Table 1. Source of hair or skin tested.

Code label	Source
	Horse *Equus caballus*
HH–1	Idaho Falls, Idaho (black hair)
HH–2	Salt River Narrows, western Wyoming (bay or red hair)
HH–3	Bedford, Wyo. (white hair)
	Moose *Alces alces*
M–1	Northwest Wyoming
	Elk *Cervus canadensis*
E–1	Northwest Wyoming
	Mule deer *Odocoileus hemionus*
D–5	Greys River area, Wyo.
D–6	Sybille area, Wyo.
D–7	Fontanelle area, Wyo.
	Antelope *Antilocapra americana*
A–1	Shirley Basin, Wyo.
A–2	Laramie Plains, Wyo.
A–3	Laramie Plains, Wyo.
A–4	Daniel area, Wyo.
	Rattlesnake *Crotalus viridis*
SL–1	Intact skin Arco, Idaho
SC–1	Cast skin Arco, Idaho (both from the same snake)

compensate for lengthwise variations along the shaft.

The hairs were then bundled into groups, with a hair of the same type being used to code and hold the bundle together. All the samples of a given run were washed in a refluxing ether bath for a minimum of 1 hour to remove surface oils and oil-held contamination. The samples were then weighed, and sealed with heat in individual polyethylene packets, all of which were then enclosed in a polyethylene "rabbit" for activation in the Materials Testing Reactor (MTR) at the National Reactor Testing Station. Our first runs indicated that an exposure time of approximately 8 hours was required for adequate analysis of longer-lived activities. Accordingly, samples were exposed to a neutron flux of 1.3 $\times 10^{13}$ neutrons cm^{-2} sec^{-1} for 8 hours and then allowed to cool for 8 hours before pulse-height analysis was started. After removal from the "rabbit" and the polyethylene packet in which they were irradiated, the samples were again sealed in a small package of "cool" polyethylene and taped to a small card which was placed on the scintillation crystal. Activities were recorded and analyzed by means of a 400-channel pulse-height analyzer (Technical Measurements Corp., model 401C) coupled to a NaI(Tl) scintillation crystal (7.5 by 7.5 cm) and photomultiplier. The crystal and photomultiplier were mounted in a steel cave, the walls of which were 27.5 cm thick.

Spectra were resolved at intervals starting immediately after cooling. The most distinctive spectra appeared after a period of approximately 250 hours. Not shown in most of these spectra are a number of shorter-lived isotopes, the most prominent being Mn^{56} and Na^{24}.

The spectra from the five different genera of ungulates were resolved 230 to 260 hours after activation (Fig. 1). For approximately the first 200 hours, most of the nuclide peaks shown here were obscured by Na^{24} peaks, and it was necessary to wait until Na^{24} had decayed enough to reveal other nuclides.

There is dissimilarity among the spectra in respect to the species of nuclide present or the proportionate amount of the nuclide or both. The scales of each spectrum are the same, but the actual position on the y-axis has been ignored to allow the comparison. Figure 2 shows within-species consistencies and variation; differences in hair color and grazing range do not affect the gamma-ray spectrum of horse hair significantly. The Wyoming horses fed predominantly on plants growing in alluvial soils derived from Paleozoic sediments, whereas the Idaho area is composed of characteristically volcanic soil of relatively recent (Cenozoic) origin. Similarly consistent patterns were found in the wild ungulates. It would appear that although differences in habitat, range, and physiological condition may produce certain differences in the amount and composition of trace elements found in the hair, these differences are superimposed on a general species or perhaps generic pattern.

GARTH S. KENNINGTON
Department of Zoology and Physiology, University of Wyoming, Laramie
CLARENCE F. T. CHING
Atomic Energy Commission, National Reactor Testing Station, Idaho Falls, Idaho

References and Notes

1. R. E. Jervis, A. K. Perkons, W. D. Mackintosh, M. F. Kerr, *Proc. Int. Conf. Mod. Trends in Activation Analysis*, College Station, Texas (1961); M. F. Kerr, *Proc. Forensic Soc. Can.* **2**, 143 (1962).
2. H. Smith, S. Forshufvud, A. Wassen, *Nature* **194**, 725 (1962).
3. M. Trotter, in *Special Cytology*, E. V. Cowdry, Ed. (Hoeber, New York, 1932).
4. E. O. Butcher, *Ann. N.Y. Acad. Sci.* **53**, 461 (1951).
5. Supported by grant from the Associated Rocky Mountain Universities, Inc., and the cooperation of the National Reactor Testing Station, Idaho Falls, Idaho. I thank the Analytical Chemistry Branch, particularly D. G. Olson, for their cooperation and encouragement, and F. Kennington and the Wyoming Game and Fish Commission Laboratory for hair samples.

