

# Quantal Nature of Synaptic Transmission<sup>1</sup>

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IT IS NOW GENERALLY ACCEPTED that transmission of excitation at chemically mediated synapses is "quantized"; i.e., transmitter substances are released in discrete "packages." Evidence in support of this view has been collected from a wide variety of preparations ranging from the invertebrate neuromuscular junction (20, 62) to synapses on spinal motoneurons of the cat (43). The purpose of this review is to summarize this experimental evidence and, more particularly, to collect the various mathematical treatments involved in the quantum hypothesis. The references cited are, for the most part, restricted to those dealing directly with quantization. For a more general discussion of synaptic transmission, the reader is referred to previous reviews and monographs (18, 21, 26, 34-36, 52).

## I. EXPERIMENTAL OBSERVATIONS LEADING TO FORMULATION OF QUANTUM HYPOTHESIS

Discovery of the spontaneously occurring miniature end-plate potentials (28) and experiments on the effects of low Ca and high Mg on transmission at the neuromuscular junction of the frog (12-14, 19, 28) led to the formulation of the quantum hypothesis by del Castillo and Katz in 1954 (14). Subsequent observations on this and other synapses have confirmed and extended the hypothesis. The crucial experiments were done on the isolated frog nerve-muscle preparation by recording intracellularly from the end-plate regions of single muscle fibers and observing the end-plate potential produced by motor nerve stimulation. The end-plate potential was reduced below threshold for initiation of a propagated muscle action potential by adding Mg to the bathing solution or reducing the concentration of Ca. When the concentration of Mg was sufficiently high (about 10 mM), or that of Ca suffi-

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ciently low (about 0.4 mm), it was observed that the end-plate potential had a minimum amplitude and that there were many "failures" of response. The minimum response, which is referred to here as the *unit potential*, was identical in size and shape with the spontaneously occurring miniature end-plate potentials. Thus it was proposed (14, 28, 29) that the unit potential was the basic building block for the end-plate potential—i.e., that the normal end-plate potential was built up of a large number of unit potentials appearing synchronously in response to a nerve stimulus.

The possibility that a unit potential could be produced by a single acetylcholine (ACh) molecule was rejected for several reasons (28, 29). First, if single molecules were involved, externally applied ACh should have produced a marked increase in frequency of spontaneous miniature end-plate potentials. Instead, application of the drug produced a smooth, graded depolarization. Second, *d*-tubocurarine and prostigmine produced graded changes in miniature end-plate potential amplitude and time course, which would not be expected if single molecules were involved. Finally, a quantitative estimate placed the number of molecules necessary to produce a single miniature end-plate potential at about  $10^4$ . More recent estimates range between  $10^4$  and  $10^8$  (e.g. 18, 34, 42). The first argument is equally valid in rejecting the idea that the quantization was postsynaptic (see also 20). Thus it seems clear that the unit potential is produced by a package of  $10^2$ – $10^4$  ACh molecules released from the nerve terminals. The origin of the package is necessarily subcellular (34). One alternative would be that the appearance of a unit potential was associated with activity in a single branch of the presynaptic nerve terminal. According to this view, Mg would reduce the quantum content of an end-plate potential by blocking transmission at individual points of bifurcation in the terminal arborization. However, increased Ca, which ought to reduce the safety factor for transmission at such points of bifurcation still further, reverses Mg block (6, 14). Furthermore, it has been shown that the number of units available for release at the frog's neuromuscular junction is probably in excess of 400 (see below). This exceeds by a factor of at least 20 the number of arborizations in the nerve terminal concerned. Finally, a recent study (38) has shown that highly localized changes in Ca concentration at end-plate regions of frog muscle fibers affect transmitter release without altering the action potential recorded extracellularly from individual nerve terminals.

## II. BASIC ASSUMPTION AND EQUATIONS

The basic assumption of the quantum hypothesis is that there is a large number ( $n$ ) of quanta stored in the nerve terminal, each with a certain probability of being released in response to a nerve stimulus. If the average of such probabilities is  $p$ , the average number of quanta released per stimulus during a series of trials is given by

