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THE ABSOLUTE LIGHT-SENSITIVITY AND SPECTRAL
THRESHOLD CURVE OF THE AQUATIC FLATWORM
DENDROCÆLUM LACTEUM

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Dendrocœlum lacteum is a limnadophil planarian with a simple and clearly defined directional response to a lateral light stimulus. Much experimental work has been done on the light responses of planarians from a behavioural point of view (Taliaferro, 1920; Ullyott, 1936; Viaud, 1950, 1951), but little attempt has been made to determine the relative thresholds for light of different wave-lengths, or to estimate the absolute threshold in energy units for light of the most effective wave-length. Viaud (1950) has done experiments on the relative effectiveness of lights of different wave-lengths in eliciting a response, but in quite different conditions and using brighter lights.

Besides the directional response to lateral light stimuli, *D. lacteum* also responds to vertical light by showing greater random mobility (Ullyott, 1936). This response is less convenient for experimental purposes, and only lateral stimuli were used for the determination of threshold responses in these experiments.

These light responses were presumably evolved as a defence against the planarian's natural enemies. If they are to be effective as such, the animal must have fairly high sensitivity. The 'eyes', however, are very small. It therefore seemed possible that the actual receptors, in spite of the simplicity of the eye, might prove to be highly efficient light detectors, with a threshold comparable to that of some of the vertebrates which have been studied, such as man, the cat, or *Xenopus*. Earlier work by Ullyott (1936) with *D. lacteum* suggested that the animals responded to a very dim light, but he did not work with narrow bands of the spectrum, and the estimate given for the absolute threshold was based on the energy of a white light of unspecified colour temperature estimated by a photocell. His estimate was about 2000 times higher than that given in this paper.

A brief communication giving some of these results has been published (Pirrenne & Marriott, 1955).

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METHODS

Dendrocoelum lacteum is a fresh-water planarian about 0.5–1 cm in length. It has two 'eyespot' about 0.08 mm in diameter at the anterior end. It is creamy white in colour, and was selected for these experiments for this reason, since it is easy to find on a dark background with a dim red electric torch—as will be seen *Dendrocoelum* is relatively insensitive to red light. The animals used came from Lake Windermere and were supplied by the Windermere Laboratory of the Fresh-water Biological Association.

The planarian eye consists of a small pigmented cup containing a number of light receptors. There is no image-forming apparatus, and the eye is probably used merely as a detector of light with directional sensitivity (Taliaferro, 1920).

D. lacteum responds to lateral light stimuli by moving away from the source.

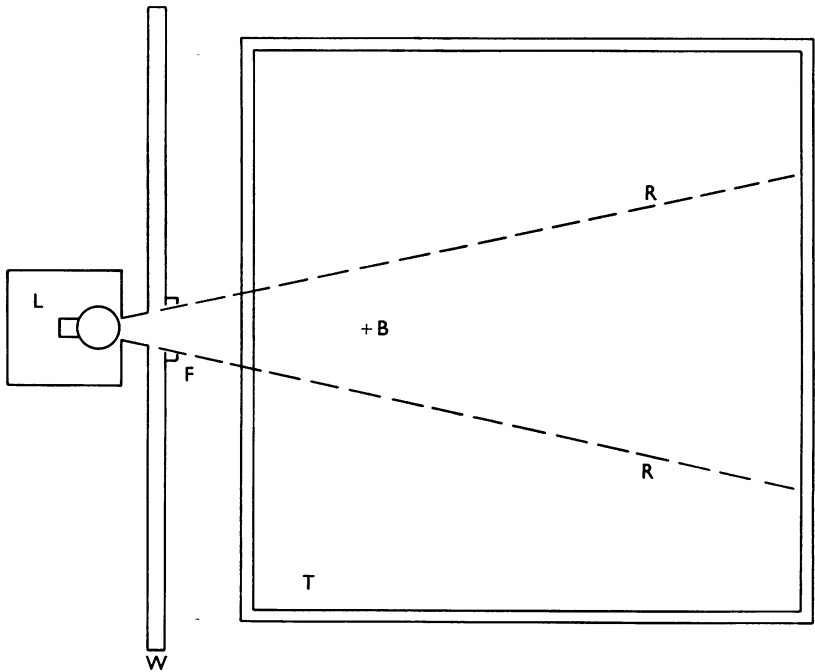


Fig. 1. Diagram of the apparatus used in the experiments. Light from the lamp L, mounted in a light-tight box, passes through two adjustable neutral wedges in the long case W, and a colour filter at F (only the wedge case and the filter holder are shown) into the Perspex tank T filled with filtered lake water. B is the point in the tank at which the experimental animal was dropped. RR are the approximate limits of the area of full illumination from the lamp L. The tank is 56 cm square.

