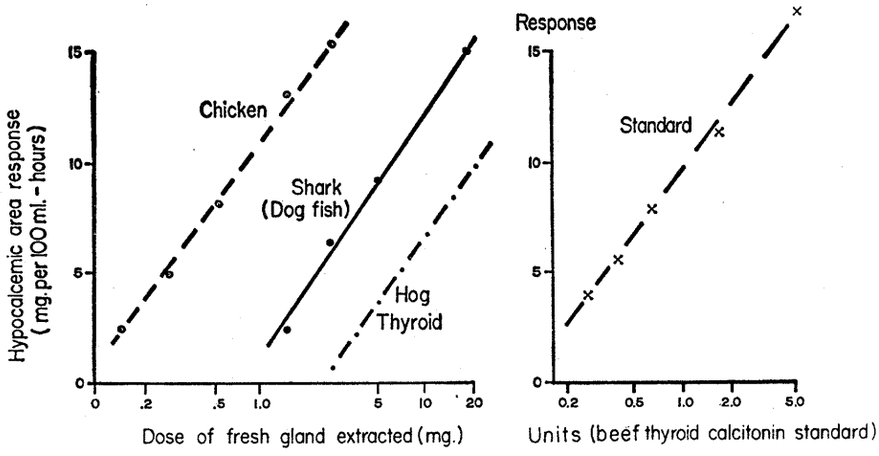

Fig. 1. Relation of hypocalcemic response to log dose of acid extracts of ultimobranchial glands of chicken *Gallus domestica* and a small shark *Squalus suckleyi*. Each point represents four to six rats. For comparison, the response to a standard preparation of calcitonin from beef thyroid and the estimated response to extracts of fresh hog thyroid (*10*) are indicated.

refer to an international standard (Thyroid Calcitonin–Standard A) prepared and made available by the Division of Biological Standards, National Institute for Medical Research, Medical Research Council, London. The relationship between response and the logarithm of the dose is shown in Fig. 1. The slopes for calcitonin from these three different sources are essentially parallel, suggesting similar biological activity. From these curves, the calcitonin extracted per gram of chicken ultimobranchial was estimated to be 130 MRC units as compared to 15 units per gram of dogfish ultimobranchial, and 2.6 to 4.3 units per gram of hog thyroid (*10*). The low value for the latter is not surprising, since ultimobranchial cells make up only 1 to 2 percent of the mass of hog thyroid.

The fall in plasma calcium level 1 hour after the injection of extract from one-tenth of a chicken ultimobranchial (0.5 mg) was 1.65 ± 0.30 mg per 100 ml; the fall after administration of extract from one-fourth of a dogfish ultimobranchial (4 mg) was 2.92 ± 0.19 mg per 100 ml. There was no detectable hypocalcemic effect from injection of extracts from 200 to 500 mg of chicken or dogfish thyroid.

The absence of detectable calcitonin in the thyroids of these animals, together with the very high level in the ultimobranchial glands, strongly suggests that in birds and elasmobranch fishes calcitonin is an ultimobranchial rather than a thyroid hormone. This is consistent with the view that calcitonin is produced by the C cells of the mammalian thyroid (*4*), and that these are of ultimobranchial origin (*5*). It is also interesting that the other calcium-regulating glands, the parathyroids, arise from a very similar embryological anlage in more anterior branchial pouches. The presence of calcitonin in the most primitive (elasmobranchs) and advanced (birds and mammals) vertebrates possessing ultimobranchial tissue suggests that this must be one of the fundamental vertebrate hormones. Indeed, phylogenetically the ultimobranchials and calcitonin appear to precede the other two important factors in calcium regulation, bone and parathyroid hormone. In mammals, calcitonin appears to act primarily on bone to inhibit osteolysis. It will be interesting to determine the action of this hormone in elasmobranchs in which there is no boney skeleton.