$$m = np$$

Experimentally,  $m$  may be obtained by dividing the average amplitude of the

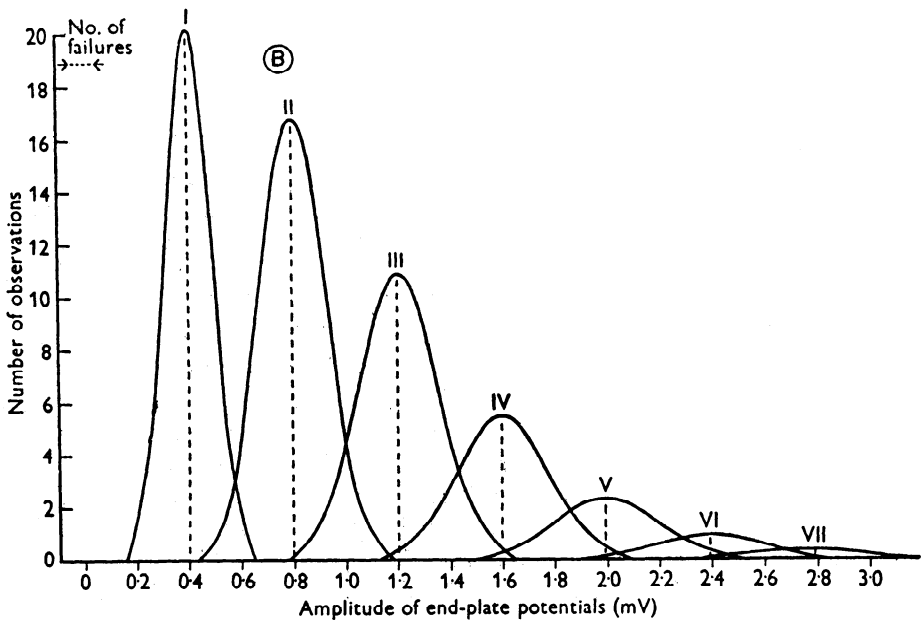
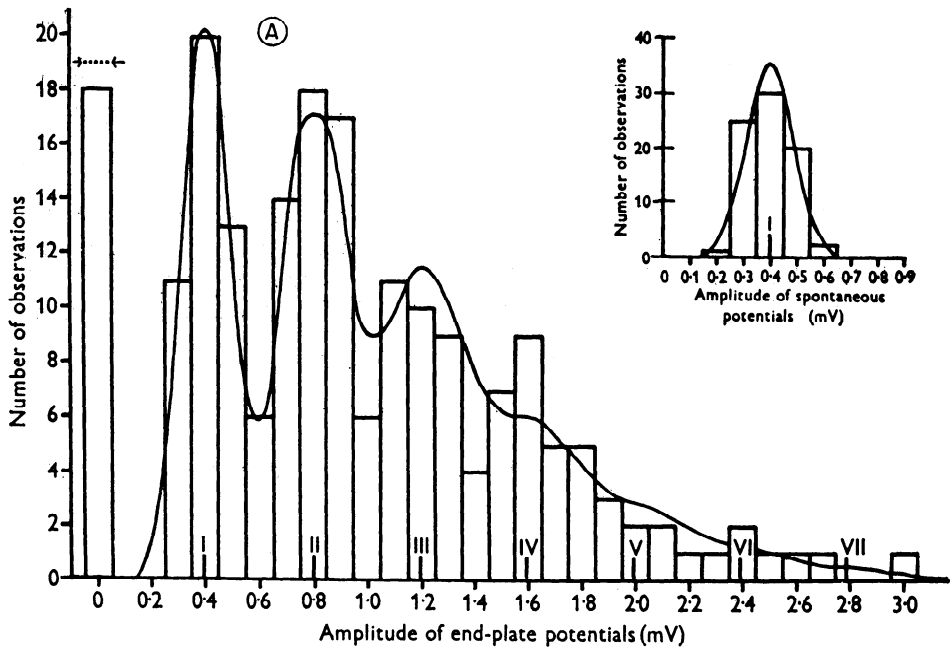


FIG. 1. *A*: Distribution of end-plate potential amplitudes at mammalian neuromuscular junction blocked with Mg. Peaks occur at multiples (I, II, III, etc.) of mean miniature potential amplitude (*inset*). First bar is number of failures. Smooth curve is theoretically expected distribution; arrows indicate expected number of failures. *B*: Method of construction of theoretical distribution in *A*.  $m$  is estimated from  $m_1 = \bar{v}/\bar{v}_1$  and used in the Poisson equation to calculate expected number of failures ( $n_0$ ), single responses ( $n_1$ ), and multiple responses ( $n_2, n_3$ , etc.). These are then distributed normally around mean amplitudes  $\bar{v}_1, 2\bar{v}_1, 3\bar{v}_1 \dots$  with variances  $\sigma_1^2, 2\sigma_1^2, 3\sigma_1^2 \dots$  and summed to produce smooth curve in *A*. [From Boyd and Martin (6)]

end-plate potentials in the series ( $\bar{v}$ ) by the average amplitude of the spontaneously occurring miniature end-plate potentials ( $\bar{v}_1$ ). This estimate of  $m$  we may call  $m_1$ . Thus

$$m_1 = \bar{v}/\bar{v}_1 \quad (1)$$

It follows from the hypothesis that during a series of trials the number of quanta in individual end-plate potentials should fluctuate in a manner predicted by the binomial distribution. If  $p$  is small, as is likely during block by Mg or low Ca, this will be approximated satisfactorily by the Poisson distribution. That is, the number of responses containing  $x$  quanta ( $n_x$ ) should be given by

$$n_x = \frac{N e^{-m} m^x}{x!}$$

where  $N$  is the total number of trials and  $x$  takes on the values of 0, 1, 2, etc. The number of "failures," for example, should be given by

$$n_0 = N e^{-m} \quad (2)$$

the number of single unit responses by

$$n_1 = N e^{-m} \cdot m$$

and so on.

