rectly in various concentrations of NaCl gave the same diagram as when NaCl was added to the distilled-water subphase.

Similar experiments were conducted with KCl being injected. Bringing the distilled water to 0.042 percent KCl (equivalent to the K^+ concentration in extracellular fluid) resulted in a drop of the mean γ_{max} from 50 to 45 dyne/cm and γ_{min} from 21.5 to 9.5 dyne/cm, but there was no appreciable change in hysteresis (Fig. 4). Reconstitution of the distilled-water subphase to 0.15 to 0.2 percent KCl or higher gave results similar to those obtained with equivalent concentrations of NaCl.

Our experiments demonstrate the importance of subphase electrolytes to the formation and behavior of surface films of lung extracts wherein the electrolyte solution provides the counterions for the surface film. Also, whereas the lung surfactants are extractable in distilled water, both the lowering of surface tension and the marked hysteresis of normal lung extracts are dependent on the ionic composition of the subphase. A high value for γ_{min} of distilled-water extracts of the lung has been reported (*5*) but the mechanism was not defined.

Electrolytes are known to lower the surface tension of monolayers of collagen (*6*) and long-chain ions (*7*). In the absence of electrolytes in the subphase of lung surfactants not only is the surface tension higher, but the compression isotherm follows the expansion isotherm more closely. A similar phenomenon was observed when 0.85 percent NaCl extracts of the lung were serially diluted (*8*). Therefore, the compression isotherm reflects conditions of greater surface concentration; that is, the greater the surface concentration, the steeper the slope of the compression isotherm, the greater the fall in surface tension for a standard reduction in area, and the wider the separation between compression and expansion isotherms. In the presence of subphase electrolyte the surface concentration of surfactants increases as indicated by the lower surface tension and the greater slope of the compression isotherm (Figs. 1 and 3). During the early phase of compression intermolecular distance decreases, and surface tension falls rapidly. With marked compression and collapse of the film (late phase) the surfactant molecules leave the surface and may enter the subphase. Upon high compression of the film, the more electrolyte present (to an optimal concentration), the more surfactant will dissolve or disperse in a flux from the surface into the subphase. Ellis and Pankhurst (*6*), working with collagen films, found that the electrolyte in the subphase was most effective in lowering the surface tension of the film when the film was highly compressed; they concluded that compression caused the surface molecules to move from the surface to the subphase. During the early phase of reexpansion, water or hydrated ions or both must move into the surface, effectively lowering the surface concentration of surfactants and increasing surface tension. Surfactants return to the surface more slowly throughout reexpansion, and gradual restoration of the original surface concentration (per square meter of surface area) takes place over the plateau of the expansion isotherm. The circulation of surfactant molecules between the surface and subphase in this way would tend to facilitate the exchange of surfactants between the alveolar cell and the alveolar surface film in a similar system in the lungs. Lung alveoli probably have a surface lining of surfactant film and subphase similar to that of lung extracts (*9*). Such films could exhibit the characteristic surface behavior of lung extracts in the presence of Na^+ concentrations found in the extracellular fluid, as simulated by the studies with 0.85 percent NaCl. The Cl^- concentration in extracellular fluid (equivalent to 0.6 percent NaCl) could also account for normal surface activity. However, the K^+ concentration of extracellular fluid (equivalent to 0.042 percent KCl) is too low to account for the normal surface activity of lung extracts.

EMILE M. SCARPELLI
KENNETH H. GABBAY
JOSEPH A. KOCHEN
Department of Pediatrics, Albert Einstein College of Medicine, New York 10061