Apparatus and experimental procedure. Up to thirty-six animals were used for each experiment. When not actually being tested, they were kept in filtered lake water in individual numbered beakers. For the experiment they were transferred one at a time to a Perspex tank illuminated from one end by a light source (Fig. 1). They were lifted on a camel-hair paint brush and dropped at a specified point near the lighted end of the tank. After 3 min they were found with a red torch and restored to their beakers. If they had moved 10 cm or more away from the light, within 20° either side of a straight line from the light, a response was recorded. Before the experiments,

all animals had been kept in complete darkness for at least 2 hr, and generally much longer. They had been starved for a considerable period before the experiments. The water temperature varied between 15.3 and 18° C.

The light source used in these experiments, an opal bulb of intensity 1.00 cd, and the arrangements for reducing the intensity by means of neutral wedges have been fully described elsewhere (Pirenne, Marriott & O'Doherty, 1957). In front of the wedges a colour filter could be mounted to isolate a narrow band of wave-lengths.

The colour filters used are listed in Table 1. Those marked Corning were combinations of Corning glass filters, the number (except for Corning 5113, which is the makers' number for a single glass filter) indicating roughly the mean effective wave-length. Those marked Ilford were gelatine filters mounted in glass issued by Ilford, and Wratten 88 was a Wratten gelatine filter issued by Kodak. The last three filters in this table were used in some preliminary experiments, but not in the main series.

TABLE 1

Filter	Mean effective wave-length (μ)	Energy transmitted (η_x)	Threshold wedge density (D_x)	Log threshold energy ($\log \bar{H}_x$)	Log $H_{2\lambda}$
Corning 0.365 μ	0.365	1.85	0	0.27	0.27
Corning 0.400 μ	0.400	2.21	0	0.34	0.34
Corning 0.435 μ	0.435	15.31	1.65	1.53	1.68
Corning 0.475 μ	0.475	8.49	1.85	1.08	1.04
Ilford 604	0.520	26.06	1.83	1.59	1.48
Corning 0.560 μ	0.560	120.33	1.80	0.28	0.21
Corning 0.600 μ	0.600	114.21	1.02	1.04	1.08
Corning 0.655 μ	(0.655)	(181.79)	(+0.5)	(2.76)	2.36
Wratten 88	(0.800)	—	—	—	—
Corning 0.515 μ	0.515	177.68	3.66	2.59	—
Corning 5113	0.420	41.95	2.82	2.80	—
Ilford 609	(0.700)	(289.74)	(+0.5)	(2.96)	—

Notes: The energies η_x and H_x are measured in arbitrary units. For the cut-off filters the estimates of the mean visually effective wave-length and of the energy transmitted, obtained as explained in the text, are given between brackets.

RESULTS

The threshold energy

All the calculations in this paper are based on the luminous intensity and colour temperature of a light source calibrated by the National Physical Laboratory, and on the optical density for lights of different wave-lengths of the colour filters, neutral wedges, and Perspex, also measured by the N.P.L. Light absorption by the water was negligible. A full description of the necessary calculations is given in Pirenne *et al.* (1957).

The threshold for any filter was taken as the level at which about one-third of the animals, or five out of seventeen in the large proportion of experiments in which seventeen animals were used, responded. Table 2 gives the results of seven experiments with the green filter Ilford 604. At the wedge setting 0.5 and 1.0, about half the animals responded; at 1.5 and 2.0, about one-third; and at 2.5 and 3.0 the response had practically disappeared. Accordingly, the threshold setting was taken as 1.75.

The exact value of the wedge density D_x , allowing for the non-neutrality

of the wedges and the slight deviations from the simple relationship between setting and density, was now obtained from the calibrations. In this case the exact value was 1.83.

