DISCUSSION

THE FOUR-FOLD TABLE AND THE HETEROGENEITY TEST

IN a recent issue of SCIENCE the interpretation of observations in a four-fold table has been discussed by E. B. Wilson,[1] and later some critical remarks to this discussion have been made by R. A. Fisher.[2] Wilson considers two samples of mice into each of which two different viruses have been injected with a result which may be written so:

	Died	Lived	Total
Sample 1	a	b	$a+b$
Sample 2	c	d	$c+d$
Total	$a+c$	$b+d$	n

The difficulty met by him is that, using different methods of analysis, he arrives at different grades of significance of the difference in death rate between the two samples. As Fisher points out, the reason is that Wilson assumes some plausible value for the death rate as known to be true ($\frac{1}{2}$ in his case). Therefore Fisher recommends the method known as "Fisher's Exact Method,"[3] since the probability P in this case is independent of the true death rate. (This independence is, however, probably only assured when applying method I, below, for the measurement of the discrepancies between the two observed samples). The cause of this independence is that of the $(a+b+1)(c+d+1)$ possible combinations of two samples with the given *sample* totals, $a+b$ and $c+d$, only those combinations having the same *marginal* totals, $a+b$, $c+d$, $a+c$ and $b+d$ are selected by Fisher as a sufficient base for the comparisons. It seems, however, that the primary base of legitimacy of this procedure of selection is that it makes the probability P independent of the unknown, true proportion p. In the following lines, therefore, it is assumed that it is necessary to compare all possible combinations of two samples having the given *sample* totals.

But other difficulties arise. The biologic question is, if the two samples may have been drawn from a common population; or, in other words, if a proportion p may, at least theoretically, be determined, such that, taking this proportion as the true one, the value of the probability P is greater than some at beforehand assigned level of significance. The number P is always made up of the sum of the probabilities of all separate combinations of two samples for which the discrepancy is as great as or greater than that in the observed two samples (in one or both tails). Therefore the first difficulty arises when choosing among different ways of measuring this discrepancy. Many such ways are possible, but consider the following three: I. The arithmetic difference between the proportions in the two samples. Greater difference means greater discrepancy. This method is used in the cited articles by Wilson and Fisher. II. The probability of the separate combinations of two samples. Smaller probability means greater discrepancy. III. χ^2 of the separate combinations of two samples. Greater χ^2 means greater discrepancy. Method I is simplest, but its logic is questionable, since it is not applicable to tables with more than two classes in each sample. When applying methods II and III, however, the answer to the question, if the probability of a certain combination of two samples is to make part of P, is dependent on the value of p.

Another difficulty is to settle which estimate of p is to be looked upon as the "best" one. Usually the maximum likelihood solution $p=\frac{a+c}{n}$, which makes the probability of the separate observed combination of the two samples to a maximum, is taken as the best one. By minimizing χ^2 we get the estimate $p=\frac{k}{1+k}$, where

$$k^2=\frac{a^2(c+d)+c^2(a+b)}{b^2(c+d)+d^2(a+b)}.$$

As a rule, however, none of these estimates gives a maximum value to P.

As a very simple example, take the four-fold table

			Total
Sample 1	0	2	2
" 2	2	1	3
Total	2	3	5

The maximum likelihood estimate is $p=.4$ and the minimum χ^2 estimate is $p=.4305$. For these and some other values of p Table 1 has been worked out.

TABLE 1

p	Probability of the separate observed combination of the two samples	χ^2 of the separate observed combination of the two samples	Probability P when the discrepancy is measured according to method		
			I	II	III
.39	.1036	2.2440	.2264	.3904	.3904
.40	.1037	2.2222	.2304	.4989	.3952
.41	.1036	2.2068	.2341	.4999	.4005
.4305 ..	.1027	2.1943	.2404	.5035	.5035
.45	.1011	2.2054	.2450	.5089	.5089
.50	.0938	2.3333	.2500	.6250	.6250
.55	.0827	2.6094	.2450	.4079	.3255
.60	.0691	3.0556	.2304	.2138	.2915

In the computation of P in Table 1, both tails have been taken as parts of P. Applying Fisher's "Exact Method" we get $P=.4$, if both tails are added, but $P=.3$ if (as Fisher himself always seems to do) only the tail of the same side as the observed samples is taken into account.

