berg,[5] there is a clumping of red cells in the smaller vessels which is probably due to loss of plasma through the highly permeable vascular wall. The red cells are stranded and silt the blood vessels forming a sludge. They do not, however, represent *true* thrombi in the beginning. A simple injection of saline enables one to wash out these erythrocytes as individual cells. Only after approximately 72 hours does organization of these cells into thrombi occur. This thrombosis ultimately leads to gangrene.

It appeared obvious therefore that therapeutic attempts to avoid gangrene after frostbite must be started before the stage of thrombosis is reached.

Ten rabbits of equal weight were exposed to chilling by the method already described. Five of the animals were treated by heparinization within four hours after exposure.[6] None of the heparinized animals developed gangrene, while in the untreated controls all areas exposed for more than 15 minutes became gangrenous. Encouraged by this result, the following experiment was done on 22 rabbits. One hind leg was exposed to an alcohol dry ice bath of −12°–20° C. for a period of 45 to 90 minutes with the leg protected by a thin boot of condom rubber. After the exposure, eleven animals were heparinized, while eleven remained untreated. Of the treated animals only two showed some slight surface lesions, while the legs of the others remained completely intact with no gangrene. All controls lost their legs by complete gangrene, including the bone.

The practical demonstration of the therapeutic value of heparinization in the prevention of gangrene was made possible by the study of artificial frostbite in human volunteers. These volunteers were recruited from patients who were being treated for subacute bacterial endocarditis at the Jewish Hospital of Brooklyn by the combination of penicillin and heparin.[7, 8] In one group the frostbite was accomplished by means of a porcelain crucible filled with dry ice and applied to the skin of the lateral aspect of the upper arm without pressure for ten minutes. An area of about 2 cm came in contact with the skin. By this method, a temperature of minus 22° C. was achieved. Heparinization was started immediately following exposure. One volunteer served as a control. The other group was subjected to local refrigeration in the same manner but for two exposures of 30 minutes each. The initial or control exposure was permitted to develop for six days before the second frostbite was induced, immediately following which treatment with the subcutaneous heparin in the Pitkin menstruum was initiated.[9, 10] The 30-minute exposure with dry ice results in temperatures considerably below minus 22 degrees Centigrade, and is comparable to the frostbite suffered by aviators in high altitude flying such as nose gunners after demolition of the plexi-glass protection or gunners attempting to un-jam guns without glove protection.

The clotting time in the treated cases stayed between 25 to 60 minutes. It was apparent from the observations in these human volunteers that all the adequately treated lesions escaped any deeper injury. One must recognize that these investigations in human volunteers are merely transition experiments which serve as added proof of the genesis of gangrene following frostbite and the validity of the therapeutic approach.

The further practical demonstration of the method's value was made possible when a frostbite case appeared at the Research Unit of the New York Medical College. A man was sent to the hospital following exposure to a temperature of 18 to 20 degrees F. for at least 14 hours while lying in the street. His hands were completely unprotected, while his feet were protected only by low shoes and thin socks. On admission his feet were ice-cold up to the knee and remained so for five hours after admission. He was heparinized by the intravenous route for five days, the clotting time being maintained between 30 and 60 minutes. He developed considerable blistering, especially on the hands, but completely escaped any permanent tissue loss. From the experience with similar exposures, one can say that this man without heparinization would probably have had more or less extensive loss of the extremities.

Experiments are under way to determine the simplest method of heparinization and the longest interval between exposure and start of therapy which would still be effective.

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ACTION SPECTRUM FOR THE PHOTOPERIODIC CONTROL OF FLORAL INITIATION IN BILOXI SOYBEAN

THE effectiveness of light applied to Biloxi soybean leaves during the middle of the dark period to prevent

[5] L. Kreyberg and L. Rotnes, *Acta. Path. Microb. Scand.*, 11: 162, 1932.
[6] K. Lange and L. J. Boyd, *S. G. and O.*, 80: 346–350, 1945.
[7] L. Loewe, P. Rosenblatt, H. J. Greene and M. Russell, *Jour. Am. Med. Asn.*, 124: 144–149, 1944.
[8] L. Loewe, *Bul. N. Y. Acad. Med.*, 21: 59–86, 1945.
[9] L. Loewe, P. Rosenblatt and J. Lederer, *Proc. Soc. Exp. Biol. and Med.*, 50: 53, 1942.
[10] L. Loewe and P. Rosenblatt, *Am. Jour. Med. Sci.*, 208: 54–63, 1944.

floral initiation has been determined at various wave-lengths. The results of such determinations constitute the action spectrum from which knowledge is obtained about photoreactions of unknown compounds that regulate floral development.

