tidis, and *Sporotrichum schenckii*) incubated on the horse serum agar (pH 7.0) at 37° C also were inhibited by 1.0-mg/ml concentrations of di-phenyl-pyraline and pyribenzamine. The growth of *C. albicans* was not affected by either drug.

Results obtained from these preliminary studies suggest that both di-phenyl-pyraline and pyribenzamine possess properties which are inhibitory to pathogenic fungus species and that their activities, therefore, are not limited to the alleviation of allergic manifestations incited by these organisms. The results also suggest that further clinical study is imperative to determine the effectiveness of these drugs *in vivo*.

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Triphenyltetrazolium Chloride in Tissue Culture[1]

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A number of reports have recently appeared in the literature describing a tetrazolium salt (2,3,5-triphenyltetrazolium chloride) as a possible indicator of viability of plant and animal tissues. Lakon (*3*), Cottrell (*1*), and Porter, Durell, and Romm (*4*) have used this compound to test the viability of seeds and wood cuttings, and Straus, Cheronis, and Straus (*6*) employed this salt to demonstrate reducing enzyme systems in living normal and neoplastic mammalian tissues.

In studies of the nucleic acids and of the effect of folic acid and its analogues on tissue cultures, it is occasionally important to determine cytologically the number of living cells present in a particular culture. Since the cytoplasm and nuclei of living cells do not stain with any of the vital dyes, and dead cells stain only diffusely, the use of tetrazolium as an indicator of cellular viability suggested itself. It is the purpose of this paper to report the failure of 2,3,5-triphenyltetrazolium chloride as an indicator of cell viability of tissue grown *in vitro*.

The method employed in testing this compound consisted of growing chick-heart fibroblasts under perforated cellophane (*2*) in a medium composed of equal parts of human fetal (umbilical cord) serum and Simms salt-diluted ultrafiltrate (*5*). After 72 hr, when sufficient migration and outgrowth of fibroblasts had occurred, the cultures were placed in a 1% solution of the tetrazolium salt in 0.9% NaCl. They were then examined grossly and microscopically, every 20 min for 6 hr, for evidence of reduction of the colorless tetrazolium to the red insoluble formazan. In none of the 16 cultures tested was there any observed reduction of the salt. Cultures left in this solution for 48 hr also gave negative results.

The failure of whole, uninjured, rapidly growing embryonal cells to reduce tetrazolium chloride indicates that this compound is not necessarily a measure of cellular viability. The action of tetrazolium chloride depends on combination with dehydrogenases which cause a reduction of the dye to its colored form. Apparently the failure of the compound to penetrate through the living cellular membranes is responsible for the negative results obtained.

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A Modification of the Hardy-Weinberg Law

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The Hardy-Weinberg law, as it is customarily specified or understood, applies to populations which are indefinitely large, which are panmictic, and in which gene frequencies are the same in both sexes. If, in addition to these conditions, gene frequencies are constant, genotype frequencies will also be constant through succeeding generations (*2–6*).

Whether gene frequencies are constant or not, the primary distribution (*primary* being used in the same sense as applied to sex ratio) of mean genotype frequencies for two alleles A and a, with respective frequencies p and q ($q=1-p$), is written $p^2AA:2pqAa:q^2aa$ when A and a are autosomal and ♀♀ $(p^2AA:2pqAa:q^2aa)$ + ♂♂ $(pAY:qaY)$ when they are sex-linked. But the frequency of one of the two alleles, say q, may be very small, and when it is sufficiently small it is evident that generations will occur in which homozygous recessives will not be produced; regardless of the smallness of q, the term q^2 then represents the probability of occurrence of genotype aa, but not its frequency, which of course is *0*. Under this condition the genotypic distribution for a pair of autosomal genes A, a becomes $(p-q)AA:2qAa$, which provisionally may be called a limiting distribution. This can readily be seen, since under panmixis the proportions of pA and qa that recombine will be equal and will be limited by the lesser of the two gene frequencies, in this case q. Hence the frequency of Aa is $2q$, while that of AA is $1-2q=p-q$. When sex-linkage is involved the distribution becomes, obviously, ♀♀$(\overline{p-q}AA:2qAa)$ + ♂♂$(pAY:qaY)$, and the ratio of female carriers to male exhibitors is $2q:q=2:1$, rather than $2pq:q=2p:1$ as in the Hardy-Weinberg distribution.

