3 and 4. Poor egg production on days 6 to 11 was traced to difficulties in mitosis and differentiation. Improvement on later days was a reflection of the unsusceptibility of interphase oogonia. Multiple sublethal doses that interfere with successive waves of differentiation are necessary to eliminate egg deposits after the 15th day.

Similar patterns of damage were obtained from other metabolite analogs. These include 6-diazo-5-oxo-L-norleucine, aureomycin, and halogenated deoxyuridines, suggesting that in the adult holometabolous insect the gonad is the target for interference with either protein or nucleic acid synthesis.

Difficulties in embryonic development, reflected as a low hatchability, are an excellent indication of nucleoprotein abnormality. For example, in the methotrexate experiment, hatchability was reduced to about 40 percent, while in the arsenite experiment, hatchability was consistently above 90 percent, not differing significantly from control values. As a destructive measure for insects, death of offspring can be even more important than a temporary numerical decrease in gametes or adults. Von Borstel (*4*) has repeatedly emphasized the importance of induced genetic lethals, particularly those of the dominant type, for promoting population collapse of insect pests.

Although not generally appreciated, genetic lethals have been used successfully in the eradication of the screwworm. This is implied by Knipling when he writes about "sterile" sperm competing with normal sperm (*5*). Accordingly, current attention in the search for potent chemisterilants, which need not be broadcast for pest control, seems properly concentrated upon the antimetabolites, and also upon alkylating agents which may have a related mode of action (*6*).

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24 June 1963

Salt Incorporation in Natural Ices

During a mid-January (1963) cold spell at Socorro, New Mexico, the freshly frozen ice on several small, shallow lakes had a surface appearance similar to that observed by Knight (*1*) on a number of Arctic lakes. Some areas of the ice surface appeared quite white or milky, possibly because of entrained air bubbles, presumably with the *c*-axes of the ice crystals vertically oriented, while the remaining portion appeared to be relatively clear ice of a darker color with the *c*-axes horizontally oriented.

Ice grown in the laboratory against a cold metal freezing block will invariably have the *c*-axis of the crystal oriented perpendicular to the block and parallel to the direction of the thermal gradient. The growing surface of such ice selectively incorporates ions from the melt into its developing structure (*2*).

Under carefully managed conditions, this incorporation of ions maintains, in the nascent layers, a space charge sufficient to develop a potential barrier of over 200 volts across the water-ice interface. Although such electrical manifestations are not likely to be observed in relatively impure terrestrial waters, the advancing *a-b* plane of an ice crystal, under a great variety of conditions, is effective in the transport of ions into the ice (*3*). These considerations led us to suspect that the lake ice with *c*-axes vertical would have a larger salt concentration than the lake ice with *c*-axes horizontal.

Pairs of ice samples were taken from two lakes which appeared to have very pronounced patterns of both types of ice. The samples of ice were rinsed four times with cold distilled water immediately after collection and were allowed to melt gradually at room temperature. The results of a chemical analysis for the major ions in the melted ice (Table 1) show that the ice grown under conditions of a vertically oriented *c*-axis contained more salt (2.5 to 10 times) than the other sample. The ice from Lake I appeared to be uniformly 6 cm thick in the areas sampled. The lake water had originated from a 18.3-m (60-ft) well about 3.3 km (2 miles) from the Rio Grande River. Lake II was formed by waters of the Rio Grande, and the ice was approximately 4 cm thick. Since the pairs of ice samples gathered were from ice of the same thickness, the rate of ice growth may be assumed to be the same.

Table 1. Analyses of ice samples from two lakes (in milliequivalents per liter). In clear ice, the *c*-axis is horizontal; in milky ice, the *c*-axis is vertical.

Ion	Lake I		Lake II	
	Clear	Milky	Clear	Milky
$Ca^{++} + Mg^{++}$	0.04	0.14	0.20	0.38
Na^{+}	.02	.33	.14	.34
K^{+}	.002	.012	.008	.016
HCO_3^{-}	.02	.30	.14	.36
Cl^{-}	.015	.12	.053	.11
SO_4^{--}	.02	.44	.13	.45
Total solids*	.05	.84	.34	.83

* Estimated by conductivity.

The increased salt content in the ice grown with a vertically oriented *c*-axis can only be explained on the basis of high surface energy and unsatisfied bonding, as indicated in the effect of Workman and Reynolds.

The results give further evidence that the freezing of terrestrial waters is an important geochemical process (*4*).