### III. EXPERIMENTAL TESTS OF QUANTUM HYPOTHESIS

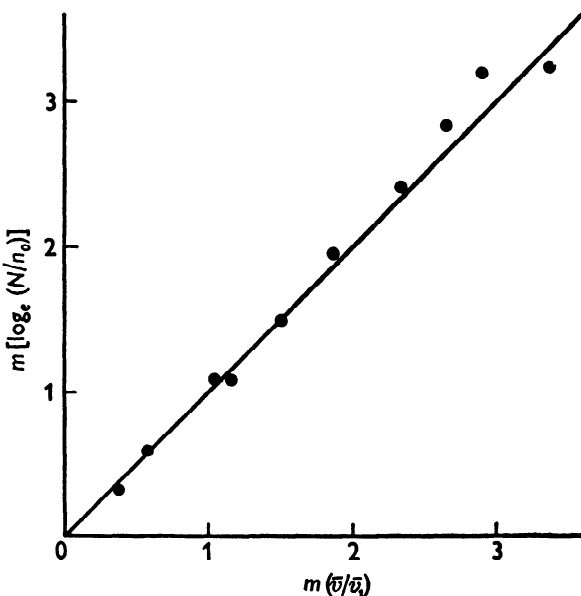
The applicability of the Poisson distribution to the quantal release of transmitter has been tested in three ways. The most complete and exacting test is a comparison of the theoretically expected distribution of synaptic potential amplitudes with that observed experimentally. The procedure is illustrated in Figure 1. A large number (about 200) of responses to nerve stimulation are recorded and a histogram of their amplitudes constructed (Fig. 1A). A similar number of spontaneous miniature potentials are also recorded and the mean ( $\bar{v}_1$ ) and standard deviation ( $\sigma_1$ ) of their amplitudes calculated. Equation 1 is then used to estimate  $m$ . Knowing  $m$ , the expected number of failures, single unit responses, etc., may be calculated from the Poisson equation. The expected single unit responses are distributed normally around a mean amplitude  $\bar{v}_1$  with variance  $\sigma_1^2$ , the double unit responses around a mean  $2\bar{v}_1$  with variance  $2\sigma_1^2$  and so on, as shown in Fig. 1B. The individual distributions are then summed to produce a smooth curve, as shown in Figure 1A, which may be compared with the experimental histogram.

The second test is concerned only with the number of failures in a series. This should give an estimate of  $m$ , which we will refer to as  $m_0$ . It follows from equation 2 that

$$m_0 = \ln (N/n_0)$$

For any given experiment, the estimates  $m_1$  and  $m_0$  should agree. The results from one set of experiments are presented in Figure 2, where one estimate is plotted against the other.

FIG. 2. Comparison of  $m$  estimated from  $m_1 = \bar{v}/\bar{v}_1$  and from  $m_0 = \ln(N/n_0)$  in 10 experiments. Estimates fall reasonably close to straight line indicating agreement. [From Boyd and Martin (6)]



The third test arises from the property of the Poisson distribution that its standard deviation ( $\sigma$ ) is equal to the square root of its mean (i.e.,  $\sqrt{m}$ ). It is, however, more convenient to work with the coefficient of variation ( $CV = \sigma/m$ ) rather than the standard deviation. If the quantum contents of individual synaptic potentials in a series are distributed according to the Poisson equation, then the coefficient of variation of the quantum content distribution should be equal to  $1/\sqrt{m}$ . Since the coefficient of variation is independent of the unit of measurement, the coefficient of variation of the *amplitude* distribution should be identical, except that allowance must be made for the fact that the unit potentials themselves are not of uniform size. This should increase the observed CV by a factor  $\sqrt{1 + (cv)^2}$ , where  $cv$  is the coefficient of variation of the unit potential amplitude (4, 22). Thus

$$\frac{CV}{\sqrt{1 + (cv)^2}} = \frac{1}{\sqrt{m}} \quad (3)$$

Experimental results are compared with this theoretical relation in Figure 3,  $m_1$  being used as an estimate of  $m$ . In practice  $\sqrt{1 + (cv)^2} \lesssim 1.05$  (3, 5, 20, 22, 28, 44, 51) and has been ignored. Equation 3 may be rewritten to provide a third estimate of  $m$  ( $m_2$ ) given by

$$m_2 = \frac{1 + (cv)^2}{(CV)^2} \quad (4)$$

The three tests outlined above were first carried out at the frog's neuromuscular junction (14). Subsequently, one or more of them were applied at the mammalian neuromuscular junction (6, 45), crayfish neuromuscular junction (20), sympathetic ganglion of the frog (4), chick's ciliary ganglion (51), and synapses on