6 December 1965

Complement-Fixing Antigens in Hamster Tumors Induced by the Bryan Strain of Rous Sarcoma Virus

Abstract. *Hamster tumors transplanted subcutaneously from primary intracranial tumors which developed after inoculation of the Bryan strain of Rous sarcoma virus, contained virus-specific tumor antigens indistinguishable from those induced by the Schmidt-Ruppin strain.*

The Rous sarcoma virus (RSV) was first shown to be oncogenic in mammals by Svet-Moldavsky who induced sarcomas in rats with the Carr strain of this virus (*1*). Ahlstrom and Forsby (*2*) reported the induction of tumors with the Schmidt-Ruppin (S-R) strain (*3*) of RSV in hamsters, mice, rats, guinea pigs, and rabbits. Huebner *et al.* (*4*) showed the presence of specific avian leukosis complement-fixing (CF) antigens in hamster and guinea pig tumors induced by the S-R strain. These tumors elicited CF antibodies which were specifically reactive with antigens found in the homologous virus-free hamster tumors, with antigens in virus-containing chicken tumors induced by both the S-R and the Bryan strains of RSV, and also with tissue cultures of chicken-embryo fibroblasts (CEF) infected with various strains of avian leukosis viruses (*4, 5, 6*). The group-specific antigens in infected tissue cultures were smaller than complete avian-tumor virus particles (*4, 5*), and hamster tumor antibodies directed against them did not neutralize infectious S-R strain RSV (*4*). Vogt *et al.* (*7*) have presented evidence that the avian leukosis antigens in RSV-transformed CEF tissue cultures free of infectious virus ("nonproducer cells") were induced by the integration of genetic material from the "defective" (*8*) Bryan strain of RSV, which is incapable of replicating complete infectious particles in the absence of a helper virus. These authors concluded that the group-specific antigens are not part of the virus envelope but may be internal viral components common to all avian tumor viruses (*7*).

Rabotti *et al.* recently succeeded in producing gliomas, leptomeningeal sarcomas, and choroid plexus papillomas by intracranial inoculation of the Bryan strain of RSV into young hamsters, mice, rabbits, guinea pigs, and dogs (*9, 10*). We now report the presence of specific avian leukosis complement-fixing antigens in subcutaneous tumors

Table 1. Antibody to avian sarcoma and leukosis antigens in representative serums from hamsters bearing serially transplanted Bryan-strain tumors. Results are given as reciprocals of complement-fixing titers of serums against the antigen (used at 4 to 8 units) indicated.

Antigens	Serum numbers			
	52901	53115	52900	53449
Hamster tumor antigen				
Bryan strain	>80	40	40	20
S-R strain	>80	80	40	40
Chicken tumor antigen				
Bryan strain	>80	40	40	20
S-R strain	>80	40	40	10
Chicken-embryo fibroblast tissue culture antigen				
Bryan strain	>80	40	40	20
S-R strain	>80	20	20	20
RIF*	>80	20	10	20
AMV†	>80	20	20	20
AEV‡	>80	10	20	10
Uninoculated CEF	0	0	0	0

* RIF, Resistance-inducing factor (avian visceral lymphomatosis virus). † AMV, Avian myeloblastosis virus. ‡ AEV, Avian erythroblastosis virus.

transplanted in hamsters from primary gliomas induced (*9*) with the Bryan strain.

The methods for transplanting tumors in newborn and weanling NIH Syrian hamsters for serum collections, and for preparing CF antigens from hamster and chicken tumors as well as from CEF cultures infected with several strains of avian leukosis virus, have been described (*4–6, 11*). The CF test (microtechnique) was that described by Sever (*12*). The serums used in CF tests to detect sarcoma and leukosis antigens were standardized pools collected from hamsters carrying tumors induced by the S-R strain of RSV (*4*).

