pared to the T value obtained from 1-year intervals) is by about an order of magnitude. The few studies of island bird turnover published to date (*2, 3*) (those for the California Channel Islands, Karkar Island, and Mona Island) primarily used survey intervals of several decades and yielded turnover estimates of 0.1 to 1.7 percent per year. These values can now be appreciated to be underestimates by considerable factors (*13*). The ongoing turnover studies of the California Channel Islands and other islands at 1-year intervals (*5, 6*) are yielding the considerably higher rates of 0.2 to 20 percent per year, mostly 1 to 10 percent per year, depending on area and other island properties. Thus, turnover studies of bird communities should be based on repeated censuses at 1-year intervals (*14*).

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$$I_i(t) = [\lambda_i/(\mu_i + \lambda_i)]\,[1 - e^{-t(\mu_i + \lambda_i)}]$$
and Eqs. 2b and 7 and the second equation of (*11*) are correspondingly modified.
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10. $$\overline{CVI}_i(t) = [I_i(1 - I_i)]^{1/2}/I_i$$
$$\overline{CVE}_i(t) = [E_i(1 - E_i)]^{1/2}/E_i$$
with $I_i(t)$ and $E_i(t)$ given by Eq. 2.
11. The relation is
$$\overline{CVT}(t) = A^{1/2}/(\sqrt{2}tS^*T(t)$$
with $T(t)$ given by Eq. 7, and A defined by
$$A = \sum_i \mu_i\lambda_i[\mu_i + \lambda_i]^{-2}[1 - (1 - \mu_i - \lambda_i)^{2t}]$$
With the help of Eq. 9, it may be seen that for $t \to \infty$ this reduces to
$$\overline{CVT}(t \to \infty) = [2\sum_i \mu_i\lambda_i/(\mu_i + \lambda_i)^2]^{-1/2}$$
Together with Eqs. 3 and 5, this gives the limiting result, Eq. 11.
12. For the four species that bred in all 29 census years, λ_i cannot be calculated. However, this uncertainty does not affect turnover calculations, since μ_i for these species is 0.
13. Lynch and Johnson have repeatedly claimed that the estimates of turnover rates for island bird communities (in *2, 3*) based on survey intervals of several decades are seriously in error by being overestimates [N. K. Johnson, *Condor* **74**, 295 (1972); J. F. Lynch and N. K. Johnson, *Abstr. Cooper Ornithol. Soc.* (1973), Abstr. 44; *Condor* **76**, 370 (1974)]. Our analysis suggests that the opposite is true; for more detailed refutation of Lynch and Johnson's claim, see (*4*).
14. Accurate censuses of insular faunas at annual intervals are rarely available for groups other than birds. How should one correct for the in-and-out effect and estimate the true turnover rate if an island has been censused only a few times? We suggest calculating T from all pairwise combinations of censuses and fitting a straight line through a graph of $\log T$ versus $\log t$. The ordinate intercept then approximates $T(1)$ within wide confidence limits.
15. Data were extracted from records of the Farne Island Committee of the National Trust by T. Reed and G. Hickling. Carrion crow was omitted because its nesting is usually prevented by the resident warden as a matter of policy.
16. We thank T. Reed, G. Hickling, and many others, for information on island bird censuses, and G. F. Oster, for helpful discussion. Supported by NSF grant BMS 7510464 and the Lievre Memorial Fund.

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Antiserums to Neurons and to Oligodendroglia from Mammalian Brain

Abstract. *Two specific antiserums were produced, one to rat neurons and one to lamb oligodendroglia isolated in bulk from brain. On the basis of immunofluorescence and absorption studies with bulk isolated cells, the antiserums were produced to specific surface components. The antiserums are useful as markers for cell identification and for studying proteins in plasma membranes.*

Two antiserums, one specific to rat neurons and the other to lamb oligodendroglia, isolated in bulk from normal brain, have been produced (*1*). The specificities of the antiserums were determined by both immunofluorescence of the respective target cells and absorption of the antiserums by isolated cell populations from different tissues. Recent methods for the bulk isolation of the three major cell types from brain (*2*) and the subsequent maintenance of neurons and oligodendroglia (*3*) have made the production and characterization of these antiserums possible. Our studies differ from previous attempts to study cell markers in which whole regions of brain such as corpus callosum or cerebellum (*4*) have been used to produce antiserums, as well as subcellular fractions [myelin synaptosomes, or synaptic membranes (*5*)], cell lines derived from neuronal or glial tumors (*6*), or soluble brain proteins (*7*). Of the soluble proteins, the glial fibrillary acidic protein does seem specific for astroglial cytoplasm (*8*).