Application of the Hardy-Weinberg law to determining primary distribution of genotype frequencies requires not only that the population be indefinitely large, panmictic, and with gene frequencies equal in both sexes, but also that the relation between the population number N (breeding population) and the gene frequency q be such that $q \geqq N^{-0.5}$. Whether a Hardy-Weinberg or a "limiting" distribution obtains actually depends upon this relation between q and N, rather than upon the absolute size of either (Dahlberg [*1*] and Hogben [*3*] have discussed a similar though not identical situation). It follows that, on the average, the point in the possible values of q at and above which the Hardy-Weinberg distribution applies, and below which it does not, is given by log $q = 0.5$ colog N. When log $q < 0.5$ colog N, genotype frequencies take the form of a limiting distribution, the Hardy-Weinberg distribution then being a probability distribution but not a frequency distribution, with respect to the pertinent genotypes.

The consequences of this relation between population size and gene frequency are at present being worked out for a number of different genetic conditions.

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A Simple Pulsating Perfusion Apparatus

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Descriptions of a number of perfusion devices are available in the literature. Many of these are highly complex, adapted to special purposes, or inefficient. We have found that a very satisfactory perfusion apparatus can be simply prepared using a Sterling Automatic Pipette.

The most important parts of a pulsating perfusion device are the valves to control the direction of flow of the blood, and a mechanism to simulate the pumping of the heart. The delivery mechanism of the automatic pipette effectively embodies both these features. The pipette used by us was a Sterling Automatic Pipette, Model 4-3 M (Ivan Sorvall, New York). The amount of perfusate delivered per stroke can be regulated from 0.5 ml to 3.0 ml by adjustment of the stroke regulator. In order to control the rate of pulsation we attached the delivery mechanism of the pipette, by means of a reduction gear, to a variable speed motor. We have found, however, that the perfusion pressure can be adequately adjusted by means of the stroke regulator when delivery rate is 50 strokes per min.

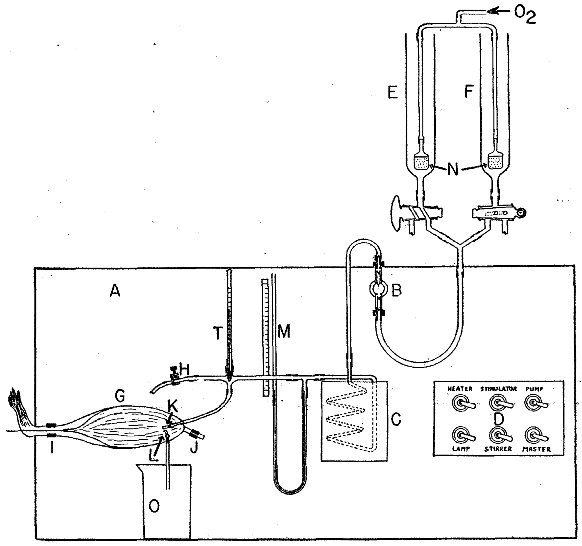

FIG. 1.

Fig. 1 presents diagrammatically a successful adaptation of this pump for the perfusion of an isolated dog hind leg, using defibrinated blood. The leg was prepared as described by Huston, Martin, and Dille (*1*). The leg was kept in good condition for several hours during investigations of the effects of certain drugs on the somatic neuromyal junction.

The tubing and clamps for the leg are attached to a perpendicular board, A. The motor for the pump and the apparatus for the constant temperature water bath are placed behind the board. Just the head of the pipette, B, and a small chamber of the water bath, C, protrude through holes in the board. In this way the work area is kept free of apparatus. The electrical equipment is controlled by switches on plate D.

The blood is stored in two reservoirs, E and F; one is for normal and the other for experimental blood. Oxygenation is accomplished by bubbling oxygen through small Mandler-type filters in the reservoirs. A two-way stopcock at the bottom of each reservoir provides a bypass.

The blood passes from the reservoir to the pump, B, and to the leg, G, through a coil in a constant temperature water bath, C. The perfusion pressure is recorded on a manometer, M; and the temperature on the thermometer, T. H is a bypass for rapid withdrawal of blood from the system.

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