DISCUSSION

PHYSIOLOGY OF THE NERVOUS SYSTEM

IN discussing some criticisms by Dr. Alexander Forbes of his book on the "Physiology of the Nervous System," Professor Fulton, in SCIENCE of August 4, 1939, makes the following statement with deliberate emphasis: "I know of no evidence fit for critical examination that would place the liberation of acetylcholine as a *primary* event essential for synaptic transmission, in any division of the nervous system, central or peripheral." In the case of the peripheral part of the system, with which the direct evidence yet available is almost entirely concerned, the statement seems to call for some comment.

The validity of the fundamental observation, that acetylcholine is released at ganglionic synapses by preganglionic impulses, was challenged by Lorente de Nó,[1] and Professor Fulton's book seemed to show that he had accepted its dismissal. I take the view that it has been effectively reinstated by MacIntosh,[2] whose evidence, as I think Professor Fulton would agree, must stand until it has been directly answered. If this fundamental observation is correct, it means that acetylcholine is released at the synapses by preganglionic impulses, in such concentration that it must directly excite the ganglion cells to the discharge of postganglionic impulses, or, to use Professor Fulton's phrase, its liberation must be a *primary* event essential for the synaptic transmission. It has rather hastily been assumed that, if acetylcholine were thus to act as transmitter, its disappearance during the refractory period, necessary to prevent repetitive discharge, could only be due to cholinesterase. Consequently, a whole literature has grown around observations on the ganglion with eserine, the largely negative character of which has, I think, been given undue importance by advocates of the electrical theory. The inadequacy of evidence for a particular mode of its disappearance does not, strictly, weaken that for the appearance of acetylcholine, in stimulating concentration, at the synaptic endings of preganglionic fibers. To dismiss such evidence, put forward by observers who are at least experienced and responsible, as not "fit for critical examination," might seem a little severe; but it will be noted that Professor Fulton's statement might mean, literally, not that he repudiates such evidence, but that he is not aware of its existence.

It may be admitted, further, that the possibility that the acetylcholine liberated at a synapse might act only as a sensitizer, necessary to render an electrical transmission effective, has been considered as an alternative, even in publications from my own laboratory. If I understand Professor Fulton, he might regard this idea as more worthy of consideration, since the function of acetylcholine in such a system, though essential, might be regarded as not *primary*—if, indeed, it would be proper to apply that term to one of several conditions essential to an effect. My chief concern, in any case, is with the evidence that the liberation and the activity of acetylcholine are in some way essential to transmission, at peripheral synapses and motor nerve endings. Even this, I think, would not be accepted by some of the distinguished contributors to the illuminating "Symposium on the Synapse,"[3] quoted by Professor Fulton in support of his own present belief. Through Professor Fulton's kindness I had an early opportunity of seeing this important document. Professor Erlanger and Dr. Lorente de Nó, in particular, there present a case for a purely electrical theory of synaptic transmission, which undoubtedly merits a critical examination. Dr. Bronk and Dr. Forbes make contributions to this in the same symposium, where they urge, from different points of view, the necessity of considering at least a chemical participation in, or a chemical aspect of, the synaptic phenomena. It is not likely, however, that either of them could have been fully acquainted with the number of facts now available, which suggest a singular significance in synaptic transmission for the release of acetylcholine, and for which a purely electrical theory appears to afford no explanation. Many of the observations in question are quite recent, and some, indeed, have not yet been published. Perhaps I may be allowed to offer a short list of some of them, as a footnote to the symposium and a modest contribution to the critical examination of the electrical theory.

(1) Nicotine (Feldberg and Vartiainen[4]) and Curarine (Brown and Feldberg[5]) annul the response of ganglion cells to preganglionic impulses and to acetylcholine, without affecting their response to K-ions in the case of Curarine, and without interfering with the release of acetylcholine by preganglionic impulses. These statements are equally applicable to the action of Curarine on voluntary muscle (Dale, Feldberg and Vogt[6]); Brown, Dale and Feldberg[7]; Brown[8]). Buchthal and Lindhard,[9] applying Curarine directly to the motor end plate of a lizard's muscle fiber, have found that it becomes insensitive simultaneously to motor nerve impulses and to application of acetylcholine, while K-ions similarly applied and direct electrical stimulation of the end plate are still normally effective in causing contractions of the fiber.

(2) After section of a preganglionic nerve, release of acetylcholine by preganglionic impulses and synaptic

[1] Lorente de Nó, *Am. Jour. Physiol.*, 121: 331, 1938.
[2] MacIntosh, *Jour. Physiol.*, 94: 155, 1938.
[3] "Symposium on the Synapse," *Jour. Neurophysiol.*, 2: 361–472, 1939.
[4] Feldberg and Vartiainen, *Jour. Physiol.*, 83: 103, 1934.
[5] Brown and Feldberg, *Jour. Physiol.*, 86: 10 P., 1936.
[6] Dale, Feldberg and Vogt, *Jour. Physiol.*, 86: 353, 1936.
[7] Brown, Dale and Feldberg, *Jour. Physiol.*, 87: 394, 1936.
[8] Brown, *Jour. Physiol.*, 91: 4 P., 1937.
[9] Buchthal and Lindhard, International Congress of Neurologists, Copenhagen, 187, 1939.

transmission disappear together (MacIntosh[10]), at a time when conduction in the preganglionic fibers is still unimpaired (Bacq and Coppée[11]).