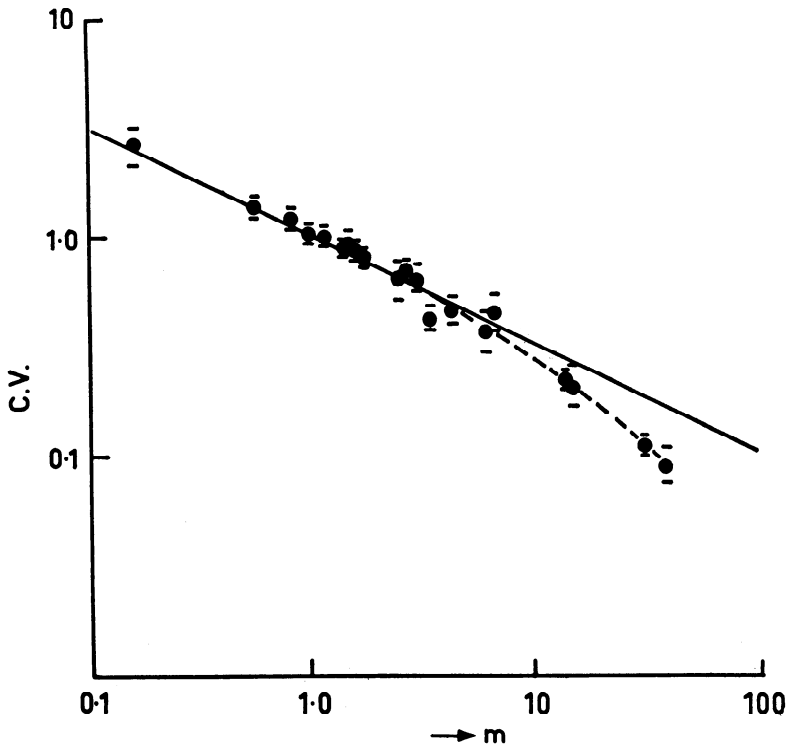


FIG. 3. Relation between mean quantum content, estimated by  $m_1$ , and coefficient of variation of end-plate potential amplitude distribution. Experimental points deviate from expected theoretical relation for large values of  $m_1$ . [From del Castillo and Katz (14)]

the spinal motoneuron of the cat (43). In all cases there has been very good agreement between experiment and theory, provided  $m$  was small.

#### IV. MODIFICATIONS OF HYPOTHESIS WHEN $m$ IS LARGE

It was observed by del Castillo and Katz (14) that the agreement between experimental results and theoretical predictions became unsatisfactory when  $m$  was greater than about 10. Specifically, the experimentally observed amplitude fluctuations were smaller than those expected theoretically so that the amplitude histograms were more compressed on the voltage scale than expected and CV was less than its expected value of  $1/\sqrt{m}$ . It was suggested that this disparity might be due either to failure of the Poisson distribution to apply for large values of  $m$  or to "non-linear summation" of the unit potentials. It was subsequently shown that nonlinear summation could account entirely for the discrepancy at the amphibian neuromuscular junction (50).

There is, however, reason to expect deviation from the Poisson distribution as  $m$  becomes large. If the increase in  $m$  is associated with an increase in  $p, n$  remaining

constant, then the Poisson approximation to the binomial distribution will no longer be accurate, and the difference between the two distributions will become apparent. In the binomial distribution, the expected number of failures, single and multiple unit responses in  $N$  trials are given by the successive terms of the expansion of  $(p + q)^n$ , viz:

$$\frac{n_x}{N} = \frac{n!}{(n-x)!x!} p^x q^{(n-x)}$$

where  $p = m/n$  and  $q = 1 - p$ . Now CV is given not by  $1/\sqrt{m}$ , but by  $\sqrt{1/m - 1/n}$ . However, this reduction in coefficient of variation was not sufficient to account for the disparity observed by del Castillo and Katz, even when a conservative value of 200 was selected for  $n$ . A second possible reason for deviation from a Poisson distribution is that when  $m$  is large a certain fraction of the population may have a very high probability of release and respond regularly, while the remaining population may respond only occasionally. In this case, CV would be reduced still further, being given by

$$\sqrt{1/m[1 - (\text{var } p)/p] - 1/n}$$

where  $\text{var } p$  is the variance of the probability distribution in the population (14). While  $(\text{var } p)/p$  could, of course, be selected to fit the results of any given experiment, there is no indication that such a correction is applicable.

As mentioned above, the main reason for the apparent deviation from a Poisson distribution appears to be nonlinear summation of the unit potentials. If the effect of a quantum of transmitter is to produce a conductance change in the postsynaptic membrane (2, 16, 27), then the amount of potential change contributed by each quantum will decrease as the number of quanta (i.e., total depolarization) increases. This will have two effects on the relation between  $m$  and CV. First, the quantum content will be underestimated by  $m_1$ ; second, the scale of the amplitude fluctuations will be reduced, so that CV will be smaller than expected. Thus, CV will appear smaller than  $1/\sqrt{m}$ , both factors contributing to the disparity. Corrections may be made to both CV and  $m$  to allow for this effect. If  $V_0$  is the difference between the resting membrane potential and the equilibrium potential for the synaptic potential, then the corrected estimate of  $m$  is given by (5):

$$m_1 = m(1 - \bar{v}/V_0)^{-1} \quad (5)$$

and CV by (47):

$$CV' = CV(1 - \bar{v}/V_0)^{-1} \quad (6)$$

In estimating  $V_0$ , the equilibrium potential may be taken as approximately -15 mv (i.e., inside negative) (2, 16, 27, 50, 53, 59). It has been shown (Fig. 4) that when these corrections are made  $CV' = 1/\sqrt{m_1}$ , as would be expected for a Poisson distribution. It should be noted that the correction depends not on  $m$  but on the depolarization ( $\bar{v}$ ). Consequently, if *d*-tubocurarine has no effect on  $m$ , then the quantity  $1/\sqrt{m_1}$  obtained before curarization should be equal to the uncorrected CV obtained after curarization sufficient to reduce  $\bar{v}$  to a small fraction of  $V_0$ . This has also been shown (Fig. 4).

