stage 3 was signaled by the appearance of high-voltage slow waves (greater than 100 μv) with some 14-cy/sec spindling; stage 4 covered that part of the record where waves larger than 100 μv in the 1- to 2-cy/sec range occurred at a rate greater than two per 10-second sample of record.

The results showed in general that all subjects displayed an electroencephalograph sleep record and learned to respond appropriately on both microswitches without returning to the electroencephalograph waking state. Moreover, the data indicated that patterned activity could occur when the subject was in any electroencephalograph sleep stage, including stages 2, 3, and 4, although most of the evidence was accumulated from stage 1B. Figure 1 shows responding during the four stages of sleep as defined above.

Of particular interest is the occurrence of responding during the time-out periods. Such responding occurred in sequences lasting from 2 to 20 seconds and resembled the performance of the subject during normal activity periods in rate and pattern. All subjects exhibited this behavior, although its occurrence was relatively infrequent and was seen only in the early sessions. The corresponding electroencephalograph stage was classified as 1B or 2 in all cases but one. Behavior of this type may have occurred because the experimental conditions appeared ambiguous to the subjects in the beginning trials. In keeping with Jasper's notion, it may very well be that the subject must learn not to respond to "irrelevant stimuli" (*1*, p. 320).

These observations of organized behavior during sleep, together with studies involving discriminative responses previously reported, lend support to the idea that the "higher mental states" continue to operate at some level of the nervous system even during *deep* sleep. Perhaps such complex behavior does not require an alert and active brain as defined by the electroencephalograph record, but perhaps it may be that our means of assessing levels of sleep and concomitant activity need to be revaluated.

ALLEN M. GRANDA
JOHN T. HAMMACK

Walter Reed Army Institute of Research, Washington, D.C.

References

1. H. H. Jasper, in *Reticular Formation of the Brain*, H. H. Jasper *et al.*, Eds. (Little, Brown, Boston, Mass., 1958), p. 319.
2. E. V. Evarts, *Federation Proc.* **19**, 828 (1960).
3. I. Oswald *et al.*, *Brain* **83**, 440 (1960).
4. B. F. Skinner, *Science and Human Behavior* (Macmillan, New York, 1953).
5. W. Dement and N. Kleitman, *Electroencephalog. and Clin. Neurophysiol.* **9**, 673 (1957).

25 January 1961

Control of Osmotic Pressure of Culture Solutions with Polyethylene Glycol

Abstract. Experiments with kidney beans indicate that Carbowax polyethylene glycol, molecular weight 20,000, upon purification, may be used as an agent to control the osmotic pressure of plant nutrient solutions without the hazard of interference with normal metabolic processes. With the sodium electrode and the thermocouple psychrometer, interaction between ions and Carbowax is shown to lead to a slight dissociation of the latter.

In the course of studies dealing with the aqueous environment of plant and animal organisms, it has become necessary to discern between effects due to the total level of solute (osmotic effects), and those due to the ionic composition at any such level (ionic composition effects). Only under conditions where an osmotic agent which does not interfere with normal metabolic processes is involved is it possible to separate osmotic effects from ionic composition effects.

In the present study, 13 different compounds were selected for examination on the basis of their chemical structure. A majority consisted of polymers used in the plastics industry. As a first step, the compounds were screened according to solubility or miscibility, viscosity, and resistance to chemical as well as biological activity. Subsequently, the compounds that passed these tests—dimethylsilicone (*1*), Elvanol 51-05 polyvinyl alcohol (*2*), polyvinylpyrrolidone NP-K30 (*3*), and Carbowax polyethylene glycols with molecular weights of about 4000, 6000, and 20,000 (*1*)—were investigated with regard to their effect on plant growth. Solution culture studies involving dwarf red kidney beans (*Phaseolus vulgaris* L.) indicated that all of the compounds were toxic when applied in their commercial form in concentrations equivalent to 1 atm of osmotic pressure.

The order in which the aforementioned compounds are listed is one of decreasing toxicity. In the case of Carbowax of molecular weight 20,000, spectrographic analysis (*4*) indicated the presence of large amounts of aluminum and magnesium. Purification of the polyvinylpyrrolidone and the two polyethylene glycols of highest molecu-

Table 1. Total fresh weight per plant of red kidney beans (average of four plants) grown in nutrient solutions containing either an excess of ions (in excess were the ions Na^+, Mg^{++}, and Cl^-; Ca^{++} and Mg^{++} were present in a ratio of 3:1, and the value of the ratio of Na^+ to $(Ca^{++} + Mg^{++})^{\frac{1}{2}}$ was 3.1 mmole$^{\frac{1}{2}}$ liter$^{-\frac{1}{2}}$ for all solutions) or one of three osmotic agents of high molecular weight [carbowax of molecular weight 20,000 (C20M) and 6000 (C6M), and polyvinylprrolidone (PVP), all purified by dialysis].

