weight is an accurate indication of function in the degenerating testis. There was no relationship between body weight and testis weight within a stage or among individuals on the same light regime. Likewise, body weight and photoperiod were not related. Thus, we are dealing with a response elicited specifically from the reproductive system by the photoperiodic treatments.

Past experimental studies of the mechanism of photoperiodic control of reproduction have used birds almost exclusively (*10*). A photoperiodic response suitable for rigorous experimental manipulation has not been available in a laboratory mammal. With further work, aimed at identification and elimination of the sources of the wide variance observed in our data, it should be possible to use the photoperiodic response of adult hamster testes as a means of experimentally studying photoperiodism in mammals.

SUZANNE GASTON
MICHAEL MENAKER
Department of Zoology,
University of Texas, Austin

References and Notes

1. J. Aschoff, *Stud. Gen.* **8**, 742 (1955); D. S. Farner, *Ann. Rev. Physiol.* **23**, 71 (1961).
2. J. Hammond, Jr., *Vitamins Hormones* **12**, 157 (1954).
3. T. H. Bissonnette, *Physiol. Zool.* **14**, 379 (1941).
4. R. Ortavant, P. Mauleon, C. Thibault, *Ann. N.Y. Acad Sci.* **117**, 157 (1964).
5. "LD 8:16" refers to a 24-hour light-dark cycle composed of 8 hours of light followed by 16 hours of darkness.
6. R. A. Hoffman and R. J. Reiter, *Science* **148**, 1609 (1965).
7. Four-week-old hamsters were obtained from the Morris Hamster Farm, Morris, Pa., in December (Fig. 1A) and in March (Fig. 1B), where they had been maintained indoors under natural conditions of light. Upon arrival, they were placed 6 per cage in separate light-tight boxes. Each of the boxes was provided with a light [Ken Rad, 4-watt, cool-white fluorescent (F4T5/cw)] with an intensity of about 400 lux at the floor of the cage which was controlled by a clock. The animals had free access to food (Wayne lab-blox) and water.
8. The hamsters whose juvenile response is shown in Fig. 1A responded similarly to the adults shown in Fig. 1B after removal of one testis and another month of photoperiodic treatment. A photoperiodic response is indicated in that average testis weight decreased in the hamsters on less than 12.5 hours of light per day, whereas an increase in average testicular weight was seen on longer photoperiods. However, the response in this case is complicated by compensatory hypertrophy of the remaining testis and thereby awaits further data for interpretation.
9. The testes were preserved in Bouin's, dehydrated in an ethanol series and xylene, embedded in Paraplast sectioned at 10 μ, and stained in Mayer's hemalum and eosin.
10. D. S. Farner, *Amer. Sci.* **52**, 137 (1964).
11. During histological processing four tissues were lost; as a result, the numbers of animals represented in Fig. 1A are different.
12. Supported in part by NSF grant GB 3806; and by NIH training grant 5-T1-CM-836-03. We thank Arnold Eskin for valuable discussion.

14 September 1967

Cholinergic Binding Capacity of Proteolipids from Isolated Nerve-Ending Membranes

Abstract. *The capacity for binding dimethyl* d-*tubocurarine-C^{14} was studied in isolated nerve-ending membranes from cerebral cortex and myelin. After treatment of the membrane with organic solvents most of the radioactivity was recovered in the extract. Preliminary evidence indicates that dimethyl* d-*tubocurarine-C^{14} is not bound to lipids or glycolipids. While the proteolipids of myelin have a low binding capacity, the results obtained with the nerve-ending membranes rich in acetylcholinesterase suggest that the cholinergic receptor may be a special type of proteolipid.*

Advances in cell fractionation of the brain have led to the separation of different types of nerve-ending membranes and to a study of their receptor properties by the use of cholinergic blocking agents. Those membranes which are richer in acetylcholinesterase also have a higher binding capacity for dimethyl *d*-tubocurarine-C^{14}, H^3-alloferin (alcuronium chloride), and hexamethonium labeled with C^{14} on the methyl groups. For example, in those separated in a gradient at 1.0*M* sucrose (that is, M_1 1.0) the uptake of dimethyl *d*-tubocurarine-C^{14} is seven times higher than that in the total particulate fraction of the cerebral cortex (*1*). The treatment of such membranes with the non-ionic detergent triton X 100 leads to the solubilization of most of the acetylcholinesterase (*2*), but in the sediment the binding capacity of the original membranous fraction is not reduced (*3*). The detergent also produces a considerable solubilization of proteins; however the proteolipids, as defined by Folch (*4*) by their solubility in a mixture of chloroform and methanol (2 : 1), are recovered in the residue (*5*). Electron-microscopic study of this residue reveals that most of the nerve-ending membrane disintegrates but that the junctional complex, composed of the two synaptic membranes and other macromolecular components, persists intact (*6*). These findings opened the possibility of isolating the receptor substance having the binding capacity for cholinergic blocking agents.