References and Notes

1. E. S. Brown, R. P. Johnson, J. A. Clements, *Proc. Soc. Exp. Biol. Med.* **95**, 180 (1957); ———, *J. Appl. Physiol.* **14**, 717 (1959); S. Bondurant, *J. Clin. Invest.* **39**, 973 (1960).
2. K. von Neergaard, *Z. Ges. Exp. Med.* **66**, 373 (1929).
3. Fractionation of lung extracts in our laboratory suggests the presence of more than one lung surfactant.
4. M. E. Avery and J. Mead, *Amer. J. Diseases Children* **97**, 517 (1959); C. Collier, *Federation Proc.* **22**, 339 (1963).
5. D. F. Tierney and R. P. Johnson, *Federation Proc.* **22**, 339 (1963).
6. S. C. Ellis and K. G. A. Pankhurst, *Trans. Faraday Soc.* **50**, 82 (1954).
7. J. Powney and C. C. Addison, *ibid.* **33**, 1243 (1937).
8. We have taken lung extracts prepared by the standard procedure and diluted them with 0.85 percent NaCl: 1 : 2, 1 : 4 . . . . . 1 : 64 dilutions. With increasing dilutions there is a narrowing of the surface tension–area diagram as the compression isotherm moves closer to the expansion isotherm. As expected, γ_{min} and γ_{max} increase with increasing dilutions.
9. R. E. Pattle, *Physiol. Rev.* **45**, 48 (1965).
10. We thank Dr. G. Colacicco, Department of Biochemistry, for assistance in the preparation of the manscript and F. Taylor for technical assistance. Supported by a grant-in-aid from the New York Heart Association and PHS grants HE 08378 and HE 07234.

19 April 1965

Pineal Gland: Influence on Gonads of Male Hamsters

Abstract. *Exposure of male hamsters to cycles of 1 hour of light and 23 hours of darkness causes atrophy of the gonads. Pinealectomy prevents this atrophy, but has no effect on animals exposed to light-dark cycles of 16 : 8. Likewise, removal of both eyes induces gonad atrophy which is prevented by pinealectomy. These data emphasize the importance of the pineal gland in the regulation of photoperiodic influences on the gonads.*

Recent investigations (*1*) show that exposure of male hamsters to low temperatures (6°C) and long periods of light [light:darkness (LD) 14:10, in hours] or room temperatures (22°C) and short periods of light (LD 2:22) induce testicular atrophy. The combination of low temperatures and short periods of light results in additive effects. While the effects induced by cold are not surprising, since hamsters are hibernators and normally show reduced reproductive activity in preparation for and during hibernation (*2*), the changes induced by short periods of light were of particular interest since so little information on mammalian responses to photoperiods is available for comparison. In view of the increasing awareness that the pineal gland is a mediator in the effects of light on reproductive functions (*3*), studies were initiated to determine the influence of this gland on the gonads of hamsters exposed to various amounts of light and darkness.

Forty-nine male hamsters weighing 84 to 135 g were used. Twenty-nine animals were pinealectomized by techniques described elsewhere (*4*) and 20 animals were subjected to comparable bleeding and trauma in the same area, but the pineal gland was left intact.

Half of each group was placed, one per cage, in an isolated room maintained at 22°C ± 1°C with LD cycles of 1 : 23 and the remainder were placed, one per cage, in a similar temperature-controlled room with LD cycles of 16 : 8. Standard laboratory pellets (Purina) and water were available at all times. The animals were killed 30 days later and the carcasses, gonads, and adrenal glands were weighed.

The pineal glands of the sham-operated animals were stained with Harris's hematoxylin and the nuclear diameters of 100 randomly chosen cells per gland were measured at a magnification of 1070 diameters by means of a Leitz microprojector. To measure the possible effects of light in the absence of the eyes, a group of animals was subjected to bilateral orbital evisceration (enucleation). Some were pinealectomized and others received sham-operations. These animals (three per cage) were subjected to the LD cycle of 16 : 8 for 30 days.

In this report we consider only the weights of the body, testes, and adrenal glands of the four groups and the diameter of the nuclei of the pineal gland cells. The data were subjected to a two-factor analysis of variance. Significant differences between means were obtained by calculating the least significant difference which is the value by which any two means must differ to be significantly different at the 95-percent level of probability.

Pinealectomy alone had no effect on the final body weights of animals subjected to either photoperiod when the weight of pinealectomized animals were compared with those of the sham-operated controls. Combined pinealectomized and sham-operated animals subjected to LD 1 : 23, however, had final body weights which were significantly different (less) from their corresponding groups in the long photoperiod (Table 1). The mechanism by which this effect is mediated is unknown. Since the hamster is nocturnal, perhaps the added period of darkness increased the activity and metabolic rates of the animals.

