melted with oil and wax. These creams emulsified easier than borax creams, the creams were more jelly-like and translucent, especially when first prepared, and the creams were stiffer (due to the stearic acid content).

Creams were prepared with white wax, spermaceti and hydrogenated castor oil. The first was well emulsified and stiffer than I (made with borax). This (II) was used as a standard for comparison of triethanolamine creams. The cream prepared with spermaceti was satisfactory when first prepared, but after standing two days was yellow colored and water had separated. The hydrogenated castor oil cream emulsified readily and was free from the "grainy" consistency of the borax cream. It was about the same consistency as the wax cream (II) when first prepared, but after standing for two days was much stiffer.

Creams were prepared using equal parts of white wax and hydrogenated castor oil and with equal parts of spermaceti and hydrogenated castor oil. In each case the cream was stiffer than with either base alone. The wax-hydrogenated castor oil cream was softer than the spermaceti-hydrogenated castor oil cream when first prepared, but after two days both creams had stiffened and were about the same consistency. Creams prepared with one part of white wax to three of hydrogenated castor oil and vice versa were softer than those made with equal parts.

Creams were prepared using a mixture of equal parts of white wax and hydrogenated castor oil with the following results:

Amount Used.	Stiffness when Prepared.	After Two Days.
20 Gm.	Much stiffer than II	Much stiffer than II
16 Gm.	Stiffer than II	Stiffer than II
12 Gm.	Slightly softer than II	Same as II
10 Gm.	Softer than II or I	Same as I
4 Gm.	Much softer than I or II	Slightly softer than I

CONCLUSIONS.

1. Hydrogenated castor oil (m. p. 86° C.) is unsatisfactory for a borax cold cream when used alone, but is very satisfactory when triethanolamine stearic acid is used as emulsifier.

2. Creams prepared with hydrogenated castor oil are stiffer than those prepared with white wax or spermaceti.

3. A mixture of equal parts of hydrogenated castor oil with white wax or spermaceti possesses greater hardening power than any of the bases alone.

4. The total solidifier content of cold creams may be reduced by 40% by using a mixture of equal parts of hydrogenated castor oil with wax.

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THE APPLICATION OF STATISTICAL METHODS TO PHARMACEUTICAL RESEARCH. V. HOW MANY ARE ENOUGH?*

BY JAMES C. MUNCH.[1]

"When it is not in our power to determine what is true, we ought to act according to what is most probable" (14). Gauss' Law of Errors states that the probability of an error of observation having a magnitude "x" is $y = h\, e^{-h^2x^2}dx$. The probability integral derived from this law, when "P" represents the probability of occurrence of any particular error (1, 8, 10, 14, 15, 17) is

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[1] John Wyeth & Brother Inc., Philadelphia, Pa.

$$P = \frac{2}{\pi}\int_0^{hx} e^{-h^2x^2}d\,(hx).$$

If P is taken as 0.5, the resultant value is that which the next observation is equally likely to meet or to exceed. If this "Probable Error" (PE) is taken about the Mean, it indicates the distribution of results in a large number of observations: 25 per cent will be expected to exceed Mean plus PE; 25 per cent will lie between Mean plus PE and Mean; 25 per cent between Mean and Mean minus PE; and 25 per cent will be less than Mean minus PE. It is obvious that the next observation is as apt to fall within the range of values of Mean plus or minus PE as to fall without this range.

In studies on statistically large numbers of animals, the scatter of individual observations has been found to approach a normal frequency curve. Solving the probability integral, various chances of occurrence of errors as multiples of PE have been determined (6, 17, 20, 24, 30). The most useful values from these integrations are shown in Table I. The first column shows the quotient obtained by dividing

TABLE I.—ODDS AGAINST OCCURRENCE OF DEVIATIONS.

k.	Odds (to 1).
1.0	1
1.48	2
2.0	5
3.0	21
3.25	35
3.5	50
3.82	100
4.15	200
4.4	333
4.6	520
4.9	1000
5.2	2000
5.5	5000
6.0	19,000
7.0	435,000
7.4	1,750,000
8.0	1,500,000,000

TABLE II.—APPLICATIONS OF FORMULA:

$$N = 2\left(\frac{k \times PE}{Diff.}\right)^2.$$

k.	PE.	$Diff.$	N.
2.0	10	10	8
3.0	10	10	18
3.82	5	5	29
	5	10	7
	10	38	2
	10	10	29
	10	5	117
	10	1	2918
	20	10	116
4.9	10	10	48
	10	5	192
	20	10	192
	20	5	768

any particular deviation by PE, or is a column of multiples of PE. The second column shows the odds against the occurrence of a deviation as great as or greater than this multiple. For example, the odds are 5 to 1 against the occurrence of a deviation greater than 2 PE; 100 to 1 against the deviation 3.82 PE; 1,500,000,000 to 1 against a deviation of 8 PE, etc.