Nerve-ending membranes rich in acetylcholinesterase (fractions M_1 0.9 and M_1 1.0) from cerebral cortex and myelin (fraction M_1 0.8) from white matter or brain stem were treated with dimethyl *d*-tubocurarine-C^{14} (Table 1) and the binding capacity was determined (*1*). The control pellet was extracted with a mixture of chloroform and methanol (2 : 1) (*4*), and the radioactivity in the residual pellet and the extract was measured. Treatment with these organic solvents completely inactivated acetylcholinesterase and removed most of the radioactive material from the residual

Table 1. Uptake of dimethyl *d*-tubocurarine-C^{14} and proteins in submitochondrial fractions of the central nervous system of the cat. The fractions in 0.32*M* sucrose were incubated for 15 minutes at 22°C (*1*) with dimethyl *d*-tubocurarine-C^{14} (25,000 count/min per milliliter) and then centrifuged at 100,000*g* for 30 minutes. The sediments were then washed three times with sucrose for 5 minutes each. Samples of the control pellet and of the extract were measured in a Nuclear-Chicago scintillation counter. In each case the counts per minute were related to the protein content determined in each tube. Subfractions containing nerve-ending membranes from the cerebral cortex (M_1 1.0 and M_1 0.9) (*1*) were compared with fractions containing myelin (M_1 0.8) from white matter (experiments 1 and 2) or midbrain (experiment 3).

Fraction	Content	Control pellet		Chloroform-methanol (2 : 1) extract		Ratio b/a
		Protein (mg/g)	Protein (count/min per mg) (a)	Protein (mg/g)	Protein (count/min per mg) (b)	
		Experiment 1				
M_1 1.0	Nerve-ending membranes	1.32	10.460	0.13	140.307	14.0
M_1 0.8	Myelin	25.20	5.838	7.20	18.850	3.2
		Experiment 2				
M_1 1.0	Nerve-ending membranes	0.93	16.744	0.08	160.100	9.5
M_1 0.8	Myelin	17.90	6.948	9.80	14.734	2.1
		Experiment 3				
M_1 0.9	Nerve-ending membranes	0.68	19.617	0.12	141.458	7.2
M_1 0.8	Myelin	8.00	12.788	5.00	18.092	1.4

pellet, which under the electron microscope appeared as an amorphous mass. However, there was a 7.2- to 14-fold increase of specific activity in the extract of the nerve-ending membranes (Table 1). In myelin, the control pellet and the extract had a much smaller uptake of dimethyl *d*-tubocurarine-C^{14}.

These results suggested that the binding capacity of the nerve-ending membranes was in some of the chemical constituents extracted with the organic solvent used. The material extracted with chloroform and methanol and a blank of 0.32*M* sucrose were washed with five volumes of distilled water. Only 5 percent of the radioactivity was recovered in the upper, watery phase of the brain extract; 99.8 percent was found in the blank. This led us to think that the gangliosides were not involved in the binding of the cholinergic blocking agent. To separate the lipids and proteolipids we passed the extract through a Sephadex G-25 column equilibrated with a mixture of chloroform, methanol, and water (60:30:4.5), which retains practically all of the nonlipid contaminants (*7*). In a control experiment, the column absorbed 98 percent of the free dimethyl *d*-tubocurarine-C^{14} dissolved in a mixture of chloroform and methanol (2 : 1) or in an extract of 0.32*M* sucrose alone. On the contrary, most of the radioactive material of the extract of nerve-ending membranes passed through the Sephadex G-25 and was recovered in the effluent. This finding differs from that recently observed by Proulx (*8*) for the uptake of γ-aminobutyric acid in brain homogenate. He observed a retention of the aminoacid by the Sephadex G-25 and postulated a rather labile type of binding. Our results may be interpreted as indicating a firmer type of binding for dimethyl *d*-tubocurarine-C^{14}. To determine whether this compound binds to the lipids or the proteolipids, we studied the effluent by thin-layer chromatography on silica gel with a mixture of chloroform, methanol, and ammonia (14:6:1) (*9*) or of chloroform, methanol, acetic acid, and water (25:15:4:2) (*10*). We found that with both methods practically all of the radioactive material remained together with the proteolipids at the point of origin.

These experiments suggest that the capacity of the junctional complex to bind dimethyl *d*-tubocurarine-C^{14} is probably due to a protein which has the solubility properties of the proteolipids. This receptor protein may be a portion of the proteolipid protein of the nerve-ending membrane, which in itself is a small proportion of the total protein (*11*). The fact that myelin has a high content of proteolipids but a very small binding capacity for dimethyl *d*-tubocurarine-C^{14} is also in line with this interpretation and indicates that the receptor protein may be a special kind of proteolipid present in some portion of the nerve-ending membrane. The results of the treatment with triton X-100 (*3, 5*) mentioned in the introduction suggest that such a proteolipid may be localized in the junctional complex of the synapse (*12*).