Pinealectomized animals subjected to either photoperiod and sham-operated animals subjected to LD 16 : 8 ("normal" controls) had similar gonad weights. Histological analysis showed that the testes of these animals were normal. Sham-operated animals subjected to LD 1 : 23, however, showed complete testicular atrophy and loss of all spermatogenic activity. This difference in total and relative weight was found to be highly significant ($p < .001$).

Table 1. Effects of two different LD cycles and of the pineal gland on the weights of the body, testes, and adrenal glands (Adr.) in hamsters. L.S.D., least significant difference.

No. of animals	Mean body weight (g)		Mean gland weights (mg/100 g body wt)	
	Orig.	Final	Testes	Adr.
	Pinealectomized; LD 1 : 23			
16	112.8	103.6*	2177	17.2
	Sham-operated; LD 1 : 23			
13	110.4	97.7*	493*	16.0
	Pinealectomized; LD 16 : 8			
10	119.4	123.2	2100	17.6
	Sham-operated; LD 16 : 8			
10	120.1	116.8	1838	14.8
L.S.D.	12.0	11.1	300	3.7

* These values are significantly different from others in the column.

While the weights of the adrenal glands of sham-operated animals exposed to LD 16 : 8 were less than those of the other three groups, the difference was not significant.

Measurements of nuclei of pineal gland cells from sham-operated animals showed that short photoperiods caused an increase in the mean diameter (5.90 ± 0.16 μ as opposed to 5.62 ± 0.13 μ). Further, the distribution frequency of nuclear size showed a shift to larger sizes. These effects, however, were not statistically significant.

Bilateral enucleation plus pinealectomy of animals exposed to LD 16 : 8 had no effect on gonad weight or histology. Bilateral enucleation of sham-operated animals, however, resulted in a highly significant atrophy of the gonads [335 mg as opposed to 1957 mg (*5*); $p < .001$].

The results indicate that the effects of short periods of light on gonads of normal hamsters are mediated by the eyes and the pineal gland. Regression of the gonads of sham-operated animals subjected to LD cycles of 1 : 23 can be prevented by removal of the pineal glands. Removal of the eyes, even with exposure to LD cycles of 16 : 8, results in atrophy of the gonads which also can be prevented by pinealectomy.

If we may extrapolate from recent data on the effects of light on reproductive functions of female rats, darkness causes the pineal gland to release melatonin, a hormone which suppresses gonad activity and function. Long periods of light act to inhibit both the synthesis and release of melatonin (*6*). A similar system undoubtedly functions in the hamster. This is supported by the observations of Mogler (*7*) that the nuclear diameters of the pineal gland cells and interstitial cells of the gonad vary inversely with environmental changes. Further, recent information shows that pinealectomized male hamsters do not undergo as great nor as rapid a gonadal atrophy in the winter months as do the controls (*8*); unfortunately the environmental conditions of the experimental animals were not reported.

Investigators of pineal gland functions have long been restricted by the lack of information concerning the effects of pinealectomy. Maintenance of pinealectomized animals in normal photoperiods produces only subtle effects in the reproductive organs of female rats. Responses of male reproductive organs have been less satisfactory as a measure of pineal gland activity or function, although enlarged seminal vesicles and ventral prostates (*9*) and testes (*10*) have been measured in young pinealectomized rats. One of us recently suggested (*1*) that the hamster is a continuous breeder only because the warmth and long photoperiods of the laboratory mask the seasonal character of its reproductive cycle normally induced by winter environments of low temperatures and short periods of light. The sensitivity of the hamster to manipulations of the environment, in this case, the photoperiod, would seem to make it an especially valuable animal for studies of the interrelationships among the pineal gland-hypothalamus-pituitary gland and gonads. Our data support the concept that the pineal gland has the important function of regulating gonadal activity so that it is compatible with certain changing environmental conditions.

