Reports

Ascites Induced in Mice by Staphylococcus

Abstract. Ascitic fluid containing high titers of antibody was induced in mice by *Staphylococcus*. Two to three intraperitoneal inoculations of 0.3 ml of a killed suspension of *S. aureus* mixed with complete Freund's adjuvant, given at 5-day intervals, produced ascites in 100 percent of the mice tested.

An adequate supply of potent antibody is indispensable for studies in basic immunology. This report describes a method for producing ascitic fluid containing high titers of antibody against *Staphylococcus aureus* strain 18 in mice (*1*).

Munoz, using paraffin oil mixed with *Mycobacterium phlei* as an adjuvant and egg albumin or bovine serum albumin as the immunizing agent, produced ascitic fluid containing good antibody titers in 50 percent of the mice tested (*2*). Herrmann and Engle, using sarcoma 180 cells and influenza A and Newcastle disease viruses, produced ascitic fluid in mice containing antibodies against these viruses (*3*).

Male mice of the National Institutes of Health general-purpose Swiss stock, weighing 20 to 25 g when used, were given two to three injections of antigen intraperitoneally at 5-day intervals. For the preparation of the antigens, a 20-hour culture of *Staphylococcus aureus* 18 grown in trypticase soy broth was treated in three different ways. (i) Cultures of live organisms were adjusted to give a concentration of 1×10^4 cells per milliliter. (ii) Cultures of organisms were concentrated to a level of 4×10^9 cells per milliliter and exposed to a temperature of 60°C for 1 hour on two successive days. (iii) Cultures were adjusted to deliver 4×10^9 cells per milliliter, formaldehyde was added, to a final concentration of 0.5 percent, and then the cultures were allowed to stand for 24 hours prior to use.

Each antigen preparation was inoculated into 16 mice, with each mouse receiving 0.3 ml of a mixture of equal parts of the prepared *Staphylococcus* antigen and Freund's complete adjuvant. The total amount of antigen inoculated into each mouse varied from 0.6 to 0.9 ml. Ascites appeared in 100 percent of the mice irrespective of the antigen preparation used. Repetition of this experiment yielded similar results in a separate investigation involving 60 additional mice.

In the mice receiving live antigen, ascites appeared during the third or fourth week after the primary inoculation. Formaldehyde and heat-killed antigens produced ascites in 1½ to 2 weeks, after the first injection. In each instance, the quantity of ascitic fluid obtained with an 18-gage needle varied from 2 to 12 ml per mouse. The total yield of ascitic fluid for each group of 16 mice after two to three taps at weekly intervals was 50 to 60 ml with the live organisms, 110 to 120 ml with the heat-killed antigen, and 90 to 100 ml with formaldehyde-treated culture. Freund's complete adjuvant, when mixed with uninoculated sterile trypticase soy broth, produced ascites in four of 16 mice 4 weeks after the primary inoculation.

When no further ascitic fluid could be obtained from the mice, the animals were exsanguinated. The titer of antibody in the serum was determined and compared with the titers of antibody contained in the ascitic fluid at successive taps. Antibody titers measured by the slide agglutination method were essentially the same for the ascitic fluid and the blood serum of each group of mice and varied from 1/2000 to 1/4000. There was no increase in the titer of antibodies of the ascitic fluid following booster injections given 6 to 7 weeks after the primary inoculation.

Another experiment, with a different strain, *S. aureus* 2 (*4*), showed that it, too, can induce the production of ascitic fluid containing antibodies in mice. In addition, the elimination of *Mycobacterium butyricum* in Freund's adjuvant had no effect on the number of mice affected with ascites, the volume of ascitic fluid formed, the level of antibody produced, or the duration of antibody response.

Abdominal adhesions may occur after two or three withdrawals of ascitic fluid, and difficulty in "tapping" the mouse may be encountered. These adhesions appear to be less severe if ascites is produced after two to three injections and may be dependent upon the quantity of the inoculum used and the time interval between doses.