EDUARDO DE ROBERTIS
SARA FISZER
EDUARDO F. SOTO
Instituto de Anatomía General y Embriología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina

References and Notes

1. E. De Robertis, M. Alberici, G. Rodríguez de Lores Arnaiz, J. M. Azcurra, *Life Sci.* **5**, 577 (1966); J. M. Azcurra and E. De Robertis, *Int. J. Neuropharmacol.* **6**, 15 (1967).
2. S. Fiszer and E. De Robertis, *Brain Res.* **5**, 31 (1967).
3. E. De Robertis, J. M. Azcurra, S. Fiszer, *ibid.*, p. 45.
4. J. Folch-Pi and M. Lees, *J. Biol. Chem.* **191**, 807 (1951); J. Folch-Pi, in *Brain Lipids and Lipoproteins and the Leucodystrophies*, J. Folch-Pi and H. Bauer, Eds. (Elsevier, New York, 1963), p. 18.
5. E. Lapetina and E. De Robertis, unpublished observations.
6. E. De Robertis, *Science* **156**, 907 (1967).
7. M. A. Wells and J. C. Dittmer, *Biochemistry* **2**, 1259 (1963).
8. P. R. Proulx, *Rev. Can. Biol.* **25**, 285 (1966).
9. L. Seminario, E. Soto, T. De Cohan, *J. Chromatogr.* **17**, 513 (1965).
10. V. P. Skipski, R. F. Peterson, M. Barclay, *Biochem. J.* **90**, 374 (1964).
11. E. Lapetina, E. F. Soto, E. De Robertis, *J. Neurochem.*, in press.
12. Supported by NIH grant NB-06953-02.

14 September 1967

Distribution of Chromatids at Mitosis

Abstract. *The distribution of labeled chromatids at the second mitosis after labeling with tritiated thymidine is random in both* Vicia faba *(the broad bean) and* Potorous tridactylis *(the rat kangaroo). This finding is contrary to that predicted by the hypothesis that chromatids containing "grandparent" polynucleotide templates segregate from those containing "parent" templates.*

Lark, Consigli, and Minocha (*1*) have recently suggested that the segregation of chromatids at mitosis is nonrandom, that is, that all chromatids containing subunits synthesized in the previous generation segregate together. Thus, at the second mitosis after labeling with tritiated thymidine, all labeled chromatids would proceed to one pole, and all unlabeled chromatids would go to the other. This possibility, suggested by experiments on bacteria (*2*), was apparently confirmed by the frequency distribution of grain counts over nuclei at various times in primary cultures of mouse embryos, and in cultures of hamster cells. Lark and his co-workers recognized that the formation of sister-strand exchanges between labeled and unlabeled chromatids would tend to disrupt the pattern. The hypothesis has since been extended to *Vicia faba* (the broad bean) and *Triticum boeoticum* (wheat) (*3, 4*). The phenomenon might, therefore, be general.

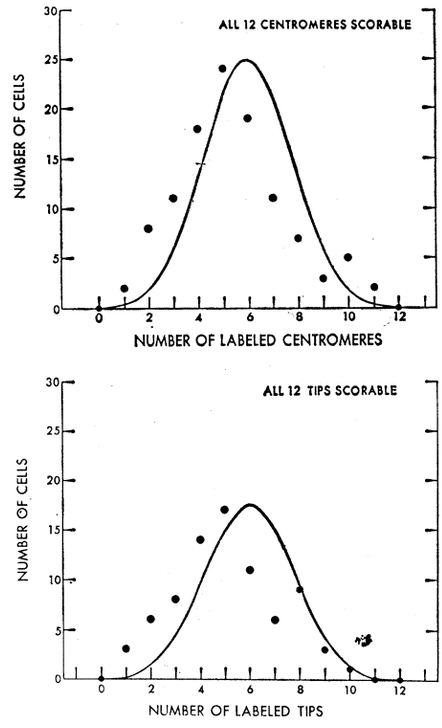

Fig. 1. Frequency distribution at the third mitosis after labeling of *V. faba* root tips. The line is the binomial expected from a random segregation of all 12 elements if the probability of being labeled is 0.5. (Top) The frequency of cells having 0,1,2 . . . 12 labeled centromeres. (Bottom) The frequency of cells having 0,1,2 . . . 12 labeled chromosome tips.

The suggestion of complete nonrandom segregation of chromatids is contrary to the usual assumptions that have been made (for example, *5*), and is contrary to our previous qualitative observations. To obtain quantitative results from experiments that avoid the