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27 May 1963

Electronarcosis and Evoked Cortical Responses

Abstract. *Evoked response data and electrocorticograms were recorded in macaque monkeys under the influence of electronarcosis currents sufficient to render the animals unresponsive to peripheral nerve stimulation. The data were obtained from chronically implanted electrodes in the sensory cortex as well as depth electrodes directed to thalamic and reticular loci. At the levels of current used, the amplitude characteristics of the evoked response data were not appreciably modified.*

Electronarcosis can produce sufficient unresponsiveness to stimuli to permit surgical procedures and has been used with success in both animals and man (*1*). However, the influence of electronarcosis on the electroencephalogram and upon evoked cortical responses has not been assessed previously because the currents used to produce anesthesia have a magnitude several hundred times greater than those

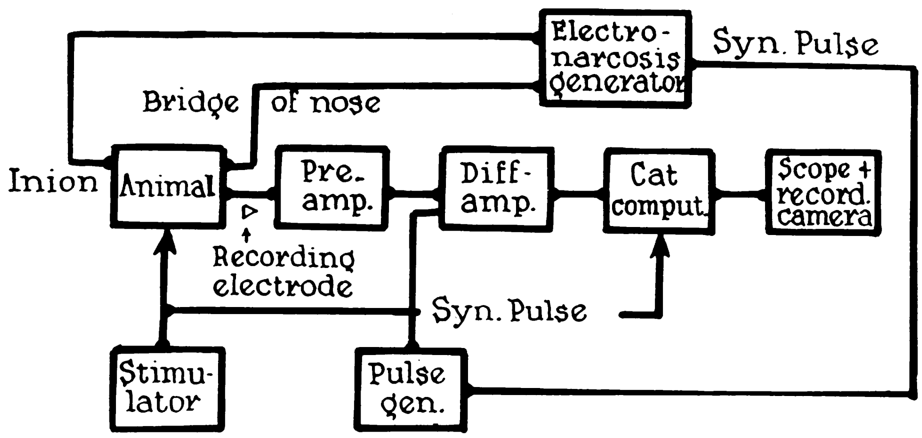

Fig. 1. Instrumentation used to record evoked response in the presence of narcotizing currents.

ordinarily recorded from the cerebral cortex.

By the use of computer and other electronic techniques, it has been possible to obtain satisfactory recordings of the "spontaneous" and evoked cortical activity during the passage of the narcotizing current. Macaque monkeys with chronically implanted superficial and depth electrodes were used for the experimentation.

The electronarcosis was produced by 100-cy/sec rectangular currents of 2.5 msec duration biased above zero voltage level. A direct current of 5 ma and an averaged alternating current of approximately 3 ma were used to obtain the composite wave forms. Two current electrodes were used in the experiment and were applied between the inion and bridge of the animal's nose.

Figure 1 illustrates the instrumentation used to record averaged evoked response data in the presence of electronarcosis. A low-level, high-gain preamplifier was used to pick up the composite evoked and electronarcosis voltage at the recording electrodes. Since the time phase of the narcotizing currents was known it was possible to trigger the pulse generator which was designed to produce a pulse approximately 180 deg out of phase with the narcotizing current. These two wave forms were then fed into a difference amplifier. Since it was difficult to completely balance out the narcotizing current it became necessary to employ a menmotron averaging response computer to average out the residual noncoherent background noise. A sync pulse from the stimulator was used to initiate the computer which proceeded to sample and store the input data. After a sufficient number of sweeps an averaged wave form of the evoked response was obtained. Clynes and Kohn have described the operation of the average response computer (*2*).

The same instrumentation was used to obtain electrocorticogram data from the implanted electrodes with the addition of an electronic low pass filter having a low pass characteristic which was flat from 0.02 to 25 cy/sec. A conventional pen recorder was used to obtain the data from the filter. The cortical electrodes were placed over primary motor, primary sensory, and frontal areas, while the depth electrodes were directed toward various loci within the reticular formation and thalamus.

The first observations were concerned with evoked cortical responses from the postcentral gyrus after radial nerve stimulation. These short latency responses were similar to those obtained when barbiturate anesthesia was employed. Evoked motor responses from stimulation of the motor area were similar with both electrical and barbiturate anesthesia. Electroencephalographic observations were made, and under electronarcosis slow voltage waves were recorded.

Depth electrodes were introduced and chronically implanted. Long-latency cortical responses were recorded at the frontal cortex with stimulation of the electrodes to the reticular formation. Figure 2 illustrates the type of records obtained when the depth electrodes in the pontine reticular formation were stimulated six times per second. The recordings were obtained from implanted electrodes in the frontal cortex. The first tracing, *a*, is the control (no electronarcosis) which was averaged over 360 sweeps. Record *b* illustrates the response with the electronarcosis currents set at 3 ma a-c and 5 ma d-c, and record *c* is the barbiturate response. The stimulus which was delayed by 10 msec is present in each figure.

