in mice after prolonged immunization with BSA are almost certainly different from those associated with acute transplantation disease; moreover, quite different chemical and biological tests with a purified protein antigen and complex isoantigens, respectively, are compared.

Although current thinking is dominated by the inferential assumption that a sequence, lymphocyte → plasmocyte → humoral antibody, affects nearly all immune reactions, we are attracted to the hypothesis that cellular immunity—whether enzymic or referable to a separate class of endoantibodies—is associated with small lymphocytes. Other recent studies (*8*) suggest that lymphocytes and histiocytes function as specifically immune cells under conditions where serum antibodies alone are ineffective or actually interfere with cellular immunity. Since functional heterogeneity in populations of small lymphocytes is apparent (*2, 9*), it is probably erroneous to equate function with cell morphology as such. Moreover, small lymphocytes appear to be deficient in cytoplasmic organelles such as lysosomes that could be expected to cause the digestion or dissolution of antigen or other cells. Thus the supposition that a small lymphocyte has to turn into something different before it engages in immunological responses continues to be attractive (*10*).

DONNA L. VREDEVOE*
W. H. HILDEMANN†
Department of Medical Microbiology and Immunology, University of California, Los Angeles

References and Notes

1. W. H. Hildemann, W. D. Linscott, M. J. Morlino, *Ciba Foundation Symp. on Transplantation* (Churchill, London, 1962), pp. 236–263.
2. J. L. Gowans, *Ann. N.Y. Acad. Sci.* **99**, 432 (1962).
3. R. E. Billingham, *Science* **130**, 947 (1959); M. Simonsen, *Progr. Allergy* **6**, 349 (1962).
4. W. H. Hildemann, *Transplantation*, in press.
5. D. L. Vredevoe, in preparation.
6. R. S. Farr, *J. Infectious Diseases* **103**, 239 (1958).
7. N. A. Mitchison, *Symposium on Mechanisms of Immunological Tolerance* (Czechoslovak Acad. Sci., Prague, 1962), pp. 245–255.
8. G. D. Snell, H. J. Winn, A. A. Kandutsch, *J. Immunol.* **87**, 1 (1961); J. S. Najarian and J. D. Feldman, *Ann. N.Y. Acad. Sci.* **99**, 470 (1962); L. J. Journey and D. B. Amos, *Cancer Res.* **22**, 998 (1962).
9. M. Holub, *Ann. N.Y. Acad. Sci.* **99**, 477 (1962).
10. Supported in part by research grant CA-04027 from the National Institutes of Health and by contract Nonr-233-(68) with the Office of Naval Research.
* Present address: Department of Medical Microbiology, Stanford University, Stanford, Calif. † Present address during sabbatical leave: Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

12 July 1963

Isolated Mammalian Eye: A Method for Quantitative Evaluation of Autonomic Drugs

Abstract. *The enucleated rodent eye can be mounted for study in vitro, in a chamber that contains electrodes suitably placed to enable the severed postganglionic nerves to the iris to be stimulated. The technique provides a simple quantitative method for the study of drugs which act at the parasympathetic neuro-effector junction.*

For many years the iris has been used as a pharmacological tool in the analysis of drug action at the autonomic neuro-effector junction. Studies of the mammalian eye in vitro have been confined to experiments with isolated excised iris, iris strips, or iris segments (*1*). In numerous other studies, the pupillary diameter of the eye has been measured *in situ* after local or systemic administration of a drug (*1, 2*). While the results of these experiments have been informative, the isolated iris is relatively fragile, not easily prepared, and its response is not easily determined quantitatively.

Recently, a simple method was described by which drug effects can be evaluated quantitatively by measuring the pupillary diameter of intact eyes enucleated from either the rat, mouse, or guinea pig (*3*). The reactivity to drugs of this pupillary neuro-effector system in vitro was found to be remarkably stable and reproducible. While the technique is applicable to adrenergic drugs, it offers particular advantages in assessing drug action at the parasympathetic neuro-effector site. It is free of the interpretive problems inherent in the use of such systems as the isolated gut with its included ganglion cells and intrinsic innervation; and it overcomes the objection to methods such as measurement of blood pressure, contraction of the urinary bladder, or salivary secretion in the intact animal, where the significance of results is compromised by uncertainties relative to varying drug distribution, drug elimination, and homeostatic mechanisms. It provides a simple quantitative method that permits maintenance of drug concentration, preserves the integrity and reactivity of the mammalian parasympathetic pupillary mechanism, and eliminates extraneous stimuli. This method has now been extended to include electrical stimulation of the postganglionic parasympathetic and sympathetic nerves which control pupillary size.