Biloxi soybean forms flower primordia at all actively growing buds when the entire plant or a single leaflet is subjected to a minimum dark period of 10.5 hours for two or more daily cycles. These dark periods must be continuous because interruptions as brief as 30 seconds with about 15 foot-candles of light prevent the formation of flower primordia, even though the photoperiod is favorable. Dark periods much longer than the minimum can also be made photoperiodically ineffective by brief periods of illumination provided none of the resulting dark periods exceeds 10.5 hours.

A study of the effect of various wave-lengths of visible light on the dark period interruption for Biloxi soybean has been made with a specially designed spectrograph. This instrument is unique in that the spectrum is wide enough to permit irradiation of fully expanded leaflets while maintaining spectral purity at high intensity. It is a two-prism spectrograph, with the slit illuminated by a 10-kilowatt D.C. carbon arc. A spherical mirror of 2 m focal length serves as a lens giving F14 for the collimator and F90 for the telescope. The dispersion at 5,000 A. is 15 A. per cm; the energy can be as great as 10^3 ergs per sec. for an effective slit width of 100 A. at 5,000 A.; and scattered light in the spectrum is less than 1.0×10^{-3} of the incident light. The spectrum measures about 6 feet between the limits of 4,000 and 7,000 A. and has a height of 3 inches in the focal plane, which is 12 m from the prisms. Definite stations at which individual leaflets were held during irradiation were established on a board placed in the focal plane. The position of the spectrum was fixed with reference to these stations in a given experiment but was variable from one experiment to another. In some of the experiments the leaflets were placed flat on the board and normal to the light, in which case a single leaflet subtended about 40 A. at 4,000 A. and 200 A. at 6,000 A. In others they were placed on wedges which held the leaflets at angles of 30° to the light beam so as to reduce the width of the band being investigated.

Most experiments included five treatments differing in energy of irradiation. Energy was varied by varying the time of exposure at constant intensity, since it was found by preliminary experiments in several regions of the spectrum that at a given wave-length the minimum energy required to prevent floral initiation, at the middle of the dark period, was independent of the variations of time and intensity of irradiation so long as their product was a constant. That is, the reciprocity law held for the periods and intensities used.

The following description shows how a typical experiment was conducted. Soybean plants that were known to be vegetative were selected from a large population that had been grown on 16-hour photoperiods in the greenhouse. These were moved to controlled environment rooms where they were conditioned with several more 16-hour photoperiods. Following this all leaflets were removed from the plants except the center leaflet of the most recent fully expanded compound leaf, which in most cases was the third. During the experimental period leaves developing above this point were removed before they expanded. The photoperiod was then decreased to 10 hours and the interruptions were arranged so that they occurred during a period of one hour extending

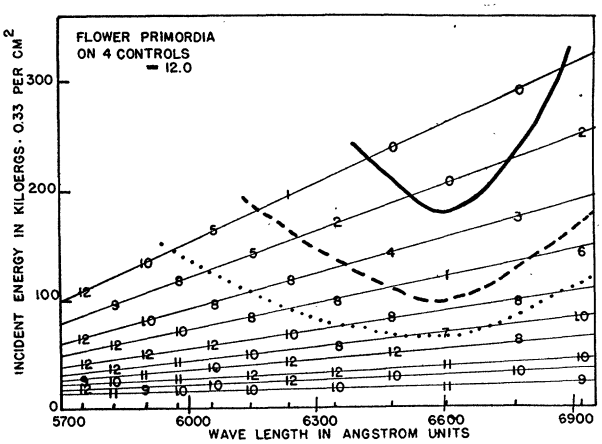

FIG. 1. Action spectrum in the yellow-red region. Cross lines indicate the energies used, and the figures on these lines show the number of flower primordia on 4 soybean plants following treatment. The three curves show the gradation of effect with decreasing energy at a specific wavelength.

30 minutes before and after the middle of the 14-hour dark period, with the shorter periods of illumination nearest the middle. Previous experiments had shown that the effectiveness of brief interruptions varied somewhat over the hour period and this factor was taken into account. In all experiments the plants were removed from the control rooms in the dark and the leaflet of each plant was placed in its position on the board and then irradiated.

Exposure times, in the red region, for instance, in a particular experiment, were 1, 2.5, 5, 9 and 13 min., and each was repeated with four lots of plants along the spectrum. With soybeans each lot was treated for 6 consecutive days, after which the plants were re-

turned to the 16-hour photoperiod. Floral initiation was determined by dissection eight days later.