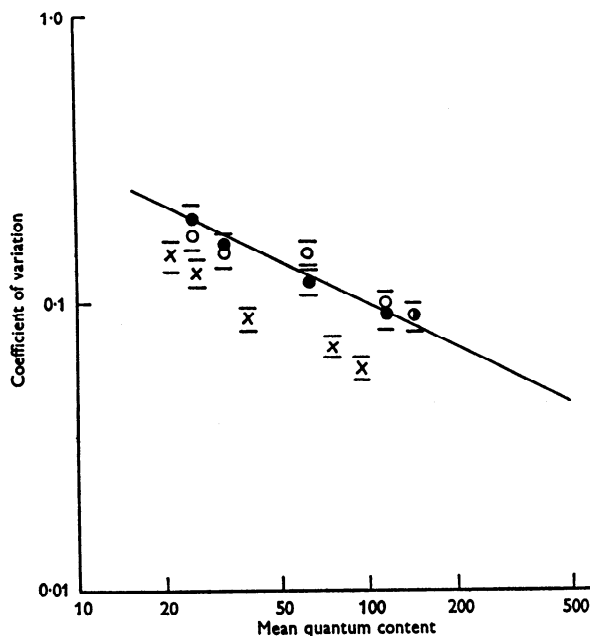


FIG. 4. Relation between mean quantum content, estimated by  $m_1$ , and coefficient of variation. Discrepancy between experimental points (x) and theoretical relation (straight line) is removed by allowing for "nonlinear summation" of unit potentials (o) or by curarizing to reduce end-plate potential amplitude (●). [From Martin (50)]

It is of interest that  $m_1$  and  $m_2$  may be used to estimate the correct value of  $m$  without use of the factor  $(1 - \bar{v}/V_0)$  (51). From equations 4 and 6

$$m_2' = m_2(1 - \bar{v}/V_0)^2$$

and from 5

$$m_1'^2 = m_1^2(1 - \bar{v}/V_0)^{-2}$$

if  $m_1' = m_2' = m$ , then

$$m_1'^2 m_2' = m^3 = m_1^2 m_2$$

and

$$m = \sqrt[3]{m_1^2 m_2} \quad (7)$$

In a recent study of quantization of the monosynaptic excitatory postsynaptic potential in spinal motoneurons, Kuno (43) has noted a disparity between CV and  $1/\sqrt{m}$  that cannot be explained satisfactorily by any of the considerations outlined above. When a single afferent fiber was stimulated, the resulting excitatory postsynaptic potential always had a mean quantum content of about unity and fluctuated as predicted by a Poisson equation. When the number of fibers stimulated was increased, deviations from the Poisson distribution became apparent for  $m \geq 3$ . The deviations were similar to those seen at the neuromuscular junction. Since the amplitude of the response was small, correction for nonlinear summation was negligible. The assumption of a binomial distribution was inadequate to explain the discrepancy and, in any case, is unsatisfactory since increasing the number of afferent fibers stimulated should have increased  $n$ , not  $p$ . Finally, since  $m$  varied little from fiber to fiber (when only one was stimulated) it seems safe to



assume that  $p$  was relatively constant also. Thus increasing the number of fibers stimulated should not have invariably introduced one or more with a regularly responding population. The most likely explanation appears to be that there was some kind of interaction between the afferent terminals.

#### V. ACCURACY OF ANALYSES AND ESTIMATION OF ERRORS

The degree of confidence that may be attached to the various tests of the quantum hypothesis may be estimated by computing the standard errors (SE) of the parameters used. It should be noted that these calculations do not take into account consistent experimental errors in measurement of individual values. Some sources of experimental error are discussed at the end of this section.

With regard to the first test, the agreement between the experimentally observed amplitude distribution and that expected theoretically (Fig. 1) can be assessed adequately by visual inspection. If one feels compelled to express this in terms of a number, the  $\chi^2$  test may be used; however, in general the test is less discriminating and will only detect disparities that are more than obvious to the eye. For example, in Figure 1A it is apparent that although the fit is good, it could be improved by selecting a slightly smaller value for  $\sigma_1$ . Nevertheless, the  $\chi^2$  test indicates that the fit is significant at better than the 99 % level ( $\chi^2 = 11.50$ ,  $f = 29$ ).

The standard errors of the various quantities used in the calculations are given below:

$$SE(m_0) = \sqrt{\frac{1-p_0}{Np_0}} \quad \text{where } p_0 = n_0/N \text{ (4, 22, 31)}$$

$$SE(m_1) = \frac{m_1}{\sqrt{N}} \sqrt{(CV)^2 + (cv)^2} \text{ (see 41)}$$

$$SE(m_2) = \frac{m_2}{\sqrt{N}} \sqrt{4(CV)^2 + 2} \text{ (22, see 41)}$$

$$SE(CV) = \frac{CV}{\sqrt{2N}} \sqrt{2(CV)^2 + 1} \text{ (41)}$$

$SE(m_0)$  is given incorrectly in reference 22 due to the inadvertent omission of the square root sign. The coefficient of variation of  $m_0$  ( $SE(m_0)/m_0$ ) is minimal when  $m_0 \approx 1.6$  ( $p_0 = 0.2$ ) and, for  $N = 200$ , exceeds 10 % when  $m_0$  is outside the limits  $0.7 \leq m_0 \leq 3.0$ . If  $(cv)^2$  is taken as 0.1,  $SE(m_1)/(m_1)$  is about 4.6 % for  $m_1 = 3$  and decreases asymptotically to about 2.2 % as  $m_1$  increases. The coefficient of variation of  $m_2$  is about 13 % for  $m_2 = 3$  and asymptotically approaches 10 % as  $m_2$  increases, again assuming  $N = 200$ .

