by the cell in that stage. However, λ is a measure of the growth rate and must be assumed to be constant.

The biologic variation can be taken into account by considering the "partial mitotic index," $m_i/n_i = \lambda_i T_i$. The total mitotic index becomes:

$$\frac{m}{N} = \frac{\Sigma m_i}{\Sigma n_i} = \frac{n_i \lambda_i T_i}{\Sigma n_i}. \quad (1)$$

If T_i is the same for all cells in the tissue mass, the mitotic index becomes:

$$\frac{m}{N} = \frac{T}{N}\{n_1\lambda_1 + n_2\lambda_2 + \cdots + m_r\lambda_r\}, \quad (2)$$

indicating again that the index is proportional to the product of T and the volume rate of growth of the tissue. The biological variation, *i.e.* the distribution of cells into the numbers n_1, n_2, n_3 .....can be measured by measuring the volumes of cells in early prophase. This will give a frequency distribution of volumes which, when compared with the frequency distribution of volumes of all cells in the tissue, should give a measure of the fraction of cells in the so-called "resting stage." This fraction is of interest in exponentially growing tissue masses because it indicates the possibility that the growth constant, λ, for the entire tissue mass is less than that for the cells which actually contribute to the growth. Values of λ = 1.33 days^{-1} lead to a volume-doubling time of 0.3 days. If a fraction of all cells are resting, the growing cells must double their volumes (on the average) in a time less than 0.3 days.

In the case of a transplantable mammary adenocarcinoma (*2*, *5*), for small tumor volumes (less than 0.5 cc.), the volume growth of all cells can be considered approximately equal. Here Wright's hypothesis may be generalized and applied to the distribution of cell volumes to measure the rate of growth of the tumor mass. According to the hypothesis, the number of cells, Δn, having volumes in the interval, Δv, for a time, Δt, is given by

$$\frac{\Delta n}{N} = \frac{\Delta t}{L}, \quad (3)$$

where L is the intermitotic time and N is the total number of cells. If the frequency distribution of volumes is $\phi(v)$ then $\Delta n = \phi(v)\,\Delta v$, and the intermitotic time is

$$t = \frac{L}{N}\int_{v_0}^{v} \phi(v)\,dv. \quad (4)$$

Here v_0 is the smallest volume in the distribution, and t is a function of v. Equation 4 is the inverse of the tissue growth function, $v = V(t)$, and permits the determination of volume growth by means of the distribution function, $\phi(v)$. It can be shown that for exponential growth in the ideal case the function $\phi(v)$ is of the form $1/v$. Preliminary integration of the volume distribution of cells published (*2*) shows that Equation 4 leads to a logarithmic relation between t and v with a characteristic constant, λ = 0.44 days^{-1}, where the measured value was λ = 0.37 days^{-1}. Improved techniques for measuring the cell volume distribution are being developed. The method must be tested on tumors of various ages to ascertain the possible effect of "resting cells" on the volume distribution.

It should be emphasized that this use of Wright's hypothesis applies only to a homogeneous, dedifferentiated group of cells having uniform growth rate.

References

1. Brues, A. M., and Marble, B. B. *J. exp. Med.*, 1937, **65**, 15-27.
2. Hoffman, J. G., Goltz, H. L., Reinhard, M. C., and Warner, S. G. *Cancer Res.*, 1943, **3**, 237-242.
3. Hunt, A., and Walker, A. C. *Amer. J. Physiol.*, 1945, **143**, 226-234.
4. Marshak, A., and Walker. A. C. *Amer. J. Physiol.*, 1945, 226-2340.
5. Reinhard, M. C., Goltz, H. L., and Warner, S. G. *Cancer Res.*, 1945, **5**, 102-106.
6. Wright, G. P. *J. roy. micro. Soc.*, 1925, 414.

Germination and Free Fatty Acid in Individual Cotton Seeds

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Seeds from cotton that has been exposed to wet weather in the field are likely to be lower in viability and to contain higher percentages of free fatty acids than those from seed cotton harvested without unfavorable exposure (*3*, *4*). Similar observations have been made of cottonseed stored under conditions of high moisture or temperature (*4*). Conventional methods of approach to the relationship of free fatty acid content to germination would require that a sample of several hundred grams of cottonseed for the free fatty acid determination and another sample of several hundred seed for germination tests be drawn from each lot tested. When sufficient data were obtained, statistical methods could be used to study the relationship between the two variables. A second approach to the problem consists of the application of microchemical methods to the analysis for the free fatty acid content (*2*) of part of the nongerm portion of a single seed and the germination of the remainder of the seed.

In order to establish whether the free fatty acid content of the nongerm end of a hulled cotton seed was correlated with that of the germ end, 50 seeds were carefully peeled and cut approximately in half; each half was weighed and placed in a numbered, small, glass-stoppered Erlenmeyer flask. To each flask 5 ml. of petroleum ether (American Oil Chemists' Society, Specification H 2-41) was added and allowed to stand for about 30 minutes to soften the seeds. The seeds were then ground by means of a glass rod with a flattened end. Any material adhering to the rod was washed into the flask by means of an additional 5 ml. of the petroleum ether. The flasks were then stoppered and allowed to stand for about 16 hours with occasional shaking. After the extraction was completed, 10 ml. of neutralized alcohol containing m-cresol purple indicator was added and the mixture immediately titrated with 0.005 N alcoholic KOH. During the titration the effect of atmospheric carbon dioxide was eliminated by bubbling a stream of carbon dioxide-free air through the titration flask. The free fatty acid content is calculated as per cent oleic acid by multiplying the milliequivalents of alkali used by 28.2 and dividing the product by the weight

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of the portion of kernel extracted. These values may be put in terms of the free fatty acid in the oil by multiplying by 3, since the kernels contain approximately one-third oil.