The threshold density was similarly calculated for each filter used in the experiments. The threshold energy H_x , in arbitrary units, is then given by:

$$\log H_x = \log \eta_x - D_x,$$

where η_x is the energy with wedges out (Table 1), so that, for Ilford 604, $\log H_x = 1.42 - 1.83 = \bar{1}.59$. The quantities $\log H_x$ were used to obtain a first approximation to the sensitivity curve. The sensitivity at any wave-length is defined as the reciprocal of the threshold energy.

TABLE 2. Number of responses in various experiments using the filter Ilford 604 with different wedge settings

Approximate optical density (\log_{10} units)	Number of responses/number of trials
0.5	9/17
1.0	8/17
1.5	4/17, 4/11*
2.0	5/17
2.5	2/17
3.0	0/17

Approximate threshold density 1.75.

* Animals from 20 m deep.

Threshold values for different filters

Table 1 shows, for each filter, the energy transmitted, the wedge density for threshold, and the resulting threshold energy. The first column gives the identification number of each filter, the second column the mean effective wave-length transmitted. The third column gives the energy transmitted by the filter in arbitrary units. The figures in brackets in this column are for filters of the cut-off type, which transmit infra-red radiation. In these cases energy has been calculated only for wave-lengths up to 0.70 or 0.72 μ , to give a rough estimate of energy in the visually effective range. This does not affect the validity of the final approximation to the sensitivity curve.

The threshold wedge density is given in column 4. No animal ever responded, even with wedges fully out, to Wratten 88. Corning 0.655 μ and Ilford 609 produced responses from only a few of the most sensitive animals even with wedges fully out; it was estimated that about three times more light would be needed to reach 'threshold', as defined for these experiments.

The logarithms of the threshold energy H_x , defined as $\log \eta_x - D_x$, is given in column 5. The values are reasonably consistent for the main series of experiments, although two filters used in preliminary experiments, Corning 5113 and Corning 0.515 μ , gave surprisingly low thresholds.

The threshold energy discussed here refers, of course, to the lowest level at which the animal *responds*. This gives an upper limit to the threshold of

'vision', the lowest level at which a nervous excitation occurs. In all discussion relating to visual pigments it will be assumed that the thresholds measured correspond to the same amount of photolysis of a single visual pigment. This assumption seems reasonable for the very simple eye of *Dendrocœlum*.

The complete absence of response with Wratten 88 showed the relative insensitivity of the animals to infra-red radiation, and confirmed the absence of light leakage from the apparatus. Further, the result confirmed the low probability of false responses—apparent responses although the light was not detected—since no animal out of nineteen responded. In another experiment in which the filter was replaced by a piece of opaque cardboard, one animal out of twelve 'responded'. In another, using Ilford 604 with a wedge setting of 3.0, none out of seventeen responded.

The use of colour filters, rather than light from a monochromator, for experiments of this type is subject to the criticism that there may be unsuspected side bands, radiation transmitted at quite a different wave-length from the main spectral band, which may affect the response. Here all filters had been calibrated throughout the visible range. In view of the results with Wratten 88 and the other red filters, infra-red radiation was obviously unimportant. Further, the source emitted so little ultra-violet radiation that even if some filters had side bands in this region, the ultra-violet transmitted could have no appreciable effect after passing through the wedges. It will be noted that to reach the threshold for Corning 0.365 μ the wedges had to be moved completely out of the beam. At shorter wave-lengths the emission by the electric bulb becomes very low and the Perspex sheets absorb heavily. The possibility of unsuspected side bands in the visible was checked (Wald, 1945*b*); no significant bands were found.

Near the animals' threshold they could be seen in the water only with great difficulty, when the light happened to strike them at a suitable angle. The source, however, was clearly visible and appeared coloured, and light could be seen in the tank. This applied to all wave-lengths except the ultra-violet, where the animals are relatively much more sensitive than man. In this case, near the animals' threshold the lamp could be seen only with averted vision and appeared greyish and blurred; light could scarcely be seen in the tank.