As before, the animals were killed and the eyeballs were enucleated with blunt curved surgical scissors, care being taken to separate the retrobulbar structures cleanly from the eyeball. In this way, the removal of the ciliary ganglion was assured and most of the ganglion cells that might have been situated along the course of the short ciliary nerves were excluded. Consequently, it is reasonably certain that electrical stimulation of the posterior half of the eyeball almost solely excited the postganglionic nerve fibers. Mice of strain C57 were selected because the pigmentation of the iris enables the diameter of the pupil to be observed and measured. The eyeballs were mounted in appropriately sized sockets in the base of a Lucite chamber designed to fit the mechanical stage of a microscope. The chamber contained 20 ml of Krebs-Ringer solution, buffered with bicarbonate and bubbled with a finely dispersed mixture of 95-percent O_2 and 5-percent CO_2, which maintained the *p*H at 7.4. The temperature was kept at 37°C by means of a heating lamp controlled by a mercury-in-glass thermoregulator dipped into the chamber.

The eyeball holder was a modified form of the previous design, each socket being provided with two silver electrodes, one in the form of a button touching the most posterior part of the eyeball, the other a ring in contact with the equator of the eyeball (Figs. 1 and 2). These electrodes were connected, through terminals on the outside of the chamber, to a square wave stimulator.

After a 30-minute stabilization period, pupillary diameter was determined with a microscope containing a micrometer disk in the ocular. The measurement was repeated after electrical stimulation, or after replacement of the plain Krebs solution with fresh Krebs solution containing a drug, or after both. The "response" was the ratio of the diameter at the end of a period of

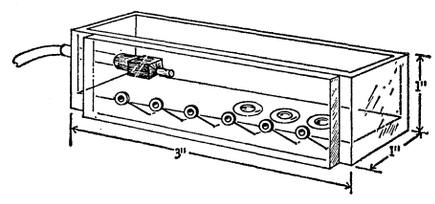

Fig. 1. Diagram of Lucite chamber. The gas was dispersed by passing it through a section of swab stick inserted in the drilled-out hub of a hypodermic needle.

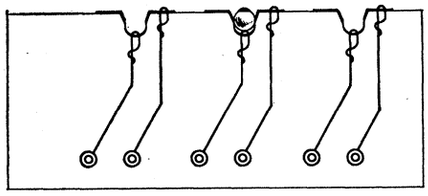

Fig. 2. Schematic view of sockets of a Lucite chamber with associated electrodes. The terminals were connected to the sockets with braided, stainless steel, surgical suture, running in milled channels (subsequently filled with epoxy plastic) in the bottom of the chamber. The silver electrodes were composed of repeated layers of silver printed-circuit ink.

electrical stimulation or after a 30-minute period of drug exposure to the control diameter.

Stimulation of the preparation produced constriction of the pupil. The maximum response was obtained with 8 volts or more, measured between the electrodes. Low frequency stimulation, 1 to 3 pulses per second, produced twitch-like contractions of the sphincter. Frequencies above 3 per second produced a sustained contraction, the degree of which was proportional to the frequency up to a maximum response at 20 pulses per second. In the maximum response, the diameter of the pupil was constricted to 50 percent of that in the control; such a response was achieved in 3 to 5 seconds, and was maintained for the duration of stimulation. Repeated periods of stimulation every 15 minutes for 7 hours decreased the average diameter of the control by 14 percent, and increased the average response by 28 percent. These changes were approximately linear with time over the 7-hour period, indicating excellent stability. Periods of stimulation could be repeated every 4 to 5 minutes, producing similar responses and allowing ample time between each period for return to the initial pupillary diameter of the control. Neither varying the pulse duration from 0.1 msec to 5 msec, nor reversing polarity of the electrodes, had any effect on the response. The routine stimulus parameters adopted were: duration of stimulation, 5 seconds; pulse duration, 0.1 msec; frequency, 20 pulses per second; voltage measured at electrodes, 12 volts.

The effects of various prototypic drugs were as would be expected in a parasympathetic neuro-effector preparation stimulated through the postganglionic nerves. Atropine sulfate ($1.28 \times 10^{-6}M$) not only blocked the constrictor response, but many experiments showed a slight but significant dilation on electrical stimulation. Presumably, this was due to an unmasking of the effect of concomitant sympathetic stimulation which was normally concealed by the preponderance of constrictor strength in the mouse iris. The adrenergic blocking agent phenoxybenzamine hydrochloride ($6.4 \times 10^{-7}M$) abolished this dilation after atropine, and in the absence of atropine, intensified the stimulus-induced constriction by blocking the physiological antagonism of the pupillary dilator muscles.

