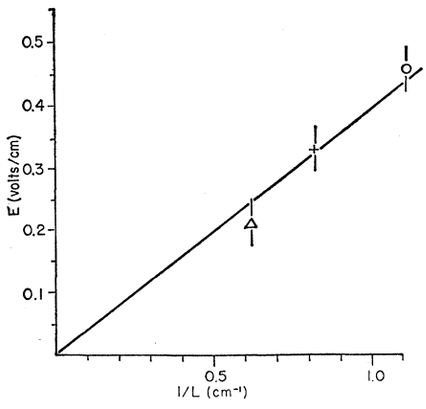

Fig. 2. Threshold field intensity as a function of the reciprocal of the length of the planarian. The theoretical curve was obtained with Eq. 7. Experimental points were computed from the data of Fig. 1.

The results are shown in Figs. 1 and 2. The data show satisfactory agreement with the predictions of the theory.

Although in the present discussion and study only two major size classes have been delineated, there probably are other neurons of intermediate length. Experiments were performed (*8*) in which a single, monophasic, square-wave pulse was used as the stimulus. The pulse duration was set at various values, and the kind of behavioral response to a pulse with the amplitude adjusted to response threshold was observed. Pulses of short duration evoked a small local contraction restricted to the head region. Pulses of intermediate duration evoked contraction of the anterior quarter of the planarian. Longer pulses evoked a longitudinal contraction of the entire planarian.

The assumption that these contractions were the result of excitation of the neurons of the affected region by the stimulus pulse permits another test of the theory. Since the values for D for the various ions which might plausibly be involved in excitation are all approximately 10^{-5} cm^2/sec, values for the chronaxies, t_c, to be anticipated for various neuronal lengths can be calculated from Eq. 5. For lengths of 2, 6, 10, and 50 μ the values of t_c would be 0.35, 3.1, 8.8, and 219 msec, respectively. In a general way these values correspond to the neuronal lengths predominating in the regions which contracted.

The correspondence between the quantitative predictions of the theory and experimental results suggest that the theory is essentially correct. The theory predicts also that the sets of neurons subjected to excitation depend in a critical, and known manner on the waveform of the electrical stimulus used as the unconditioned stimulus. In our experiments the pulse trains employed had a pulse separation equal to pulse duration. Although this particular pulse-train configuration was more or less arbitrary as far as testing the theory is concerned, it was chosen for comparability with the Barnes and Katzung experiments (*4*), in which a similar waveform, but different frequency, was employed. The lengths of the planarians employed in their study was not reported but, on the basis of the intensities of electric field which they used as stimuli and the results of the present study, it would appear that their subjects must have been much smaller than either our own or those normally used. Therefore, the distribution of neuronal sizes would be different, and consequently the pattern of primary excitation produced by the same stimulus frequency and waveform would be different. The discrepancy between their original results and those later obtained in Calvin's laboratory (*3*) might be accounted for on such a basis. The situation is analogous to higher-animal experiments in which intracranial stimulation is the reinforcement, except that here the relations between waveform and distribution of neuronal sizes are functionally analogous to electrode placement in determining which regions of the central nervous system will be excited. Whether a particular laboratory obtained the reported behavioral modifications or not would thus have depended upon their more or less fortuitous choice of stimulus waveform in relation to planarian size. The Harvard inductorium has probably led to the most consistent success precisely because its waveform is "dirty"—that is, it contains a mixed-frequency spectrum. For shooting in the dark at an unknown target a shotgun is better than a rifle. Learning in a planarian, as in any other animal, probably entails activation of some specific subset of neurons in a specific temporal order. In the absence of certain knowledge about this requisite subset and order, the probability of producing them is improved, though only inefficiently, by activating a variety of neuronal subsets in a multiplicity of firing orders.

JAY BOYD BEST
ERROL ELSHTAIN
Department of Physiology, Colorado State University, Fort Collins

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Catecholamines in Human Plasma

The data on catecholamines and consequently the conclusions in the report by J. F. O'Hanlon, Jr. (*1*), are open to question for the following reasons:

1) The indicated values for plasma noradrenaline (NA) and adrenaline (A) in his subjects are about 8 and 2 times as high, respectively, as the values now accepted as normal (*2*). In fact, the NA values are in the range which we have seen only in patients with pheochromocytoma and neuroblastoma.

2) The validity of the method used for the analyses of the catecholamines has been seriously questioned (*3*), the 1959 rebuttal by Weil-Malherbe (*4*) and Manger (*5*) notwithstanding. In fact, Weil-Malherbe, whose method O'Hanlon used, now recognizes that his previously reported values for plasma catecholamines in humans were much too high, and has modified his method with a additional resin treatment of the aluminum oxide extract. As a consequence, the values he now reports are in the accepted normal range. The points are emphasized by Weil-Malherbe himself in the publication (*6*) cited by O'Hanlon. Until about 5 years ago there may have been some question about the upper limit of catecholamines in normal human plasma. Sufficient data are now available, however, including those of Weil-Malherbe (*6*), to show that this figure is less than 1.5 μg/liter for the combined values of NA (not more than

1.0 μg) and A (not more than 0.5 μg).

