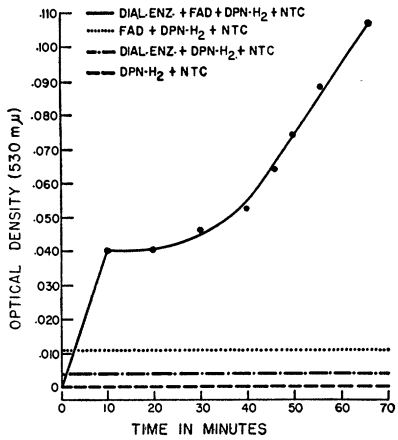

FIG. 2. Reactivation of bacterial oxidase after dialysis in dilute HCl. The system consisted of 0.5 ml bacterial enzyme, 24 μg FAD, 100 μg neotetrazolium, 1 ml *M*/10 pyrophosphate buffer pH 7.8, and distilled water; incubated for 10 min before the addition of 1000 μg reduced DPN to make a final volume of 3 ml. Control systems are indicated.

centrations of flavin adenine dinucleotide (FAD) were added in order to determine the rate of oxygen uptake as a function of the flavin concentration. The flavin content of the hydrolyzed bacterial oxidase was then determined in the same manner. It was capable of reactivating the D-amino acid oxidase apoenzyme. The nonhydrolyzed enzyme failed to reactivate the oxidase.

Although the data indicated the presence of FAD, it seemed essential to demonstrate the necessity of this coenzyme for enyzmatic activity. By dialyzing the bacterial enzyme for 24 hr in the presence of dilute HCl and potassium monobasic phosphate, similar to the method of Warburg and Christian (*9*), an apoenzyme was prepared which was inactive in the presence of reduced DPN and neotetrazolium (Fig. 2). When FAD was incubated with this apoenzyme, the system was reconstituted, and about 60% of the original activity was recovered. Neither reduced DPN nor FAD, alone or combined, could reduce the neotetrazolium.

By limiting the addition of flavin (Fig. 3), it was found that the amount of diformazan produced is a function of the concentration of added FAD. Experiments were performed in which the rate constant in

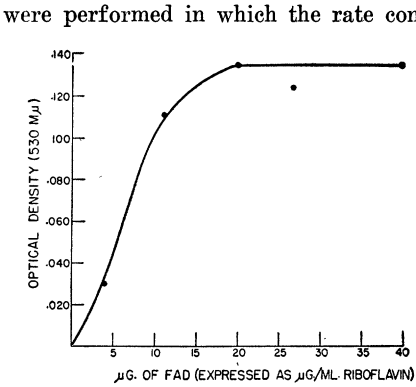

FIG. 3. Effect of various concentrations of FAD on bacterial oxidase after dialysis in dilute HCl. (Conditions same as in Fig. 2.)

mole^{-1} min^{-1} liter was determined for the oxidation of the bacterial enzyme with neotetrazolium and oxygen (*10*). The bacterial enzyme was found to have a greater turnover when neotetrazolium was used as an electron acceptor. The rate of reaction of neotetrazolium was similar to the rate of reaction of ordinary redox dyes such as methylene blue.

In bacteria the reduction of tetrazolium salts has been shown to occur in discrete granules (*11*). *In vitro* this reduction with isolated bacterial enzymes can be accomplished by flavin enzymes. Neither DPN-dehydrogenases nor reduced DPN, per se, can reduce these salts to the formazan state.

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The Grafting of Large Monocotyledonous Plants

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The grafting of plants has been a common horticultural practice for many centuries. For almost as great a stretch of time two ideas have been associated with this practice: first, that monocotyledonous plants cannot be grafted; and second, that leaves cannot be grafted. As late as 1947, Transeau, Sampson, and Tiffany (*1*) stated, "Neither twig grafting nor budding is possible in stems without cambiums." For successful union it was stated by Eames and McDaniels (*2*) that the cambium of the scion must be united with that of the stock. Similar expressions may be found in dozens of textbooks.

In 1946 La Rue and Reissig (*3*) reported that leaves of numerous species could be grafted and found that fleshy leaves required a technique different from that used with thin ones.

