Previous determinations of action spectra of phototropism in plants have led to the following generalizations: (a) the photoreceptor consists of one or more yellow pigments, since the response is limited to wavelengths shorter than 500 m\(\mu\), and (b) \(\beta\)-carotene and riboflavin are considered the most probable candidates for the photoreceptor, since they are universally present in plant tissues and their absorption spectra coincide reasonably well with the action spectra. The absorption spectrum of \(\beta\)-carotene in organic solvents shows a principal peak at 445, a smaller one at 475, and a shoulder at 420 m\(\mu\). These closely match the action spectrum obtained by Johnston (8) for Avena in which he found a principal peak at 440 m\(\mu\) and a secondary one at 475 m\(\mu\). Galston and Baker (7) obtained peaks at 450 and 470 m\(\mu\) and Bunning (3) at 445 and 480 m\(\mu\), with a slight shoulder about 430 m\(\mu\). Riboflavin has a single, broad absorption peak in the region of 445 m\(\mu\) and a near-ultraviolet peak at 370 m\(\mu\). Bunning (2, 3, 4) has proposed a dual pigment hypothesis in which \(\beta\)-carotene is a passive absorber which attenuates the radiant energy in its passage to the distal side of the coleoptile, while some other photochemically active pigment such as riboflavin is the actual photoreceptor.

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3. Research was supported in part by funds provided by the National Science Foundation Grant G-3514.
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5. Deceased, April 8, 1958.

In rapidly growing shoots, auxin is a limiting factor for cell elongation and, therefore, various hypotheses have been presented based on the photoxidation of indoleacetic acid or active auxin sites. In vitro, \(\beta\)-carotene is not a strong photosensitizing agent. On the other hand, riboflavin is a very strong photosensitizer. These considerations led Galston and Baker (7) to study the photoinactivation of indoleacetic acid in pea epicotyl breis. The spectral sensitivity of the inactivation had a broad peak at 440 m\(\mu\), with a sharp rise beginning in the near-ultraviolet. Because of instrumental difficulties, they were unable to extend the observations below 400 m\(\mu\). On the basis of these and other results, they proposed the possibility that a flavoprotein might be the photoreceptor system.

The principal criticism of any conclusions which are based upon the use of cellular macerates is that the structural organization of the cell is destroyed and pigments and substrates which might otherwise not be associated with one another may be brought together in close proximity and carry out completely abnormal reactions. This variable behavior of a photosensitizing pigment has been amply demonstrated in the case of the so-called “photodynamic action” of chlorophyll. Chlorophyll is well known for its role as the photoreceptor pigment in carrying out photosynthesis in the intact chloroplast. However, chlorophyll from tissue breis, in the presence of oxidizable substrates and oxygen, behaves as any other fluorescent pigment in accelerating substrate oxidation. The classic example is the hemolysis of the red blood cell (1).
The present study is an attempt to contribute to a resolution of the question of carotenoid versus flavin participation in phototropism by extending the action spectrum into the near-ultraviolet. Because of the relatively great difficulty of obtaining sufficient intensity with high spectral purity in the near-ultraviolet, none of the action spectra, except for those reported by Curry and Thimann (6), have been extended into the near-ultraviolet with any reasonable degree of resolution. Although both riboflavin and β-carotene have somewhat similar absorption bands in the blue, that for β-carotene in organic solvents continues to fall to a minimum in the near-ultraviolet, whereas that of riboflavin in aqueous solutions rises to a maximum at about 370 μm. Also, β-carotene has three bands in the visible while riboflavin has only one. These characteristics should make it possible to discriminate between β-carotene and riboflavin if either one or the other is acting solely as the active pigment in the phototropic system.

