loidal particle. This mechanism by-passes the soil solution and postulates that hydrogen ions on the roots exchange directly for cations on the clay. The theory for cation exchange by contact is supported by theoretical considerations (*4*). A detailed account of both theories discussed briefly here was given recently by Jenny (*2*).

One difficulty in appraising these two mechanisms arises from the fact that ions exist in both the liquid phase, or soil solution, and in the solid phase, or soil colloid. From the data already presented, the possibility was considered that it might be possible to identify from which of the two phases the plants absorbed Zr and Nb. This prospect was explored experimentally in the following way. Five-gram samples of soil containing radioactive Zr and Nb with an activity comparable to that in which the plants were grown, were leached with a liter of each of the following solutions: Hoagland's solution in equilibrium with atmospheric CO_2, giving a pH of 5.0, and a similar solution saturated with CO_2, giving a pH of 3.9. Distilled water, under the same two CO_2 pressures, was also used as a leaching agent. Of the two leaching agents, Hoagland's solution was considered the more reasonable facsimile of what is understood by a soil solution. The use of CO_2 is an attempt to simulate the respiratory excretion of roots.

The leachings were evaporated to dryness and measured for activity on the Geiger counter. No activity was detected with any of the leaching solutions. From this it was concluded that no Zr and Nb were present in the soil solution and therefore the CO_2-soil solution theory was inadequate to explain the uptake of these radioactive substances by plants from soils.

However, from the leaching experiment with organic acids (Table 1) it is entirely within the realm of possibility that a soil solution theory incorporating as an important feature the excretion of organic acids by plant roots could very nicely explain the absorption of Zr and Nb. Such a theory might conceivably account for the uptake from soils of such ions as iron made available by complex formation with the same organic acids as were used in our leaching test. It is common practice in the water culture of plants to supply iron in the form of citrate or tartrate. So the organic acid theory offers a possible explanation, even though it has not been advanced very seriously as a mechanism for ion uptake. For instance, a recent review of organic acids in plants (*7*) considers their role as intermediates in respiration, as agents in the maintenance of cation-anion balance, and as participants in protein metabolism, but no mention is made of their possible excretion by roots in connection with ion availability.

Of course, it is not strictly necessary that plants themselves excrete organic acids, inasmuch as microorganisms in soils could perform this function just as well, to their mutual advantage. The biological excretion of organic acids has been demonstrated (*8*) and their function in increasing the availability of phosphate and potassium in soils has already been considered (*9*). Obviously, the possibilities of organic acids require serious consideration and investigation in relation to ion availability.

Another mechanism for explaining these results is offered by the contact theory. According to this idea, it is not necessary to have ions in solution in order for plants to absorb them. All that is required for an exchange of ions is the intermingling of the electric double layers between two colloidal particles. In this connection, some interesting results were obtained by Sengupta (*5*). He was able to remove Zr^{95} and Nb^{95} from clay by synthetic cation exchange resins such as Amberlite IR-100. This behavior would be analogous to the activity of a root acting in accordance with the contact exchange concept.

It is concluded that insofar as the absorption of radioactive Zr and Nb is concerned, the CO_2-soil solution theory does not offer a satisfactory explanation. The only possible mechanisms are offered by the contact theory or by a soil solution theory postulating the excretion of organic acids by plant roots or microorganisms growing in the same environment.

References

1. Jacobson, L. and Overstreet, R. *Soil Sci.*, 1948, **65**, 129.
2. Jenny, H. *Symposium on plant nutrition,* Madison: Univ. of Wisc., 1949. In press.
3. Jenny, H. and Overstreet, R. *J. phys. Chem.*, 1939, **43**, 1185.
4. Overstreet, R. *Soil Sci.*, 1945, **59**, 265.
5. Sengupta, K. Ph.D. thesis, Univ. of Calif., 1949.
6. Tompkins, E. R. *et al. J. Amer. chem. Soc.*, 1947, **69**, 2769.
7. Vickery, H. B. and Pucher, G. W. *Ann. Rev. Biochem.*, 1940, **9**, 529.
8. Virtanen, A. I. *et al. Suomen Kemist.*, B, 1946, 19 (7/8), 83. (Abstract.)
9. Waksman, S. A. *Principles of soil microbiology.* Baltimore: Williams & Wilkins, 1932.