At present, it is not possible to obtain all three cell types from one brain sample. Neurons and an astrocyte fraction were isolated from 12-day-old rat brain (*1*); oligodendroglia were isolated from the subcortical white matter of lamb, calf, or human autopsy tissue (*1*). The procedure involves incubation of minced brain tissue in a trypsin solution, dissociation of the tissue through several screens of 100 and 200 mesh, and separation of the cells on discontinuous sucrose gradients. With rat brain, a mixed cell layer was also obtained which contained all three cell types. The cells were identified by both phase and electron microscopy and have been biochemically characterized (*9*). The neurons are more than 90 percent pure; the cells are large, 15 to 40 μm in diameter, with a large nucleus, a frequently visible nucleolus, and abundant cytoplasm. The astrocytic layer is only 60 percent pure, being contaminated with cell fragments trapped by the elaborate processes. Astrocyte cell bodies are smaller, 10 to 30 μm in diameter, with nuclei of irregular shape, and with scant cytoplasm. The oligodendroglia are round cells, 7 to 10 μm, with a small round nucleus surrounded by a rim of cytoplasm; the fraction is more than 90 percent pure by particle count.

Neurons obtained from the gradients were washed three times in 50 percent fetal calf serum, *p*H 6.0, to remove residual trypsin activity. Oligodendroglia were washed three times in 5 percent fetal calf serum, with a gradual shift in *p*H from 6 to 7.0. Both cell types were maintained in glass flasks at 37°C in a moist incubator (90 percent air and 10 percent CO_2), in Dulbecco's high glucose tissue culture medium (*10*) with requirements specific for each cell type. The cells remained morphologically intact during these manipulations.

For the production of antiserums, two rabbits were each injected intravenously with 70×10^6 to 120×10^6 freshly isolated and serum-washed rat neurons that were then washed in Dulbecco's phosphate-buffered saline (PBS) (*10*) and suspended in 1 ml of PBS. Oligodendroglia could not be injected intravenously owing to their clumping in serum, which caused death of the rabbits before the injections were finished. Thus oligoden-

droglia were washed in cell medium without albumin, pH 6.0, and were injected subcutaneously in 1 ml of Freund's complete adjuvant (*10*). Both series of injections were repeated at 2-week intervals for three injections. Two weeks after the final set, the rabbits were bled; the serum was collected, inactivated by heating at 56°C for 30 minutes, and stored at −20°C in small vials.

The antiserums were tested for evidence of antibody activity by indirect immunofluorescence with the use of suspensions of target cells that had been maintained overnight. Neurons or oligodendroglia were washed in PBS, suspended in serial dilutions of antiserum (total volume, 200 μl), and incubated for 30 minutes at room temperature. The cells were again washed, 50-μl portions of a 1:10 dilution of fluorescein-conjugated goat antiserum to 7*S* rabbit globulin (*10*) were added (total volume, 200 μl), and the mixtures were incubated for a further 30 minutes at room temperature. After a final washing, the cells were identified first by phase microscopy, and then examined for fluorescence with a Zeiss fluorescent microscope with a halogen light source. The cells are not fixed during this procedure.

Neurons characteristically showed a dull yellow autofluorescence, even in the absence of serum. In contrast when the same cells were incubated with the antiserum to neurons at dilutions of 1:80 and 1:160, they exhibited a bright green surface fluorescence. At a dilution of 1:270, the fluorescence was marginal. Bulk-isolated maintained mouse neurons also reacted with this antiserum.

Negative results were obtained with controls of the conjugate alone, nonimmune rabbit serum, or antiserum without conjugate. As is indicated in Table 1, the fluorescence observed with maintained neurons was absorbed by isolated neurons, but was not absorbed by cells from other tissue (such as liver, red cells, or thymus), or by rat myelin (see method of absorption, Table 1).

When freshly isolated rat astrocytes or oligodendroglia in the mixed layer were used as target cells with serial dilutions of antiserum to rat neurons, no fluorescence was observed with either astrocytes or oligodendroglia. Only neurons exhibited fluorescence. As was mentioned above, the astrocyte fractions contain only 60 percent intact astrocytes, with the remainder consisting of cell fragments (*9*). This cellular debris tends to aggregate in any serum; these "clumps" may become highly fluorescent. Absorption of the antiserum to neurons with 4 mg (dry weight) of astrocytes (more than twice the weight of neurons needed to absorb the antiserum) did not change the binding of antiserum to neurons. However, this binding was removed by absorption with greater concentrations of astrocytes (8 mg, dry weight). Both the absorption by the concentrated astrocyte fraction and the fluorescence of the clumped fragments could be explained by contamination of this fraction by neuronal fragments. It is also possible that a second set of antigens, proteins common to brain, were present, but at lower concentrations. However, more precise biochemical methods are needed to determine the presence of shared antigens.