Physostigmine, in a dose ($1.28 \times 10^{-6}M$) which had no effect on the resting diameter of the pupil, caused the iris to be sensitive to stimulation of the parasympathetic nerves, increasing the constrictor response from 80 to 53 percent of the diameter in the control. Cocaine hydrochloride ($1.28 \times 10^{-3}M$) blocked the pupillary constriction produced by nerve stimulation, but not that produced by the action of the parasympathomimetic drug carbamylcholine. The ganglionic-blocking drugs hexamethonium ($6.4 \times 10^{-3}M$) and tetraethylammonium ($1 \times 10^{-2}M$) had no effect on the preparation (*2*; *4*).

WILLIAM T. BEAVER

Department of Pharmacology, Cornell University Medical College, New York 21

References and Notes

1. See review by F. Poos, *Ergeb. Physiol. Biol. Chem. Exptl. Pharmakol.* **41**, 882 (1939); I. Lowenfield, *Doc. Ophthalmol.* **12**, 185 (1958).
2. P. Pulewka, *Arch. Exptl. Pathol. Pharmakol.* **168**, 307 (1932); H. R. Ing, G. S. Dawes, I. Wajda, *J. Pharmacol. Exptl. Therap.* **85**, 85 (1945); D. R. Bennet, D. A. Reinke, E. Alpert, T. Baum, H. Vasquez-Leon, *ibid.* **134**, 190 (1961).
3. W. T. Beaver and W. F. Riker, *ibid.* **138**, 48 (1962).
4. Supported by U.S. Public Health Service graduate research training grant 2G-99.

6 June 1963

Cortisol from Human Nerve

Abstract. *Cortisol was found in myelinated nerve fibers of the lumbosacral plexus (2.0 to 6.0 μg per gram of tissue) and in the sympathetic chain with dorsal root ganglia (0.2 to 0.4 μg per gram of tissue).*

Peripheral nerve tissue, spinal cord, and brain are known to contain large quantities of cholesterol (*1*). Little is known of other possible steroids in these tissues. We have now found that nerve tissues contain relatively large amounts of cortisol (17-hydroxycorticosterone).

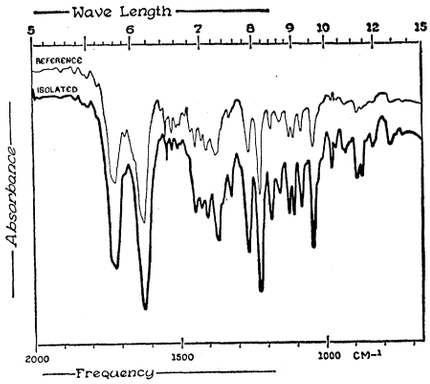

Fig. 1. Infrared spectrum of cortisol isolated from nerve. The acetate was incorporated into a 1.5-mm disk of potassium bromide.

Myelinated nerve fibers of the lumbosacral plexus and the thoracolumbar sympathetic chain which included unmyelinated fibers and dorsal root ganglia were obtained at autopsy. After an average post-mortem period of 10 hours, the tissues were homogenized in a blender with water. After the protein was precipitated with four volumes of acetone, in a manner described for adrenal tissue (*2*), and filtered off, the acetone solution was concentrated by evaporation and the aqueous residue was extracted with a mixture of chloroform and ethyl acetate (1:1); the extract was washed with 5-percent sodium bicarbonate and water. The organic solvents were removed by evaporation and the residue was partitioned between 70-percent methanol and heptane for removal of fats. The 70-percent methanol solution was concentrated by evaporation and the residue was dissolved in chloroform; this chloroform solution was dried over sodium sulfate and the chloroform was evaporated. The residue was subjected to paper chromatography in a toluene–propylene glycol system for three days. The product eluted from the paper chromatogram showed properties identical to those of reference cortisol as follows: an ultraviolet light absorption maximum at 240 mμ; a positive reaction with the blue tetrazolium reagent, specific for the alpha ketol group; absorption spectrum of the Porter-Silber phenylhydrazine reaction product showing a maximum at 410 mμ; the characteristic mobility of the acetate on the paper chromatograms developed in the toluene–propylene glycol system; and the absorption spectrum in sulfuric acid showing maxima at 240, 284, 390, and 475 mμ.