New Method for Studying Electrical Orientation and Relaxation Effects in Aqueous Colloids: Preliminary Results with Tobacco Mosaic Virus

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Various investigators have observed the double refraction effects produced by the application of sinusoidal electric fields to suspensions of anisometric colloidal particles. Lauffer (*3*) studied by a visual method the birefringence produced in aqueous tobacco mosaic virus (TMV) solutions by 60-cycle sinusoidal fields. Mueller (*4*) and Norton (*5*) observed in bentonite aquasols birefringence which varied in magnitude and sign with the frequency of the applied sinusoidal voltage and the concentration of the sol. Although the phenomena are similar to the Kerr effect (*2*), a number of interesting anomalies have been reported which suggest the existence of orienting mechanisms other than those due to permanent or induced dipoles.

It seems clear that data obtained with sinusoidal fields

are difficult or impossible to interpret where different kinds of orientation may occur during a single cycle. Heating and electrophoretic effects are troublesome, particularly with biological materials. The apparatus described here utilizes an electronic switching circuit capable of producing pulses of alternate polarity[1] as shown in Fig. 1A, to minimize heating and polarization effects, and square waves (Fig. 1B), for certain experiments described below.

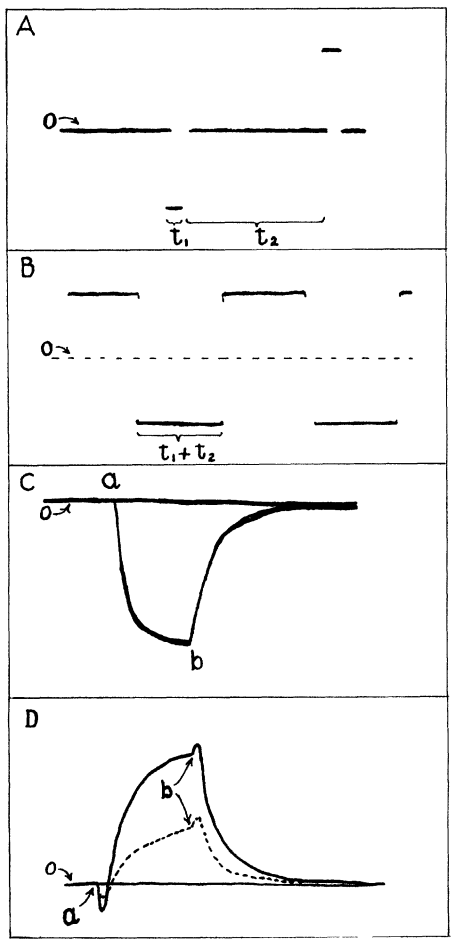

FIG. 1. Oscillograms of (A) pulses and (B) square waves generated across the cell by electronic switching circuit. In our experiments, $t_2 = 9t_1$; $t_1 = 3$, 0.3, or 0.03 msec. Rise time in all cases was one μsec. (C) The photosignal from 0.073% TMV solution. Linear sweep; sweep time = 10 msec; 238 v/cm pulse applied at *a* and removed at *b*, 3 msec later; applied voltage zero otherwise. (D) The same as (C) but with 1.4% TMV (solid curve) and 2.75% TMV (dashed curve). The relaxation is exponential in (C) but not in (D).

In the optical systems employed previously, the electric field was applied transversely to a cell containing the solution between crossed nicols oriented at 45° to the field, and an increase in light intensity was produced by birefringence of either sign. In the present apparatus, a $\lambda/4$ plate is interposed between the cell and the analyzing nicol, which is rotated slightly from the crossed position, with the result that light intensity increases when birefringence is positive, but decreases when it is negative. The transmitted light beam is focused upon an electron-multiplier phototube. A d-c wide band-pass amplifier converts photocurrent variations into vertical deflections of an oscilloscope beam, which is synchronized with the applied pulses for photography of the build-up and decay of the birefringence. The rectangular cell, $10 \times 5 \times 2.5$ mm, contains two bright platinum plane electrodes.

Because of random fluctuations in the photocurrent due to stray light transmitted by the crossed nicols, and the function relating the signal strength to the angle between the directions of polarization and of the analyzer, the maximum sensitivity (signal-to-noise ratio) is realized for small values of double refraction, other factors constant, when the analyzer is rotated from the crossed position by an angle α_m given by the equation

$$\sin\alpha_m \approx (I_s/I_o)^{\frac{1}{2}}, \qquad (1)$$

where I_s is the stray light intensity and I_o is the total intensity. This ratio was determined experimentally for our optical system and gave $\alpha_m \approx 10°$. The sensitivity achieved in this way was shown to be greater than any sensitivity achievable without the $\lambda/4$ plate.