If the correction factor for nonlinear summation is used, the standard errors of  $m_1'$ ,  $m_2'$ , and  $CV'$  are given by the same expressions with the corrected values substituted for the uncorrected ones. For example,

$$SE(m_1') = \frac{m_1'}{\sqrt{N}} \sqrt{(CV')^2 + (cv)^2}$$

If the weighted mean of the uncorrected values is used to obtain  $m = \sqrt[3]{m_1^2 m_2}$

then

$$SE(m) = \frac{m}{3} \sqrt{4 \left[ \frac{SE(m_1)}{m_1} \right]^2 + \left[ \frac{SE(m_2)}{m_2} \right]^2}$$

Experimental errors may arise in three ways. First, it may be necessary to measure small potentials on a relatively thick base line. This may lead to difficulty in distinguishing the smallest unit responses from failures (11, 51). In addition, measurements made with a low signal-to-noise ratio will increase the coefficient of variation of the amplitude distribution. Second, recording 200 or more responses at a low repetition rate (say 4/min) may take about an hour. During this time the mean amplitude of the response may increase or decrease, due either to a gradual change in  $m$  or to a change in potential or resistance of the postsynaptic membrane. In this case, CV will be increased and  $m$  may be grossly underestimated by  $m_2$ . Before using such data for calculations, the responses should be divided into about 10 sequential groups and the group means compared, to ensure that there has been no drift (14). The error introduced by drift in mean amplitude may be reduced by calculating the variance from a linear regression on the series (rather than from the mean) and using this variance and the mean to obtain the coefficient of variation (8). Finally, if the correction factor  $(1 - \bar{v}/V_0)$  is used, over- or undercorrection may result, depending on the value selected for  $V_0$ . High-resistance micro-pipettes with relatively large tip potentials tend to underestimate resting membrane potentials, leading to overcorrection (51).

#### VI. ESTIMATES OF $n$ AND $p$

Whereas any of the methods outlined above may be used to obtain a reasonably accurate estimate of  $m$ , it is more difficult to assign values to  $n$  and  $p$ . It may be estimated from Figure 4 that the failure of the Poisson distribution to approximate the binomial distribution could have been detected if  $n$  were less than about 400 (50), thus providing a lower limit for  $n$ . In the same series of experiments,  $m$  was found to be about 100 in normal bathing solution, so the upper limit for  $p$  was about 0.25.

An estimate of  $n$ , similarly involving the difference between a Poisson and binomial distribution, may be obtained from one experiment by Blackman et al. on frog sympathetic ganglion (4). When the Ca concentration in the bathing solution was increased from 1.8 to 5.4 mM, the mean synaptic potential amplitude was increased by a factor of about 1.4. The mean quantum content, however, estimated by  $m_2'$ , was increased by a factor of about 2.5; i.e., the decrease in CV' was greater than expected. This disparity was removed by recalculating  $m_2'$ , assuming a binomial distribution with  $n = 48$  quanta. Since  $m$  in normal solution was about 22,  $p$  would be of the order of 0.46. However, the authors point out that the standard error of  $m_2'$  was very large and the estimate can only be regarded as giving an approximate order of magnitude for  $n$ .

A more accurate estimate of  $p$  may be obtained from two-shock experiments in which the amplitude of the second response is depressed, provided one is prepared to accept the hypothesis that the depression of the second response is the re-

sult of depletion of transmitter by the first. The method was used by Liley and North (47) to calculate the "release factor" (i.e.,  $p$ ) at the mammalian neuromuscular junction. A similar depression was noted at the frog neuromuscular junction (after a brief period of facilitation) by Takeuchi (56), and the constant  $D_0$  appearing in his tables is again equivalent to  $p$ . In both cases, the amplitude of the second end-plate potential returned to the control value exponentially as the interval between the two shocks was increased. The mathematical treatment from which  $p$  is derived may be summarized as follows.

Let the number of quanta available for release under resting conditions be  $n$ . If the conditioning shock produces an end-plate potential of quantum content  $m$ , then  $n$  will be reduced to  $n' = n - m$ . If the reduction in amplitude of the test response is due to the reduction in  $n$ , then  $n$  must return to its resting value exponentially. At any time  $t$  its value will be given by  $n_t$ , where

$$n - n_t = me^{-kt}$$

$1/k$  being the experimentally observed time constant. If, during the period of depression,  $p$  is constant and equal to its resting value,

$$np - n_t p = mpe^{-kt}$$

i.e., at time  $t$ , the quantum content of the test response ( $m_t$ ) will be given by

$$m - m_t = mpe^{-kt}$$

Ignoring nonlinear summation, the amplitudes of the conditioning response ( $v$ ) and test response ( $v_t$ ) may be assumed to be proportional to their quantum contents. Then

