A complement fixation test with this rabbit antiserum, comparable to the complement fixation tests for avian (*17*) and murine (*18*) leukemia viruses has now been developed (*19*).

With regard to the natural transmission of viruses of this type, the most important mode is probably vertical (*20*), that is, from one generation of hosts to the next, although horizontal transmission (between unrelated individuals) is also prominent in chickens (*21*) and has been observed in mice (*22*).

In this context the salivary gland was positive for FeLV antigen in the one LSA case tested (Table 1). Because soluble antigen of murine leukemia virus occurs in the milk of carrier mice (*23*), we looked for FeLV antigen in the milk of 27 normal lactating cats; but none was found in unconcentrated milk.

Reports of clustering of LSA cases among unrelated cats of single households speak for case-to-case transmission (*24*). In one household of ten cats under study, two littermates and two unrelated cats developed LSA. In the four cases FeLV antigen was present. Another cluster comprised two unrelated Abyssinian cats with LSA; a lymph node biopsy from a third apparently healthy Abyssinian in the same household (above) had FeLV antigen and characteristic C-type particles.

WILLIAM D. HARDY, JR.
GAYLA GEERING
LLOYD J. OLD
ETIENNE DE HARVEN
Sloan-Kettering Institute for Cancer Research, New York 10021
ROBERT S. BRODEY
SUSAN MCDONOUGH
School of Veterinary Medicine, University of Pennsylvania, Philadelphia 19104

References and Notes

1. W. F. H. Jarrett, E. M. Crawford, W. B. Martin, F. Davie, *Nature* **202**, 567 (1964); W. F. H. Jarrett, W. B. Martin, G. W. Crighton, R. G. Dalton, M. F. Stewart, *ibid.*, p. 566; H. M. Laird, O. Jarrett, G. W. Crighton, W. F. H. Jarrett, D. Hay, *J. Nat. Cancer Inst.* **41**, 879 (1968); C. G. Rickard, personal communication; W. D. Hardy, Jr., G. Geering, L. J. Old, unpublished observations.
2. G. T. Kawakami, G. H. Theilen, D. L. Dungworth, R. J. Munn, S. G. Beall, *Science* **158**, 1049 (1967).
3. C. G. Rickard, L. M. Barr, F. Noronha, E. Dougherty, J. E. Post, *Cornell Vet.* **57**, 302 (1967); O. Jarrett, H. M. Laird, G. W. Crighton, W. F. H. Jarrett, D. Hay, *Leukemia in Animals and Man* (Basel and Karger, New York, 1968), pp. 244–254.
4. G. Geering, W. D. Hardy, Jr., L. J. Old, E. de Harven, R. S. Brodey, *Virology* **36**, 678 (1968).
5. G. Geering, L. J. Old, E. A. Boyse, *J. Exp. Med.* **124**, 753 (1966).
6. A. Gregoriades and L. J. Old, *Virology* **37**, 189 (1969).
7. E. A. Eckert, R. Rott, W. Schäfer, *ibid.* **24**, 426 (1964).
8. H. Bauer and W. Schäfer, in *Subviral Carcinogenesis*, Yohei Ito, Ed. (Nissha, Kyoto, Japan, 1966), pp. 337–352.
9. R. C. Nowinski, L. J. Old, D. H. Moore, G. Geering, E. A. Boyse, *Virology* **31**, 1 (1967); L. J. Old, E. A. Boyse, H. F. Oettgen, E. de Harven, G. Geering, B. Williamson, P. Clifford, *Proc. Nat. Acad. Sci. U.S.* **56**, 1699 (1966); J. M. Kirkwood, G. Geering, L. J. Old, *Biology of Amphibian Tumors*, M. Mizell, Ed. (Springer-Verlag, New York, in press). In comparing several versions of the microimmunodiffusion technique, we did not find that agar in lower concentration (0.7 percent) or the template method with cadmium sulfate as a developing agent [M. A. Fink, W. F. Feller, L. R. Sibal, *J. Nat. Cancer Inst.* **41**, 1395 (1968)] facilitated detection of FeLV antigen.
10. The breed incidence of feline lymphosarcoma in our series is as follows:

Breed	No. cases	FeLV antigen-positive cases
Domestic	18	13
Siamese	10	8
Persian	2	2
Abyssinian	1	1
Burmese	1	1
Maltese	1	0