ROGER A. HOFFMAN
RUSSEL J. REITER
Physiology Division, Chemical Research and Development Laboratories, Edgewood Arsenal, Maryland 21010

References and Notes

1. R. A. Hoffman, R. J. Hester, C. Towns, *Comp. Biochem. Physiol.*, in press.
2. C. Kayser, *Rev. Can. Biol.* **16**, 303 (1957); R. A. Hoffman, in *Handbook of Physiology*, sec. 4, *Adaptation to the Environment*, D. B. Dill *et al.*, Eds. (Wiliams and Wilkins, Baltimore, 1964), ch. 24, p. 379; R. A. Hoffman, *Ann. Acad. Sci. Fennicae Ser. A. IV* **71**, 201 (1964).

3. J. I. Kitay, *Endocrinology* **54**, 114 (1954); R. J. Wurtman, M. D. Altschule, U. Holmgren, *Am. J. Physiol.* **197**, 108 (1959); R. J. Wurtman, W. Roth, M. D. Altschule, J. J. Wurtman, *Acta Endocrinol.* **36**, 617 (1961); R. J. Wurtman, J. Axelrod, E. W. Chu, *Science* **141**, 277 (1963); C. J. Meyer, R. J. Wurtman, M. D. Altschule, E. W. Laso-Wazem, *Endocrinology* **68**, 795 (1961).
4. R. A. Hoffman and R. J. Reiter, in preparation.
5. For unknown reasons, three of these six pinealectomized animals had one damaged testis which resulted in a lower than normal mean gonad weight.
6. R. J. Wurtman, J. Axelrod, E. W. Chu, *Ann. N.Y. Acad. Sci.* **117**, 228 (1964).
7. R. K. Mogler, *Z. Morphol. Oekol. Tiere* **47**, 267 (1958).
8. J. C. Czyba, C. Girod, N. Durand, *Compt. Rend. Soc. Biol.* **158**, 742 (1964).
9. W. W. Roth, *Am. Zool.* **4**, 53 (1964).
10. L. Thieblot and S. Blaise, *Ann. Endocrinol.* **24**, 270 (1963).
11. This work was supported, in part, by the U.S. Army Edgewood Arsenal Chemical Research and Development Laboratories In-House Laboratory Independent Research Program. In conducting this research, we adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

9 March 1965

Transport of Sodium in Plant Tissue

Abstract. *Two mechanisms are implicated in the absorption of alkali cations by barley roots. Mechanism 1 has a high affinity for potassium, but its affinity for sodium is so low that, in the presence of even a low concentration of potassium (1 mM), sodium absorption by this mechanism is all but abolished. Mechanism 2 has a much lower affinity for alkali cations and is not highly selective; it transports sodium as well as potassium.*

Roots of barley, *Hordeum vulgare*, absorb potassium ions through the operation of two clearly distinguishable transport mechanisms, one highly specific for potassium (and rubidium) and indifferent to sodium, the second one competitively inhibited by sodium (*1*, *2*). In the work described here, we demonstrated that the second mechanism effects the absorption of sodium by this tissue. We shall first recall our earlier findings concerning the transport of potassium.

Mechanism 1 of potassium transport is effective at very low external potassium concentrations. It has an apparent Michaelis constant of about 0.02 m*M* and operates at nearly the maximum theoretical rate at a potassium concentration of 0.2 m*M* (*1*). Sodium competes very ineffectually with potassium in this process. At an external potassium concentration of 1 m*M* (at which concentration mechanism 1 operates at the maximum velocity) sodium even in 20-fold excess fails to compete significantly with potassium (*2*).

At higher concentrations of potassium, up to 50 m*M*, a second mechanism with much less affinity for potassium comes into play (*1*). It differs in several respects from mechanism 1; one important difference is that sodium strongly and competitively inhibits the absorption of potassium in mechanism 2 (*1*).

