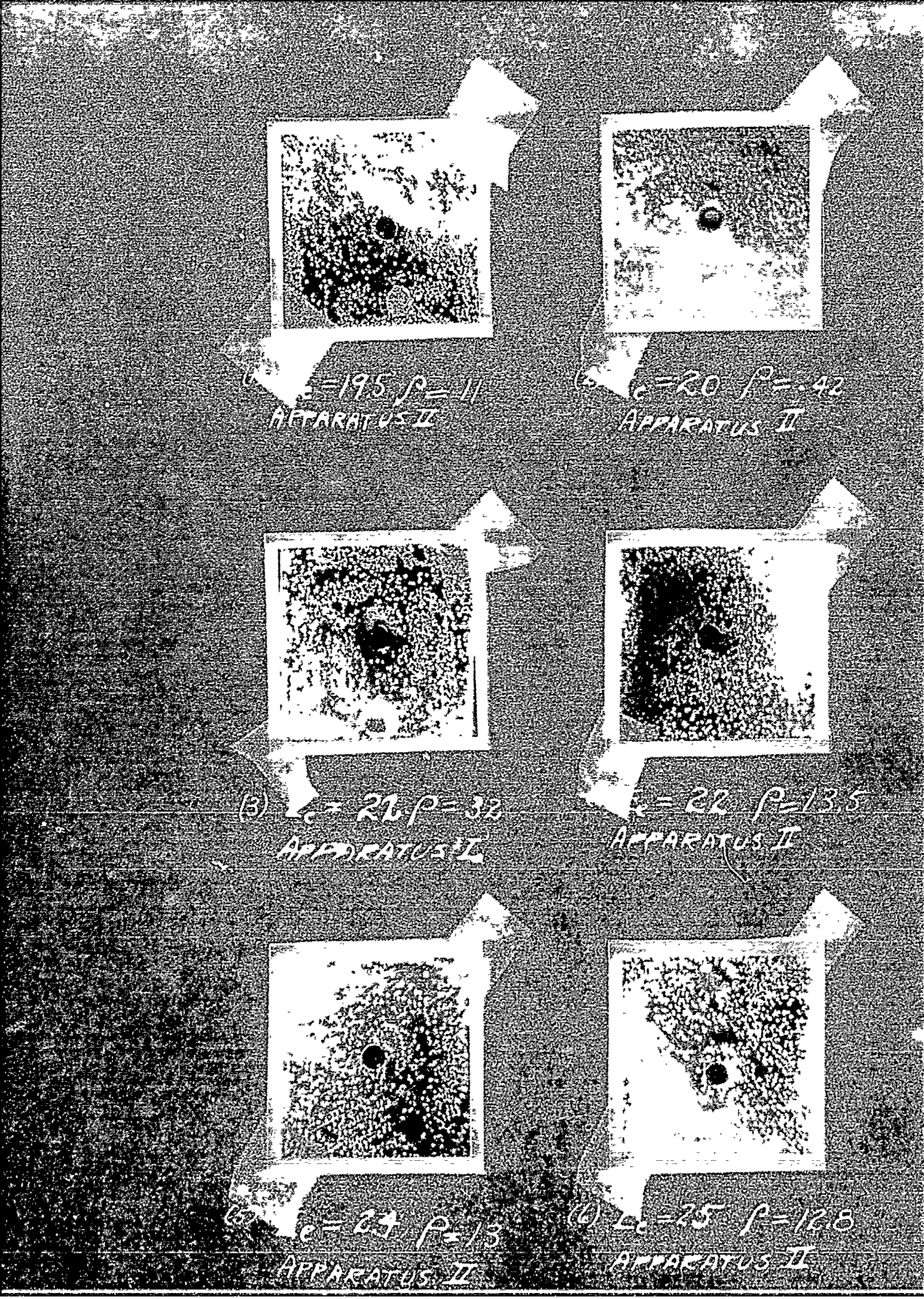

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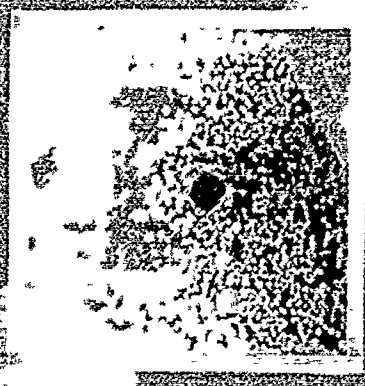
(a) $z=28$ $p=3.75$
Apparatus I.