Fifty-five percent of the LSA cases occurred in cats that were not inbred (domestic), while 45 percent occurred in inbred (pedigreed) cats. The latter group, however, certainly does not represent 45 percent of the population at risk, suggesting that inbreeding may render the cat more susceptible to viral LSA. This is another similarity to the murine and avian leukemia viruses. Our figures and those of others [C. R. Dorn, D. O. N. Taylor, H. H. Hibbard, *Amer. J. Vet. Res.* **28**, 993 (1967)], after correction for the frequency of breeds in the population at risk, show that Siamese cats have the highest incidence of LSA.
11. Two of these cases were provided by C. Rickard.
12. R. Dutcher, unpublished observation.
13. There are strong indications that feline infectious peritonitis has a viral etiology. The disease has been transmitted by cell-free filtrates [B. C. Zook, N. W. King, R. L. Robison, H. L. McCombs, *Pathol. Vet.* **5**, 91 (1968); J. M. Ward, R. J. Munn, D. H. Gribble, D. L. Dungworth, *Vet. Rec.* **83**, 416 (1968); W. D. Hardy, Jr., unpublished observation]. Electron microscopy of tissues from diseased cats (Zook *et al.* and Ward *et al.*) has shown the presence of virus particles ranging from 70 to 100 nm occurring within the cisternae of the endoplasmic reticulum. The particles we observed in the one out of eight cats with infectious peritonitis (Table 2) form by budding at the plasma membrane and bear a close resemblance to FeLV. The presence of FeLV antigen in this case (Table 1) adds strength to this conclusion.
14. L. J. Old, E. A. Boyse, G. Geering, H. F. Oettgen, *Cancer Res.* **28**, 1288 (1968).
15. Passage FeLV was inoculated intraperitoneally into newborn beagle puppies, one of which developed lymphosarcoma (with characteristic FeLV particles) at 7 months of age. [J. A. Moore and J. R. Mitchell (State of Michigan Department of Public Health) and C. Rickard (New York State Veterinary College, Cornell University), unpublished observation.]
16. D. Armstrong, *J. Virol.* **3**, 133 (1969).
17. P. S. Sarma, H. C. Turner, R. J. Huebner, *Virology* **23**, 313 (1964).
18. J. W. Hartley, W. P. Rowe, W. I. Capp, R. J. Huebner, *Proc. Nat. Acad. Sci. U.S.* **53**, 931 (1965); J. W. Hartley, W. P. Rowe, W. I. Capp, R. J. Huebner, *J. Virol.* **3**, 126 (1969).
19. In collaboration with P. Sarma and R. J. Huebner, unpublished results.
20. L. Gross, *Oncogenic Viruses* (Pergamon, New York, 1961).
21. H. Rubin, A. Cornelius, L. Fanshier, *Proc. Nat. Acad. Sci. U.S.* **47**, 1058 (1961).
22. T. Aoki, E. A. Boyse, L. J. Old, *J. Nat. Cancer Inst.* **41**, 97 (1968).
23. R. C. Nowinski, L. J. Old, E. A. Boyse, E. de Harven, G. Geering, *Virology* **34**, 617 (1968).
24. R. Schneider, F. L. Frye, D. O. N. Taylor, C. R. Dorn, *Cancer Res.* **27**, 1316 (1967); W. D. Hardy, Jr., J. E. Meincke, W. V. Hobbie, unpublished observations (1968).
25. Supported by NCI grant CA 08748 and grants from the John A. Hartford Foundation, Inc., and the New York Cancer Research Institute, Inc. We acknowledge the assistance of Drs. J. E. Whitehead, L. Abrevaya, G. Robinson, W. F. Lucker, W. Stockman, W. O'Reilly, L. Friedman, and Mr. W. Smith of the Henry Bergh Memorial Hospital of the ASPCA, New York; of Drs. J. E. Meincke, A. Palminteri, W. Hobbie, E. Peterson, L. Sanders, E. Grano, G. Gedon, R. Imhoff, R. Shomer, E. Baker, P. Thouin, H. Millar, and J. Whitefield, private veterinary practitioners, and of Dr. R. Marshak, University of Pennsylvania, School of Veterinary Medicine.

17 June 1969; revised 18 August 1969 ■

Ribonucleic Acid Metabolism of a Single Neuron: Correlation with Electrical Activity

Abstract. *The giant neuron of the abdominal ganglion of* Aplysia californica *incorporates tritiated uridine into RNA at a constant rate at rest. This rate increases under synaptic stimulation, the increase being directly proportional to the number of action potentials produced by the neuron. Multineuronal samples from stimulated ganglia failed to show an increase in incorporation.*

The relationship between the electrical activity of nervous tissue and its RNA metabolism has been a major problem of neurochemistry, and with recent evidence tending to correlate changes in neuronal RNA metabolism with learning, this relationship takes on added significance (*1*). The majority of investigations in this area have found increased RNA production following electrical activity, but the results are somewhat ambiguous, and the relationship has not been precisely quantified (*2*).

