The method used for relabeling is essentially a repetition of the original process. After the unreacted fluorescein derivatives have been dialyzed away, the labeled protein is simply shaken overnight again with the proper amount of dried isocyanate. Since the protein solution is already in approximately 1 gram percent concentration, no more saline is added. However, buffer is again added (10 percent of the original volume of the globulin).

In our opinion, the main advantages to be obtained from the use of dried isocyanate in the manner described above are (i) that the isocyanate can be prepared centrally in either commercial or noncommercial laboratories and can then be sent out to smaller research or diagnostic laboratories for actual use, and (ii) that an antiserum can be made to stain more intensely by relabeling, without danger of loss of protein content as a result of denaturation.

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Mortality of Aquatic Insects and Fishes Caused by Use of Latex Tubing in Experimental Apparatus

A commonly used brand of latex rubber tubing was employed in the construction of apparatus for experiments on the dissolved oxygen requirements of certain aquatic insects under varying conditions of flow. A unit of the apparatus consisted of a glass tube through which water was continually recirculated by means of a small centrifugal pump. About 10 ft of ½-in. latex tubing provided the connections between the tube, a 5-gal bottle in which the dissolved gas content of the water was adjusted, and the pump. The test animals were held in the tube in a capsule of stainless-steel screen. The total volume of water in use at any one time was about 20 lit. During any one experiment there was no exchange of the water. Test water temperatures were about 20°C.

Table 1. Mortality associated with latex tubing in standing water tests.

Test No.	Animal	No.	Test conditions	Animals dead in 24 hr	48 hr	72 hr
1a	*A. pacifica*	10	Latex tubing		3	7
1b	*A. pacifica*	10	(control)		0	0
2a	*A. californica*	10	Latex tubing		5	6
2b	*A. californica*	10	(control)		0	0
3a	*L. reticulatus*	10	Latex tubing	0	0	5
3b	*L. reticulatus*	10	(control)	0	0	0
4a	*O. tshawytscha*	10	Latex tubing	10		
4b	*O. tshawytscha*	10	(control)	0		
5a	*O. tshawytscha*	10	Latex tubing	10		
5b	*O. tshawytscha*	10	(control)	0		
6	*A. californica*	10	Plastic tubing		0	0

In numerous trials it proved to be impossible, with the use of this apparatus, to keep more than a small percentage of the nymphs of *Acroneuria pacifica* and *Acroneuria californica* (Plecoptera) alive and in good condition, even under "control" conditions, for periods of several days. Usually, within 24 hours, some mortality occurred, and all or most of the surviving nymphs were in sluggish condition. Generally, in 2 or 3 days, about half of the nymphs were dead or moribund.

Subsequent testing of individual parts of the apparatus for harmful effects on the nymphs indicated that only the latex tubing could be responsible for the trouble. Confirmatory tests were conducted, in 1-gal glass jars, in which the two species of *Acroneuria*, fingerling king salmon (*Oncorhynchus tshawytscha*), and immature guppies (*Lebistes reticulatus*) were used. In these experiments a piece of latex tubing several feet long was coiled in the test jars filled with water. The animals were then introduced into the test water, which was kept well aerated and in continuous circulation by rising air bubbles. Appropriate controls were used. As Table 1 shows, no losses occurred among the control animals, but in all cases there was considerable mortality among the test animals, of all species, in the jars that contained the tubing. A piece of plastic tubing was used in a similar jar test. No deaths occurred, and the stonefly nymphs used appeared to be normally active after 3 days.

When about 1500 ml of water per minute flowed continuously and without recirculation or reuse through 10 ft of the latex tubing and into a glass tube containing stonefly nymphs, no difficulty was experienced in keeping the animals alive and in good condition for several days.

As a result of these experiments, the original apparatus was reconstructed and the plastic tubing, which had been found to be apparently harmless, was used; in the course of tests performed thereafter, with this apparatus, no unexpected mortality of stonefly nymphs occurred.

Although widely used and apparently entirely suitable for most experimental purposes, latex rubber tubing evidently should be used with caution in experiments involving the recirculation of water in closed systems where there is no continuous exchange of water and where species of animals sensitive to the constituents of the tubing are utilized. Even though mortality might not result from such use of the tubing, the results of sensitive physiological tests might be markedly influenced.

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Synthesis of "On-Off" and "Off" Responses in a Visual-Neural System

The most distinctive feature of the discharge of impulses in the vertebrate optic nerve in response to a light stimulus is the marked activity elicited by changes in the level of illumination. The early records of Adrian and Matthews from the whole optic nerve (*1*) demonstrate a strong burst of activity when the light is turned on, a continuing discharge at a lower rate as long as the light remains on, and, upon the cessation of light, a renewed burst which gradually subsides.

Hartline (*2*) has shown that this composite response results from individual fibers whose activity differs markedly: some fibers discharge regularly as long as the light shines; others discharge only briefly when the light is turned on and again when it is turned off, with no activity during steady illumination; still others respond only when the light is turned off. These complex responses, observed in third-order neurones, have been ascribed by Hartline (*2*) and Granit (*3*) to the excitatory and inhibitory interactions of retinal structures interposed between these neurones and the photoreceptors rather than to special properties of the photoreceptors themselves.