Calderini (*4*) reported, more than a hundred years ago, that he had made successful grafts of rice on barnyard grass. He claimed that by this means he had produced a new strain of rice with superior characteristics which, thereafter, were transmitted by the seeds. The latter statement, however, brings into question the validity of the entire paper. Plotnikov (*5*) made grafts in grains, and Monakima and Sölevey (*6*) grafted

FIG. 1. Longitudinal section of a sugar-cane stem, showing union between scion and stock, 30 days after grafting. Some vascular connections between stock and scion are now established.

monocotyledonous bulbs. La Rue (*7*), without knowledge of the work of the three preceding investigators, grafted species of *Tradescantia, Zebrina,* and *Commelina.* He proved that the vascular bundles of stock and scion could unite when pieces of broken intercalary meristems on stock and scion were fastened in contact with each other.

In spite of the preceding records of success, no one, so far as the authors are aware, has successfully grafted any of the larger monocotyledons. Although cambiums are not usually found in monocots, or are limited in amount and confined to unusual locations, such as bulbs, corms, etc., other meristems do occur. The most important of these in this study was the intercalary meristem, a region just above the node which retains its meristematic qualities long after the rest of the stem is mature. By appropriate manipulation of this structure, successful grafts of bamboo (*Bambusa longispiculata* Gamble ex Brandis), sugar cane (*Saccharum officinarum* L.), guinea grass (*Panicum maximum* Jacq.), and Merker grass (*Pennisetum purpureum* Schum.) were obtained. On the average about 3% of the grafts were successful.

The method used was very simple. The shoot was grasped firmly and broken with a quick jerk. The young, thin-walled cells in the intercalary region are weaker than the adjacent mature thick-walled cells, and the rupture occurs in this region. The same stem may be replaced, or a stem of the same size from another plant of the species may be inserted. An exact fit is very important, but trimming the edges of the graft proved deleterious. In most of the plants tested, the leaf sheath served to support the graft. It was advantageous to tie the grafts firmly to ensure even, continuous contact. Best results were obtained with young, vigorous material.

Histological sections were made at various stages of the graft union and stained with fast green and safranin. At the junction of stock and scion a week after grafting there is a thin dark layer, which is probably formed in part by the remnants of cells injured in rupturing the stock and scion and in part by oxidation on the surfaces of cells torn apart but with walls left intact. Resorption of this contact layer occurs first between the ruptured vascular bundles and is more or less limited to these areas. Cell proliferation occurs simultaneously along the edges of both stock and scion, and a band of cambiumlike tissue is sometimes formed. In successful grafts, this layer is usually limited to a few tiers of cells. These parenchyma cells differentiate into short, lignified tracheids, with scalariform thickening, and reunite the vascular bundles. It is apparent that survival of the graft is dependent on the successful resorption of the contact layer, at least between the broken vascular bundles. Definite vascular connections were first observed about 4–6 weeks after grafting (Fig. 1).

During this period the scion remains green, but no elongation occurs. New growth occurs from terminal or axillary buds. In sugar cane, the scions sometimes formed roots, giving a fictitious appearance of successful union. After a successful graft begins to grow it sometimes continues at about the same rate as on intact plants, although on others it may be very slow. For example, one of the bamboo grafts grew approximately 10 ft in 8 months, whereas another stayed green but grew hardly at all over the same period. Grafted sugar-cane and guinea grass plants flowered at the same time as ungrafted plants.

Microscopic examination showed that in the rejoining of stock and scion, there was seldom perfect juxtaposition of the vascular bundles, and the reunion often took place in a curve, rather than a straight line. This is a clear demonstration of the influence of the vascular tissue on the proliferation and differentiation of parenchyma cells. Differentiation occurs both upward and downward, although mostly in a downward direction.

On the Solomon Island ivyarum, *Scindapsus aureus* (Lindl. & Andre) Engl., a large tropical liana, a different technique was used. This species does not have an intercalary meristem, but the actively growing tips remain more or less meristematic throughout the youngest 3 or 4 internodes. Such tips were grafted by inarching, and the unions were made in a transverse rather than a longitudinal direction. Other grafts on this plant were made by rupturing the young inter-

node as in the previously described experiments, but since these plants have no leaf sheaths, the graft was supported with waxed paper tubes slipped over the stump and scion, or with bamboo splints, which served to hold the broken edges in contact until union occurred. This suggests that other monocotyledonous species may also be grafted, even though they may lack an intercalary meristem.