With the use of maintained lamb oligodendroglia as the target cell and the antiserum produced to oligodendroglia, the glial cells exhibited bright green surface fluorescence at the titrations indicated in Table 2. Similar results were obtained with oligodendroglia isolated from calf or human brain. No surface fluorescence was observed when the antiserum was absorbed with isolated oligodendroglia. However, absorption with rat neurons, lamb liver or red cells, lamb brain gray matter, or lamb myelin did not alter the surface fluorescence of the glial cells.

As judged by immunofluorescence, the antiserums did not appear to contain antibodies against any of the components used during the cell isolation (trypsin, albumin, or fetal calf serum) since each antiserum would be expected to bind in a similar manner with all three cell types; this binding was not observed. Moreover, myelin does not appear to share the same surface components with oligodendroglia, since repeated absorptions with myelin did not change the specificity of the antiserum. This result is in accord with the differences previously found in the biochemical compositions of oligodendroglial plasma membranes and myelin (*11*).

Thus these antiserums may be used to aid in the identification of cell types in

Table 1. Immunofluorescent staining of rat neurons with antiserum to neurons. The absorption studies were performed as follows. Portions of antiserum (1 ml) were sequentially absorbed with 3-ml fractions of packed dissociated cells from rat liver, then by thymus cells or red cells. Liver and thymus were dissociated by gentle mechanical teasing of the cells in PBS and washed several times with PBS. Absorptions were performed by suspending the packed washed cells in serum at room temperature for 30 minutes and then removing the supernatant serum after centrifugation. Different portions of serum were absorbed with freshly dissociated cells to ensure consistent results. Separate unabsorbed portions (0.1 ml) were absorbed with the following: 0.12, 0.24, and 0.47 mg of myelin protein; 0.5, 1, and 2 mg (calculated dry weight) of neurons; and 0.5, 1, 2, and 4 mg (calculated dry weight) of astrocytes. The immunofluorescence assay is described in the text. Each concentration gave the same result; the titrations before and after absorptions were the same. In this qualitative study, + indicates positive surface fluorescence and − indicates no surface fluorescence.

Treatment of antiserum	Fluorescence with antiserum diluted	
	1:80	1:160
None	+	+
Absorbed with fractions from rat		
Liver	+	+
Liver and red blood cells	+	+
Liver and thymus	+	+
Myelin	+	+
Astrocyte fraction	+	+
Neurons	−	−

Table 2. Immunofluorescent staining of lamb oligodendroglia with antiserum to lamb oligodendroglia. Portions of antiserum were sequentially absorbed with lamb liver and red blood cells as indicated in Table 1. Separate 0.1-ml unabsorbed portions were absorbed with dissected lamb brain gray matter (10 volumes); 1.2 mg of rat myelin protein; 1.56, 3.12, and 6.24 mg of lamb myelin protein; and 250×10^6 and 490×10^6 isolated lamb oligodendroglia. The results shown were the same for each concentration; titrations were the same before and after absorptions. +, Positive surface fluorescence; −, no surface fluorescence.

Treatment of antiserum	Fluorescence with antiserum diluted		
	1:80	1:160	1:270
None	+	+	+
Absorption with			
Liver	+	+	+
Liver and red blood cells	+	+	+
Gray matter	+	+	+
Rat myelin	+	+	+
Lamb myelin	+	+	+
Oligodendroglia	−	−	−

vitro; to identify and study differences between abnormal and normal cells in vitro; to study any changes that may occur in cell surface components during development; and, potentially, to sort specific cell types by means of a fluorescent activated cell sorter, a technique now used with lymphocytes (*12*). Surface labeling of the proteins of the cells, by standard membrane probes, can now be used to determine the nature of the antigens responsible for the specific antiserum produced.