The relative spectral sensitivity curve

To obtain a spectral sensitivity curve from data of threshold energies for a series of filters, it is necessary to estimate a first approximation to the curve, and then to improve it by further successive approximations. The calculations are described in Denton & Pirenne (1954). This method is capable of giving a very accurate curve, provided the wave-bands transmitted by the filters lie fairly close together, the true curve is a smooth one, and the thresholds are

accurately known. In the present experiments the thresholds are not accurately known; they are subject to errors of about 0.5 log unit. It is therefore not worth while attempting to estimate the curve with great accuracy, but the range of sensitivity between different parts of the spectrum is so great that it is possible to determine the general shape of the curve.

It should be realized that each experiment, at a single intensity, represented half a day's work, the determination of one point of the spectral sensitivity curve requiring several days. Close spacing of the intensity levels was thus not practicable.

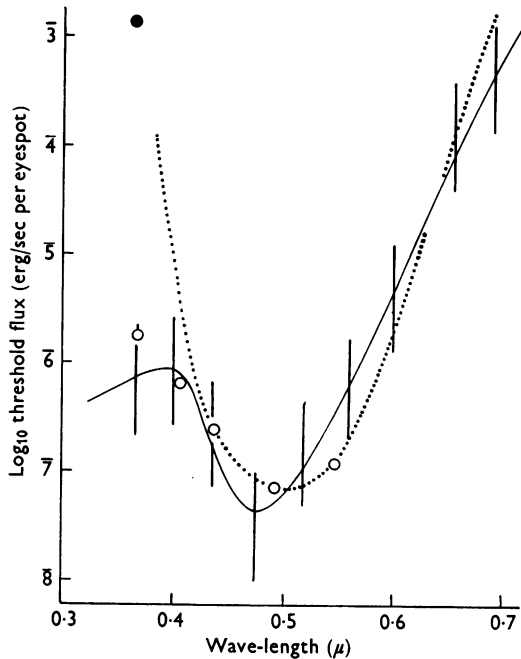


Fig. 2. The spectral sensitivity of *Dendrocaelum lacteum*. The vertical lines show the experimental results; the centre of each represents the calculated mean threshold H_x in erg/sec per 5.03×10^{-6} cm², taken as the area of the 'eyespot', and the ends plus and minus the approximate s.e. The solid curve is the final approximation to the response curve ($H_{2\lambda}$, see text). The dotted line is the C.I.E. human scotopic response curve (arbitrarily placed on the ordinate axis), and the black circle is an extension of this curve due to Wald. The open circles represent Wald's measurements of threshold for the human aphakic eye; except for the short wavelengths, these subjects have the same spectral sensitivity as normal subjects.

Fig. 2 shows the second approximation, $H_{2\lambda}$, plotted against λ . In this case, $H_{2\lambda}$ gave a very good fit, and no further approximation was necessary. Fig. 2 also shows the C.I.E. (*Commission Internationale de l'Éclairage*) human scotopic response curve, and points obtained by Wald (1945*a*) for the human aphakic eye—that is, for subjects whose lenses had been removed.

Biological variations

For each filter, experiments were done at intervals of 0.5 log unit. Roughly speaking, throughout the range in which some of the animals responded and some did not, an increase in luminance of 0.5 log unit gave two more animals responding in the experiments in which seventeen or eighteen animals were used. For example, as is shown in Table 2, for the filter Ilford 604 no animal out of seventeen responded at wedge setting 3.0, while nine out of seventeen responded at 0.5.

A full statistical analysis of the experimental data to obtain the standard error of the threshold estimate for any filter was not practicable, but it is fairly easy to get a rough idea of the accuracy of these estimates. It is quite possible that a threshold value might be in error by 0.5 log unit in either direction; an error of 1 log unit, however, is most unlikely. It seems reasonable, therefore, to say that the standard error of any point on the response curve $H_{2\lambda}$ is about 0.5 log unit. This estimate is on the conservative side; in fact the standard error is probably considerably less.

*The absolute threshold of response of Dendrocœlum lacteum
in terms of illumination*

The light source used was calibrated by the N.P.L. and found to give a candle-power, at the voltage at which it was run, of exactly 1.00 cd. The planarians had to move 10 cm away from the light before a response was recorded. At the mid point of this movement, when they had moved 5 cm, they were approximately 30 cm from the light source. The threshold values were taken at this point. The threshold illumination was defined as the illumination in the tank on a plane normal to the beam of light.