It is doubtful that these techniques will soon find practical application, unless a greater percentage of success can be achieved, but they should be useful for certain types of investigation.

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Tables for Use in Fourfold Contingency Tests[1]

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Statistical tests are becoming more and more commonly used by professional research workers, technologists, and "occasional investigators" such as medical practitioners. Many of these workers, however, find the arithmetic irksome, and, when dealing with small samples, they are perturbed by the possibility that the simple familiar tests may be misleading. For some tests both these problems can be solved by tables that demand little or no computation by the user. The possible objection to such tables, that they will tend to reduce the investigator to a statistical automaton, does not, we think, apply to tables more than to tests that require calculation. Repetition of arithmetic does not increase insight into the meaning of tests, and reduction of arithmetic may allow emphasis to be placed where it should always be placed, on sampling and inference.

In order to solve the two problems reliability of verdicts and reduction of arithmetic, tables for use in contingency tests and in the estimation of binomial confidence limits were published in an article on statistical methods for medical research workers by the National Research Council of Canada in 1948 (*1*). That article is now out of print, but copies are still requested by workers in many branches of applied science. Revision and extension of the tables have therefore been undertaken, and the first two of the new series are presented here.

Purpose. These two tables are primarily for the comparison of equal samples, with individuals classified as *A* and not-*A*, and arranged in a fourfold contingency table; for instance: animals wounded by the same method and randomly allocated to two diets for comparison of fatality rates; two successive differential blood counts from the same patient to show a change in the neutrophil leukocyte percentage; samples of an industrial product made by two slightly different machines and examined for proportions defective; samples of tagged fish or birds liberated in different localities or under different conditions, for comparison of proportions subsequently recovered.

In each instance, if the investigator wishes to make allowance solely for random sampling (chance) variation he will ordinarily apply the chi-square contingency test, introducing for greater accuracy Yates' correction for continuity; or he may use an equivalent form of the standard error of the difference between two proportions. Owing to the smallness of samples or skewness of distributions, one or more of the expected values in the χ^2 calculation may be small, and the smaller the expected value the less dependable is the χ^2 test, even with Yates' correction. The investigator can apply certain empirical rules (*2*) to determine the safety of the test, and if it is unsafe he can do a further calculation and use Table VIII of Fisher and Yates (*3*). If that is insufficient he can calculate the exact probabilities (Fisher [*4*], Sec. 21.02).[2]

Our tables can be substituted for all these methods, even the initial χ^2 calculation, when the samples are equal and when, as is usually the case, the investigator requires only an assessment of significance at the conventional 5% and 1% levels—i.e., when the standards are $P = 0.05$ and $P = 0.01$, where P is the probability for χ^2 or the corresponding (two-tailed) exact probability.

Method of using the tables. Let us imagine two samples, *V* and *W*, each containing 30 individuals. *V* is composed of 17 *X*s and 13 *Y*s; *W*, 20 *X*s and 10 *Y*s. For ease in entering our tables, we form a contingency table, with the order of the samples changed, thus:

Sample	*Y* (*A*s)	*X* (not-*A*s)	Total (*N*)
(1) *W*	10	20	**30**
(2) *V*	13	17	30

The *W* sample, having the greater discrepancy between *X* and *Y*, is placed in the upper line and becomes Sample (1); and in that sample the smaller value, 10,

[1] This article is part of a biometrical tables project conducted in the New York University Division of Medical Statistics. For grants-in-aid of the project we are much indebted to the Abbott Laboratories, the Milbank Memorial Fund, Parke, Davis & Company, Chas. Pfizer & Co., and the Squibb Institute for Medical Research. For carrying out nearly all the computations we wish to thank Catherine Mescal and Joan M. Hansen.

[2] Sometimes this method is incorrectly referred to as "the exact chi-square" method. The distinction can be illustrated in an elementary way by a sampling experiment (*2*).