For any setting α, the relation between the change in light intensity ΔI and the optical retardation δ, in radians, of the ray vibrating along the electric field was found to be:

$$\Delta I = I_o \left[\frac{(2 \sin\alpha + \delta \cos\alpha)^2}{(4+\delta)^2} - \sin^2\alpha \right]. \qquad (2)$$

For $\alpha = 10°$ and small values of δ, in which we are primarily interested, this reduces to:

$$\delta = 5.85 \frac{\Delta I}{I_o}. \qquad (3)$$

By suitably calibrating the amplifier and oscilloscope and adjusting the photomultiplier current to a predetermined level, values of $\Delta I/I_o$ are obtained. This procedure compensates for any variations in the light source (a battery-operated tungsten lamp) or electron-multiplier sensitivity. Values of the optical retardation for the mean wavelength of the useful light are calculated from the above equations.

Fig. 1C shows an oscillogram typical of the results obtained with dilute TMV solutions.[2] Alternate positive and negative pulses separated from each other by a long period at zero potential (Fig. 1A) were applied to the electrodes of the cell, while the amplified photocurrent was introduced to the vertical plates of the oscilloscope. It can be seen that both the onset and removal of the voltage pulse are followed by an apparently exponential rise or fall of the birefringence of the solution. The time constant of the exponential curves is 0.6 msec, with the birefringence decaying to zero after the removal of the pulse. Since the photograph was a time exposure covering many pulses, it can be seen that the effect of

[1] This apparatus was developed before the authors became aware of the work of Benoit (*1*), who obtained pulses by means of a motor-driven interrupter.

[2] We are indebted to Dr. Howard Schachman of the Virus Laboratory for a purified sample of tobacco mosaic virus.

both positive and negative pulses is exactly the same. The birefringence in both cases is positive, i.e., the slow ray has its electric vector oriented parallel to the impressed electric field. Concentrations as low as 0.03% behaved similarly.

In Fig. 1D are sketched two of the curves observed with more concentrated virus solutions for a 3-msec pulse. The birefringence varies with time in a complex manner, first becoming slightly positive, then decreasing to zero

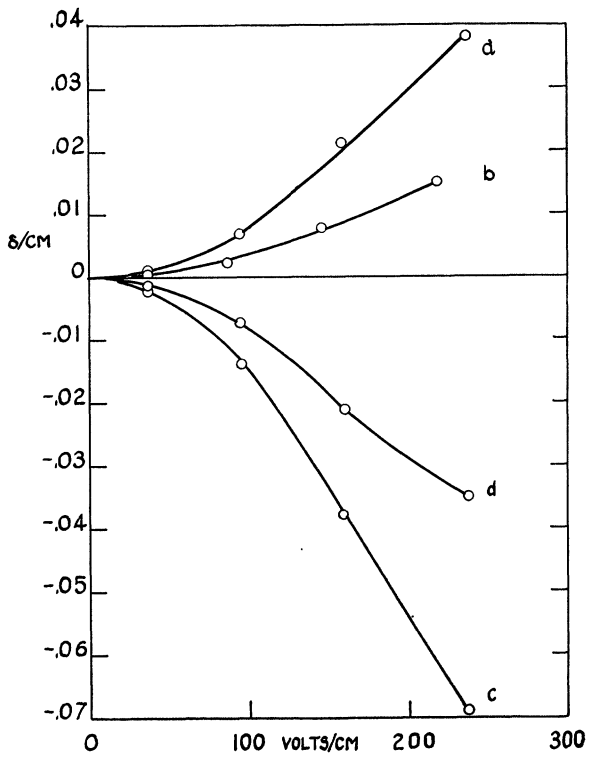

FIG. 2. Peak values of optical retardation in radians per centimeter of solution versus applied field strength: (a) 0.073% TMV, 1.5×10^{-4}M buffer; (b) 0.073% TMV, 1.0×10^{-3}M buffer; (c) 1.4% TMV, 1.5×10^{-4}M buffer; (d) 2.75% TMV, 1.5×10^{-4}M buffer.

and becoming negative, and finally becoming slightly more negative after the field is removed and before decaying to zero. The build-up and decay do not follow exponential curves and occur somewhat more slowly than in dilute solutions.