The absolute threshold is calculated by estimating first, the energy flux emitted by the light source, and then the proportion transmitted by the Perspex, the colour filter, and the neutral wedges at their threshold setting. This gives the threshold illumination in erg/cm².sec, and it is easy, if desired, to convert this figure to quanta of light of a wave-length corresponding to the maximum transmission of the filter.

Ilford 604 (see Tables 1, 2) was used for these calculations; the maximum transmission is at 0.52 μ , or a little above the estimate of the most effective wave-length. More experiments were done with this filter than with Corning 0.475 μ , and the value obtained with Ilford 604 is therefore a safer estimate.

The threshold illumination may thus be calculated as 2.95×10^{-3} erg/cm².sec, or 7.67×10^8 quanta/cm².sec. The planarian 'eyespot' has a diameter of about 0.08 mm. Thus the number of quanta per 'eyespot' per second at threshold is $\pi \times 4^2 \times 10^{-6} \times 7.67 \times 10^8 = 39\,000$.

The area of the planarian 'eyespot' is roughly that of a circle of diameter

16' of arc on the human retina, which would cover about 800 rods on the densest part of the retina. Such an area responds almost like that corresponding to a point source.

Experiments with eyeless animals

To interpret physiologically the value of the absolute threshold, it was necessary to determine how the response was mediated. Experiments with an allied species (*Planaria maculata?*) (Taliaferro, 1920) had shown that the animals possessed some skin sensitivity to light, but that the characteristic response depended on the two small 'eyespot' at the anterior end. To confirm that the 'eyespot' were, in *Dendrocoelum lacteum*, really the relevant receptor organs, it was necessary to carry out experiments on animals which had had their eyes removed.

These experiments proved rather difficult to carry out because the eyes regenerated in about 9 days. However, it was eventually shown that animals with eyes removed lose the power of responding, and that this power returns when the eyes regenerate. It seems, therefore, that it has been established beyond reasonable doubt that the response is mediated through the eyes.

DISCUSSION

The form of the response curve of Fig. 2 is similar to the sensitivity curves of the known visual pigments. The relative insensitivity to infra-red is a common feature of all such curves, and there are strong theoretical reasons why this should be so (Fick, 1879; Pirenne, 1951). There is a minimum energy for response about 0.475μ , the curve rises to rather higher values at the blue end of the spectrum, and to very high values at the red end. The curve given by Viaud (1950) was obtained under quite different conditions. The minimum of this curve is at 0.530μ , but the accuracy of the curve $H_{2\lambda}$ is not high, and it cannot be determined whether the difference in minima is a real one.

The C.I.E. human scotopic response curve is also shown in Fig. 2 for comparison. This curve extends down to 0.380μ . The black dot at 0.365μ is an extension of the curve based on the results of Wald (1945*a*). Bearing in mind the magnitude of the experimental errors, the two curves are similar in general shape for wave-lengths greater than about 0.450μ , but at the blue end of the spectrum there are very large differences. At 0.360μ in the near ultra-violet the difference in sensitivity, relative to the maximum sensitivity, is over 3 log units, or about six times the standard error. This difference is probably rather underestimated, owing to absorption of the ultra-violet in the glass of the bulb. The planarians are relatively much more sensitive than man in this part of the spectrum.

The relative insensitivity of the human eye to ultra-violet radiation is due largely to absorption in the eye media, and particularly in the yellowish

lens (Wald, 1945*a*; Stiles, 1948). Subjects whose lenses have been removed are much more sensitive to blue and near ultra-violet. Wald (1945*a*) carried out experiments on several such subjects, and obtained the points of the spectral response curve for the aphakic human eye which are shown in Fig. 2 (open circles). This curve is the same as the C.I.E. curve for wave-lengths above 0.52μ , but has lower values than the C.I.E. curve for the shorter wave-lengths.

Wald (1945*a*) points out that the relatively high sensitivity of many of the lower animals to ultra-violet radiation is probably due primarily to a difference in the absorption in the eye media, and not to a difference in the spectral sensitivity curve of the pigment involved, and it therefore seems reasonable to compare the results obtained with the curve for the aphakic human eye. In fact, this curve fits the data fairly well, nowhere differing from the experimental curve by more than about 0.5 log unit, which is within the limits of experimental error.