D. H. Copp
D. W. Cockcroft
Yankoon Kueh

Department of Physiology,
University of British Columbia,
Vancouver 8, Canada

References and Notes

1. D. H. Copp, E. C. Cameron, B. A. Cheney, A. G. F. Davidson, K. C. Henze, *Endocrinology* **70**, 638 (1962).
2. P. F. Hirsch, G. Gauthier, P. L. Munson, *ibid.* **73**, 244 (1963).
3. G. V. Foster, A. Baghdiantz, M. A. Kumar, H. A. Soliman, I. MacIntyre, *Nature* **202**, 1303 (1964).
4. G. Bussolati and A. G. E. Pearse, *J. Endocrinol.* **37**, 205, (1967).
5. A. G. E. Pearse and A. F. Carvalheira, *Nature* **214**, 929 (1967).
6. A. F. Carvalheira and A. G. E. Pearse, *Histochemie* **8**, 175 (1967).
7. W. E. Camp, *J. Morphol.* **28**, 369 (1917).
8. D. H. Copp, *J. Lab. Clin. Med.* **61**, 1029 (1963).
9. ——— and A. V. Kuczerpa, *Proc. Can. Fed. Biol. Soc.* **10**, 113 (1967).
10. P. L. Munson and P. F. Hirsch, *Clin. Orthopaed.* **49**, 209 (1967).
11. Work supported by a grant from the Medical Research Council of Canada.

25 September 1967

Photoperiodic Control of Hamster Testis

Abstract. *The response of the testes of juvenile and adult hamsters to various photoperiods was examined. The testes of juvenile animals reached maturity regardless of the light cycle on which the animals were raised. However, the testes of adult hamsters required at least 12.5 hours of light per day to maintain spermatogenesis and prevent degeneration. This is one of the few demonstrations of a response suitable for study in investigations of the photoperiodic control of testicular function in a laboratory mammal.*

Seasonal breeding patterns in the field have long been observed and are well documented for most mammalian species (*1*). To insure production of the young at the time of year most conducive to survival, accurate synchronization of reproductive activities with the environment is essential. Most attempts to explain the observed correlation of reproductive activity with season invoke day length as the controlling factor. For, of all the environmental cues which indicate seasonal change, the duration of light per day is the most reliable.

The controlling role of light has been demonstrated by extensive experimental work on the timing of estrus in several mammalian species (*2*). However, only a few studies have considered photoperiodic influences on the male. Bissonnette (*3*) subjected male goats, which normally breed on the short days of autumn, to a short photoperiod in the spring. As a result of this treatment mature sperm were produced at a time when the seminal epithelium is normally quiescent. Ortavant *et al.* (*4*) showed that testis weight and sperm production in the ram was maximum on a photoperiod of LD 8:16 (*5*). Photoperiods shorter or longer than 8 hours resulted in loss of testicular weight, as well as in decrease in spermatogenesis. Several studies have