(3) When Locke's solution containing no glucose is perfused through a ganglion, continued stimulation of the preganglionic nerve rather rapidly exhausts the mechanism of synaptic transmission, and the output of acetylcholine fails with it; both being promptly restored when glucose, lactate or pyruvate is added to the perfusion (Kahlson and MacIntosh[12]).

(4) Perfusion of a ganglion with Locke's solution lacking calcium, while it renders nerve fibers and ganglion cells abnormally excitable, in particular by K-ions, stops the release of acetylcholine by preganglionic impulses, and therewith synaptic transmission, both again being promptly and simultaneously restored by addition of calcium to the perfusion (Harvey and MacIntosh[13]).

Many observations by other workers could be cited, such as the important, though less direct, evidence of Cannon and Rosenblueth,[14] which Dr. Forbes mentioned. Even this limited selection, however, from those with which I have been directly in touch, seems to me to contain points worthy of critical examination by a supporter of a purely electrical theory, who, unless he can show that they are not valid as facts, should be prepared to explain how that theory accommodates them.

H. H. DALE

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THE USE OF PRONTOSIL AS A VITAL DYE FOR INSECTS AND PLANTS[1]

RECENT studies by the writer on the effect of sulfanilamide compounds on a leafhopper vector of a virus disease have resulted in some incidental observations of some general interest.

One of these compounds, Neoprontosil,[2] has proved to be a useful vital stain for both plant and insect. Young corn seedlings, with or without the roots cut off, take up the dye with extreme rapidity, it being a matter of seconds for the leaves to be visibly streaked with red. After a few hours, the entire plant may show the red color diffused throughout or the lower leaves only may be entirely colored, with color on the upper leaves showing only as streaks in some of the fibrovascular bundles.

When nymphs and adults of *Peregrinus maidis* Ashm., the corn leafhopper, were caged on these dye-saturated leaves, the presence of the dye in the insect could be easily observed through the body wall, after feeding a day or two.

The experiment was tried of caging three or four of the insects in a cage which limited feeding of the group to a portion of the leaf tissue less than 1 sq. cm in area. Under these circumstances, great variation in the amount of dye visible in the individual insects could be observed, varying from no outward evidence at all to complete diffusion through the body of the insect. An insect showing no evidence of coloration from the outside may still have ingested the dye, as may be seen from colored defecations. This variation in amount of dye absorbed may be due either to variations in the amount of feeding by individual insects or to differences in the permeability of the intestinal tracts of individuals of the same species or to the specific plant tissues reached by individual insects. This last alternative is not confirmed by experiments wherein the insects were fed on solutions of the dye through membranes. Again, similar differences in amount of visible dye were to be found, insects caged on a single membrane showing all the degrees of color variation from none to a dense red diffused color.

Solutions up to 5 per cent. strength have been used, with one per cent. sucrose added for food, when the dye was administered through membranes.

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FORMATION OF A LARGE ALCOHOL BEAD

A SLIGHT vacuum was applied to the residual lime mass in the preparation of absolute alcohol. The alcohol came over quite rapidly and formed a somewhat warm layer. As the rate of distillation became less, the drops of alcohol distillate were cooler and formed beads very readily on the surface of the liquid in the receiver. A short while later the surface of the alcohol had risen to touch the tip of the delivery tube, and a bead about 2.5 cm in diameter and hemispherical in shape was seen to be attached to the delivery tube. Distillation was proceeding at the rate of about 140 drops per minute, and the bead kept increasing in size, until at the end of 18 minutes it measured about 7.5 cm in diameter. The bead was in constant rhythmic pulsating motion, changing its shape from that of a wide shallow saucer-like object to that of a narrow deep bowl. It seemed to be much more highly refractive than the bulk of the alcohol distillate. Finally the bottle became so full of alcohol that distillation had to be stopped, and when the receiver was disconnected, the bead burst.

Thus, in the time during which the bead was observed, the volume increased from 4.1 cm^3 to 110.5 cm^3, and the total stable existence of this bead becomes

10 MacIntosh, *Jour. Physiol.*, 92: 22 P., 1938.
11 Bacq and Coppée, *Jour. Physiol.*, 92: 17 P., 1938.
12 Kahlson and MacIntosh, *Jour. Physiol.*, 96: 277, 1939.
13 Harvey and MacIntosh. In course of publication.
14 Cannon and Rosenblueth. *Am. Jour. Physiol.*, 119: 221, 1937.

1 Published with the approval of the director as Miscellaneous Paper No. 29 of the Pineapple Experiment Station, University of Hawaii.

2 Winthrop Chemical Company, Inc., 170 Varick Street, New York, N. Y.