The value $\frac{\text{DEVIATION}}{PE}$ may be designated as "k." The PE of a single observation is the deviation expected when the next single observation is made. PE of the Mean, representing the deviation to be expected if another set of the same number of measurements should be made upon the same material under the same conditions, is obtained by dividing $PE_{sing.}$ by the square root of the number of samples analyzed.

A number of studies have been made to determine the relationship between

dose and effect (2, 3, 4, 5, 6, 7, 9, 12, 13, 16, 18, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30). In certain types of assays, such as toxicity, the "all or none" law may be employed. A lethal dose may be defined as the dose killing all test animals. A sub-lethal dose may be defined as that dose killing a certain percentage of the animals injected. By using a sub-script indicating the number of animals tested, and another sub-script indicating the percentage giving any desired degree of effect ($_nED_{\%}$), this standard curve relationship may be broadened to cover various quantitative bioassays. Many investigators prefer the Median Lethal dose, $_nLD_{50\%}$.

The number of animals required for an assay depends upon three factors: the degree of certainty to be placed in the results ("k"); the probable error of the assay (PE); and the difference which is considered to be discriminatory ("*Diff.*"). Of the variety of formulas for determining "how many are enough?" that formula suggested by Haynes and Judd (11) and further developed by Denny (6) for calculating the number of fruits required for adequate analytical samples, seems the simplest and most readily understood:

$$N = 2\left(\frac{k \times PE}{Diff.}\right)^2$$

When two of the four variables in this equation are arbitrarily made constant, the third variable will vary as a function of the fourth variable. If three variables are fixed, the expression becomes invariant. To illustrate the application of this formula, a series of examples have been worked out, with the results in Table II.

How many animals will be required for an assay in which the degree of certainty sought is 100:1, PE is 10 per cent, and the difference to be detected is 10 per cent? Substituting these values in the equation,

$$N = 2\left(\frac{3.82 \times 10}{10}\right)^2 = 2 \times (3.82)^2 = 29$$

When PE and *Diff.* are identical, and cancel out, the equation is simplified to:

$$N = 2\,k^2$$

when PE and *Diff.* are not identical, the longer formula must be used.

CONCLUSION.

A formula has been developed to determine the number of animals required to produce a specified degree of assurance that a particular difference in experimental results is statistically significant.

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PHYTOCHEMICAL STUDY. SEED OF THE MAGNOLIA GRANDIFLORA.*

BY ST. ELMO BRADY.[1]

The magnolia tree is one of the most interesting and beautiful of the trees which are embraced in the flora of North America. In the southern part of this country it grows to a magnificent height and is prized highly as an ornamental tree primarily for its beautiful, well-scented white flowers. No complete chemical examination has hitherto been made of the seed of this tree, and the constituents of the fatty oil have been only imperfectly known. Most of the published works have been confined chiefly to the examination of the volatile oil of the leaves and bark. Proctor (1) made an examination of the fruit of the *Magnolia tripetala* and reported the isolation of crystals during the course of several experiments without indicating anything as to their composition. Rawlins (2) examined the leaves of the *Magnolia glauca*, L., and recorded the presence of crystals, calcium oxalate, volatile oil, and extracts which possess a bitter taste, impart fluorescence to chloroform, and after boiling with sulfuric acid reduce Fehling's solution. Randolph (3) made a study of the bark of *Magnolia grandiflora* and recorded the following constituents: tannin, starch, saccharine and coloring matter. Greshoff (4) examined the bark of several species of the magnolia and reported the presence of a bebeerine-like alkaloid.

The present study is the first of a series which is being carried out in this laboratory on the *Magnolia grandiflora*. Interests in the tree grew out of the reported statements that the bark has been used, domestically, in infusion or decoction for the treatment of rheumatism and malaria; and tinctures of the bark in brandy or whisky are said to produce cures in chronic cases of chills and fevers

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