METHODS AND MATERIALS

IRRADIATION EQUIPMENT: The general plan of the irradiation equipment is given schematically in figure 1. The equipment was housed in a system of four cabinets containing: (a) the source and filters, (b) the grating monochromator, (c) holders for the Avena seedlings, and (d) source and holders for shadowgraphing. Various sources were tried in preliminary experiments, including both incandescent projection lamps and a 1 KW xenon arc. A modified automatic-feed, 1 KW cored-carbon projection arc produced from two to three times the intensity of the xenon arc in the near-ultraviolet. By careful adjustment, it was possible to obtain very satisfactory stability over the irradiation times that were used. All of the final work reported here was carried out with the carbon arc. The arc was of the reflector, axial-trim type operating at 60 volts, 15 amperes DC. The mirror was removed and the arc turned around and mounted at about 45 degrees to the optical axis so that the image of the crater of the positive carbon could be focused with a plano-convex quartz lens on the monochromator slit. The quartz lens was 7.5 cm in diameter and had a focal length of 10 cm.

Wavelengths longer than 625 μm were removed by an aqueous solution filter (F1) of hydrated copper sulfate (200 g/l, 5 cm pathlength). A second solution filter (F2) of 3 cm pathlength usually was used to further reduce scattered energy. This filter consisted of various dye solutions (11) as given in table 1. The composition was varied to accommodate the wavelength range in use.

The monochromator was designed and constructed in the laboratory shop. It was of relatively simple construction, consisting of a Littrow mounting with a double slit. The energy from the source entered the upper slit and the rays diverged to the parabolic off-axis mirror, M, which directed a parallel beam to the grating, G. The dispersed rays were then reflected back to the parabolic mirror and brought to a focus on the lower slit. The monochromatic energy was then reflected by the mirror, M', into the irradiation chamber. The grating was rotated by a sine linkage driven by a precision screw, coupled to a revolution counter. The length of the sine linkage arm was so adjusted that the revolution counter read directly in millimicrons. Calibration with a mercury arc spectrum demonstrated that the system could track with an accuracy of better than 1.0 μm throughout the visible and near-ultraviolet. The dispersing element was a Bausch and Lomb 100 × 100 mm, 600 lines per mm replica, plane grating, blazed for the near-ultraviolet. The linear dispersion (dl/dλ) was 3.0 × 10⁶ (0.30 mm/μm). The intensity of the emergent beam was controlled by adjusting the width of the double slit system and by varying the filter densities. The widths of the entrance and exit slits were varied from 1.0 to 4.0 mm, producing band widths of from 3 to 12 μm for the minimum and maximum intensities used.

A standard blue source (S4) was used to induce curvatures in a set of control seedlings to insure that the physiological behavior was the same for each

![Figure 1. Schematic diagram of monochromator and irradiation cabinets.]

- F1 — Aqueous CuSO₄ · 5H₂O filter (5 cm pathlength)
- F2 — Aqueous dye filter (3 cm pathlength)
- G — Plane grating (100 × 100 mm)
- L — Quartz condensing lens (6 cm diameter, 10 cm focal length)
- λ — Revolution counter (read directly in μm)
- M — Parabolic mirror (15 cm diameter, 1 m focal length)
- M' — Plane mirror (20 × 20 mm)
- N — Holder for seedlings and photographic film (5 × 6 × 25 cm)
- S — Carbon arc
- S1 — Standard blue source
- S4 — Green source for shadowgraphing

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TABLE I

Composition of Aqueous Dye Filters

<table>
<thead>
<tr>
<th>Wavelength Range (μm)</th>
<th>Concentration (mg/L)</th>
<th>Dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>350-390</td>
<td>50</td>
<td>New Fuchsin</td>
</tr>
<tr>
<td>400-450</td>
<td>170</td>
<td>Victoria pure blue BO</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Crystal violet extra pure APNX</td>
</tr>
<tr>
<td>460-480</td>
<td>60</td>
<td>Victoria pure blue BO</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>8-hydroxy-quinoline sulfate</td>
</tr>
<tr>
<td>490-520</td>
<td>170</td>
<td>Pieric acid</td>
</tr>
</tbody>
</table>

experiment. The source was a 75-watt, 120-volt projection lamp operating at 90 volts AC from an electronic voltage regulator. The beam passed through a diffusing glass plate, a 1.0-cm cell of 10% hydrated copper sulfate and a crystal-violet gelatin filter (11). This system isolated a band of wavelengths from about 400 μm to 500 μm.