Results of an experiment covering the region from 5,700 A. to 6,900 A. are shown in Fig. 1. Cross lines on the graph indicate the ten variations of energy used in the experiment. The figures on these lines are the numbers of flower primordia produced on four plants and their positions along the lines correspond to the wave-length at the middle of the station where they were irradiated. In this experiment there was a gradation of effect with decreasing energy at a specific wave-length. Results of an experiment in the green to violet region of the spectrum are shown in Fig. 2. The efficiency of radiation for interrupting the dark period in this experiment decreases from the red to 4,600 A. About 800 kiloergs per cm² are required to prevent formation of flower primordia in this region, while 30 to 50 kiloergs per cm² are sufficient in the red. Below 4,600 A. effectiveness increases and another maximum is reached near 4,000 A.

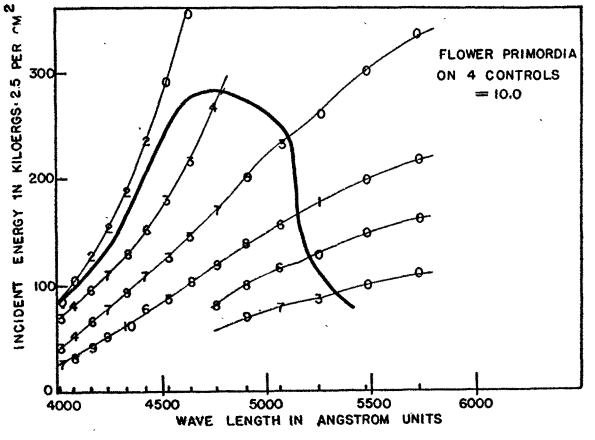

FIG. 2. Action spectrum in the green-violet region.

Results for many experiments have been combined in a curve, covering the region from 3,800 A. to 7,200 A. (Fig. 3). The region from 7,200 to 20,000 A. has also been investigated, but floral initiation was not inhibited beyond 7,200 A. Floral initiation can be suppressed by interruption of the dark period with light of sufficient energy from any region of the visible spectrum, but there are two regions of maximum efficiency, one in the yellow, orange and red and the other in the violet near 4,000 A. The response curve was very accurately determined from 4,000 A. to 5,100 A. and from 5,500 A. to 7,200 A. and the incident energy required to prevent floral initiation is accurate within each region, but difficulty was encountered in connecting the two. The energies indicated for the blue-violet are uncertain by a possible twofold factor relative to those in the red. This was a result of using different lots of plants for the two regions and of the necessity for irradiating longer times in the blue-violet than in the red and over periods removed by as much as 30 minutes from the middle of the dark period.

The over-all response curve has striking similarities to the curve for photosynthetic utilization of carbon dioxide. In particular it shows the same action limit in the red and two maxima, one in the red and the other in the blue. The curve indicates that the chloroplast pigments of the leaf are associated with the dark period interruption reaction. Carotenoids apparently are not involved in the light absorption responsible for the reaction, since their maximum absorption is in the range of minimum effectiveness and they are transparent in the red. It is likely that the action spectrum is due to a porphyrin-like material which is probably chlorophyll. The action spectrum is not identical with that for carbon dioxide utilization, as might be anticipated, but the significance of the differences between the two require detailed analysis.

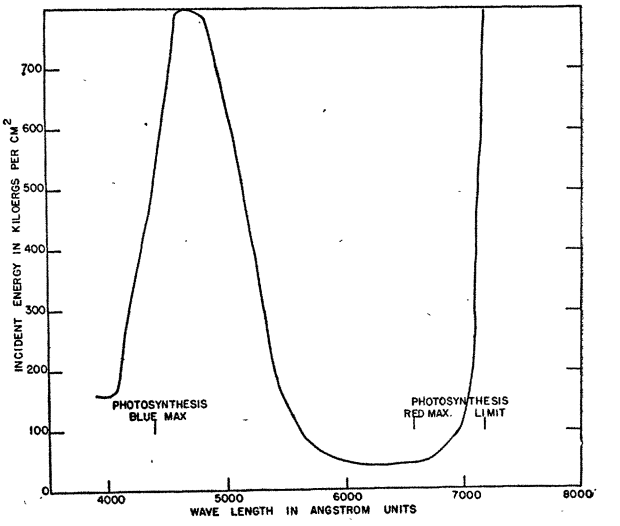

FIG. 3. Composite action spectrum based on 21 experiments. Any point on the curve shows the amount of energy that must be applied to a Biloxi soybean leaflet during the middle of the dark period to prevent flower initiation. The points of maximum utilization of CO_2 in photosynthesis and the limits of this reaction, as shown by Hoover,[1] are included for reference.