Further studies are in progress to determine the effect of various staphylococcal components; the importance of strain, age, and sex in mice; the optimal immunization regimen; and the role of different adjuvants in the production of ascitic fluid containing antibodies in mice.

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References and Notes

1. *Staphylococcus aureus* strain 18 was recently isolated from an abscess in a patient at the National Naval Medical Center, Bethesda, Md.
2. J. Munoz, *Proc. Soc. Exptl. Biol. Med.* **95**, 757 (1957).
3. E. C. Herrmann, Jr., and C. Engle, *ibid.* **98**, 257 (1958).
4. *Staphylococcus aureus* strain 2 was isolated from sputum from a patient with pneumonia at the U.S. Public Health Hospital, Staten Island, New York.

16 October 1958

Phototropic Equilibrium in Phycomyces

Abstract. When illuminated by two light sources, the positively phototropic sporangiophores of *Phycomyces blakesleeanus* come into a state of phototropic equilibrium. In this state, the sporangiophores assume an orientation determined by the light fluxes falling upon opposite sides of their light-sensitive zones. Under certain conditions, the sporangiophores show tropic oscillations about the equilibrium direction.

Sporangiophores in their mature fast-growing stage were illuminated by two horizontal (*1*) light beams which made angles with each other of from 180° to 30°. When the intensities of the beams are equal, the sporangiophores assume a direction of growth that bisects the angle between the beams. Such a stable equilibrium position is often maintained for more than 12 hours, during which time the sporangiophores grow a distance of more than 3 cm. If, however, the beams

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper. (Since this requirement has only recently gone into effect, not all reports that are now being published as yet observe it.)

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

make an angle of 180° with each other, the equilibrium is indifferent rather than stable. In this case the sporangiophores continue to grow in whatever direction they were started.

When the light beams are unequal in intensity and make an angle of less than 180° with each other, the equilibrium is stable as before, but the direction of growth is now shifted toward the more intense beam. This shift in equilibrium position was measured for various relative beam intensities and various angular beam separations. The results of this work have been reported in detail elsewhere (*2*), and hence it will suffice here to summarize the findings. In all cases the direction of equilibrium is that direction for which the light-sensitive region intercepts an equal amount of flux from each beam on opposite sides of the sporangiophore.

The calculation of the amount of flux received by the sensitive zone from a single beam of parallel light is based on the following considerations: (i) The phototropically sensitive region has been estimated by Cohen and Delbrück (*3*) to extend from 0.5 to 2.0 mm below the sporangium. (ii) The sensitive zone is not always fully illuminated by an incident beam, since for certain angles of incidence the terminal sporangium casts its shadow on parts of this region. Thus, the portion of the sensitive zone that is illuminated is a simple geometrical function of the angle of incidence of the beam and of the dimensions of the sensitive zone and sporangium. (iii) The flux intercepted by the illuminated part of the sensitive zone is proportional to the intensity of the beam and to the sine of the angle between the beam and the axis of the sensitive zone. It is interesting to note that the resultant law, which applies to *Avena* coleoptiles in phototropic equilibrium (*4*), is identical to the equal flux law given above if the shading action of the sporangium is neglected.