Serums from hamsters carrying subcutaneous transplants in the second subpassage of a glioma induced with the Bryan strain showed specific CF antibodies to homologous hamster tumor antigens, to S-R strain hamster tumor antigens, to antigens prepared from chicken wing-web tumors induced by the S-R and Bryan strains, and to avian leukosis virus antigens grown in CEF tissue cultures (Table 1). No reactions were observed with the virus-induced hamster tumor antigens of adenovirus type 7 or type 12 (*11, 13*), CELO virus (*14*), SV-40 virus (*15*), polyoma virus (*16*), the sodium cholate-induced FSa-3 hamster tumor antigens (*17*), or uninoculated tissue culture preparations of normal chicken-embryo fibroblasts. In general, studies of the antibodies in the Bryan strain hamster serums confirmed previous observations in other virus-induced tumor systems in hamsters (*11*) which indicated a correlation between antibody titers and the tumor sizes and lengths of time they were borne by the hamsters.

The reactivities of the Bryan strain hamster tumor antigens and antiserums were compared in box titrations with those produced by the S-R strain (Table 2). The CF reagents that were used included a pool of serums collected from hamsters carrying the sixth-passage subcutaneous transplants of a Bryan virus-induced tumor, a standard pool of serums from hamsters carrying transplanted tumors induced by the S-R strain (*4*), and antigen preparations made from the respective transplanted tumors. Although the antigen titers of the Bryan strain tumors were generally lower than those found in tumors of the S-R strain, the reactions appeared to be equally specific (Table 3).

Attempts to recover RSV from antigenic Bryan strain tumors were unsuccessful: (i) No tumors developed when fresh cells from several tumors in the first subcutaneous hamster transplantation were inoculated into chicks free of resistance-inducing factor (RIF). (ii) Extracts of six tumors with the highest CF titers, when tested directly with standard serums from hamsters bearing the S-R strain tumor, were negative when examined in COFAL tests (*6*) for infectious avian leukosis virus; and foci failed to form when roller tubes of primary CEF inoculated with these extracts were held for 33 days. (iii) All attempts to produce tumors in chicks with cell-free extracts of primary and transplanted hamster tumors have been unsuccessful.

Prior to the work of Rabotti *et al.* (*9*), the "defective" (*8*) Bryan strain was felt by many investigators to be incapable of inducing transformation of mammalian cells in vivo. The presence in these hamster tumors of CF antigens which are identical to those found in S-R strain tumors is substantial evidence that the Bryan strain RSV induced a genetic alteration during transformation of hamster cells in vivo although no virus has yet been recovered from these tumors.

Thus, despite the marked immunological (*18*) and biological differences between the Bryan and Schmidt-Ruppin strains of RSV, the tumors induced in hamsters by both these viruses contained CF antigens which were indistinguishable from each other. These antigens were also shared with those in chicken tumors induced by both of the sarcoma strains and in tissue cultures of several avian leukosis viruses.

Table 2. Presence of common antigens in Bryan strain and S-R strain RSV hamster tumors as shown by parallel box titrations of pooled tumor antigens tested with pooled serums from tumor-bearing hamsters.

Serum dilutions	Tumor antigen dilutions							
	Bryan strain				S-R strain			
	2*	4	8	16	2	4	8	16
Bryan strain serums								
10*	4†	4	2	1	4	4	4	2
20	4	4	2	0	4	4	4	2
40	4	4	1	0	4	4	4	1
80	4	4	1	0	4	4	3	1
S-R strain serums								
10	4	4	4	4	4	4	4	4
20	4	4	4	4	4	4	4	4
40	4	4	4	3	4	4	4	4
80	4	4	4	1	4	4	4	3

* Reciprocal of dilution. † Degree of complement fixation (range: 0–4).

Table 3. Specificity of representative hamster tumor antigens serially transplanted from primary tumors induced by Bryan strain RSV. Results are given as reciprocals of antigen titers against pooled serums (4 to 8 units) from hamsters bearing the indicated tumors.

Tumor antigens	Hamster tumor antiserums		
	Bryan RSV	S-R RSV	Control*
T–10073	4	>16	0†
T–10198	8	16	0
T–10199	4	8	0
T–10200	4	8	0

* Adenovirus 7, adenovirus 12, SV-40, polyoma, FSa-3. † No reaction at antigen dilution of 1:2.