Employing 3-msec pulses, the peak values of birefringence were determined as a function of field strength for solutions of various virus and buffer concentrations. In all experiments a phosphate buffer, pH = 7, was used. Data are shown in Fig. 2.

If it is assumed that the rotations of the rodlike virus particles may be characterized by a rotational diffusion constant, D, a quantitative expression may be obtained for the time constant of the exponential decay. In this case the Brownian motion of the particles should follow the diffusion equation (*6*):

$$\frac{1}{D}\frac{\partial f}{\partial t} = \frac{1}{\sin\theta}\frac{\partial}{\partial\theta}\left[\sin\theta\left(\frac{\partial f}{\partial\theta} - \frac{Mf}{kT}\right)\right], \qquad (4)$$

where f is the distribution density of rods with respect to θ, the angle between the electric field and the axis of the rod, t is the time, M is the torque caused by the field, and k and T have their usual meanings. When the field is zero, corresponding to the relaxation between pulses in our experiments, M is zero and the solution of the equation is found by standard methods to be

$$f = a + bP_1(\cos\theta)e^{-2Dt} + cP_2(\cos\theta)e^{-6Dt} + \ldots, \qquad (5)$$

where the $P_n(\cos\theta)$ are the Legendre polynomials and a, b, and c are constants. Further investigation shows that the first term of this series that contributes to the polarization tensor characterizing the double refraction is the one involving $P_2(\cos\theta)$. Hence the double refraction is exponential in time, with a time constant of $1/6D$, unless higher order terms are unexpectedly important. Now using the formula of Perrin (*6*), simplified for $a \gg b$:

$$D = \frac{3kT}{16\pi\eta a^3}\left(-1 + 2\ln\frac{2a}{b}\right), \qquad (6)$$

where $2a$ is the length of the particles and $2b$ is the diameter (here assumed to be 2800 A and 150 A respectively), one may calculate that the time constant should be 0.3 msec, compared with 0.6 observed. The disagreement is somewhat larger than would be expected.

The length of the particles calculated from the relaxation time, assuming $a/b = 19$, is 3660 A.

It is of considerable interest to determine the nature of the mechanism causing the birefringence. Four possibilities by which the particles may be oriented with respect to the electric field have been considered: (1) permanent dipoles, (2) induced dipoles, (3) viscous drag of the surrounding medium on particles moving by electrophoresis, and (4) distortion of the ionic atmosphere surrounding the particles.

If permanent dipoles were responsible for the observed orientation in dilute solutions one would expect the anomalous Kerr effect discovered by Raman and Sirkar (*7*), i.e., a decrease of δ with increase of frequency. Our calculations, based on equation 4, show that this effect would be very evident with square waves (Fig. 1B) at frequencies of the order of the reciprocal of the observed decay time constant. Experiments, however, showed that the peak birefringence remained constant within experimental error as the frequency of applied square waves was increased well beyond this point. This has led us to discount permanent dipole orientation as the mechanism responsible for the positive birefringence of dilute solutions. An attempt to treat the phenomena on the basis of a torque arising from translational electrophoretic motion of the anisometric particles in a viscous medium fails because of the well-known tendency of anisometric bodies consistently to orient themselves *across* the direction of motion.

It has been shown (*8*) that the torque on a dielectric ellipsoid within a dielectric medium will be in a direction to orient the ellipsoid along the applied electric field, whether the dielectric constant of the medium is greater or less than that of the ellipsoid. Thus, induced dipoles would give positive birefringence, as observed with dilute solutions. Since the induced dipoles reverse their directions when the field is reversed, the torque remains un-

changed and the birefringence with square waves should be equal to the steady state value with an equivalent steady field, and this is consistent with our observations. Because the conductivity of the dispersing medium will affect the ionic atmosphere and the electric field in the vicinity of a particle, a change of birefringence with buffer concentration is to be expected. The observed result, a decrease in birefringence with increasing buffer concentration, is illustrated in Fig. 2, curves a and b. The complex nature and reversal of sign of the birefringence at higher concentrations (curve c) might be due to interactions between the particles and their ionic atmospheres.

Whatever may be the orienting mechanisms involved, it is clear that the birefringence and its decay are very sensitive to the size and shape of the particles. For this reason, the method shows promise of application in aggregation studies and other investigations in which the size of large colloidal particles is important.

