q is relatively fixed in Italy, the ABO incompatibility load was plotted against the Rh incompatibility load for the 90 provinces, giving a correlation coefficient -0.278 ($P < .01$). But omitting the three Sardinian provinces with low ABO and Rh loads, the correlation coefficient became -0.431 ($P < .001$). Thus on the whole, there is an inverse correlation between the ABO and Rh incompatibility loads, so that if the ABO load is high the Rh load is low, and vice versa, but in Sardinia, with high gene frequencies for thalassemia and G6PD deficiency, the ABO and Rh loads are both low. Thus it seems likely that complex regulatory mechanisms at the population level adjust gene frequencies to prevent the overall genetic load from becoming excessive.

Based on 33 points, a similar significant negative correlation coefficient ($P < .01$) was found from Mourant's (*8*) maps of the gene frequencies of blood groups in Europe (with Sardinia omitted). Two extreme populations deserve mention. First, the frequency of Rh-negative individuals in the Basque country is 30 to 40 percent, which is close to the frequency for the maximum possible Rhesus incompatibility load which occurs when the proportion of Rh-negative individuals is 45.45 percent. The ABO load, on the other hand, is very low in the Basque country, so a high Rh load is associated with a low ABO load. Secondly, in Lapland where $p \approx 0.5$ (Lapland being the only part of Europe where p is greater than 1/3), a relatively high ABO load is associated with a low Rh load. Thus these two populations do not disagree with the hypothesis. The most divergent population is, in fact, in Sardinia.

In ten Jewish populations, the lowest incidence of Rh-negative individuals is in the Kurdish Jews (*9*) which have both thalassemia and G6PD deficiency genes at a high frequency (*10*). The Jews also give a significant negative correlation coefficient ($P < .02$) between the ABO and Rh incompatibility loads (*11*). In many other parts of the world information is sparse, but in many of the regions where malaria is, or was, endemic the frequency of Rh negatives is extremely low or even zero (*8*).

Thus the evidence from Europe, in particular, is strongly suggestive of a regulatory mechanism at the population level adjusting gene frequencies so that the overall genetic load is not excessive. Such a mechanism would operate even apart from any specific fitness interactions between loci. Presumably the result of the regulatory systems will be the attainment of an optimum genetic load, since zero genetic load implies no evolution, and too great a genetic load would lead to extinction.

It is not suggested that these conclusions invalidate the uses of blood groups in physical anthropology. The broad blood group gene frequency variations are no doubt based on migration and differential sensitivities to disease, but within a specific region it can be argued that the regulatory mechanisms proposed will be important as shown by the evidence for the importance of malaria as an environmental control mechanism.