Thus, despite the marked immunological (*18*) and biological differences between the Bryan and Schmidt-Ruppin strains of RSV, the tumors induced in hamsters by both these viruses contained CF antigens which were indistinguishable from each other. These antigens were also shared with those in chicken tumors induced by both of the sarcoma strains and in tissue cultures of several avian leukosis viruses.

M. J. CASEY
Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland
G. F. RABOTTI
Laboratory of Viral Carcinogenesis, National Cancer Institute
P. S. SARMA
Microbiological Associates, Bethesda
W. T. LANE, H. C. TURNER, R. J. HUEBNER
Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases

References and Notes

1. G. J. Svet-Moldavsky, *Nature* **182**, 1452 (1958).
2. C. G. Ahlstrom and N. Forsby, *J. Exptl. Med.* **115**, 839 (1962).
3. K. H. Schmidt-Ruppin, *Strahlentherapie* **41**, Sonderband 3, 26 (1959).
4. R. J. Huebner, D. Armstrong, M. Okuyan, P. S. Sarma, H. C. Turner, *Proc. Natl. Acad. Sci. U.S.* **51**, 742 (1964).
5. D. Armstrong, M. Okuyan, R. J. Huebner, *Science* **144**, 1584 (1964).
6. P. S. Sarma, H. C. Turner, R. J. Huebner, *Virology* **23**, 313 (1964).
7. P. K. Vogt, P. S. Sarma, R. J. Huebner, *ibid.* **27**, 233 (1965).
8. H. Hanafusa, T. Hanafusa, H. Rubin, *Proc. Natl. Acad. Sci. U.S.* **49**, 572 (1963).

9. G. F. Rabotti, W. A. Raine, R. L. Sellers, *Science* **147**, 504 (1965).
10. G. F. Rabotti, unpublished data.
11. R. J. Huebner, W. P. Rowe, H. C. Turner, W. T. Lane, *Proc. Natl. Acad. Sci. U.S.* **50**, 379 (1963).
12. J. L. Sever, *J. Immunol.* **88**, 320 (1962).
13. R. J. Huebner, M. J. Casey, R. M. Chanock, K. Schell, *Proc. Natl. Acad. Sci. U.S.* **54**, 381 (1965).
14. P. S. Sarma, R. J. Huebner, W. T. Lane, *Science* **149**, 1108 (1965).
15. P. H. Black, W. P. Rowe, H. C. Turner, R. J. Huebner, *Proc. Natl. Acad. Sci. U.S.* **50**, 1148 (1963).
16. K. Habel, *Virology* **25**, 55 (1965).
17. J. G. Fortner, A. G. Mahy, R. S. Cotran, *Cancer Res.* **21** (6) pt. 2, 199 (1961); A. B. Sabin, H. M. Shein, M. A. Koch, J. F. Enders, *Proc. Natl. Acad. Sci. U.S.* **52**, 1316 (1964).
18. P. S. Sarma, R. J. Huebner, D. Armstrong, *Proc. Soc. Exp. Biol. Med.* **115**, 481 (1964).
19. Supported in part by the NCI field studies.
13 January 1966

Synaptic Activity in Motoneurons during Natural Stimulation of Muscle Spindles

Abstract. *Synaptic activity evoked in cat motoneurons during stretch stimulation of muscle spindles in the homonymous muscle has been studied by intracellular recording. A class of miniature excitatory postsynaptic potentials evoked by such physiologic stimulation results from activity in single group Ia spindle afferent fibers, and the criterion for identification is a predictable pattern of rhythmic occurrence of the synaptic potentials. Statistical examination of the amplitude distributions of this class of miniature synaptic potentials suggests that some group Ia fibers liberate a relatively large number of quantal excitatory postsynaptic potential units per fiber impulse.*

Intracellularly recorded synaptic activity, evoked in a motoneuron by stretch stimulation of muscle spindle receptors in the muscle innervated by that motoneuron, consists of a complex series of membrane potential variations or wavelets, the aggregate of which is usually impossible to analyze visually into discrete components (*1*). However, in favorable circumstances when individual wavelets can be examined in isolation, they correspond in wave form to the familiar excitatory postsynaptic potential (EPSP) which results from simultaneous electrical activation of a large population of group Ia spindle afferent fibers in a muscle nerve (*2*, *3*). It is reasonable to suspect that stretch-evoked wavelets may indeed be miniature EPSP's that result from activity in single group Ia fibers and thus represent unitary components of the volley-evoked EPSP. Kuno has, in fact, demonstrated that an electrically evoked EPSP can be fractionated into such all-or-none unitary components by limiting the stimulus to one or a few group Ia afferents (*4*).