(b) $z=28$ $p=2.2$
Apparatus I.

(a) $z=29$ $p=1.32$
Apparatus II

(b) $z=29.5$ $p=1.8$
Apparatus I.

(a) $z=29.5$ $p=7.5$
Apparatus I.

(b) $z=30$ $p=7.5$
Apparatus II.

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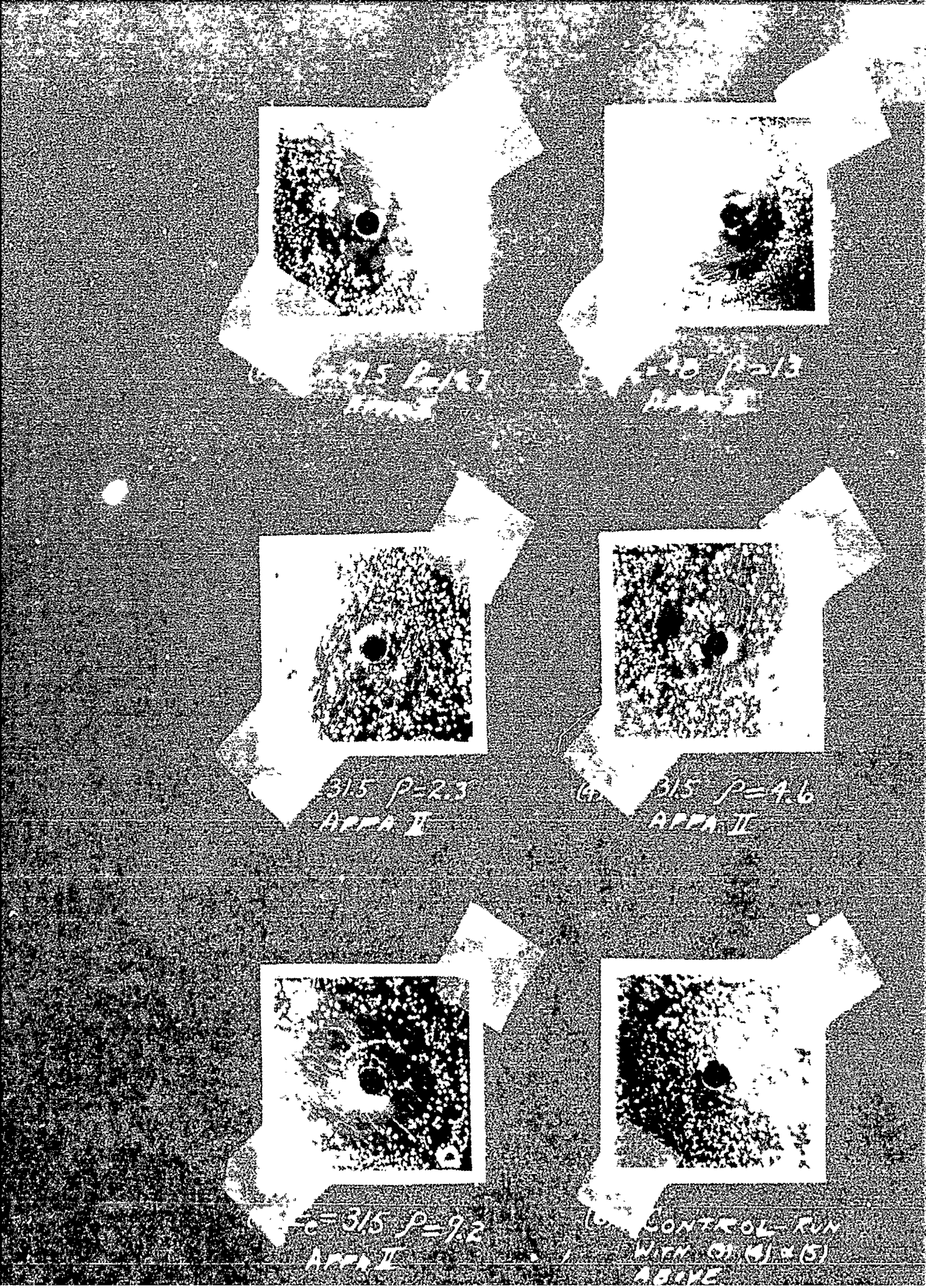

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