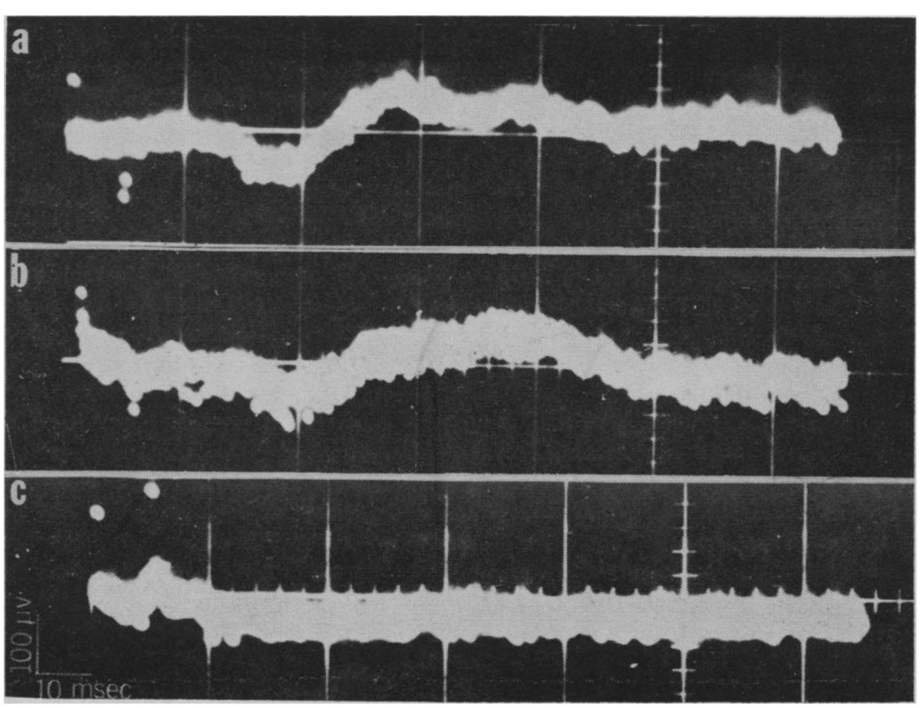

Fig. 2. Recordings made in frontal cortex with six-times-per-second stimulation of the pontine reticular formation. *a*, Control (no electronarcosis); *b*, evoked response with electronarcosis currents at 3 ma a-c and 5 ma d-c; and *c*, barbiturate response.

These data indicate that responses

were blocked by the intravenous injection of barbiturates but not by electronarcosis sufficient to permit stimulation of peripheral nerves. However, preliminary experimental results indicate that larger values of current may be capable of reducing the evoked responses at the cortex (*3*).

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3 May 1963

Labeled Oxygen: Increased Diffusion Rate through Soil Containing Growing Corn Roots

Abstract. *The diffusion of oxygen labeled with oxygen-18 through cores of wet soil increased significantly when roots of growing corn seedlings penetrated the cores. The increase was small or absent when roots grew through a layer of soft wax or a layer of water-saturated 0.5-mm glass beads, indicating that the diffusion increase was a joint effect of the roots and air-filled pores in the soil. A possible mechanism is suggested.*

This report describes an unexpected effect observed while developing techniques for studying soil aeration, which entailed the use of oxygen-18. Moist Webster silty clay loam was packed into vertical Plexiglas cylinders which were then closed at both ends, leaving an air space above and below the soil (Fig. 1). Soil cores thus formed were 8.0 cm high, 7.6 cm in diameter, and had a bulk density of about 1.3 g/cm^3. A 4-cm layer of 5-mm glass beads was placed on top of the core to provide a medium in which 18 pregerminated corn seedlings (*Zea mays* var. Iowa 4570) were planted without disturbing the soil. Openings in the Plexiglas cylinder permitted the passage of equal streams of air above and below the soil core at a slow rate (3.7 ml/min). The oxygen of the upper air stream was enriched so that for every 100 atoms of O^{16} it contained about 1.2 atoms of O^{18}. The lower air stream contained O^{18} in its natural abundance—that is, 0.2 atom percent. Both air streams were produced by a special train described elsewhere (*1*). Samples were taken daily from the air streams and from the air spaces just above and below the soil for several days, during which the primary roots of the seedlings grew through the soil and emerged from the bottom of the core. The ratio of O^{18} to O^{16} in these samples was measured with a mass spectrometer.