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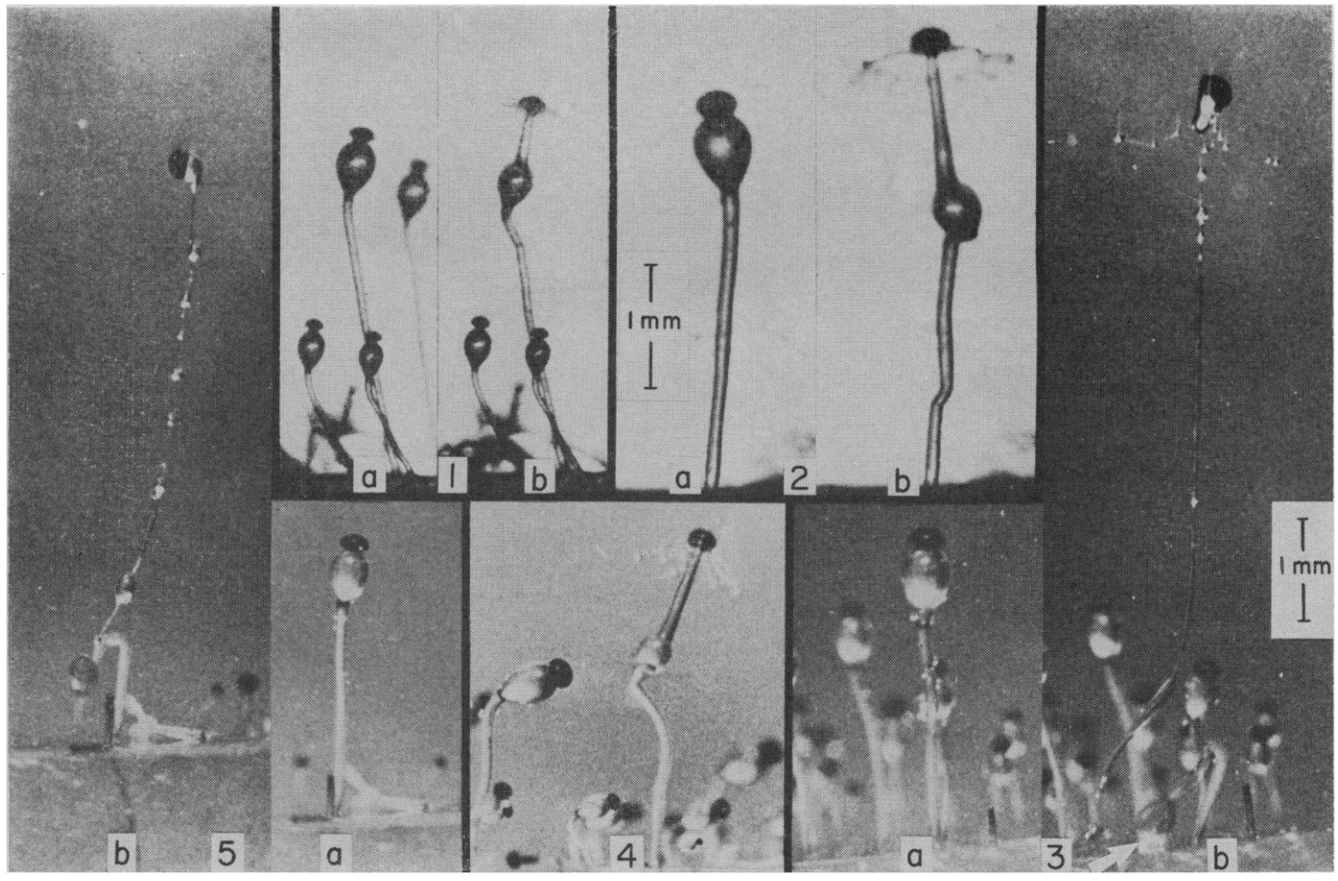
Sporangium Discharge in Pilobolus: A Photographic Study

Abstract. *Stages in the discharge of sporangia by the fungus,* Pilobolus kleinii, *were photographed by means of a high-speed electronic flash triggered by a photocell. The photographs confirm that the sporangium is propelled by a jet of cell sap. The jet is deflected from the sporangium and attains a considerable length before it breaks into droplets.*

The forcible discharge of the mature sporangium of *Pilobolus* is a dramatic and characteristic feature of asexual reproduction in this genus of coprophilous Phycomycetes (*1*). The structure of the sporangiophore and the discharge of the sporangium have been described by Buller (*2*) and Ingold (*3*), but recent improvements in techniques of high-speed photography have made it possible to record and study stages in the process of discharge itself.

To photograph the discharge, *Pilobolus kleinii* van Tiegh. was grown in 60-mm petri dishes on a synthetic medium (*4*). Cultures were maintained at 24°C in darkness for 4 days; they were then subjected to a daily alternation of 12 hours of light and 12 hours of darkness to induce synchronous formation of sporangiophores. The fungus began to discharge sporangia about 3 hours after the beginning of the third light period. A group of sporangiophores was cut from a culture a few hours before the beginning of discharge and placed in a small glass chamber together with moist blotting paper to maintain a high relative humidity. This chamber, with the sporangiophores oriented horizontally, was placed on the stage of a compound microscope. Light from a small illuminator fitted with a red filter and a diaphragm to limit the diameter of the field was focused on a sporangium by the condenser of the microscope. The objective of the microscope was focused on the same sporangium with the aid of a beam-splitter and telescope from a Leitz Micca attachment. A cadmium selenide photocell (Clairex CL-3) was mounted at the focus of the ocular. This photocell was connected in series with a 22.5-v battery and the primary of a small transformer, the secondary of which was connected to an amplifier whose output was applied to the grid of a thyratron (*5*). The thyratron triggered the flash unit, a General Radio Strobotac (model 1531A) (*6*), which was used at the high intensity setting and gave a flash with a duration of 3 μsec. In some cases, a nerve stimulator (Grass SD-5) was interposed between the thyratron and the flash unit to permit a delay of 200 μsec between the time of discharge of