A green source (S2) was used for shadowgraphing. This consisted of a small incandescent lamp with an image of the filament focused on a shutter with a condensing lens. A type B green safelight gelatin filter (12) transmitting from 520 to 580 μm was placed in front of the aperture. This system produced an intensity of relatively inactive green energy at the film strip of 70 μW/cm². An exposure of 10 seconds produced distinct images with Kodak ortho type II film. It was found that a 10-minute exposure to the green safelight did not produce any measurable curvatures in the Avena seedlings.

Irradiances above 1 μW/cm² were measured with a vacuum thermopile which was calibrated frequently against a standard lamp from the National Bureau of Standards. The thermopile had a calcium fluoride window and operated directly into a Liston-Becker type modulation amplifier. A strip chart recorder was used to record the deflections. While it was not essential that a recorder be used, it greatly facilitated the evaluation of thermal drift in the thermopile system during measurement. Irradiances below the range of the thermopile were measured with a vacuum photoelectric cell having an S-5 photocathode. The photocell was mounted in a small aluminum case containing a 45-volt heating-aid battery. The system was then connected with shielded cable to a DC electrometer amplifier (General Radio Type 1230-A). The photoelectric cell was calibrated at each wavelength with the thermopile.

Biological Material: Husked oat seed (Avena sativa, Victory 2020) were soaked one hour in distilled water and imbedded on the surface of 1.0% distilled water-agar slants in 10 x 75 mm test tubes. They were grown for 48 hours in a red irradiation cabinet at 10 μW/cm² (625 to 1000 μm) to inhibit elongation of the first internode and then transferred to a dark cabinet for 17 to 22 hours. Fifteen seedlings were rigorously selected over the safelight for uniform straightness and length of 20 ± 2 mm, and the tubes secured in wooden holders with rubber bands. Each holder contained five tubes for the blue irradiated controls and 10 for the monochromatic irradiation. The seedlings were mounted 13 mm apart at an average distance of 150 cm from the exit slit. The growing room, including the treatment cabinets, was maintained at 25.0 ± 0.5° C and 90 ± 5% relative humidity.

Procedure: The upper 5 mm of the 10 coleoptiles were irradiated for a constant exposure time of 30 seconds to the monochromatic energy variables. The upper 5 mm of the controls were exposed for one minute to the standard blue source which produced an average curvature of 25.0 ± 1.8°. The holders were irradiated at an angle of 45° to the respective incident beams (fig 1). This procedure introduced an irradiance variation for the seedlings at both ends of about ± 10% from the axial or mean value. Since the variation was the same in each experiment and the values were averaged, this factor was neglected in the calculations. After irradiation, the holders were rotated an additional 90°, placed in the adjacent cabinet, and shadowgraphed. This procedure made it possible to obtain shadowgraphs of the curvatures at right angles to the coleoptile bending plane without having to rotate the seedlings individually in the holder. Ninety minutes after the first exposure, a second shadowgraph was made on the same film. The angles of curvature were measured from the pair of shadowgraphs with a grid marked to the nearest degree. The curvatures given in figure 2 should be multiplied by cos 45° to obtain actual curvatures. Since this factor does not alter the slopes of the response-log energy curves or the relative values of the action spectra, it was not carried through the calculations.

