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.

nounced electromotive activity of acetylcholine. No other substance in such diminutive concentrations is known to produce perceptible electromotive effects on second-class conductors.

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THE DETERMINATION OF THIAMIN BY THE YEAST FERMENTATION METHOD

A RECENT note in SCIENCE by H. H. Bunzell[1] described experiments on yeast fermentation in which only an 8 per cent. stimulation of fermentation rate was caused by thiamin, whereas a 106 per cent. stimulation was produced by an extract of wheat germ. Observations such as these naturally cast doubt upon the reliability of the yeast fermentation method for the determination of thiamin.[2,3] In view of the widespread use of the latter method it was considered desirable to show how Bunzell's experiments differ from the published procedure.[3]

His description of the fermentation medium mentions a "nutrient" solution. This term does not occur in our paper,[3] and thus there is no way of knowing exactly what his "nutrient" solution contained. However, on the basis of our experience with fermentation it is probable that his "nutrient" solution *did not contain ammonium ions* as required by the published procedure.[3]

Without ammonia in the medium thiamin causes a very slight stimulation and, conversely, without thiamin ammonia causes only a slight stimulation. The combination of the two in maximum amounts, however, causes a 100 per cent. increase in fermentation rate. This circumstance might explain Bunzell's results with the wheat germ extract since it has been shown[4] that various amino acids, etc., have an effect equivalent to ammonium ions.

Bunzell's difficulties recall the experience of Smythe,[5] who, observing a remarkable stimulation of fermentation due to an extract of bull testicle, finally isolated ammonium chloride as the active factor. Smythe made the additional mistake of obtaining his yeast from the small cakes sold in grocery stores. Such yeast is too rich in thiamin to show any stimulation of fermentation when thiamin is added to the medium.

[1] H. H. Bunzell, SCIENCE, 93: 238, 1941.
[2] A. S. Schultz, Lawrence Atkin and C. N. Frey, *Jour. Am. Chem. Soc.*, 59: 2457, 1937.
[3] Lawrence Atkin, A. S. Schultz and C. N. Frey, *Jour. Biol. Chem.*, 129: 471, 1939.
[4] A. S. Schultz, L. Atkin and C. N. Frey, *Cereal Chem.*, 16: 648, 1939.
[5] C. V. Smythe, *Enzymologia*, 6: 9, 1939.

If the published procedure for the determination of thiamin[3] is followed with ordinary attention to detail, a satisfactory determination of the thiamin content of wheat germ will be obtained.

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CONTROL OF RED SPIDER (TETRANYCHUS TELARIUS) BY PHTHALIC GLYCERYL ALKYD RESIN

THE common red spider (*Tetranychus telarius* L.), commonly found on greenhouse-grown plants and on many field crops, is extremely difficult to control. The ineffectiveness of many insecticides which have been recommended for control of red spiders may be ascribed to their lack of ovicidal action. Furthermore, chemicals which possess ovicidal properties are often injurious to cultivated plants, especially those grown in greenhouses.

In the course of an investigation, totally unrelated to the problem of red spider control, the writers observed that when a 2 per cent. phthalic glyceryl alkyd resin in water was applied to plants heavily infested with red spiders, the latter quickly disappeared. Microscopic examination of infested leaves showed large numbers of dead red spiders in all stages of development and masses of spiders' ova which had turned yellow and become shriveled after five days. Further examination of the ovicidal properties of phthalic glyceryl alkyd resin showed that it possesses a remarkable insecticidal efficiency. No injury was observed on plants tested experimentally under greenhouse and field conditions. Concentrations less than 2 per cent. (but not less than 1 per cent.) were effective on adults but not on ova; above 2 per cent. the margins of the leaves were burned.

The following plants were sprayed with beneficial results and without injury to the leaves: alfalfa (*Medicago sativa* L.), almond (*Prunus communis* Fritsch. and *P. nana* Stokes), apple (*Pyrus malus* L.), apricot (*Prunus armeniaca* L. and *P. mume* Sieb. & Zucc.), begonia (*Begonia octapetala* L'Her., *B. tuberhybrida* Voss., *B. semperflorens* Link and Otto, *B. haageana* Wats., and *B. rex* Putz.), *Coleus blumei* Benth., florists cyclamen (*Cyclamen indicum* L.), *Gardenia veitchii* Bailey, *Pelargonium sp.*, grape (*Vitis vinifera* L.), *Hydrangea hortensis* Smith, India rubber plant (*Ficus elastica* Roxb.), ivy (*Hedera helix* L.), poinsettia (*Euphorbia pulcherrima* Willd.), plum (*Prunus americana* Marsh), rose (*Rosa sp.*), snapdragon (*Antirrhinum majus* L.), strawberry (*Fra-