Figs. 1 to 5. Sporangium discharge in *Pilobolus kleinii*. In Figs. 1, 2, 3, and 5, *a* shows the appearance of the sporangiophore before discharge; *b*, the same sporangiophore during discharge. Figs. 1, 2, and 4, no added delay; Figs. 3 and 5, 200 μsec time delay between discharge and flash. Flash duration, 3 μsec. Figs. 1 and 2, about × 17; Figs. 3, 4, and 5, about × 14.

the sporangium and the flash. For photography by reflected light, the arc of the flash tube was located about 3 cm from the sporangiophores. For photography by transmitted light, the sporangiophores were located immediately in front of a pair of condensing lenses, with a combined focal length of 28 mm, which focused the arc on the camera lens. Photographs were taken with a 35-mm camera fitted with an extension bellows. The equipment was set up in a darkroom so that the shutter of the camera could be left open. A blue filter (Wratten No. 47) was used to prevent fogging of the film by the red triggering light. Tri-X film was used for reflected light and Plus-X film for transmitted light, with lens openings of *f* 11 and *f* 22, respectively. Both types of film were processed with Diafine developer.

The photographs (Figs. 1 to 5) show clearly that the sporangium is propelled by a jet of cell sap. Sometimes this jet appears to be cylindrical in its early stages, but usually it tapers (Figs. 1, 2, and 4). This tapered shape suggests that the opening in the sporangiophore must dilate as the liquid is ejected. The jet may attain a considerable length before it breaks into droplets; the jet shown in Fig. 3, for example, is over 7 mm long, but it has begun to break up at its distal end, and the nodular appearance of the main body indicates that it is on the verge of breaking into droplets; the jet shown in Fig. 5 has broken into droplets. The basal part of the jet shown in Fig. 3 was thrown into a distinct curve by the movement of the recoiling sporangiophore.

A "skirt" is observable beneath the sporangium during early stages of discharge. In some photographs taken with reflected light, the skirt appears light (Fig. 4), but in all photographs made with transmitted light it appears dark (Figs. 1 and 2). This skirt appears to consist of liquid deflected from the sporangium. Its diameter increases as the sporangium moves away from the sporangiophore, and finally the liquid coalesces into droplets (Figs. 2 and 4). Some of these droplets accompany the sporangium on its flight (Fig. 3), but many of the smaller droplets probably evaporate quickly. Doubtless, the shape of the concave under-surface of the sporangium formed by the columella contributes to the efficiency of energy transfer between the jet and the sporangium by deflecting the liquid backward.

In most of the photographs taken with time delay between the discharge and the flash, the sporangium appears asymmetrical with respect to the axis of the jet (Figs. 3 and 5); hence the sporangium and the drop which accompanies it may go end over end on their flight. The muzzle velocity of the sporangium was estimated crudely by subtracting the average distance traveled by sporangia in photographs taken with no delay from the average distance traveled by sporangia shown in photographs taken with a time delay between discharge and flash. The value obtained, 16 m/sec, agrees well with values obtained by mechanical (*7*) and electronic (*4*) methods.

As the jet is discharged, the sporan-

giophore first recoils (Figs. 1, 2, and 4) and then collapses (Figs. 3 and 5). In some cases the subsporangial swelling appears to contract irregularly (Fig. 4), but in others it maintains its general shape even after it has been thrown to the surface of the medium (Fig. 3*b*, arrow).

It is not clear whether the liquid that constitutes the jet comes entirely from the subsporangial swelling. The appearance of some cylindrical jets, and Buller's observation (*2*) that the orange protoplasmic ring from the base of the subsporangial swelling may sometimes be found among discharged sporangia adhering to a target, suggest that the liquid may come, at least in part, from the stipe of the sporangiophore. However, two lines of evidence suggest that the liquid comes mostly from the subsporangial swelling. First, it is difficult to see how a tapered jet could be formed by the stipe, and second, the crimp apparent in the stipes of some sporangiophores during early stages of discharge (Figs. 2 and 4) would seem to prevent a rapid movement of liquid up the stipe. Moreover, the volume of the subsporangial swelling is more than adequate to accommodate all of the liquid in even a long jet. The volume of the subsporangial swelling in Fig. 3, for example, is approximately 0.08 mm^3, but the total volume of the jet and visible droplets is less than 0.06 mm^3.

The photographs not only confirm Buller's deduction (*2*) that the sporangium of *Pilobolus* is propelled by a jet of cell sap which breaks into droplets, but they also reveal unsuspected details of the behavior of the jet, the sporangium, and the sporangiophore during discharge.