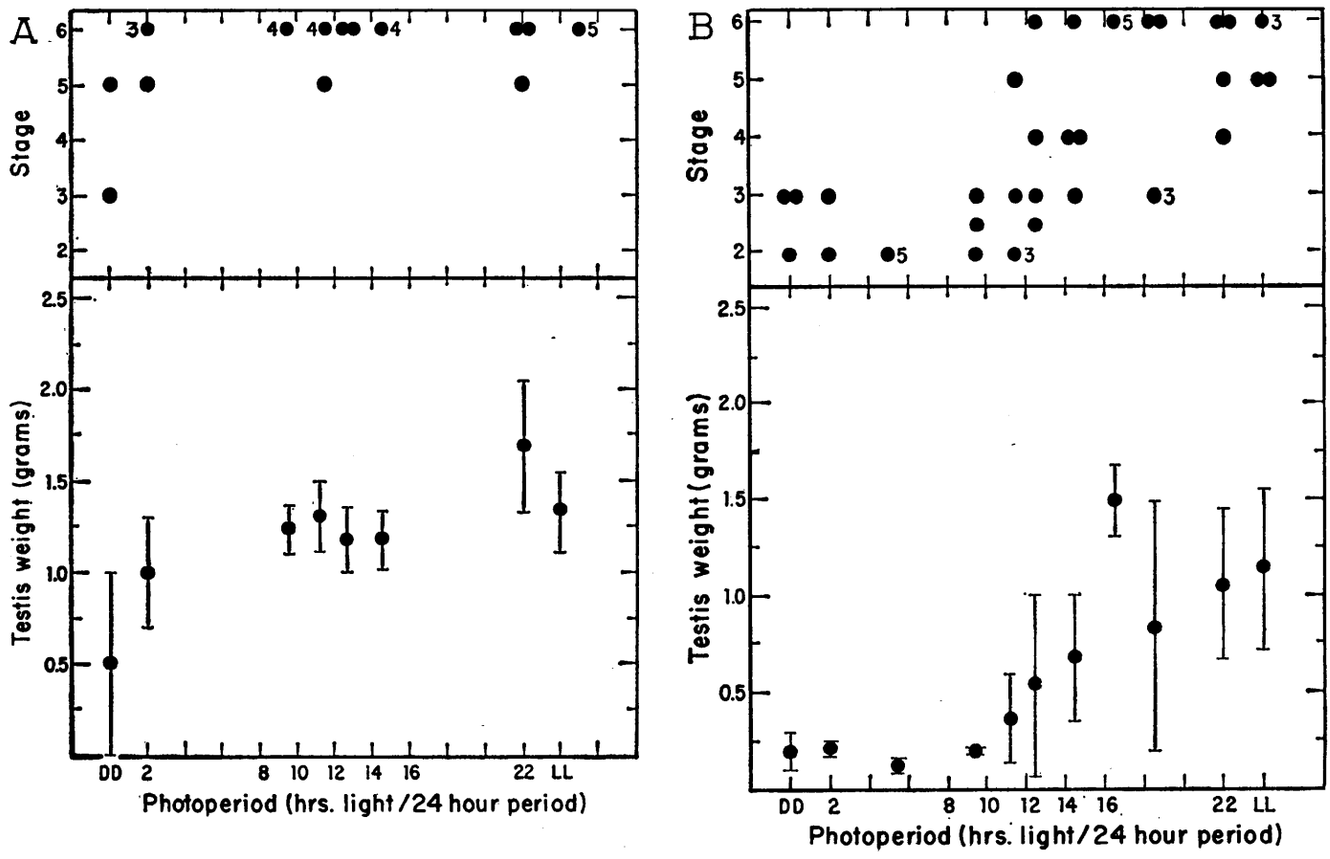

Fig. 1 (above). Response of hamster testes to different photoperiods. The abscissa for the stage data (see text) is identical to that against which testis weight is plotted. Points for testis weight are group means, and the bars above and below them are standard deviations. The number accompanying some of the points of stage data indicates the number of animals at that stage. (A) Response of the right testis of juvenile hamsters after 1 month of treatment. (B) Testicular response of adult hamsters after 6 weeks of treatment. Testis weight is an average of the weights of left and right testis. (*11*).