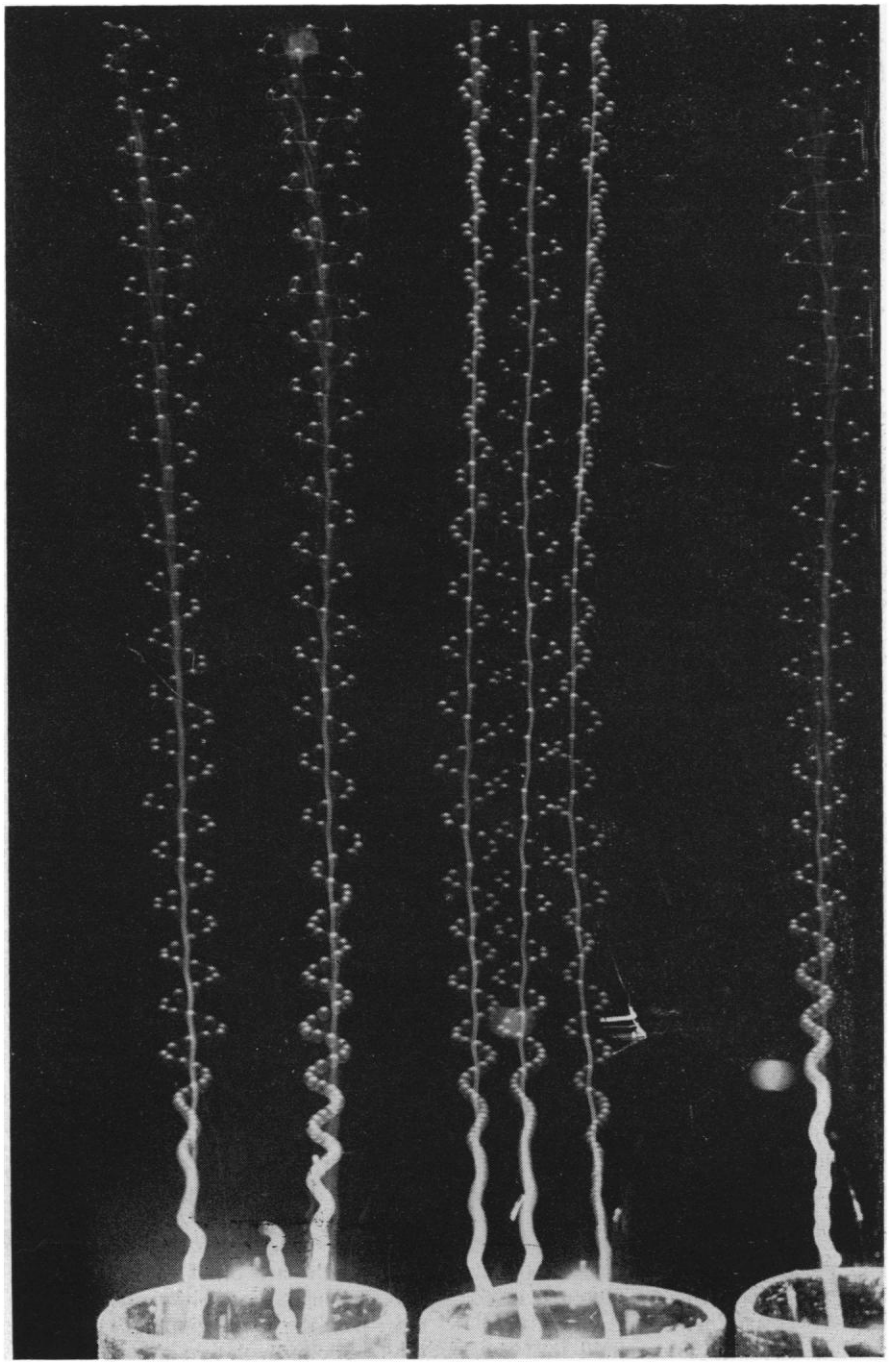

Fig. 1. Multiple-exposure photograph of a group of sporangiophores oscillating about their stable equilibrium points. The light beams were of equal intensity and were separated by an angle of 60°. Exposures were made every 5 minutes by red light, which is phototropically ineffective. The scale of the photograph is indicated by the size of the glass vials, which were 12 mm in diameter.

The most striking property of sporangiophores in stable equilibrium is the phenomenon of oscillation. Figure 1 is a time-lapse photograph of several sporangiophores in stable phototropic equilibrium. Here the sporangiophores are in a state of phototropic oscillation. in which regular rhythmic bending take place alternately to the right and left Each sporangium is recorded in the photograph as a wavy trail of dots as it is carried back and forth by the oscillating and growing sporangiophore.