Single neuron preparations would seem to be ideal for such studies, since they allow precise monitoring of electrical activity and minimizing of errors due to the production of RNA by nonneuronal elements. However, so far these preparations have also given conflicting results; Fischer *et al.* have re-

Table 1. Incorporation ratios for the single neuron R2, and the multineuronal samples from the left and right sides of the ganglion. Entries are given as mean ± standard deviation (number of experiments in parentheses).

Experimental conditions	Tissue sample		
	R2	Right	Left
4 hours, unstimulated	2.28 ± 1.06 (2)	2.91 ± 1.47 (3)	2.34 ± 0.54 (3)
9 hours, unstimulated	4.60 ± 1.81 (5)*	4.48 ± 1.83 (5)	4.53 ± 2.02 (5)
9 hours, stimulated	8.94 ± 1.30 (5)*	5.85 ± 2.64 (4)	6.32 ± 2.55 (5)

* Significantly different by two-tailed *t*-test (P, .01).

ported positive results with the squid giant axon (*3*), but Edstrom and Grampp could find no correlation in the lobster stretch receptor (*4*).

The giant neuron [R2 of Frazier *et al.* (*5*)] of the abdominal ganglion of the mollusk *Aplysia* offers several unique advantages for such studies. Its electrical behavior is well known (*6*), and its large size (soma diameter, 400 to 800 μ) allows easy dissection of the soma from the ganglion and the gathering of data on intracellular nucleotides as well as RNA by conventional extraction techniques. In the present experiments, this cell was used to determine the effect of synaptic activation on the rate of RNA synthesis as reflected by the incorporation of tritiated uridine.

Aplysia californica were kept in recirculating seawater at 15°C. The abdominal ganglion was dissected from the animal and pinned to the bottom of a recording chamber containing 1.7 ml of incubation medium (0.2*M* uridine + 50 unit/ml each of streptomycin and penicillin in Millipore-filtered seawater). The chamber temperature was regulated at 15.0° ± 0.1°C. The connective tissue sheath overlying the ganglion was slit medially to facilitate exchange with the medium.

A suction electrode on the left pleurovisceral connective nerve was used to stimulate R2 synaptically. Another suction electrode on the right connective nerve allowed recording of R2's axon spike, which is readily identifiable by virtue of its large size (*6*). Identification of the spike was confirmed in other animals in which the soma spike was recorded intracellularly.

After the stimulus parameters sufficient to activate R2 were determined, 20 μc of uridine-5-^{3}H (New England Nuclear) in 0.2 ml of incubation medium was added to the solution in the chamber. For control runs R2 was stimulated once per hour as a check on the viability of the preparation; in experimental runs it was stimulated maximally for 5 to 10 minutes every half hour.

At the end of the incubation period (4 or 9 hours), the sheath was stripped from the ganglion and the soma of R2 was dissected out into a mortar of 20-μl capacity, ground with a pestle, and transferred to a specially designed Millipore-filtering chamber. This had a capacity of 0.1 ml, incorporated a 0.22-μ filter, and allowed pressure filtration directly into scintillation vials.

In addition, groups of neurons were plucked from the right and left sides of the ganglion and extracted concurrently with R2. The extraction procedure was a modification of the method of Munro and Fleck (*7*), adapted to filtration and small volumes. After transfer of the homogenate to the filter, the mortar and pestle were rinsed with 20 μl of a yeast RNA carrier solution, and the wash was transferred to the filter. The homogenate was treated with cold acid to extract free nucleotides, including RNA precursors. Subsequent alkaline hydrolysis resulted in a solution of RNA-derived nucleotides. The radioactivity in each of these fractions was determined by liquid-scintillation counting. The procedure is quantitative: in preliminary experiments with yeast RNA the recovery was 97 ± 7 percent. Carry-over of radioactivity between fractions was eliminated by washing until the counts in the wash declined to background.

To establish that the counts appearing in the alkaline hydrolysate were actually derived from RNA, I made use of the fact that alkaline hydrolysis of RNA results in a mixture of 2′- and 3′-mononucleotides, whereas all other uridine nucleotides in the cell are expected to exist as the 5′-phosphates. Three ganglia were incubated under control conditions and fractionated, and their alkaline hydrolysates were combined and applied to a Bio-Rad AG1-X10 ion-exchange column. Standards of 5′- and 2′,3′-UMP (uridine monophosphate) were added and the columns were eluted with a gradient of formic acid–ammonium formate. The results presented in Fig. 1 show negligible contamination of the RNA fraction by 5′-UMP (*8*).