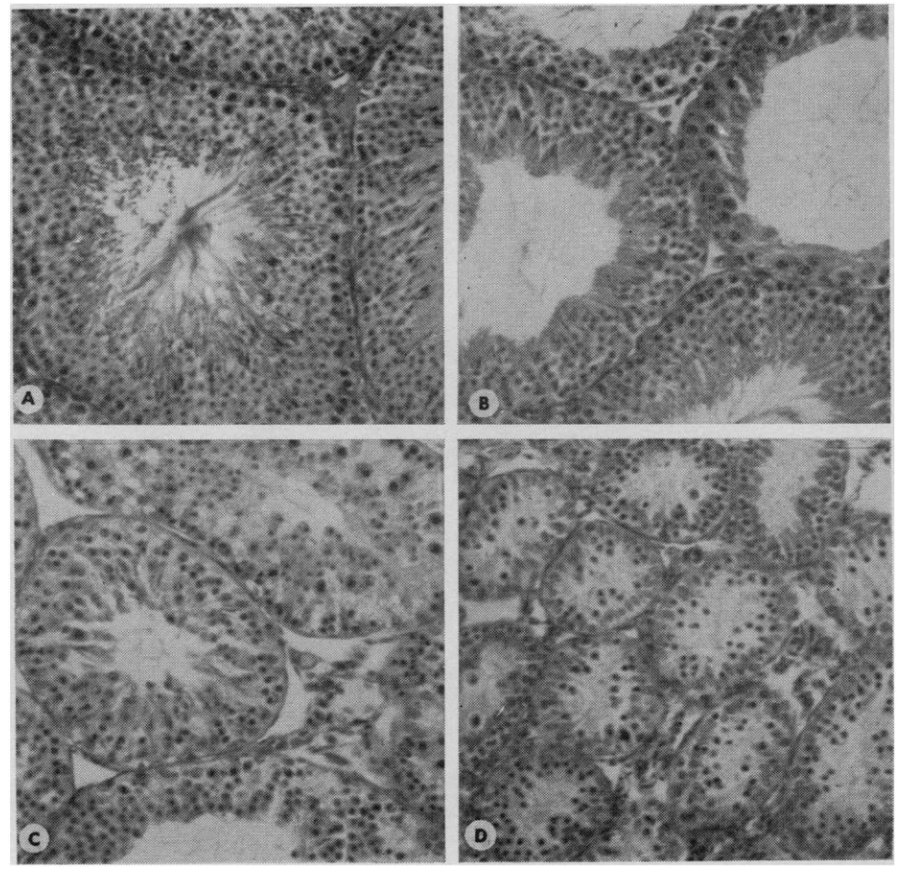

Fig. 2 (right). Representative sections from normal and degenerate testes (about × 160). (A) Stage 6; full spermatogenesis. (B) Stage 5; fewer sperm are being produced, and sloughing of seminal epithelium into the lumen is evident in about 50 percent of the tubules of a given section. (C) Stage 4; there is loss of ordered cell division within the seminal epithelium, absence of spermiogenesis, shrinkage of tubules, and shrunken appearance of interstitial cells. (D) Stage 2; the lumina have disappeared, the primary spermatocyte is the predominant germ cell, and further tubule shrinkage has taken place.

shown testis function in ferrets to be photoperiodically controlled. Comparisons of effects of full photoperiods with those of skeleton regimes in the male ferret have indicated that LD 2:10:2:10 can effectively replace an LD 14:10 cycle in maintaining reproductive activity (*2*). Hoffman and Reiter (*6*) have shown that LD 16:8 maintained spermatogenesis whereas LD 1:23 produced testicular involution and loss of spermatogenic activity in hamsters. Such studies establish the influence of light on the reproductive function of the mammals examined. However, the way in which light effects the synchronization of breeding cycles is as yet undetermined. A description of the reproductive response to a range of different photoperiods is preliminary to an examination of the mechanisms of photoperiodic control.

Four-week-old male hamsters were subjected to experimental photoperiods (*7*). Figure 1A shows the testicular response of these hamsters after a 1-month exposure to various photoperiods. The testes of most of the hamsters attained adult weight and full spermatogenic activity (Fig. 2A) on all light regimes. Apparently juvenile hamsters are able to attain reproductive maturity regardless of the photoperiod on which they are raised. Indeed, spermatogenesis is found even on LD 2:22 and in constant darkness.

Fig. 1B illustrates the very different testicular response of intact adult hamsters to different photoperiods. These animals were maintained in the laboratory until after they reached sexual maturity (at approximately 10 weeks of age) on a photoperiod of LD 13:11. They were then transferred to the experimental regimes for 6 weeks and subsequently killed. The relationships of testis weight and histological stage of spermatogenic activity to the experimental photoperiods show clearly that maintenance of testis size and spermatogenic activity are photoperiodically controlled. The testes of hamsters receiving less than 12.5 hours of light per day were small and histologically degenerate, whereas testes of most of the animals maintained on longer day lengths were relatively large and functional. These data indicate a critical day length of about 12.5 hours for the photoperiodic control of testis maintenance in adult hamsters (*8*).

Histological evaluation of the degree of degeneration was based on a minimum of 20 serial cross sections (*9*) from the middle of the right testis from each animal. Between 250 and 300 cross sections of seminiferous tubules are present in each section. Stages were assigned on the basis of the following criteria: type of germ cells present; diameter of the seminiferous tubules, appearance of the interstitial cells, and the degree of sloughing of the seminal epithelium. Degenerative changes, when present, were observed uniformly throughout a given section, as well as in all serial sections of the tissue examined. With respect to the above criteria, the histological degeneration appeared identical in all animals regardless of previous history (for example, a stage-3 testis produced by LD 2:22 is indisinguishable from a stage-3 testis from an animal maintained on LD 12.5:11.5) (Fig. 2).