Analysis of Data: The responsivity was determined from 350 to 520 μm at 10 μm intervals. In

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Fig. 2. Response-log energy curves for principal wavelengths. The abscissa is shifted one unit to the right for each wavelength. Probable errors are given for the responses at 370, 445 and 475 μm.
the vicinity of the peak regions, intermediate 5 mμ determinations were made. The average curvature of 40 to 60 seedlings was measured for four or five incident energies at each wavelength. The curvature, \( \theta \), in degrees of angle, was plotted against the logarithm of the incident energy \( E_i \) in nano-joules/cm\(^2\) (nano-joule = 10\(^{-9}\) joules/cm\(^2\)) from 3 to 30°. Regression lines were calculated by the method of least squares to give the best straight line fit, \( \theta = m \log E_i + b \). The slope and intercept constants, \( m \) and \( b \), are given in table II. The apparent threshold energy, \( E_0 \), is defined for each wavelength as the value of \( E_i \) when the curve is extrapolated to zero response, \( \theta = 0 \). The regression lines in figure 2 were drawn for only 9 of the 23 wavelength stations. The logarithmic scale of the abscissa is shifted one unit to the right for each wavelength to avoid superposition.

The action spectrum was plotted as the reciprocal of the moles of incident quanta, \( n' \), required at each wavelength to produce a given response. [Compare with quantum responsivity (10).] The action spectrum was calculated from the incident energy \( E_i \) in nano-joules/cm\(^2\) and the wavelength \( \lambda \) in mμ by the conversion factor:

\[
\text{Quantum activity} = \frac{1}{n'} = \frac{120 \times 10^9}{\lambda E_i} \text{ (cm}^2/\text{nano-einstein)} \quad (1)
\]

<table>
<thead>
<tr>
<th>WAVELENGTH Mμ</th>
<th>SLOPE, ( m ) ( / \log \left( \text{nJ/cm}^2 \right) )</th>
<th>INTERCEPT, ( b ) ( / \log \left( \text{nJ/cm}^2 \right) )</th>
<th>THRESHOLD ENERGY, ( E_0 ) ( / \log \left( \text{nJ/cm}^2 \right) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>350</td>
<td>14.9</td>
<td>-30.2</td>
<td>2.03</td>
</tr>
<tr>
<td>360</td>
<td>20.5</td>
<td>-40.8</td>
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</tr>
<tr>
<td>365</td>
<td>22.0</td>
<td>-43.5</td>
<td>1.98</td>
</tr>
<tr>
<td>370</td>
<td>22.7</td>
<td>-44.5</td>
<td>1.96</td>
</tr>
<tr>
<td>375</td>
<td>22.2</td>
<td>-43.4</td>
<td>1.95</td>
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<tr>
<td>380</td>
<td>21.4</td>
<td>-41.7</td>
<td>1.95</td>
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<tr>
<td>390</td>
<td>17.2</td>
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<td>1.92</td>
</tr>
<tr>
<td>400</td>
<td>12.9</td>
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<td>13.2</td>
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<td>1.76</td>
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<td>435</td>
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<td>17.5</td>
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<td>17.7</td>
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<td>470</td>
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<td>1.69</td>
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<td>475</td>
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<td>480</td>
<td>17.2</td>
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<tr>
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<td>14.4</td>
<td>-28.5</td>
<td>1.98</td>
</tr>
<tr>
<td>500</td>
<td>12.0</td>
<td>-27.5</td>
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</tr>
<tr>
<td>510</td>
<td>7.3</td>
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</tr>
<tr>
<td>520</td>
<td>6.2</td>
<td>-16.0</td>
<td>2.38</td>
</tr>
</tbody>
</table>

\( \theta = m \log E_i + b \), where

\( \theta \) = response (degree of curvature)
\( m \) = slope
\( E_i \) = incident energy
\( b \) = ordinate intercept
\( E_0 \) = \( E_i \) when \( \theta = 0 \)

**Results**

The action spectra obtained for 0, 5, 10 and 20° responses are given in figure 3. Maxima occur in the visible between 410 to 415, 440 to 445, and 470 to 475 mμ. A near-ultraviolet peak occurs at 370 to 375 mμ and is more evident the larger the response. The variable magnitude indicates some sort of interaction between two pigment systems. Separation cannot easily be made of the activity of the two pigment systems, flavin or carotenoid, based on the presence or absence of a near-ultraviolet peak. It also was observed that the slopes of the response-log energy curves (fig 2) are a function of wavelength and not a constant value as reported previously for phototropism in Avena (6). It is this change in slope with wavelength which is responsible for the variable height of the near-ultraviolet maximum.

