DISCUSSION

THE INTERPRETATION OF EXPERIMENTAL FOUR-FOLD TABLES

IN a note printed in SCIENCE, June 13, 1941, Dr. E. B. Wilson[1] discusses the discrepancy in the probabilities arrived at by two different methods of treating the four-fold table of experimental results, where groups of animals subjected to two contrasted treatments are recorded as having lived or died. He concludes by saying:

> Hence there is neither logical nor arithmetic likelihood that the use of χ^2 should solve well our problem of determining whether the effects of treatment in experiment and control are statistically significant. It is still true, of course, that if numbers are sufficiently large, χ^2 will give the correct probabilities, but they have to be larger than is customary in such experiments.

Dr. Wilson is eminent among statisticians, both for his practical acumen and for his logical penetration. There is no one whose opinion I would sooner seek on the usefulness of any methods published in mathematical statistics. Yet in advocating the particular method he chooses for the interpretation of data of this important class he has, I believe, overlooked a difficulty which the approach based on, and giving the exact solution for, the classical view-point of χ^2 and the four-fold table, was expressly devised to obviate.[2]

Let us consider the simple example first discussed by Wilson. Of six treated mice five have died and one lived, while of six controlled mice one has died and five lived. Wilson considers the probability that the difference between the proportion dying in the two series shall be as great as, or greater than, that observed; that is, in the present instance, the aggregate probability of the six possible experimental results:

	Died	Lived	Total	Died	Lived	Total
(a)				(b)		
Treated	6	0	6	6	0	6
Control	2	4	6	1	5	6
Total	8	4	12	7	5	12
(c)				(d)		
Treated	6	0	6	5	1	6
Control	0	6	6	1	5	6
Total	6	6	12	6	6	12
(e)				(f)		
Treated	5	1	6	4	2	6
Control	0	6	6	0	6	6
Total	5	7	12	4	8	12

in contrast with all the remaining possible results, in which the difference between the numbers dying is not so great as four in favor of the treated series.

Assuming that the chance of death is one half in each series, the total probability of getting one or other of these six results is 79 out of 4,096, or 1.9287 per cent. The basis of this assumption, which is not likely to be exactly true, is that the total number which died in both series together is just one half of the total under observation.

It is this circumstance which introduces a logical difficulty, for the probability assigned to the chosen group of possible results does not depend only on the results which constitute the group, but on the particular one of them which has been observed. Thus to the possible result (a) in which six of the treated mice die and two of the untreated, the probability $\frac{15}{4,096}$ or 0.3662 per cent. has been assigned in the calculation made above; but if this particular outcome had been observed, a different probability, namely, $\frac{3840}{531441}$ or 0.7226 per cent. would have been ascribed to it, since the chance of death would be taken to be $\frac{2}{3}$. The probabilities arrived at by this method do not, in fact, correspond with any objective frequency distribution applicable to the whole aggregate of possible experimental results. Moreover, the probabilities assigned to each particular result, if it were observed, would not add up to unity.

The method which Wilson speaks of as the use of χ^2, and which, though it is an exact arithmetical method, in which the χ^2 distribution is not employed, did arise from the study of the inadequacy of χ^2 when used with small numbers, proceeds on a different plan; from the aggregate of all possible results of the experiment we select those, seven in number, which have the same marginal totals. These are:

	Died	Lived	Total	Died	Lived	Total
A				B		
Treated	6	0	6	5	1	6
Control	0	6	6	1	5	6
Total	6	6	12	6	6	12
C				D		
Treated	4	2	6	3	3	6
Control	2	4	6	3	3	6
Total	6	6	12	6	6	12
E				F		
Treated	2	4	6	1	5	6
Control	4	2	6	5	1	6
Total	6	6	12	6	6	12
G						
Treated	0	6	6			
Control	6	0	6			
Total	6	6	12			

Now it may be shown by simple algebra that *whatever is the probability of dying,* supposing this to be the same for the treated and the controlled series, the

[1] E. B. Wilson, SCIENCE, 93: 557–560, 1941.

[2] R. A. Fisher, "Statistical Methods for Research Workers" (Section 21.02), Oliver and Boyd, Edinburgh. 1925–1941.

relative frequencies with which these seven results will occur are the same, namely, out of 924 trials for which one or other of these seven observations is made, we may expect:

Result	A	B	C	D	E	F	G
Frequency	1	36	225	400	225	36	1

The possible results arrange themselves without ambiguity in order such that A is most favorable and G least favorable to the view that the treatment has increased the probability of death. The sum of the probabilities of the outcome observed and of the one more favorable possibility is $\frac{37}{924}$ or 4.0043 per cent.