The reasons for this interpretation are many. One reason derives from the nature of these "transient" responses. Hartline (*4*) found that, in the eye of the frog, an "off" response elicited in a single fiber by illuminating one group of receptors may be suppressed by illuminating another group of receptors in the same receptive field, and Barlow (*5*) has obtained a similar suppression using stimuli outside the receptive field. Also, Kuffler (*6*) has shown that, in the cat, these diverse response "types" are labile and that, depending on the locus of illumination and the level of background illumination, a particular fiber may exhibit a variety of responses. Furthermore, the fibers arising directly from the ommatidia in the lateral eye of the invertebrate, *Limulus,* do not exhibit the diversity of response found in the vertebrate nerve. Although "off" responses have been found in the *Limulus* optic ganglion (Wilska and Hartline, *7*), the typical response of *Limulus* optic nerve fibers to stimuli of long duration is a sustained discharge while the light is on and an immediate cessation of impulses when the light is turned off (Fig. 1*A*).

The relatively simple discharge pattern typically observed in fibers of the optic nerve of *Limulus* may be greatly modified by various means. First, a pronounced afterdischarge may be obtained by proper control of exposure time, intensity of the stimulus, and state of adaptation. Second, the frequency of the discharge of impulses may be decreased by illuminating neighboring ommatidia; this inhibition seems to be mediated by a plexus of lateral interconnections immediately behind the ommatidia (Hartline, Wagner and Ratliff, and Hartline and Ratliff, *8*).

By combining these various influences on the discharge of impulses, "on-off" and "off" responses have been "synthesized" in individual fibers of the *Limulus* optic nerve (*9*). Examples are shown in Fig. 1*B*. These responses possess the properties of the analogous responses in the vertebrate eye. The "on-off" responses are characterized by a burst of activity when the light is turned on, no further activity as the light stays on, and a final burst of activity after the light is turned off. The pure "off" responses also have the properties of the vertebrate response: no discharge appears until after the light goes off, and the discharge may be inhibited by reillumination. Both types of response have been obtained over a considerable range of stimulus durations.

A very delicate balance of excitatory and inhibitory influences is required to obtain these "on-off" and "off" responses in the optic nerve fibers of *Limulus*. The responses are not obtained under ordinary conditions of stimulation and have never been obtained when only one ommatidium is stimulated. From what we know of the properties of the inhibitory effect and of the temporal characteristics of the afterdischarge, we view the "on-off" response as occurring in the following manner: the onset of light stimulates several ommatidia, one of which gives rise to the fiber whose activity is being recorded. A few impulses are discharged in this fiber before the inhibitory influences from the neighboring elements completely suppress its activity. This suppression continues until the light is turned off. If the duration of the stimulus and the relative states of adaptation of the various receptors are adjusted so as to enhance the afterdischarge of the inhibited fiber and to minimize the afterdischarge of the neighboring elements, an "off" response is obtained. To obtain a pure "off" response the latency of excitation of the element whose activity is being recorded must be longer than the latency of the inhibition exerted upon it. This can be achieved by adjusting the angle of incidence of the light so that the element whose activity is being recorded is stimulated less effectively than are the neighboring elements which inhibit it.

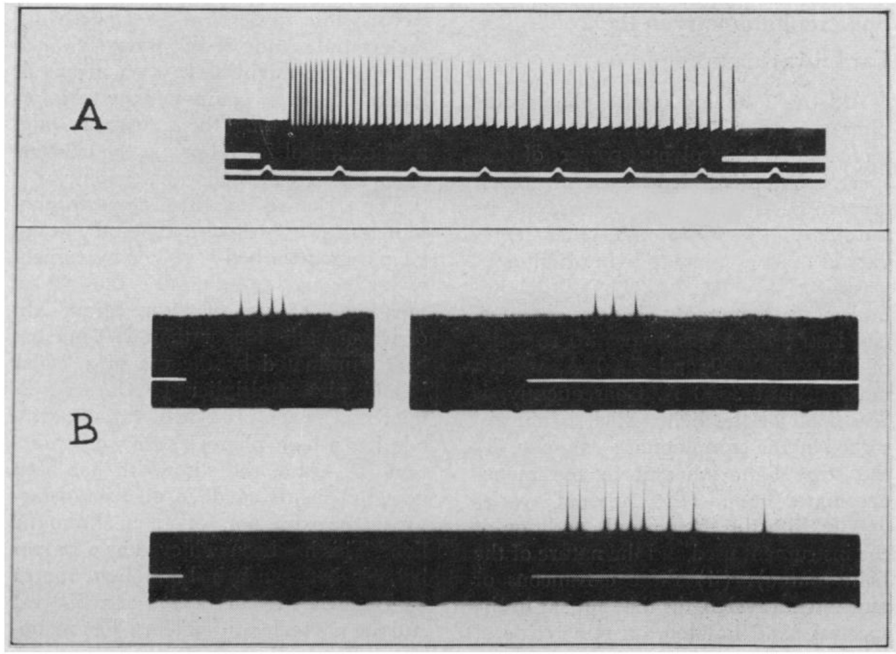

Fig. 1. Oscillograms of diverse "types" of impulse discharge patterns in single fibers of *Limulus* optic nerve. *A,* Typical sustained discharge in response to steady illumination. *B,* Upper record; a synthetic "on-off" response (approximately 1 sec was cut from the middle of this record). Lower record; a synthetic "off" response. Time is marked in 1/5-sec periods. Signal of exposure of eye to light blackens the white line above the time marker. Fibers whose activity is shown in the two *B* records gave a sustained discharge like that shown in *A* when the ommatidia from which they arose were illuminated alone.

The consequences of these experiments are twofold. (i) They lend support to the view that "on-off" and "off" responses are the result of the complex interplay of excitatory and inhibitory influences by showing that the experimental manipulation of these influences can, indeed, yield such transient responses; and (ii) they show the feasibility of using the *Limulus* preparation in the further study of these transient responses. This preparation offers distinct experimental advantages over the vertebrate preparation because the excitation and inhibition of individual receptor units may be easily and independently controlled and measured.

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