A possible explanation for these observations is that energy absorbed by the chlorophyll is transferred to a reaction leading to the destruction of a material determining floral initiation. This may be a photo-oxidation, even though the photo-response was found to be independent of oxygen pressure.

The action spectrum is being determined for *Xanthium pensylvanicum*, and its response in the red

[1] W. H. Hoover, Smithsonian Miscellaneous Collection, Vol. 95, No. 21, 1937.

has been found to be similar to that of Biloxi soybean.

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A NEW POLYSACCHARIDE FROM BLACK SPRUCE (PICEA MARIANA)

WHEN native lignin is isolated from black spruce (*Picea mariana*) with aqueous alcohol and the alcohol is distilled off under reduced pressure, a mixture of native lignin and resins separates from the remaining aqueous solution.[1] On saturating the aqueous filtrate with sodium sulfate, a polysaccharide separates as a flocculent precipitate which, after centrifuging and washing with 80 per cent. alcohol, then with absolute alcohol, and finally with ether and petroleum ether, is obtained as a light powder. After purification by prolonged electrodialysis and repeated precipitations by dropping a concentrated aqueous solution into absolute methanol, the polysaccharide is obtained as a white, nonhygroscopic powder in a yield of about 0.1–0.2 per cent. of the wood. It does not reduce Fehling solution before hydrolysis with hot dilute hydrochloric acid, but does so very strongly after this treatment. In spite of lengthy electrodialysis, it still contains 0.7 per cent. ash (determined as sulfate). It is very soluble in water, forming a slightly turbid solution similar to starch solutions. Its aqueous solution shows a slight levorotation which, after hydrolysis, changes to a strong dextrorotation. A hydrolysis curve, obtained by boiling the polysaccharide with 2 per cent. sulfuric acid, shows that a maximum reducing power of about 95 per cent. sugar (calculated as glucose) is reached after 6 hours. The presence of 0.7 per cent. MeO and a slight residue left after hydrolysis indicate that a small amount of lignin is still present which is difficult to remove because of the colloidal properties of the polysaccharide. On distillation with 12 per cent. hydrochloric acid, the polysaccharide gives 3.3 per cent. carbon dioxide, which corresponds to 13.2 per cent. uronic acid. When the polysaccharide is acetylated by heating it with a mixture of pyridine and acetic anhydride, a gelatinous suspension is formed from which an acetylated product is obtained which is insoluble in water and the common organic solvents.

A biochemical analysis[2] of the hydrolyzed polysaccharide by the method of Wise and Appling[3] shows the presence of 72.6 per cent. galactose, corresponding to 65.3 per cent. galactan. The polysaccharide also contains 13.1 per cent. arabinose (determined by the method of Wise and Peterson[4]) corresponding to 11.5 per cent. araban; glucose, mannose and xylose are absent. The presence of uronic acid, the levorotation and the insolubility of the acetylated derivative differentiate the polysaccharide from the arabogalactans isolated from certain larch species.[5,6] As the acetate of arabogalactan is soluble in organic solvents,[5] it is improbable that the polysaccharide is a mixture of arabogalactan and polyuronic acid because, in this case, the acetate should be at least partially soluble in organic solvents.

From the above analysis, it seems that the 3 components—galactose, arabinose and uronic acid—are present in the polysaccharide in a 4:1:1 molecular ratio.

A closer chemical investigation of this polysaccharide is in progress and the results will be reported at an early date.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE WATER MANOMETER FOR RECORDING INTESTINAL ACTIVITY[1]

EXPERIMENTS on intestinal activity *in situ* often fail because of unsatisfactory recording equipment. This tends to discourage performance of such experiments in student laboratories. This note describes a simple device for recording intestinal motility which has the advantage of economy, ease of construction and maintenance, and which may be readily adjusted to give a wide range of initial force distending the intestine. Furthermore, by the use of a lever, considerable amplification of changes in the manometer level may be obtained readily.

[1] F. E. Brauns, *Am. Chem. Soc.*, 61: 2120, 1939.

[1] Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

[2] The author is indebted to Dr. P. Cundy, of the Analytical Department and Mr. J. F. McCoy, of the Bacteriological Department of the Institute, for carrying out the analyses.

[3] L. E. Wise and J. W. Appling, *Ind. Eng. Chem., Anal. Ed.*, 16: 28, 1944; 17: 182, 1945.

[4] L. E. Wise and F. C. Peterson, *Ind. Eng. Chem.*, 22: 362, 1930.

[5] F. C. Peterson, A. J. Barry, H. Ukauf and L. E. Wise, *Am. Chem. Soc.*, 62: 2361, 1940; and preceding papers.

[6] E. V. White, *Am. Chem. Soc.*, 64: 2838, 1942; and preceding papers.