The fatty acid content of the germ ends varied from 0.4 to 23.6 per cent and averaged 6.9 per cent; that of the nongerm ends, from 0.4 to 23.8 per cent with an average of 7.2 per cent. The correlation coefficient was 0.79, indicating a highly significant, though not by any means perfect, correlation between the free fatty acid contents of the two ends of a cotton seed. Analysis of the composited germ ends and composited nongerm ends of several hundred cottonseed showed that the nongerm end contained 39.5 per cent oil, while the germ end contained 38.7 per cent.

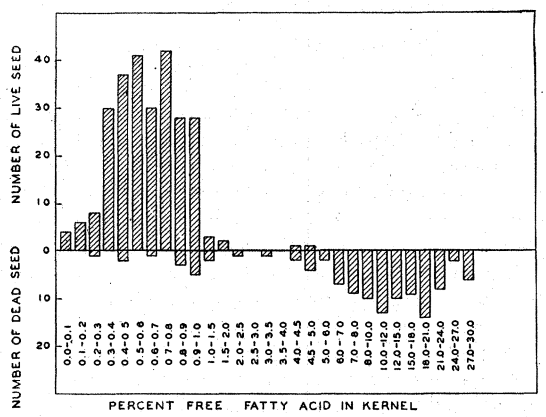

FIG. 1. Distribution of viable cottonseed according to per cent free fatty acid in kernel.

The seeds used to obtain the data plotted in Fig. 1 were selected from 10 sample lots of seed: three lots from the 1941 crop, five from the 1942 crop, and two from the 1943 crop. The seeds used were from experimental plots grown at Tifton, Georgia, Florence, South Carolina, Knoxville, Tennessee, and Baton Rouge, Louisiana, and included the following varieties: Coker's Farm Relief No. 5, Coker's 100 str. No. 3, Acala 1-13-3-1, Rowden 42A, Stoneville 37-10, and Arkansas Green Lint. The seeds were carefully hulled by hand using a razor blade. They were then cut approximately in half perpendicular to the long axis. The germ end was sterilized by dipping in a solution containing 0.25 gram of mercury bichloride dissolved in a liter of 50 per cent ethanol (*1*). After being rinsed in sterile distilled water the germ end was almost completely submerged, pointed end down, in sterile nutrient agar in a numbered test tube, and after being covered with a sterile cap the tube was placed in the dark to germinate. When growth above the agar, accompanied by root formation, was noted, germination was rated positive. If such growth was not observed within two weeks, the seed was rated dead.

The nongerm end was weighed, placed in a numbered flask, extracted and titrated as described above.

In Fig. 1 are shown the results obtained from 369 individual cotton seeds. Several dead seeds were found whose nongerm end contained less than 1 per cent fatty acid. These may have been seeds which were nonviable from some cause which did not produce an increase in acidity or seed in which the acidity development was higher in the germ end than represented by the analysis of the nongerm end. All seeds whose nongerm end contained over 5 per cent of fatty acids were dead. A striking feature of the data is that so few seeds were found whose nongerm end contained from 2.0 to 4.0 per cent fatty acid. Only two seeds were found, probably indicating a rapid rise in fatty acid content in this range. Two seeds having fatty acid content in the nongerm end in excess of 4.0 per cent were found to be viable. Since these showed some discolored spots, the value for fatty acid obtained on the nongerm end is probably considerably higher than that of the germ end. The data indicate that most (over 71 per cent) of the seeds contained less than 1 per cent of free fatty acid, whereas the fatty acid content of the others ranged from 1 to 30 per cent. In the group of seeds containing less than 1 per cent the number of live seeds was about 21 times the number of dead seeds, while in that containing from 1 to 30 per cent there were 14 times as many dead as live seeds.

References

1. ARNDT, C. H. *Plant Physiol.*, 1945, **20**, 200.
2. HOFFPAUIR, C. L., and KETTERING, J. H. *Amer. Dyestuff Rep.*, 1946, **35**, 265-266.
3. MELOY, G. S. *Oil & Soap*, 1939, **16**, 174.
4. SIMPSON, D. M. *J. agric. Res.*, 1942, **64**, 407.

Oral Efficacy of BAL in Protecting Rats Against Alloxan Diabetes

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BAL (British anti-lewisite), administered intravenously or subcutaneously as recently reported by us (*2, 3*), gave complete protection against the diabetogenic and lethal effect of alloxan in rats. The effective intravenous antidiabetic-dose-50 of BAL against a dose of alloxan otherwise producing diabetes in 100 per cent of injected animals was approximately 8 mg./kg. BAL was thus effective in appreciably smaller doses than cysteine (*1*). BAL administered intravenously or subcutaneously afforded complete and permanent protection against alloxan diabetes for 85 minutes and reduced the lethal effect of alloxan to approximately half.

Recently we have found that BAL also protects rats from alloxan diabetes by the oral route of administration, as shown in Table 1.

TABLE 1

Dose of BAL per os mg./kg.	No. of rats protected
75	0/7
110	4/7
162	5/9
237	7/7

Thus, not only is BAL effective in reducing the toxicity of alloxan, a nonmetallic compound, but it can exert its effect by the oral as well as the parenteral route.

References

1. LAZAROW, A. *Proc. exp. Biol. Med.*, 1946, **61**, 441.
2. TISLOW, R., and CHESLER, A. *Fed. Proc.*, 1947, **2**, 215.
3. TISLOW, R., and CHESLER, A. Seventeenth International Physiological Congress, Oxford, 1947, Abstracts of Communications, p. 48.

[1] With the technical assistance of Mary Ann Griffith.