References

1. BENOIT, H. *Compt. rend.*, 1949, **228**, 1716.
2. KERR, J. *Phil. Mag.*, 1875, (4), **50**, 337, 446; 1879, (5), **8**, 85, 229; 1882, **13**, 53, 248; 1894, **37**, 380; 1894, **38**, 144.
3. LAUFFER, MAX A. *J. Amer. chem. Soc.*, 1939, **61**, 2412.
4. MUELLER, HANS. *Phys. Rev.*, 1939, **55**, 508, 792.
5. NORTON, F. J. *Phys. Rev.*, 1939, **55**, 668.
6. PERRIN, F. *J. phys. Radium*, 1934, (7), **5**, 497.
7. RAMAN, C. V. and SIRKAR, S. C. *Nature*, Lond., 1928, **121**, 794.
8. STRATTON, J. A. *Electromagnetic theory*. New York: McGraw-Hill, 1941. Chapter III.

The Induction of Resistance to 4-Amino-N^{10}-Methyl-Pteroylglutamic Acid in a Strain of Transmitted Mouse Leukemia[1]

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It has been shown previously (*2, 3*) that there is a marked prolongation of the survival time of mice inoculated with transplanted leukemia Ak 4 when they are treated with 3 mg/kg of 4-amino-N^{10}-methyl-pteroylglutamic acid[2] (*6*) three times weekly to a total of ten doses. Even when treatment is continued until death, however, the mice eventually die of leukemia between the 28th and 40th day. In view of the ultimate failure of therapy observed with this and closely related substances in many clinical trials in acute leukemia, the mechanism of this eventual lack of response in mouse leukemia was deemed worthy of further investigation.

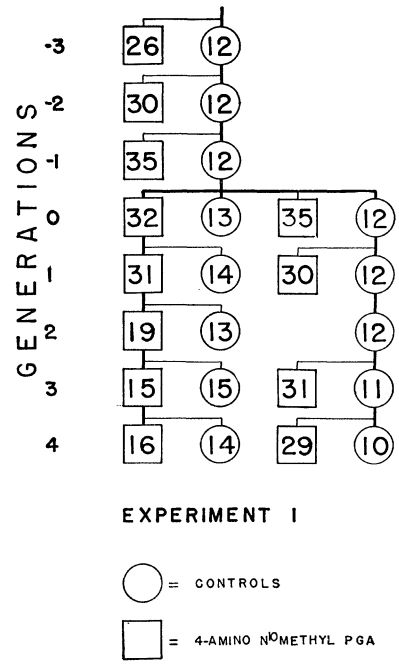

FIG. 1. Genealogy of 4-amino-N^{10}-methyl PGA-resistant strains. The figures in the circles represent the average survival time in days of groups of ten untreated mice; those in the squares the average survival time of groups of ten mice treated with 4-amino-N^{10}-methyl-PGA 3 mg/kg intraperitoneally three times weekly.

Many examples of the development of drug-fast strains in microorganisms under drug treatment *in vitro* and *in vivo* have been noted in the history of chemotherapy (*1, 4, 7*). Unpublished work elsewhere has shown a similar drug fastness to develop in cells of tumors of chloroleukemia Ak 1394 in mice treated with benzene (*5*).

The experimental studies undertaken to develop such a drug-resistant subline in mice are herewith reported. Akm mice inoculated with leukemia Ak 4 in from the 21st to the 35th transplanted generation were used in these experiments. Saline suspensions of splenic tissue, which had been obtained from mice dying of leukemia Ak 4 despite continued therapy with 4-amino-N^{10}-methyl-PGA in doses of 3 mg/kg given three times weekly, were inoculated intraperitoneally into 20 Akm mice. Forty-eight hr later these were divided into two groups of ten mice each. One group was considered as a control and received no treatment. The other group was treated with 4-amino-N^{10}-methyl-PGA in doses of 3 mg/kg given intraperitoneally three times weekly until death. The average survival time of treated and control mice was noted and transfer of the line continued through one of the treated mice. The genealogy and differing response to therapy of sublines after repeated passages through treated or untreated mice can be seen in Fig. 1.

In experiment 1, a subline of this lukemia developed complete resistance to 4-amino-N^{10}-methyl-PGA after three

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[2] We are indebted to Dr. J. H. Williams of the Lederle Laboratories for our supply of this compound.