Compartmental analysis (*2*) may be used to derive an expression for the downward rate of oxygen diffusion through the soil core. For the air space below the core,

$$a_0\rho_{10} + a_2(\rho_{12} - \rho_{r2}) - a_1(\rho_{21} + \rho_{r1} + \rho_{31}) = 0$$
$$\rho_{10} + (\rho_{12} - \rho_{r2}) - (\rho_{21} + \rho_{r1} + \rho_{31}) = 0 \qquad (1)$$

where the symbols are those defined in the legend of Fig. 1. The solution of Eq. 1 is

$$\rho_{12} - \rho_{r2} = \frac{a_1 - a_0}{a_2 - a_1}\rho_{10} \qquad (2)$$

and hereafter $\rho_{12} - \rho_{r2}$ will be referred to as ρ. Table 1 shows the values of ρ for three soil cores, together with the number of roots, R, transfixing the core, and the air-filled pores of the soil, A, at the time of sampling. Porosity was maintained at low levels by daily additions of distilled water. The value of A was determined from the weight (corrected for seedling weight) and dimensions of the core, the soil particle density (2.575 g/cm^3), and the initial moisture content (measured by oven-drying two subsamples).

Equation 3 shows the regression of ρ on the number of roots, R, and the air-filled porosity, A, as deduced by multiple-regression analysis (*3*) of the results in Table 1.

$$\hat{\rho} = -3.8 + 0.096R + 0.62A \qquad (3)$$

The regression coefficient of R is significant with a probability less than 0.025 that it is zero [$P(0) < 0.025$]. For the coefficient of A, $P(0) < 0.10$. Evidently, the presence of corn roots increased significantly the diffusion of oxygen through the soil core.

Analysis of variance showed that variation between cores was significant at the 1 percent level. Part of this variation results from different ranges of R and A for the three cores; however, part of it may result from differences in such factors as packing and soil respiration. Hence, a result of greater significance might be obtained for the regression coefficients by adjusting ρ for variation between cores. This adjustment was made by calculating $\hat{\rho}_{\bar{R},\bar{A}}$ from multiple regression analyses for each core separately, and by adjusting ρ so that $\hat{\rho}'_{\bar{R},\bar{A}} = \hat{\bar{\rho}}_{\bar{R},\bar{A}}$, where the prime indicates adjusted ρ values. Accordingly, each value of ρ for core No. 1 was adjusted by $+ 0.4$, for core No. 2 by $+ 1.6$, and for core No. 3 by $- 2.0$. The effect of this adjustment is to translate, without rotation, the separate regression planes for the cores parallel to the ρ-axis until they intersect at

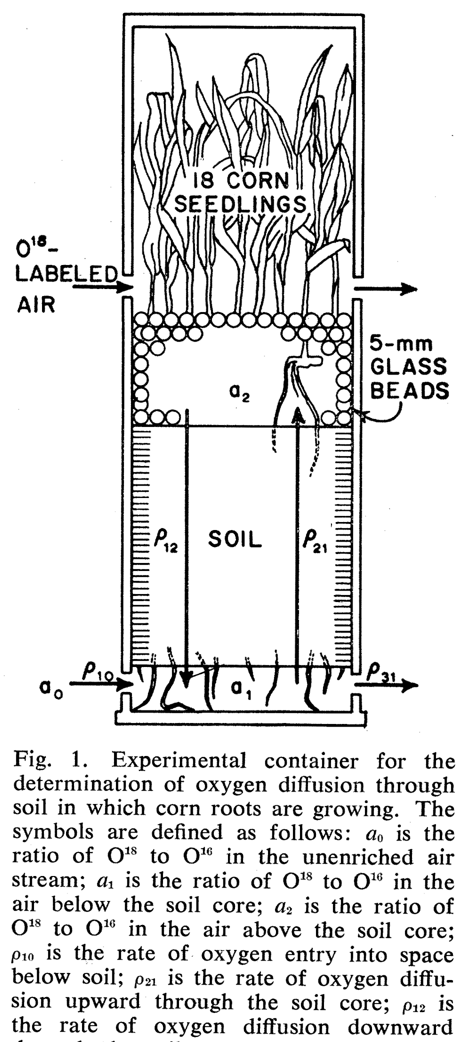

Fig. 1. Experimental container for the determination of oxygen diffusion through soil in which corn roots are growing. The symbols are defined as follows: a_0 is the ratio of O^{18} to O^{16} in the unenriched air stream; a_1 is the ratio of O^{18} to O^{16} in the air below the soil core; a_2 is the ratio of O^{18} to O^{16} in the air above the soil core; ρ_{10} is the rate of oxygen entry into space below soil; ρ_{21} is the rate of oxygen diffusion upward through the soil core; ρ_{12} is the rate of oxygen diffusion downward through the soil core; and ρ_{31} is the rate of oxygen exhaust from the space below soil. Other symbols not shown in the figure are ρ_{r1}, which is the respiratory loss of oxygen from the space below the soil caused by roots extending into this space, and ρ_{r2}, which is the respiratory loss of oxygen from ρ_{12}.