$$\frac{v - v_t}{v} = pe^{-kt}$$

When the left-hand term was plotted against time on a semilogarithmic scale the relation was linear in both preparations, except at short intervals ( $< 500$  msec) when there was facilitation at the frog neuromuscular junction and the depression was less than predicted in the mammalian preparation. These discrepancies seem most likely to be due to a transient increase in  $p$  after the conditioning shock. In both cases, the normal value of  $p$  may be estimated by extrapolating the linear portion of the curve to zero time. This gives an estimate of 0.45 for one experiment on mammalian muscle and an average of 0.14 in eight experiments with normal Ca on frog muscle. Ignoring nonlinear summation will tend to reduce the estimates, since the depression of end-plate potential amplitude will be somewhat less than the depression of quantum content. The recovery time constants were about 1.8 sec and 5.2 sec for the mammalian and amphibian preparations, respectively. If  $m$  is normally about 300 in mammalian muscle (6, 45), and about 100 in frog muscle (50, 58), then these values of  $p$  suggest that  $n$  is about 700 in both cases. A more recent estimate by Thies (61) for the mammalian neuromuscular junction is of the same order of magnitude (1000), while estimates by Elmqvist and Quastel (25), who applied similar considerations to the progressive loss of end-plate potential amplitude at the beginning of a tetanus, tend to be somewhat smaller (300–1000).

A possible objection to the two-shock analysis is that some of the depression

of the second response may be due to postsynaptic "desensitization" of the end-plate receptors. Experiments with iontophoretic application of ACh from micropipettes placed near the end plate have shown that small amounts of the drug may produce a marked reduction in sensitivity to subsequent test applications (40). However, the evidence available at present indicates that the release of several hundred quanta from the nerve terminal itself is not sufficient to produce any detectable desensitization. Thesleff (60) reported some desensitization at the mammalian end plate after brief repetitive trains of end-plate potentials, but similar experiments by Otsuka et al. (55) revealed none, even when the test end-plate potential was depressed by as much as 60 %. In addition, these authors found that the depression was increased by increasing the Ca concentration in the bathing solution (see also 48, 49) but was unaffected by anticholinesterase. Thus the depression depended on the amount of ACh released by the conditioning stimuli, not on the amount reaching the end-plate receptors. Finally, it has been shown that the depression of the end-plate potential produced by low-frequency stimulation over long periods can be accounted for entirely by a reduction in quantum content (8, 15), and more recently Thies (61) has shown that the depression seen after a single conditioning shock is, in fact, due to a reduction in the number of quanta released, not to a change in unit size.

The relatively large values calculated for  $p$  imply that maintenance of transmitter output at a finite level during repetitive stimulation is dependent on replacement of available quanta as they are released. The low  $Q_{10}$  for recovery from depression following a single impulse at the frog neuromuscular junction (56) suggests that ACh synthesis is not immediately involved. That is, replacement of the population of quanta represented by  $n$  seems to be from a larger pool by a process similar to simple diffusion. Thus it seems likely that the model proposed by Birks and MacIntosh (1) for storage and release of ACh in the sympathetic ganglion may be applied to the neuromuscular junction as well. In this model, only part of the ACh available for release (depot ACh) is in a readily releasable state. This releasable fraction would correspond to the population represented by  $n$  and might consist, for example, of those quanta situated immediately adjacent to the nerve-terminal membrane. Replacement of released quanta would be from the larger pool of depot ACh, which in turn would be maintained by synthesis. In the mammalian ganglion, the releasable fraction consisted of about 20 % of the total available ACh stored in the ganglion. However, this figure seems to be much lower at the neuromuscular junction. Various estimates of the total available ACh may be made from data presented in the literature (23, 24, 42), ranging from the equivalent of about 200,000 to 700,000 quanta. Thus  $n$  seems to be only a fraction of 1 % of the total available pool.

## VII. SPONTANEOUS RELEASE OF QUANTA

The spontaneous appearance of miniature synaptic potentials, mentioned previously, was first observed by Fatt and Katz (28) in frog skeletal muscle. Spontaneous activity of this nature has been recorded subsequently in twitch fibers of skeletal muscle from mammals (5, 7, 23, 44), birds (32), and fish (57), in slow fibers

of frogs (9), birds (32), and mammals (33), in mammalian smooth muscle (10, 54) and in invertebrate muscles (20, 62). In addition, spontaneous appearance of quanta occurs in autonomic ganglia (3, 51) and in spinal motoneurons (37, 43). Mathematical treatments of the phenomenon have been concerned with analysis of the distribution of intervals between successive discharges and the distribution of the miniature potential amplitudes.

If the discharge is a Poisson process with zero dead-time, the number of intervals ( $\Delta n$ ) whose duration falls within the time increment between  $t$  and  $t + \Delta t$  should be given by the relation

$$\begin{aligned}\Delta n/N &= e^{-t/T} - e^{-(t+\Delta t)/T} \\ &= e^{-t/T}(1 - e^{-\Delta t/T})\end{aligned}$$

where  $N$  is the total number of observations and  $T$  is the mean interval. If  $\Delta t/T$  is small, this may be approximated by (28)

$$\Delta n = N(\Delta t/T)e^{-t/T}$$

The cumulative distribution (total number of intervals less than  $t$ ) is given by (28)

$$n = N(1 - e^{-t/T})$$

Such an exponential distribution has been observed in all preparations from which miniature potentials have been recorded and is consistent with the idea that the discharge is random in nature (30)—i.e. that the intervals are independent of each other. However, even if the discharge is completely random, it may still be the summed effect of a large number of constituent units discharging regularly but independently (28) although external records from “active spots” on the end plate suggest that individual units themselves discharge in an irregular manner (17, 20, 28, 44). It should be pointed out that an exponential distribution is a necessary but not a sufficient condition for establishing that the discharge is a Poisson process. Other tests should be used to establish that successive intervals are independent of each other. Burnstock and Holman (10) introduced an important additional test by plotting the duration of each interval against that of the one immediately preceding. These were found to be independent.