**Discussion**

Phototropic curvature, caused by a unilateral stimulus of radiant energy, is the result of a difference in effect on the two sides of the coleoptile. This difference is produced by the attenuation of the incident energy in its transmittance through the coleoptile. The attenuation is due to three factors: 1) self-screening by the photoreceptor, \( A_p \), 2) neutral scattering, \( A_N \), and 3) foreign screening by inert pigments, \( A_F \). These factors can be grouped as the total absorbance of the coleoptile, \( A_T \), where \( A_T = A_p + A_N + A_F \). Absorption then produces a photoproduct, which indirectly controls cell elongation. The total amount of photoproduct depends upon the incident energy, \( E_i \), and the absorption coefficient of the photoreceptor, \( \beta \). Thus the magnitude of the difference in growth effect on each side (the response) is a function of three factors, the attenuation, the absorption coefficient of the photoreceptor, and the incident energy. This is the case, regardless of the secondary mechanism which actually regulates cell elongation, whether it is auxin redistribution, auxin...
inactivation or the destruction of an enzymatic locus necessary for auxin-regulated cell growth.

From the above considerations, a general equation describing the response can be written:

$$\theta = k A_T \left( \log E_1 + \log \beta \right)$$  \hspace{1cm} (2)

The response is proportional to the total amount of photoprotect formed and the attenuation of the energy across the tissue.

To obtain the photoreceptor absorption spectrum, the attenuation factor must be excluded. From equation 2, simply set $\theta = 0$ to get

$$\frac{1}{E_i} = \beta$$  \hspace{1cm} (3)

which is the defined action spectrum. Thus the action spectrum obtained for the threshold response will correspond to the photoreceptor absorption spectrum. The action spectra plotted from the energies required for larger responses are a mixture of effects due both to the multiplier $A_T$ and absorption coefficient $\beta$. The best procedure, theoretically, would be to measure the response over the range of the first and second positive curvatures and plot log $\theta$ instead of $\theta$ versus log $E_i$. Then for each wavelength a curve would be obtained which would be congruent with the curve obtained for every other wavelength. The attenuation factor could be precisely determined from the shift along the ordinate and the action spectrum from the shift along the abscissa. This was attempted with the present data, but it was impossible in practice to separate the two factors. Extending the measurements over the first and second positive curvatures would insure characteristic inflection points for precise comparison.

A plot of the slopes of the response-log energy curves is given in figure 4. The definite near-ultraviolet peak suggests that the curvatures produced in this region were primarily due to an increase in the attenuation. The maxima in the visible are the same as those shown in the action spectra and are presumably due to attenuation by self-screening of the photoreceptor.

A 2nd possible explanation of the 370 m$\mu$ peak is the presence of a fluorescent substance having high absorption in the near-ultraviolet. It would absorb energy and then re-emit it in the blue where it could be reabsorbed by the photoreceptor. It would thus not only increase the attenuation, but also increase the concentration of effective photoprotect. In support of this idea, we have observed that coleoptiles irradiated with high intensities of the mercury 365 line, several hundred $\mu W/cm^2$, appear to have small, irregularly shaped areas which fluoresce blue. These were observed visually in the section 0.5 to 1.0 mm below the tip with a long focal-length microscope. Attempts have been made to measure the magnitude of this fluorescence and how it is a function of the incident energy. Experimental difficulties of obtaining sufficient sensitivity while at the same time screening out all of the incident blue energy have prevented precise measurements. One of the degradation products of riboflavin, lumichrome, has a near-ultraviolet absorption peak and fluoresces in the blue. Galston and Baker (7) added lumichrome to sections of pea epicotyl for straight growth measurements, but could find no significant effects in the light. However, to obtain a 10° curvature, a difference in length of the two sides of the coleoptile of only 2.0 to 4.0% is needed. Differences of this order of magnitude would not have been detected in the straight growth type of experiment.