Osmotic pressure (atm)		Fresh wt (g per plant)			
		Osmotic agents			No osmotic agents
Ions	Osmotic agents	C20M	C6M	PVP	
		With osmotic agents			
1.4	0.5	86	54	62	
1.4	0.9	77	32	17	
2.5	0.5	64	43	42	
		With excess of ions			
1.9					83
2.3					72
3.0					63

Table 2. Properties of ionic solutions* of Carbowax polyethylene glycol of molecular weight 20,000 (C20M). Ca^{++} and Mg^{++} were present in proportions of 3:1, and the value of the ratio of Na^+ to $(Ca^{++} + Mg^{++})^{\frac{1}{2}}$ was 3.1 mmole$^{\frac{1}{2}}$ liter$^{-\frac{1}{2}}$ for all solutions.

Osmotic pressure (atm)		Mean activity coefficient of NaCl†	Osmotic pressure (% difference)‡	Relative viscosity (seconds of outflow)§	r_i
Ions	C20M				
1		0.92		9.5	
	1	1.03	+0	21.8	82
	2	1.08	+3	47.0	109
	3	1.25	+3	86.1	134
2		0.89		9.2	
	1	0.91	+2	21.0	82
	2	1.04	+4	46.4	111
	3	1.08	+3	82.2	134
3		0.87		9.4	
	1	0.94	+10	20.5	81
	2	1.08	+14	45.7	108
	3	1.11	+11	81.9	134

* The cations present were Na^+, Ca^{++}, and Mg^{++}; the anion was Cl^-. One atmosphere of osmotic pressure corresponds to solutions of about 0.024N NaCl or 0.032N $CaCl_2$. † As measured with the Beckman No. 78178V sodium selective electrode. ‡ The deviation of the osmotic pressure of mixed solutions of ions and C20M from the sum of the osmotic pressures of separate solutions of ions and C20M, as registered by the thermocouple psychrometer. § Of a 10-ml aliquot from 10-ml pipette.

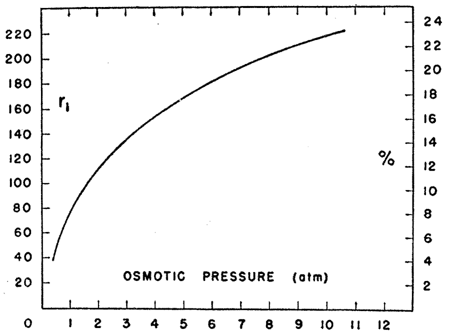

Fig. 1. The osmotic pressure of aqueous solutions of Carbowax molecular weight 20,000 as a function of the density in percent by weight, and the refractive index, r_1, of the solutions at 20°C (*12*).

lar weight—henceforth referred to as PVP, C6M, and C20M, respectively—was attempted by dialysis (*5*). Osmotically equivalent quantities of the purified products were dissolved in Hoagland's nutrient solution, which was diluted four times (Table 1). The magnitude of the aliquots taken followed from the relationship between the concentration and osmotic pressure of aqueous solutions (Fig. 1) as determined by the thermocouple psychrometer method (*6*).

The yield data indicate that nutrient solutions containing C20M produced slightly higher fresh weights of kidney beans than did isotonic solutions without C20M. This observation points to the absence of effects other than osmotic ones in the case of C20M. Less likely would be an alternative explanation implying that the C20M in the one case and the excess of ions in the other case exert an osmotic and ionic composition effect, the sum of which is of approximately equal magnitude. The lower yields, and the nonlinearity between osmotic pressure and yield, of plants grown in solution cultures containing dialyzed C6M and PVP, as compared to the yields of plants grown in isotonic solutions containing ions only, point to the physiological toxicity of these compounds.

An efflorescence of white material appearing on the upper surface of leaves of kidney beans grown in solutions containing C20M was shown by infrared analysis (*4*) to be identical to C20M. This finding suggests that C20M had passed through the plant system without undergoing any breakdown of its basic structural unit. Comparable transport of macromolecular substances through plant systems has been reported (*7*). These considerations, in conjunction with the existing knowledge on the structure of polyethylene glycols, render it improbable that C20M per se might exert any physiological effect on plant growth. The results of a more comprehensive experiment (*8*) support the viewpoint that purified C20M is physiologically inert to the extent that it can be used to control the osmotic pressure of plant culture solutions pending the effect of any interaction between ions and C20M.