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Dibutyryl Cyclic AMP Mimics Ovariectomy: Nuclear Protein Phosphorylation in Mammary Tumor Regression

Abstract. *Growth of mammary carcinoma induced by 7,12-dimethylbenz(a)anthracene is arrested by either ovariectomy or treatment with* $N^6,O^{2'}$*-dibutyryl cyclic adenosine 3',5'-monophosphate (dibutyryl cyclic AMP). When this occurs, a new nonhistone protein species becomes the predominant endogenous substrate of cyclic AMP-dependent protein kinase in the tumor nuclei. Phosphorylation of this regression-associated protein ceases when resumption of tumor growth is induced by either the injection of 17β-estradiol or cessation of dibutyryl cyclic AMP treatment. Thus phosphorylation of regression-associated protein may play a role in the regression of hormone-dependent mammary tumors.*

Studies from several laboratories have suggested that most mammalian cytoplasmic protein kinases are stimulated by adenosine 3',5'-monophosphate (cyclic AMP) and preferentially phosphorylate basic proteins, such as histones, in vitro (*1*). In contrast to the cytoplasmic enzymes, protein kinases from nuclear fractions have generally been found to be unresponsive to cyclic AMP and to prefer acidic proteins, such as casein and phosvitin, as substrates in vitro (*2*).

A mechanism that would allow cytoplasmic protein kinase to influence nuclear events has been implicated in several tissues. Redistribution of protein kinase from the cytoplasm to the nuclear fraction has been shown to occur in rat liver after stimulation with glucagon or dibutyryl cyclic AMP (*3*), in rat uterus with hormonal stimulation of adenylate cyclase (*4*), and in calf ovary in response to chorionic gonadotropins (*5*). This translocation of cytoplasmic protein kinase into the nucleus was also found in tumors regressing after dibutyryl cyclic AMP treatment (*6*). Thus a correlation exists between growth arrest in vivo and nuclear accumulation of protein kinase.

The growth of mammary carcinoma induced by 7,12-dimethylbenz(*a*)anthracene (DMBA) is arrested by either treatment with dibutyryl cyclic AMP (*7*) or by ovariectomy (*8*). Furthermore, soon after the initiation of either treatment, microsomal isozyme activity of glucose-6-phosphate dehydrogenase disappears; and the activity and amount of acid ribonuclease doubles in the neoplastic tissues (*7, 9*). Growth arrest of hormone-dependent mammary tumors induced by either deprivation of estrogen (ovariectomy) or treatment with dibutyryl cyclic AMP, may be mediated through a common mechanism that could involve phosphorylation of nuclear proteins. Therefore, we investigated the role of protein kinase–dependent phosphorylation of nuclear proteins in the growth control of a hormone-dependent DMBA-induced mammary carcinoma in Sprague-Dawley random-bred female rats.

Tumor nuclei were incubated with [γ-^{33}P]ATP to phosphorylate the nuclear proteins via intrinsic protein kinase. These nuclear proteins were then treated with 1 percent sodium dodecyl sulfate (SDS) and 1 percent mercaptoethanol and subjected to electrophoresis. As shown in Fig. 1, five major groups of protein bands were apparent with Coomassie blue staining. Similar patterns of protein bands were observed for unincubated nuclei and nuclei incubated with adenosine triphosphate (ATP) in the presence or absence of cyclic AMP. Protein species IV was found to be the major endogenous substrate for nuclei-associated protein kinase in the growing tumor (Fig. 1A), whereas in nuclei of the growth-arrested tumor (Fig. 1B) and regressing tumor (Fig. 1C) the radioactivity peaks associated with protein species IV decreased by about 40 percent, and new radioactivity peaks coincident with protein species I appeared. Injection of 17β-estradiol into the host for 5 days (5 μg/day; 200-g rat; subcutaneously) or cessation of dibutyryl cyclic AMP treatment for 5 days produced regrowth of the tumors and reversed the pattern of nuclear protein phosphorylation to that of the growing tumor. The phosphorylation of protein species I was not observed in a few DMBA tumors that grow autonomously despite ovariectomy.

To further characterize the regression-associated phosphorylation of protein species I, differential extractions of nuclear proteins were used after incubation of the nuclei with [γ-^{33}P]ATP. The protein bands and phosphorylation patterns of basic proteins isolated from the nuclei of growing and regressing tumors are shown in Fig. 2, A and B, respectively. Three major classes of protein bands were designated BI, BII, and BIII, with BIII being the fastest band migrating toward the anode. In nuclei from the growing tumor, basic protein species BIII were found to be the major substrates for nuclei-associated protein kinase, and the radioactive peak was coincident with the major radioactive peak shown in Fig. 1A (Fig. 2A). In nuclei from the regressing tumor, two prominent radioactive peaks