The amplitude and period of oscillation can be obtained directly from such a photograph. The amplitude varies somewhat and has a maximum of about 30°. The period shows a remarkable regularity from specimen to specimen; at the beginning of the oscillation, when the sporangiophore is young, the period is about 60 minutes, but as the sporangiophore grows, the period drops until it reaches a value of about 30 minutes. This transition from long period to short period is quite gradual and is unaffected by changes in the amplitude. It is a somewhat curious fact that while the period of oscillation is decreasing, the rate of growth is increasing. These two processes are so synchronized that the "wavelength" of oscillation remains constant at about 2 mm.

Careful analysis of the three-dimensional path traced out by the sporangium has revealed that this path is not a flat zigzag but rather a flattened helix. The cross section of this helix is an ellipse whose major axis is about four times its minor axis. The explanation of this ellipticity of path probably lies in the fact that the growing region of the sporangiophore twists about its own axis as it grows.

A series of experiments was also carried out to determine what effect the angle between the light beams has on oscillation. It was found that oscillation

occurs if this angle is 90°, 60°, or 30° but does not occur if it is 120°, 150°, or 180°. Oscillation does not occur if a single beam is used. It was also discovered that a sporangiophore might be under conditions favorable for oscillation and yet remain in the nonoscillating state; in this case it is only necessary to give a short tropic stimulus in order to initiate the oscillation, which is then self-sustaining.

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References and Notes

1. When the beams are in the vertical plane the equilibrium is affected by negative geotropism.
2. D. S. Dennison, Ph.D. thesis, California Institute of Technology (1958).
3. R. Cohen and M. Delbrück, *J. Cellular Comp. Physiol.*, in press.
4. O. Hagem, *Bergens Museums Arbok. Naturv. Rekke* No. 3 (1911).

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Flower Induction in Japanese Chrysanthemums with Gibberellic Acid

Abstract. Gibberellic acid, when applied in lanolin to apices of three Japanese varieties of *Chrysanthemum morifolium*, induced bolting and flowering. These varieties are not sensitive to photoperiod but require a cold treatment in order to flower. On the other hand, the varieties of *Chrysanthemum* which belong to the short-day group are not induced to flower by gibberellic acid.

The discovery that gibberellic acid (GA) induces bolting and flowering in several species (*1, 2*) has stimulated much research on the flower-inducing properties of this compound. Gibberellic acid, however, seems to be unable to induce flowering in the cocklebur (*Xanthium pennsylvanicum*) (*2*) and in short-day varieties of chrysanthemum (*Chrysanthemum morifolium*) (*3*). This situation raises the following point: Is gibberellic acid ineffective, in the cited cases, because the two species, *Xanthium pennsylvanicum* and *Chrysanthemum morifolium*, happen to be nonresponsive for genetic reasons, or is it ineffective because of the physiological short-day character which these species have in common?

In order to elucidate this question, three varieties of Japanese chrysanthemums which can be induced to flower regardless of the photoperiod were selected: Shuokan, Kinkazan, and Shin-misono (*4*). These varieties require a cold treatment near 1°C for 3 to 4 weeks in order to be able to flower, whether under long-day or short-day illumination; without a cold treatment, they may remain in a rosetted condition for almost a year.

The following procedures were carried out, with eight replications per treatment for each of the three varieties: (i) controls were kept in a greenhouse at a temperature above 15°C during the whole growing period; (ii) plants were subjected to temperatures of 1° to 5°C for 4 weeks in an outdoor cold frame, then returned to the greenhouse; (iii) plants were kept in the greenhouse but were treated once, at the growing point, with about 5 mg of a lanolin paste containing 10 μg of gibberellic acid per milligram (*5*). At all times, including the periods of cold treatment, all the plants were given long, 18-hour days by supplementing the hours of natural daylight with periods of incandescent light. From each plant the lateral shoots were removed, only one main stem being left.