3) In Figs. 1 and 2, O'Hanlon purports to show a change in one direction of endogenous A which is accompanied by a compensatory change in the opposite direction of endogenous NA. Thus, the circulating plasma catecholamines add up to approximately the same amount at each of the indicated points. This is a phenomenon which I have not seen in several hundred of our own analyses—done with a trihydroxyindole method (*7*)—in patients under conditions ranging from presurgical stress to electroshock therapy; neither am I aware of any other such data in the literature (excluding drug effects and pathology). Changes in the same direction of both amines or a change in one with little or no change in the other are more likely to occur. Furthermore, in numerous reports in the literature on the effect of a variety of stress-inducing situations on urinary catecholamines in man and laboratory animals over a time period similar to that used by O'Hanlon, there is usually an increase in both or one of the amines. Von Euler (*2*) has pointed out that "it appears highly improbable that increased urinary excretion rates of A and NA are associated with lower plasma levels. Any findings to this effect would call for a revaluation of the techniques used for the determinations."

4) The catecholamine data in the figures, particularly Fig. 2, are misleading in that there is no indication in the curves or the accompanying legends as to the range of the individual determinations at each point, or which of the values are significantly different from those of the controls. It is pointed out in the text that certain of the adrenaline values in the experimental subjects are significantly different from those of the controls. However, no such difference was found in the case of noradrenaline, and yet a similar type of curve is shown for NA as for A. It seems to me that tables and figures should contain some indication of the significance of the data, especially where curves and numbers may give the impression of a difference when in reality there is none.

A. H. ANTON

College of Medicine,
University of Florida, Gainesville

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3. See the papers cited by von Euler (*2*), for example, Holtzbauer and Vogt, 1954; Price, Linde, and Price, 1960; and Zileli *et al.*, 1958.
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5. W. M. Manger, *ibid.*, p. 289.
6. H. Weil-Malherbe, in *Methods in Medical Research*, J. Quastel, Ed. (Year Book, Chicago, 1961), pp. 130–146.
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1) The "normal" concentrations for plasma adrenaline (A) and noradrenaline (NA) cited by Anton are, in fact normal *resting* concentrations, typically found in recumbent persons after a period of inactivity. In view of the known lability of man's sympathoadrenal response to stress (*1*), it is not too surprising that concentrations I reported were higher, on the average, than normal resting values. (It will be recalled that my subjects were catheterized, isolated, partially immobilized, and in some cases made to perform a difficult vigilance task.) Still, when the 81 estimations of A and NA from my experiment (*2*) are considered separately, it can be seen that many fall within reported normal resting ranges. For A, 71.6 percent fall within the "normal" range (0 to 1.14 μg/liter) reported for the chemical method (*3*) used and 40.7 percent fall within the range cited by Anton as "normal." For NA these values are lower—45.7 percent in the range 0 to 3.10 μg/liter and 7.4 percent in the range cited by Anton.

Anton's position that the values I reported for NA are surprisingly high may be well taken. This still does not affect the conclusions of my report, since these concerned the observed relation between the subjects' performance and A. The concentrations I reported for A seem credible.

2) The chemical method used in my experiment was the 1961 modification of the method of Weil-Malherbe (*3*) which, according to Anton himself, yields values in the normally accepted range. It is unclear how the criticisms of Weil-Malherbe's earlier methods cited by Anton have direct bearing upon the method used and consequently on the results in my experiment.

Early modifications of Weil-Malherbe's method, though lacking in specificity, have been successful in measuring relative plasma catecholamine changes during stress (*4*). There is no reason to expect that the latest and apparently most specific modification of this method would be less useful in this respect.

3) In my report I was logically unable to discuss the apparent inverse relation between the experimental subjects' A and NA, since the latter trend was not statistically significant. Consequently I do not know if the relation suggested by Anton occurred. If it did, the phenomenon was characterized by transient changes in the subjects' production of A and NA during the period of stress. Such changes could only be measured over a series of plasma samples during stress, since the concentrations of catecholamines in urine reflect the subjects' *average* production during the collection period. This point has also been made by von Euler (*5*).

4) I do not agree that the figures were misleading. They were a simple statement of the data. I am unaware of any convention for stating the results of statistical tests in the legends of figures when these results are reported in the text.

After considering the criticisms I would amend my conclusions only slightly. Some factor, measured as adrenaline by an accepted chemical technique, decreased in the experimental subjects' plasma as a function of time in the vigilance task. The same factor did not change in the controls' plasma over an equivalent period.

JAMES F. O'HANLON, JR.

Human Factors Research, Inc.,
Santa Barbara, California

References and Notes

1. One example of this can be taken from M. Goodall, *J. Clin. Invest.* **41**, 2, 197 (1962). He reported that urine A concentrations were up to six times greater than the maximum normal level in men awaiting centrifugation, while noradrenaline concentrations were greater than twice the maximum normal level after that stress.
2. These data are shown in the original technical report of my experiment and are obtainable from Defense Documentation Center, Arlington, Virginia (A.D. No. 606136).
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