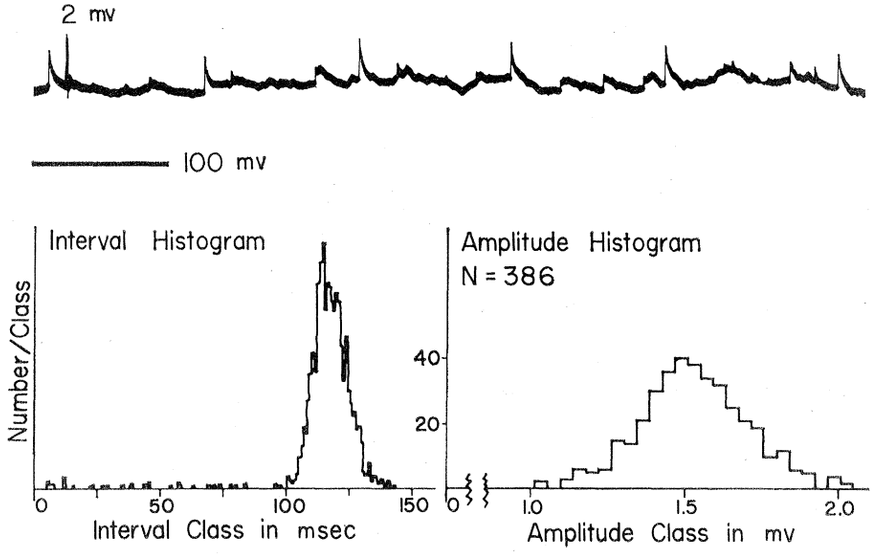

Fig. 1. (Top) High-gain intracellular potential recording from a plantaris motoneuron with the muscle slack. Note regularity of occurrence of large EPSP of typical waveform. (Lower left) Histogram of the time interval in milliseconds between successive large rhythmic EPSP's, developed over a 5-minute period on a CAT computer (*11*). The narrow peak indicates a high degree of periodicity which is quite stable. (Lower right) Histogram of the amplitude distribution of 386 successive EPSP's from the rhythmic pattern, measured from film records. Mean amplitude is 1.57 mv ± 0.16 S.D. Note the break in the abscissa. There were no failures of occurrence (breaks in the rhythmic pattern), and no constituent synaptic potential was less than 1.0 mv in amplitude.

Afferent fibers of muscle spindles discharge quite rhythmically during muscle stretch and often even without stretch; the regularity of firing is particularly evident when central nervous system control of spindle sensitivity through the gamma loop has been interrupted by section of the ventral roots (*5*). Therefore, rhythmic occurrence of stretch-evoked miniature EPSP's should be a useful criterion for identifying synaptic potentials which result from impulses in single group Ia afferent fibers. Our present results demonstrate this.

The experiments were performed on cats that had been anesthetized with pentobarbital and firmly fixed in a steel frame. The right leg was denervated completely, and only muscle nerves to the medial and lateral gastrocnemius heads and to the plantaris muscle were left intact. Tendons of these muscles were separated and arranged for independent stretch with weights. After laminectomy, ventral roots of lumbar segments L6 and L7 and sacral segment S1 were cut, and the L7 segment was explored with micropipettes (diameter of tip, 2 μ) filled with potassium citrate. Potentials from gastrocnemius and plantaris motoneurons, recorded intracellularly, were identified by the pattern of postsynaptic potentials evoked from appropriate muscle nerves (*6*). After identification, the homonymous muscle was stretched tonically with small weights, and the resulting synaptic activity was recorded photographically and on an FM magnetic-tape recorder for later analysis.

In about 90 percent of the extensor cells that we examined, muscle stretch evoked the apparently asynchronous and unpatterned barrage of miniature potentials already described (see *1*). However, in the other 10 percent (numbering 11 cells to date), careful grading of the stretch from zero to moderate amounts (about 100 g) evoked miniature EPSP's of typical wave form which were clearly unitary events and which occurred in a strikingly regular rhythmic pattern. This rhythmic pattern was recognizable primarily because most of the successive constituent