The results obtained were as follows. Two weeks after treatment with either gibberellic acid or cold, the stems of the respective plants started to elongate (Fig. 1). Later on, flower buds appeared, and 19 weeks after the beginning of the treatments the plants were in full bloom (Fig. 2). At that time the controls were still in a rosetted state and without any flowers. The controls eventually bolted and finally bloomed also, but much later —some 11 weeks after the treated plants had bloomed. Results were essentially similar in all three varieties, except that in the Shin-misono variety the plants treated with gibberellic acid bloomed 2 weeks later than the cold-treated ones. As is shown in Table 1, the cold-treated plants flowered at a lower node than those treated with gibberellic acid and had longer internodes.

These experiments show that gibberellic acid can induce bolting and flowering in varieties of chrysanthemum which normally require a cold treatment in order to flower. They indicate that it is not the species *Chrysanthemum morifolium*, as such, which is insensitive to the flower-promoting effect of gibberellic acid but rather the short-day characteristic of some of the varieties belonging to this species. This result strengthens the idea that gibberellic acid is not effective in inducing flowering in short-day plants (*6*).

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Table 1. Effect of flower-inducing treatments on three varieties of Japanese chrysanthemums.

Variety	Controls	Gibberellic acid	Cold
	Total height (cm)*		
Shuokan	17.8	95.4	85.0
Kinkazan	36.0	106.8	99.4
Shin-misono	25.2	64.0	66.6
	Average length of internodes (cm)*		
Shuokan	0.31	2.27	2.64
Kinkazan	0.49	2.07	2.42
Shin-misono	0.39	1.40	2.08
	*Average nodes to flower**		
Shuokan		42.0	32.2
Kinkazan		51.6	41.0
Shin-misono		45.6	32.0
	Number of weeks to anthesis (from beginning of treatments)		
Shuokan	30	19	19
Kinkazan	29	20	19
Shin-misono	31	22	20

* Nineteen weeks after the beginning of treatments.

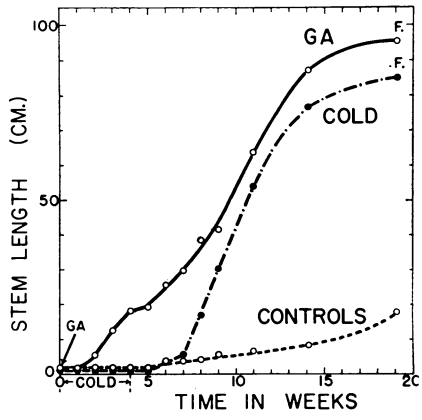

Fig. 1. Growth curves of Japanese chrysanthemums, var. Shuokan, subjected to the following treatments: (open circles and solid line) about 50 μg of gibberellic acid applied in a lanolin paste to the growing points at time 0; (solid circles) 4 weeks of cold treatment; (open circles and dashed line) controls. (Each point represents the average of eight replications.)

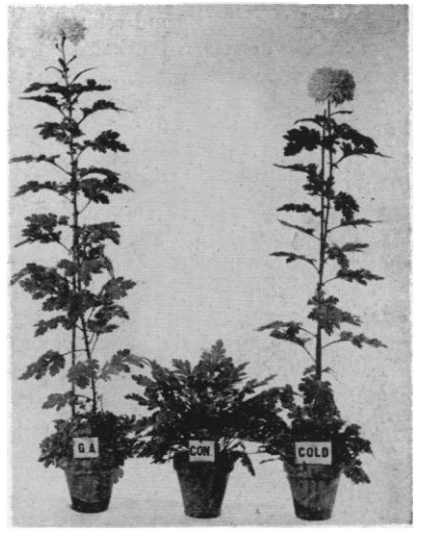

Fig. 2. Induction of bolting and flowering in Japanese chrysanthemums, var. Shuokan, 19 weeks after the application of gibberellic acid (left) or the beginning of a 4-week cold treatment (right). The control (middle) remained rosetted and vegetative.