The results were calculated as the ratio of counts in the RNA fraction to those in the acid-soluble fraction. The use of this incorporation ratio should provide a normalization for cell size, degree of exchange with the medium, specific activity of the medium, and other factors affecting the availability of labeled uridine precursor. Changes in this ratio could reflect changes in the net rate of RNA synthesis, in the rate of uridine phosphorylation, or in the production of uridine by the neu-

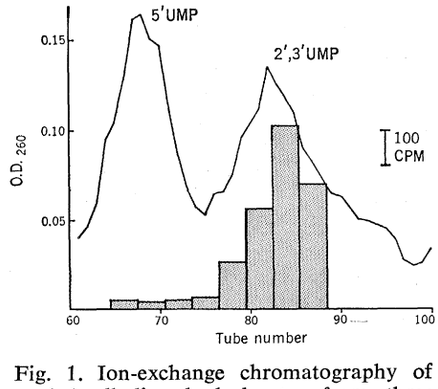

Fig. 1. Ion-exchange chromatography of pooled alkaline hydrolysates from three ganglia. Curve gives optical density at 260 nm; the peaks are from added standards. Histogram shows radioactivity from the samples. Three tubes were pooled for each radioactivity measurement.

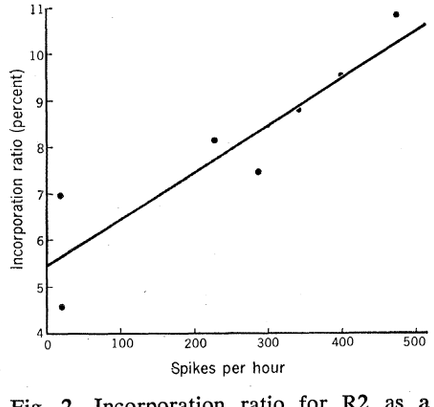

Fig. 2. Incorporation ratio for R2 as a function of impulse frequency. The two leftmost points are from unstimulated ganglia and represent spontaneous spike activity. The line is a least-squares fit to the points and has a slope of 1 percent per 100 spikes per hour.

ron. Since the specific activity of the uridine triphosphate in the cell was not measured, it is not possible to choose between these alternatives. However, the high uridine concentration in the incubation medium renders the last interpretation unlikely.

Since it has been shown that a neuron can still initiate and conduct impulses for up to 24 hours during inhibition of RNA synthesis by actinomycin D (*4*), it was necessary to establish the viability of this preparation over the duration of the experiment. Evidence for this consists of the facts that (i) although the stimulus voltage necessary to fire R2 increased during the experimental period, the neuron was still capable of being synaptically activated after 9 hours in all cases, and (ii), as seen in Table 1, the incorporation ratio is linear through 9 hours for all three tissue fractions.

Table 1 also shows that when R2 was stimulated, the ratio increased significantly. There was no change in the rate of uptake of radiouridine from the medium, that is, no change in the mean activity of the acid-soluble fraction. Therefore, the increased ratio actually represents an increase in the rate of appearance of labeled RNA.

Unstimulated R2's produced about 20 spikes per hour. Stimulated preparations were driven at 200 to 500 spikes per hour, depending on the ability of the neuron to follow the stimulus. There was a definite trend toward greater incorporation in those cells which fired more impulses. This is seen in Fig. 2, where the incorporation ratio for R2 is plotted against spike output. The relationship is clearly linear and shows a highly significant rank-order correlation.

The close agreement between the ratios for the groups of cells from the right and left sides of the ganglion, shown in Table 1, indicates the reproducibility of the extraction method, especially since the mass of cells taken from each side of each ganglion varied. The rate of RNA synthesis in these neurons did not increase significantly with stimulation, although the mean value was raised. This is to be expected, since stimulation of the left connective nerve excites many of the neurons in the ganglion (*5*), but is unlikely to fire each of them.

These data point out the disadvantages of studies on even small populations of neurons, when the electrical activity cannot be accurately specified. The demonstration of electrical-metabolic coupling reported here is a direct result of the precision attainable in single neuron analysis. The same factors contributing to that precision should make this preparation useful for investigating the mechanism by which the two processes are linked.