Observations were made pertinent to this interaction. For this purpose, both ions and C20M were dissolved in concentrations equivalent to 1, 2, and 3 atm of osmotic pressure (Table 2). With the Beckman No. 78178V sodium selective electrode (*9*), the mean activity of sodium chloride was measured in the presence and absence of C20M, and mean activity coefficients were calculated. The results indicate that an increase in the concentration of C20M raises the mean activity coefficients of sodium chloride to well above unity. It is postulated that the large C20M molecules participate in a process of solvation. The ensuing arrangement of water molecules would accord a greater activity to the cations present. The occurrence of solvation would also explain why aqueous solutions of C20M do not observe Van't Hoff's law, complicating the freezing point depression method as a way of ascertaining the osmotic pressure of the solutions (see *10*).

The interacting effect of C20M on cations is, of course, mutual. This becomes at once clear from a comparison of the results of osmotic pressure measurements of mixed solutions of ions and C20M with the results of the corresponding separate solutions (Table 2). As the ionic concentrations were increased, the measured osmotic pressure of the ionic solutions of C20M appeared to be considerably in excess of the sum of the osmotic pressures of the separate solutions of C20M and ions. Under the same circumstances, the relative viscosity of the solutions decreased slightly for any one concentration of C20M. Both phenomena may be explained on the basis of an apparent dissociation of the long-stretched C20M molecule into shorter molecules of the same basic structure under the influence of ions, the decrease of the particle size of the solute being accompanied by an increase of the particle density of the solution. This feature complicates the use of C20M for controlling the osmotic pressure of plant nutrient solutions (*11*).

J. V. LAGERWERFF
GEN OGATA
HAROLD E. EAGLE
U.S. Salinity Laboratory,
Riverside, California

References and Notes

1. Union Carbide Chemicals Co.
2. E. I. Du Pont de Nemours and Co.
3. Antara Chemicals.
4. We are indebted to Alton W. Specht, Spectrochemical Laboratory, Agricultural Research Service, U. S. Department of Agriculture, and R. T. Ambrose, Department of Chemistry, University of California at Riverside, for the spectrographic and infrared analyses, respectively, of samples of Carbowax.
5. Sacks prepared from cellulose casing, 5½ in. (inside diameter) (Visking Corp.) were filled with about 5 liters of a solution of the osmotic agent in water and suspended in running tap water for 10 days. Deterioration of the sacks due to chemical corrosion necessitated the exercise of extreme care in collecting the dialyzed solution.
6. L. A. Richards and G. Ogata, *Science* **128**, 1089 (1958).
7. H. Groenewegen and J. A. Mills, *Australian J. Biol. Sci.* **13**, 1 (1960); J. W. Mitchell, I. R. Schneider, H. G. Gauch, *Science* **131**, 1863 (1960); W. A. Jensen and A. D. McLaren, *Exptl. Cell Research* **19**, 414 (1960).
8. J. V. Lagerwerff and G. Ogata, "Plant growth as a function of interacting activities of water and ions under saline conditions," *Intern. Soc. Soil Sci. Proc.*, in press.
9. C. A. Bower, *Soil Sci. Soc. Am. Proc.* **23**, 29 (1959).
10. H. G. Applegate, *Nature* **186**, 232 (1960). The freezing point depression method yields values which are from 20 to 40 percent higher than those indicated by a thermocouple psychrometer for solutions ranging from 1 to 8 atm osmotic pressure.
11. This report is a contribution from the U.S. Salinity Laboratory, Soil and Water Conservation Research Division, Agricultural Research Service, U.S. Department of Agriculture, Riverside, Calif., in cooperation with the 17 western states and Hawaii.
12. The relationship shown to exist between the osmotic pressure and the density of C20M solutions is accurately reflected by their refractive index, r_1. Measurements have shown that, for all practical purposes, the influence of ions and of temperature on the r_1 values of C20M solutions is negligible. Therefore, r_1 measurements constitute a simple and fast reference method for obtaining the osmotic pressure value of ionic solutions of C20M, for as far as due to this compound.

18 November 1960

On the Anomalous Activity of Thyroxin Analogs in Tadpoles

Abstract. The abnormally high thyroxin-like activity of certain thyroxin analogs is due to the use of the unique test route of immersion. When thyroxin, triiodothyronine, and their propionic and acetic acid analogs are tested by injection into the tadpole, their activities are more comparable to activity levels established in rat and man.

It has been frequently reported that certain side-chain variants of thyroxin (T_4) and triiodothyronine (T_3) showed many times the activity of thyroxin when compared for their ability to induce metamorphosis in anura (*1*). For example, the compound, 3,5,3′-triiodothyropropionic acid has been variously reported to be 200 to 300 times as active as T_4. However, the abnormally high activity of these T_4 analogs does not appear in T_4 activity comparisons in other in vivo or many in vitro systems (*1, 2*). Thus the quantitative aspects of the tadpole response to certain T_4 analogs seemed to be unique.

Our continuing interest in the role of T_4 in general, and the biochemical aspects of anuran metamorphosis in par-