The three photoreceptor maxima obtained from the threshold action spectrum in the visible tend to disprove Binning's (2, 3, 4) hypothesis of a screening mechanism in which a flavin is the photoreceptor. If the flavin were the photoreceptor, the threshold curve would have had a smooth single maximum. Further evidence against the active participation of a flavin is the recent work of Mer (9) in which he could find no evidence which conclusively supports the in vivo photoactivation of auxin by a flavin.

Recently, Crane and Beinert (5) described an electron transferring flavoprotein (ETF) which has three maxima in the blue at 410, 438 and 460 m$\mu$ and a single broad peak about 370 m$\mu$. Two of the blue maxima are apparently due to the protein moiety, since the absorption spectrum of the flavin released from ETF by acid hydrolysis has a characteristic smooth single maximum in the blue. It was tempting at first to consider this as the photoreceptor on the basis of the action spectra for responses larger than 5°. However, the threshold curve again, with the absence of the near-ultraviolet peak, eliminates ETF as the probable phototropic receptor.

**SUMMARY**

Detailed action spectra of the first positive tip-curvature of Avena have been determined for 10 m$\mu$ steps from 350 to 520 m$\mu$. Steps of 5 m$\mu$ were determined in the peak regions. From the action spectra it is concluded that the active photoreceptor is a yellow pigment, probably carotenoid in nature, having absorption maxima at 410 to 415, 440 to 445, and 470 to 475 m$\mu$. A flavin photoreceptor appears unlikely, although a peak was observed in the near-ultraviolet.
at 370 m\(\mu\). Evidence is presented to show that these curvatures may be due to increased attenuation across the coleoptile tissue by an inactive secondary pigment having an absorption maximum at about 370 m\(\mu\).

The authors are indebted to Dr. Max Delbrück for his recent theoretical analysis of the phototropic response data presented in this paper.

We wish to thank Dr. Harland Stevens, U.S.D.A. Branch Experiment Station, Aberdeen, Idaho, for supplying the Avena seed.

LITERATURE CITED


EFFECT OF BICARBONATE ON THE ENZYMIC SYNTHESIS OF LONG CHAIN FATTY ACIDS\(^{1,2}\)

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The metabolic role of carbon dioxide in higher plants is complex. Photosynthetic carboxylation (11) as well as the incorporation of carbon dioxide into oxalacetate (6) and malate (10) have been studied extensively on an enzymatic level in plant materials. In addition carbon dioxide has been implicated in the regulation of several gross responses in higher plants. These effects include the induction of fermentation in aerobic tissues (2), regulation of photoperiodic induction (7), and the control of stomatal opening (3). Recently the inhibition of cytochrome \(c\) oxidation-reduction by carbon dioxide has been reported in castor bean mitochondria preparations (1).

We wish to report the requirement of bicarbonate for the synthesis of long chain fatty acids from acetate by a soluble enzyme extract prepared from avocado particle acetone powders. A similar effect has been reported by Wakil and coworkers in their soluble avian system (9).

The components required for the condensation of acetate to long chain fatty acids by the soluble avo-

cado enzyme preparation are listed in table I. By a combination of gas liquid chromatography and reversed phase kerosene paper chromatography the major product of the reaction has been identified as

![Graph](image)

**Fig. 1.** The effect of bicarbonate concentration on synthesis of long chain fatty acids. Conditions same as for table I, except KHCO\(_3\) concentration as indicated.

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1. Received May 9, 1958.
2. Supported in part by a grant from the National Science Foundation.