On the basis of these findings we made two tentative predictions. (i) In the presence of 1 m*M* potassium (enough to saturate mechanism 1), the absorption of sodium from solutions of low sodium concentrations (less than 0.2 m*M*) should be almost eliminated. Under such conditions, both mechanisms 1 and 2 would be unavailable for sodium transport. Mechanism 1 would be transporting potassium at maximum velocity and since sodium fails to compete it would fail to occupy the potassium-transporting sites of this mechanism (*1*, *2*). As for mechanism 2, its affinity for alkali cations is much too low for it to make an appreciable contribution to sodium transport at low sodium concentrations (less than 0.2 m*M*). (ii) The second prediction was that at high sodium concentrations (more than 0.2 m*M*) sodium in the presence of 1 m*M* potassium would be transported because in the high concentration range mechanism 2 comes into play, and the finding that in mechanism 2 sodium competitively interferes with potassium absorption indicates that it has affinity for the transport sites of mechanism 2 and might be transported by them.

Figure 1 shows that these predictions, based mainly on the inhibition of potassium transport by sodium, are borne out when the absorption of sodium itself is examined. In the presence of potassium at 1m*M* concentration, sodium absorption from dilute solutions (less than 0.2 m*M*) is all but eliminated. At higher concentrations sodium is absorbed; and this absorption occurs at rates which are comparable with those at which potassium (*1*) and chloride (*3*) are absorbed at the same concentrations. The sodium absorbed is not readily exchangeable with external sodium. When roots containing radioactive sodium are transferred to a solution containing nonradioactive sodium, isotopic exchange occurs very slowly.

Our second prediction was based on the finding that the increment of potassium absorption due to mechanism 2 was competitively inhibited by sodium (*1*). Although this showed that the potassium-carrying sites of mechanism 2 have affinity for sodium ions, the possibility remained that sodium ions, while displacing potassium from these sites, would not themselves be transported. Sodium might be a competitive inhibitor of potassium transport without being an alternative substrate for the transport mechanism. However, the results bear out the prediction: sodium competes with potassium and is itself absorbed.

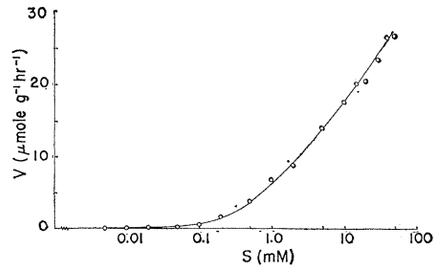

Fig. 1. Rate of absorption, *v*, of sodium labeled with Na^{22}(Na*) by excised roots of barley, *Hordeum vulgare* var. Arivat, as a function of the external concentration of Na*Cl (*S*), plotted logarithmically over the range 0.005 to 50 m*M*. Roots were cultured as described (*2*). Experimental solutions, 0.5 m*M* $CaCl_2$, 1.0 m*M* KCl, and Na*Cl as indicated, *p*H 5.7, 30°C, aerated. Absorption period, 20 minutes, discontinued by a 30-minute exposure to a cold (7°C) aerated solution of 0.5 m*M* $CaCl_2$, 5 m*M* NaCl (nonlabeled). The experimental technique was described in an earlier paper (*5*).

Thus we have confirmed the existence of two mechanisms which effect the transport of alkali cations by barley roots. Since the present work was completed we have found that mechanism 2 is itself heterogeneous. The evidence indicates the operation of a number of transport sites in mechanism 2, with different affinities for any given cation (*4*).

D. W. Rains
Emanuel Epstein
Department of Soils and Plant Nutrition, University of California, Davis 95616

References and Notes

1. E. Epstein, D. W. Rains, O. E. Elzam, *Proc. Natl. Acad. Sci. U.S.* **49**, 684 (1963).
2. E. Epstein, *Plant Physiol.* **36**, 437 (1961).
3. O. E. Elzam, D. W. Rains, E. Epstein, *Biochem. Biophys. Res. Commun.* **15**, 273 (1964).
4. E. Epstein and D. W. Rains, *Proc. Natl. Acad. Sci. U.S.*, in press.
5. E. Epstein, W. E. Schmid, D. W. Rains, *Plant Cell Physiol. Tokyo* **4**, 79 (1963).
6. Supported by grants from NSF and the Office of Saline Water, U.S. Department of the Interior.

29 April 1965