The relationship between testis weight and stage in Fig. 3 shows that

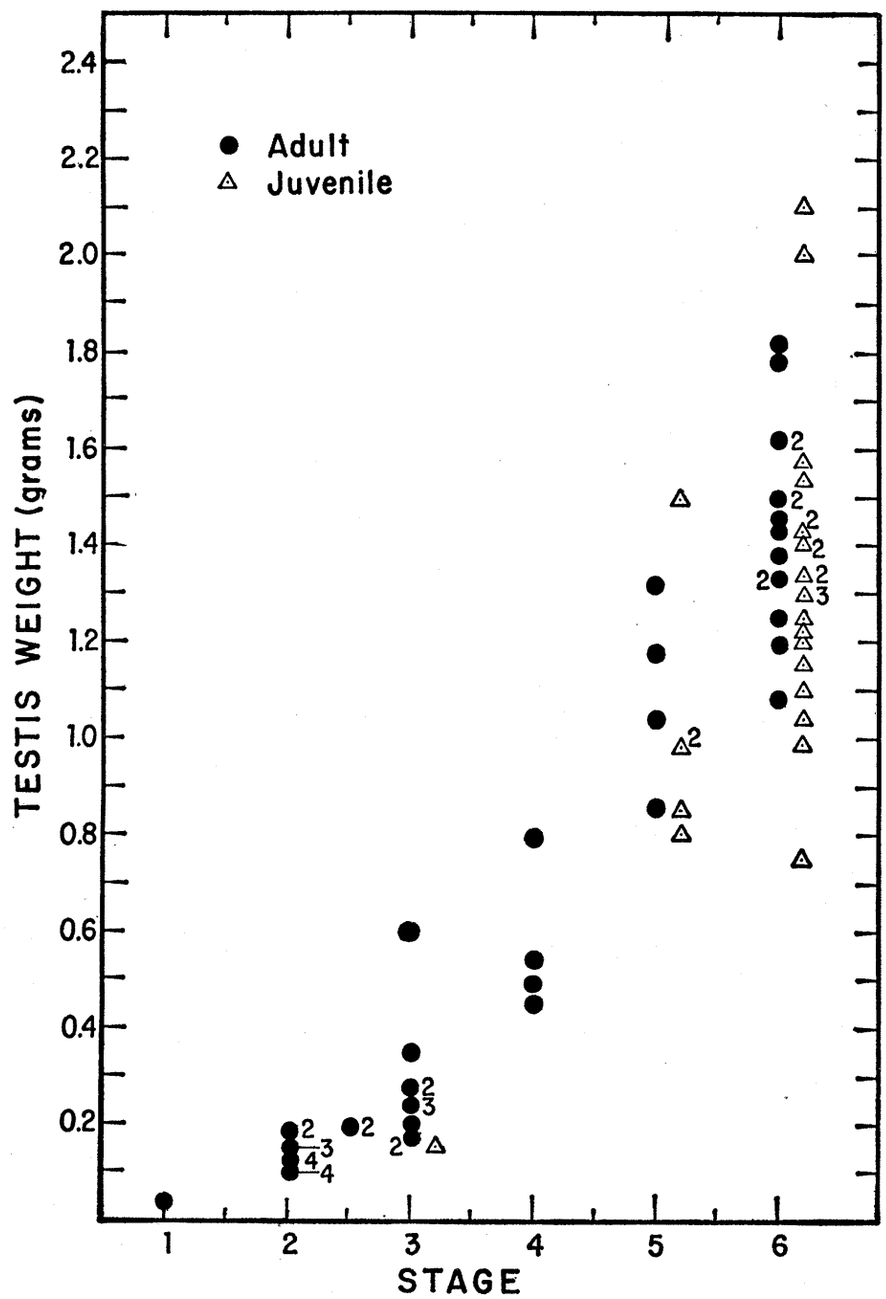

Fig. 3. Relationship between testis weight and histological stage. Numbers beside points indicate number of animals per point. Stage 1 represents a quiescent immature testis and is included for comparative purposes. This stage (resting spermatogonia only) was not observed in any degenerate adult testes.

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