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

[2] R. A. Fisher, SCIENCE, 94: 210–211, 1941.

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

It is clearly seen that for the $p:s$ chosen, $p = .50$, though greater than the maximum likelihood estimate, gives maximum value to P, whatever method used. All values of P in this example are far above the usual levels of significance. But cases may well occur where P falls short of such a level when the maximum likelihood or the χ^2-minimum estimate is used, though P may be greater than the level for other values of p, and that thus the two samples may have been drawn from a common population.

It may finally be pointed out that difficulties of quite the same kind occur when dealing with tables of higher rank than the four-fold, especially when making heterogeneity tests. It is only the limitation of available methods which is the defense of our mode of attacking the problems.

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A NEW THEORY OF THE ORIGIN AND NATURE OF LIFE[1]

OF the numerous theories which have been proposed to account for the origin of life nearly all have been hypothetical reconstructions based on biochemical considerations, but unsupported by laboratory demonstrations linking the implied syntheses with the genesis of specific structural entities which simulate, both in appearance and in behavior, forms already known to the biologist. After devoting forty-three years to the experimental investigation of this problem, I have been able to produce two principal groups of phenomena which appear to me to be significant:

1. *Colpoids.* Mix thoroughly 200 cc of very fresh, pure olive oil with 800 cc of clear, pure gasoline. Place the mixture in a flat-bottomed container (a tray of the sort used to develop photographic negatives is advantageous) and add, drop by drop, a solution of pure sodium hydroxide (12 g to 100 cc) to which has been added a gram of hematoxiline to serve as a stain. The resulting macroscopic cells exhibit lively ameboid movement, intracellular streaming and fission. Their activity is inhibited by chloroform. We are dealing here with saponification activated by osmotic currents and electric disturbances set up by the chemical reaction, but the parallel with overt phenomena of life on the protozoan level is so striking that one is compelled to seek identical explanations. This experiment was presented a number of years ago at a session of the New York Microscopical Society by Dr. C. W. Weiant and more recently was exhibited in motion pictures before the Congress of Biophysics in New York.

2. *Sulphobes.* Taking it for granted that formaldehyde is an essential stage in the synthetic activity of green plants, I made a methodical study of the action of reagents on formaline. The fumes of ammonium sulfide acting upon thin layers of formaline produce many of the structural aspects of protoplasm. Since, according to Pflügger, life is due to cyanogen and its derivatives on up to the proteins, I decided to dissolve ammonium thiocyanate in formaline, spread the material in very thin layers, and then waited several hours before making a microscopic examination. I have repeated this experiment, under varying conditions, for a period of ten years, thereby obtaining thousands upon thousands of microscopic structures with activities analogous to those of living organisms.[2] Chemical products include vestiges of starch, at least two amino-acids, a condensation product of protein character and globules of green, yellow and red pigments. The latter substances I am now investigating. They do not manifest absorption rays in the spectrum, perhaps because present in such minute quantities. Structures noted comprise cell, ameba and tissue forms of infinite variety, imitating virtually the whole microscopic world. More than 6,000 varieties, among them the counterparts of diatoms, spermatogonia, spores, chromosomes and astrospheres, direct and mitotic divisions, plasmodia, etc., have been recorded and published during the past ten years. I have sent to foreign scientists and scientific institutions more than 900 specimens preserved in Canada balsam and will gladly send additional samples without cost to any interested inquirer.

Now let it be remembered that it is possible to synthesize the thiocyanate used in these experiments by subliming sulfur in a matrass with ammonium nitrate and carbon. Sulfur alone sublimed on cold glass yields no end of cellular patterns, by virtue of its molecular polymorphism and resulting allotropic states. In view of these facts, may it not be that the emanations from volcanoes—sulfurous, cyanic and ammoniacal—have produced and continue to produce microorganisms by chemical synthesis? I intend to study the sublimates of the solfataras of Popocatepetl from this standpoint. Sulfur is present in nearly all proteins and in all living organisms and thus merits special attention in any theory of the origin of life. The particular theory offered here of course lacks confirmation. Much further research is required for that, but it is a theory which, up to a certain point, finds laboratory corroboration. It is itself the outgrowth of experimental method.

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[1] Translated by C. W. Weiant.

[2] See *Bulletin du Laboratoire de Plasmogenie*, Vol. I, and Vol. II now appearing.