The absolute threshold energy at a wave-length near to the maximum sensitivity, as given above (p. 375), is 2.95×10^{-3} erg/cm².sec, or 7.67×10^8 quanta/cm².sec, which is roughly equivalent to 1.4×10^{-3} photopic lux. Now the illumination of the ground on a clear moonless night is about 3×10^{-4} photopic lux (Le Grand, 1948), or about one-fifth of the animals' threshold, to a rough approximation. Therefore it seems that the threshold of the most sensitive animals is of the same order as the illumination received from a clear, moonless, night sky.

Ulyott (1936) stated that the absolute threshold was 'usually less than 7 erg/cm².sec...'. It is not clear from his paper how this figure was obtained. White light was used, and the intensity was varied with a rheostat, so that the colour temperature also varied. Light intensities were measured with a photo-electric cell. Probably Ulyott's value corresponds to a genuinely higher intensity, since he followed the movements of the animals by eye, and does not mention having had any difficulty in so doing, but estimates made using a photocell in this way cannot be regarded as reliable.

The absolute threshold in man for a small source continuously exposed corresponds to about 100–150 quanta entering the pupil per second (Marriott, Valerie B. Morris & M. H. Pirenne, unpublished results; for earlier estimates, see Pirenne, 1956). For the planarian, the number of quanta per 'eyespot' per second at threshold is 39 000, as shown above.

Now there is a wide range of sensitivity among planarians, and the most sensitive animals would still respond 1 log unit below the average absolute threshold defined for these experiments. (In earlier experiments with a different filter, Corning 0.515 μ , transmitting over a rather wider wave-band with maximum transmission at 0.515 μ , three out of nine animals responded at an energy level 1 log unit lower; see Table 1.) Further, the diameter of the 'eyespot' used in the calculation is the diameter of the whole pigmented area.

The area of the aperture of the eye is considerably smaller—probably by about a factor of 2. In terms of the light flux entering the ‘eyespot’, or the eye, some of the experimental animals may therefore have reached a sensitivity about 1/20 that of man.

Consequently, it seems that the planarian eye, simple as it is, is not much less efficient as a detector of light than the human eye. Of course, the actual threshold in terms of the light source intensity is some 4 or 5 log units higher but this is mainly owing to the difference in pupil size. In some animals used in these experiments the retinal mechanism itself required only about twenty times as much light as in man for a response, and there may, of course, be even more sensitive animals with a threshold comparable on this basis to that of man.

SUMMARY

1. Experiments were carried out to determine the spectral sensitivity and absolute visual threshold of *Dendrocoelum lacteum*. The photonegative response to lateral light stimuli was used as an indication of visual sensitivity. The threshold, the level at which a certain predetermined proportion of the animals failed to respond, was taken as an inverse measure of spectral sensitivity. The absolute value of the threshold was determined for light near the wave-length at which the animals were most sensitive.

2. The following conclusions were reached:

- (a) The animals are insensitive to infra-red radiation.
- (b) The sensitivity curve for visible light is roughly similar to that of the human eye from which the lens has been removed. The animals are relatively more sensitive than man (with a lens) to the blue end of the spectrum.
- (c) The animals are relatively more sensitive than man to the near ultra-violet (about 0.360μ). The calculations in this region are not very reliable, but probably the threshold energy is rather over-estimated. The shorter ultra-violet radiation was not investigated.
- (d) The response is mediated through the ‘eyespots’.
- (e) The absolute threshold for green light corresponds to an illumination of 1.4×10^{-3} photopic lux, or about 39 000 quanta per ‘eyespot’ per second. This is an average value, and some animals are much more sensitive. The efficiency as light detectors of the ‘eyespots’ themselves is possibly little less than that of the human eye, but the animal requires a much higher illumination to respond on account of the very small size of the ‘eyespots’.

The experiments described in this paper were planned and started in collaboration with Dr M. H. Pirenne, now of the University Laboratory of Physiology, Oxford. We are deeply indebted to Mr H. C. Gilson, director of the Windermere Laboratory of the Freshwater Biological Association, for facilities to carry out the earlier experiments in his laboratory and for supplying animals for the later experiments. Later experiments were carried out in the Physiology Department, University of Aberdeen, and the University Laboratory of Physiology, Oxford. The apparatus was acquired through the support of the Medical Research Council and the Nuffield Foundation.

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