Examination of the amplitude distribution of the miniature potentials suggests that in some cases there is interaction of brief duration between constituent units. In frog and mammalian skeletal muscle, the amplitudes of individual potentials are usually distributed normally about their mean with a coefficient of variation of about 30 %. However, Liley (45, 46) noted the occurrence of “giant” miniatures that appeared to be due to spontaneous multiquantal discharges. The number observed was larger than the number expected on the basis of random coincidence. The occurrence of such discharges is relatively more frequent in autonomic ganglia (3, 51), although the over-all mean frequency of the discharge is much lower. Their effect is to produce an amplitude distribution markedly skewed in the positive direction. It is, of course, possible that the large spontaneous potentials represent a separate population of large units rather than coincident discharges of two or more quanta. However, a recent analysis (51) suggests that the skewed amplitude distribution can, in fact, be accounted for satisfactorily by the assumption that the discharges are multiquantal events produced by brief, nonrandom interactions.

The analysis is based on the assumption that whenever a unit is released spontaneously, the remaining units each have a small probability of being "dragged" with it. Single discharges may then be classified as failures (no response to interaction), double discharges as single unit responses, and so on. The analysis then becomes similar to that of discharges evoked by stimulation, the number of failures, single and multiple unit responses being predicted by the Poisson equation. The number of failures ( $n_0$ ) is determined by fitting a normal curve to the peak of the distribution and a value of  $m$  for the responses obtained from the relation

$$m = \ln (N/n_0)$$

Thus  $n_1$ ,  $n_2$ , etc. may be calculated and a theoretically expected amplitude distribution drawn. In general, although the total number of observations was small, the agreement between the experimental amplitude distribution and that expected theoretically was good; i.e., the height and length of the "tail" of the distribution could be predicted from the amplitude and width of the peak. The analysis does not exclude the possibility that the miniature potentials appear as the result of spontaneous activations of a "release mechanism." In this case, the conditions are slightly different, since the number of such activations ( $N$ ) and failures ( $n_0$ ) are unknown, and  $n_1$  and  $n_2$  may be selected to give the best fit to the experimental results;  $m$  is then given by  $2n_1/n_2$ , and may be used to determine  $n_3$ ,  $n_4$ , etc. The limited amount of data available (3, 51) does not permit either approach to be rejected in favor of the other. A third possibility, suggested by Liley (46), is that the large spontaneous potentials are due to the release of units previously formed by coalescence of two or more individual quanta.

One consequence of the idea that the large responses are multiquantal discharges is that the modal value of  $v_1$ , rather than the mean, should be used in estimating  $m_1$ . The modal amplitude will, of course, depend on the impedance and resting potential of the postsynaptic element (39). Estimates of the conductance change associated with the unit potential range from  $1.4 \times 10^{-7}$  mhos in frog muscle (58) to about  $10^{-8}$  mhos in frog sympathetic ganglion (3) and about  $2 \times 10^{-9}$  mhos in avian ciliary ganglion (51) and cat spinal motoneurons (43).

#### VIII. JUNCTIONS AT WHICH QUANTIZATION HAS NOT BEEN ANALYZED

At many synapses the evidence for quantization of transmitter release rests on the observation that spontaneous miniature potentials occur. In general, analysis of the response to stimulation becomes difficult if there is multiple innervation at distributed sites on the postsynaptic membrane. This is because the response will then contain components of varying quantum content occurring at various distances from the recording site. Such a situation has been encountered in slow muscle fibers of frogs (9), birds (32), and mammals (33), in mammalian smooth muscle (10, 54), in insect muscle (62), and in frog spinal motoneurons (37). This problem has been circumvented at the crayfish neuromuscular junction (20) by recording highly localized responses externally from "active spots" on the surface of the fiber (17, 28, 44). In mammalian spinal motoneurons the problem of multiple innervation has been avoided by stimulating only one, or a few, afferent fibers (43).

## IX. CONCLUSION

There seems little doubt that transmitter release from nerve terminals is quantized. Indeed, the various lines of evidence discussed in this review are concerned not so much with demonstrating that quantization exists as with testing the hypothesis proposed by del Castillo and Katz to explain its nature, viz: that it is due to a statistical release of units from a large latent population. The experimental support for this idea has been overwhelming. As a consequence, the parameters  $n$  and  $p$  must be considered to be just as "real" as the directly measurable quantity  $m$ . Further studies concerning the synthesis, storage, and release of synaptic transmitter substances must ultimately be related to these parameters if they are to have specific meaning within the framework of the quantum hypothesis.

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