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6. I am grateful to H. E. Edgerton for suggesting the use of this unit and for other counsel.
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Ionizing Radiation: Effect on Genetic Transcription

Abstract. *Cells of* Escherichia coli *grown on maltose can be induced by the addition of thiomethyl galactoside to produce β-galactosidase. If cells are irradiated shortly after induction, the transcription of the DNA ceases, and the enzyme produced by the messenger RNA is observed to reach a maximum. From these data the calculated half-life of unstable messenger RNA is given over a temperature range from 8.1 minutes at 10°C to 0.7 minute at 45°C. The kinetics of cessation of transcription give information on both messenger RNA decay and rate of transcription. Arrhenius graphs for both these rates are given, and the activation energies measured are 11,000 calories per mole for decay and 22,000 calories per mole for transcription. This relation to temperature is characteristic of enzymatic behavior.*

It has been suggested that one important action of ionizing radiation is concerned with the transcription of the genetic message from DNA to RNA (*1*). Billen and Lichtstein (*2*) studied the effect of ionizing radiation on formic hydrogen lyase and Clayton and Adler (*3*) showed that induced catalase synthesis in *Rhodopseudomonas spheriodes* is inhibited by low doses of rays, giving experimental support to the idea. Pollard and Vogler (*4*), using *Escherichia coli* cells in which the process of induction was dependent on both permease induction as well as the measured enzyme induction, showed that there is some sensitivity to radiation. Novelli (*5*) found a reduced sensitivity as compared with colony formation, but still a considerable sensitivity.

The process of induction of an enzyme has been thoroughly studied (*5–10*), particularly by Pardee and Prestidge (*6*), Boezi and Cowie (*7*), Nakada and Magasanik (*8*), Levinthal, Keynan, and Higa (*9*), and Kepes (*10*). Their work, which supports the well known suggestion by Monod, Jacob, and Gros (*11, 12*), indicates that the transcription of the genetic message is repressed by something which can be acted on by a small molecule, the inducer, to remove repression and permit the formation of messenger RNA which then acts to make the enzyme. The messenger RNA undergoes decay, by a process which is still not clear. Very elegant measurements by Kepes (*10*) show that for the messenger RNA for β-galactosidase the half-life is 1.02 minutes at 37°C and 2.05 minutes at 25°C. The time of onset of the enzyme activity after induction was about 3 minutes.

If the process of transcription is indeed sensitive to ionizing radiation, then the irradiation of cells which have just been induced should show development of the enzyme to the extent of formation of new messenger RNA for a few minutes until transcription stops and should show the formation of the enzyme while the messenger RNA is decaying. This pattern was found by Clayton and Adler (*3*). Our experiments now amplify and extend their work, and also permit some measurements of the half-life of the messenger RNA, which are in agreement with the work of Kepes. The experimental procedure is as follows. Cells of either *Escherichia coli* B or *E. coli* 15 Thy^-Leu^- are grown in minimal medium with maltose as a carbon source. This does not repress the formation of enzyme, nor does it induce it. When the cells are at a concentration between 5×10^7 and 1.0×10^8 per milliliter they are induced by the addition of 1 ml of thiomethyl galactoside (TMG) (0.2 g/100 ml) to 20 ml cells. The concentration of cells at irradiation must be kept relatively low. At higher concentrations the cells are much less sensitive for reasons not yet wholly clear, but partly the cause is

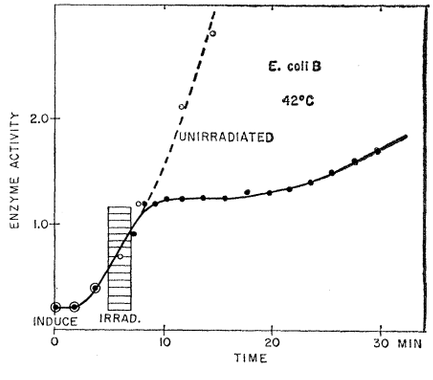

Fig. 1. A culture of *E. coli*, strain B, previously grown on maltose was induced with TMG at time zero. It was then allowed to grow at 42°C and irradiated in a Co^{60} source for 2¼ minutes, with a dose of 13,500 r. The amount of β-galactosidase in 1 ml of the culture was measured at various times for this culture and an unirradiated control. The production of enzyme continues for a short while after irradiation and then ceases. From the kinetics of cessation of production the half-life of messenger RNA can be found. Later, presumably as new DNA synthesis starts, the supply of enzyme begins to increase.