We should, therefore, judge the result significant in favor of the view that treatment had increased the death rate, though not nearly so strongly significant as if we had relied on the first method of calculation.

Using the second method, it should be noted that the particular experimental result arrived at (B) determines without ambiguity both the series of results having the same marginal totals, with which its probability is to be compared, and its ordinal position in this series. Had any other observation within the same series been made, (B) would have been assigned the same probability, the sum of the probabilities of the members of each series being always unity.

The danger of using the double binomial is very clearly brought out by Wilson's comparison, for with small numbers the probability assigned is often no more than one third or one half of that given by my method. This is no doubt due to the method assuming some plausible value for the death rate among the controls *as known to be true,* an assumption which would be justified only if the number of animals used as control were increased indefinitely. If, for example, we knew this death rate to be one in six, the probability of observing so many as five dead among the treated series, having *ex hypothesi* the same death rate, would be only $\frac{31}{46656}$ or .0664 per cent. Our ignorance of the true death rate is, however, an essential part of the logical position, and is indeed the only reason why the control series is observed at all.

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ELECTRICAL ACTIVITY OF ACETYLCHOLINE

ACETYLCHOLINE is produced by activity of the nervous system and has a stimulating action on ganglia and muscles, but the relation between acetylcholine and electrical phenomena in nerve is still obscure. Previous work[1] has shown that alkaloidal salts can produce electrical negativity when in contact with oil or lipoids. Moreover, it has been demonstrated[2] that acetylcholine modifies the electrical potential of skin in a negative direction. These findings led to the present experiments which show the production of a negative electrical potential by contact of an extremely dilute acetylcholine solution with various water-insoluble substances resembling lipoids.

In this model of electrical phenomena in nerve the oil layer (guaiacol, nitrobenzene, cresol, creosote or other substances) made contact on each side with 0.7 per cent. NaCl connected by salt bridges to beakers containing 0.7 per cent. NaCl into which dipped Ag-$AgCl_2$ electrodes leading to the E.M.F. terminals of a Leeds and Northrup thermionic amplifier (for high resistance circuits) serving as a null instrument for a potentiometer. In some experiments the surface of the oil to be treated made contact with 0.1 per cent. sodium benzoate which established a positive charge, thereby increasing the sensitivity of the layer to the negativity of acetylcholine. Mecholyl (acetyl-beta-methylcholine), acetylcholine chloride and acetylcholine bromide produced negative potentials which were proportional to the logarithm of the concentration. The highest potential obtained was 200 mv. with 0.03 per cent. mecholyl and nitrobenzene in saline. The lowest effective concentration obtained so far was one in one hundred million parts of acetylcholine chloride, which gave rise to 5 mv. (negative) on nitrobenzene in 0.1 per cent. sodium benzoate. Experiments now in progress indicate that the threshold is considerably lower than this concentration and may approach the value of 5×10^{-6} micrograms which Buchthal and Lindhard[3] reported as the threshold concentration for stimulation of the end plate by acetylcholine introduced by a micromanipulator.

The electrical negativity following acetylcholine, compared with other alkaloids,[1] is remarkable for its size, its rapidity of appearance on application and disappearance after removal and for the extremely low concentrations required. These observations may support the hypothesis that perhaps acetylcholine produces a part of the negative electrical variation in nerve. Moreover, deNo[4] has found that acetylcholine is liberated from nerve fibers as well as from synapses and Boell and Nachmansohn[5] have recently reported that choline esterase is concentrated along the surface of the axon. Regardless of the theory of the nervous impulse adopted, we wish to draw attention to the pro-

[1] R. Beutner, *Jour. Am. Chem. Soc.*, 36: 2045, 1914; *Jour. Pharm. Exptl. Therap.*, 31: 305, 1927.

[2] T. C. Barnes, *Amer. Jour. Physiol.*, 130: 557, 1940.

[3] F. Buchthal and J. Lindhard, *Jour. Physiol.*, 95: 59P, 1939.

[4] R. L. deNo, SCIENCE, 91: 501, 1940.

[5] E. J. Boell and D. Nachmansohn, SCIENCE, 92: 513, 1940.