R. W. BERRY*

Biology Department,
University of Oregon, Eugene

References and Notes

1. H. Hyden and E. Egyhazi, *Proc. Nat. Acad. Sci. U.S.* **52**, 1030 (19[illegible] J. W. Zemp, J. E. Wilson, K. Schlesinger, W. O. Boggan, E. Glassman, *ibid.* **55**, 1423 (1966).
2. L. Z. Pevzner, in *Macromolecules and Behavior*, J. Gaito, Ed. (Appleton-Century-Crofts, New York, 1966), p. 43.
3. S. Fischer, M. Cellino, P. Gariglio, I. Tellez-Nagel, *J. Gen. Physiol.* **51**, 72s (1968).
4. J.-E. Edstrom and W. Grampp, *J. Neurochem.* **12**, 735 (1965).
5. W. T. Frazier, E. R. Kandel, I. Kupfermann, R. Waziri, R. Coggeshall, *J. Neurophysiol.* **30**, 1263 (1967).
6. L. Tauc, *J. Gen. Physiol.* **45**, 1077 (1962).
7. H. N. Munro and A. Fleck, in *Methods of Biochemical Analysis*, D. Glick, Ed. (Interscience, New York, 1966), vol. 14, p. 113.
8. Chromatography was performed at the suggestion of Dr. E. Herbert and in collaboration with R. W. Morris.
9. I thank Drs. M. J. Cohen and A. G. Szent-Györgyi for their advice and encouragement. This work was supported by PHS grant R01 NBO 1624-12 to M. J. Cohen and PHS grant 671 2T1 GM336-08.
* Present address: Biology Department, Yale University, New Haven, Conn. 06520.

31 July 1969

Alpha-Naphthoflavone: An Inhibitor of Hydrocarbon Cytotoxicity and Microsomal Hydroxylase

Abstract. *Alpha-naphthoflavone inhibits the metabolism of 3,4-benzopyrene and 7,12-dimethylbenz*(a)*anthracene in hamster embryo cell cultures and protects the cells against the inhibition of cell multiplication by these carcinogens. Alpha-naphthoflavone also inhibits the aryl hydrocarbon hydroxylase activity in homogenates of induced hamster embryo cells and in liver microsomes from rats previously treated with polycyclic aromatic hydrocarbons, but not in microsomes from control rats.*

The microsomal hydroxylases are important enzymes in the metabolism of aromatic hydrocarbons and are involved in the conversion of many drugs, steroids, and carcinogens (*1*). Some of the polycyclic aromatic hydrocarbons which are carcinogenic in animals are present in the environment and are ingested by man and thus may be factors in human carcinogenesis. Studies in vitro have shown that carcinogenic polycyclic hydrocarbons are toxic for normal embryonic rodent cells and inhibit their multiplication in monolayer cell cultures. Transformed rodent cells and both normal and transformed primate cells are relatively resistant to this effect (*2*). Cells that are sensitive to the cytotoxic effect metabolize the polycyclic hydrocarbons to water-soluble derivatives (*3*) and contain the NADPH-requiring (nicotinamide adenine dinucleotide phosphate, reduced) microsomal enzyme system, aryl hydrocarbon hydroxylase (AHH) (*4, 5*); in these cells the enzyme is also readily inducible (*5*). In cells that are resistant to the cytotoxicity, both the metabolic conversion and the enzyme system are markedly reduced or absent (*3, 6*). We have found that α-naphthoflavone (ANF) inhibits both AHH activity and the cytotoxicity induced by carcinogenic polycyclic hydrocarbons.

Primary monolayer cultures of Syrian hamster embryo cells were initiated by treating 11- to 14-day-old embryos with trypsin and were grown in Eagle's basal medium supplemented with fetal calf or calf serum (10 percent). All experiments were done with 8- to 48-hour-old secondary or tertiary subcultures in which the cell densities were 4×10^4 to 8×10^4 cell/cm^2. The ANF (Eastman Kodak, or Aldrich) (*7*) was dissolved in dimethyl sulfoxide, and the polycyclic hydrocarbons (Eastman Kodak) were dissolved in dimethyl sulfoxide or acetone; these solutions were further diluted with culture medium. The final concentrations of dimethyl sulfoxide and acetone in the cell cultures never exceeded 0.5 and 0.1 percent, respectively.

The metabolism of 3,4-benzopyrene to water-soluble derivatives was measured by adding tritium-labeled benzopyrene (Amersham/Searle; specific activity, 4 c/mmole) to hamster embryo cultures and extracting the medium, at specified times, with four volumes of water and 20 volumes of a mixture of