

We noted that all three viruses gave turbid plaques on BN1 lawns, which suggested that some cells were immune to lytic infection. From such turbid plaques on BN1 lawns infected with MVL1, two resistant clones were isolated and designated BN1 v^{r1} and BN1 v^{r2} . Although selected for resistance to MVL1, both clones were also resistant to MVL52 and MVG51. Similarly, cells selected for resistance to MVL52 or MVG51 are also resistant to the other viruses. Adsorption studies show that the viruses do adsorb to the resistant cells. The possible lysogenic nature of these clones is indicated by preliminary studies which show that virus release from BN1 v^{r2} can be induced by ultraviolet irradiation.

Four antibiotic-resistant cell populations were selected by the addition of antibiotics to the liquid culture medium. The cells isolated were resistant to (per milliliter) 400 μ g of naladixic acid (BN1 Na1^r), 100 μ g of kanamycin (BN1 Kan^r), 40 μ g of novobiocin (BN1 Nov^r), or 500 μ g of streptomycin (BN1 Sm^r). When the three viruses were plated on these cells, it was found that BN1 Na1^r and BN1 Kan^r supported viral growth, but none of the viruses was able to form plaques on lawns of BN1 Nov^r or BN1 Sm^r. The plaques on BN1 Na1^r were clear, as opposed to the turbid plaques on the parental BN1; hence, BN1 Na1^r was used as the indicator lawn for measurements of virus titer. The mechanism of resistance to virus infection of BN1 Nov^r and BN1 Sm^r, which were selected for antibiotic resistance, is not understood. Furthermore, the virus-resistant strains BN1 v^{r1} and BN1 v^{r2} have proved to be resistant to streptomycin. This suggests that perhaps Sm^r, v^{r1} , and v^{r2} may be ribosome protein mutations.

The viruses are able to form plaques on lawns which are 2 to 13 hours old. By 24 hours, a lawn is no longer able to support viral growth. No plaques were ever seen on uninfected lawns. However, if old lawns (over 24 hours) were washed and plated on 6-hour lawns, a few plaques were sometimes seen. The numbers are too small to allow a statistical estimate of this frequency; for example, four of the washes from 36 old BN1 lawns gave rise to plaques on young BN1 lawns. Hence, host-carried virus must be considered in studies of Mycoplasmatales viruses. However, such viruses must not interfere with titer measurements due to the low frequency of spontane-

ous virus release, the time required for virus growth, and the short period during which the cells are susceptible to lytic attack. These same reasons probably account for the stability of the virus-cell relationship and for the difficulty in isolating the viruses.

In an effort to find other hosts, the ability of the three viruses to form plaques was examined on lawns of 73 Mycoplasmatales strains, consisting of 62 *A. laidlawii*, one *M. hominis*, one *Mycoplasma* sp. (from goat), five *M. gallisepticum*, and four *Mycoplasma* sp. strains avian serotype I. Plaques were observed only on 13 *A. laidlawii* lawns. The titers of the three viruses on six of these lawns, relative to their titers on BN1 Na1^r lawns, is in Table 1. These data indicate a similar host range for MVL1 and MVG51, which is different from that of MVL52.

When lawns of the 13 strains, chosen for ability to grow MVL1, were washed, eight of the strains gave plaque-forming units. These data are summarized in Table 2. These eight new viruses give plaques on lawns of BN1 Na1^r but not on BN1 v^{r1} , BN1 v^{r2} , or BN1 Sm^r.

Of the 14 *A. laidlawii* strains examined thus far, we have isolated viruses from nine, or about 65 percent, of the strains. We do not know whether viruses could be isolated from the remaining strains by the use of other indicator lawns and we do not know whether such a high percentage of other species are carrying virus. Strain MVG51, isolated from a goat source, shows that other species can carry a virus. The original host of MVL1 is

unknown. We also do not know whether the 11 Mycoplasmatales viruses (Table 2) are all different. At least three of them have different properties, although the three all appear to have the same morphology. Micrographs have also shown that MVL59 and MVL60 are also rod-shaped particles of similar size.

The prevalence of Mycoplasmatales viruses means that they must now be considered in investigations of *Mycoplasma* pathogenicity, both as etiologic agents and for their possible involvement in lysogenic conversion of the host *Mycoplasma*.

ALAN LISS

JACK MANILOFF

Departments of Microbiology and
Radiation Biology and Biophysics,
University of Rochester,
Rochester, New York 14620

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Radiohalos: Some Unique Lead Isotope Ratios and Unknown Alpha Radioactivity

Abstract. *Previously unreported lead isotope ratios, that is, values for the lead-206/lead-207 ratio ranging from about 20 to 60, primarily radiogenic in origin but unsupported by uranium decay, have been determined in the inclusions of certain polonium halos by means of ion microprobe techniques. Evidence for radiogenic lead-208 unsupported by thorium decay may also be inferred from the existence of a composite polonium halo type with rings from the radioactive precursors of lead-208. Several new dwarf halo sizes seem to indicate the existence of unknown, very low-energy alpha-emitters. Furthermore, the three-ring "X halo" also provides evidence for an unknown series of genetically related alpha-emitters with energies in the range from 3 to 7 million electron volts.*

An intriguing aspect of the study of radioactive halos (radiohalos) is the occurrence of unusual halo varieties (1-3), some of which remain unasso-

ciated with known α -emitters. There has been speculation of late that certain of these variant halos may be related to the existence of superheavy

elements (4), and, although no definite evidence presently exists in support of this hypothesis, investigations of a variety of radiohalo types have revealed several new results that may be summarized as follows: (i) The ion microprobe mass spectrometer has been utilized to determine the Pb isotope ratios in the halo inclusions of radiohalos previously associated with a Po isotope decay sequence terminating with ^{206}Pb . Several factors, including values for the $^{206}\text{Pb}/^{207}\text{Pb}$ ratio ranging from about 20 to 60, suggest a unique history for the Pb in the Po halo inclusions as compared to that of previously observed lunar or terrestrial Pb (5, 6). (ii) A new type of composite radiohalo has been found with rings attributable both to the ^{218}Po decay sequence and to ^{212}Po and possibly ^{212}Bi . (iii) In addition to the dwarf halos (5.2 and 8.6 μm in radius) found by Joly (1), I have discovered a variety of dwarf halo sizes ranging from 1.5 to 11 μm in radius. If we assume a radioactive origin, the extremely small dwarf sizes (1.5 to 2.5 μm) correspond to α energies, E_α , of approximately 1 Mev, less than that of any known α -emitters.

In the past, identification of various radiohalo types was primarily dependent upon a correlation of the halo ring radius with a specific α energy derived from a range-energy relation for the host mineral in which the halos occurred. This technique was successful in identifying radiohalos that could easily be ascribed to α decay in the halo inclusion from the ^{238}U and ^{232}Th decay chains as well as radiohalos that apparently matched the sequential α -decay patterns from ^{218}Po (three rings), ^{214}Po (two rings), and ^{210}Po (one ring). Although the ring structure of this latter group of radiohalos seemed perfectly compatible with the proposed Po isotopic designation (2), some questions have arisen over this identification because of the half-lives of the respective isotopes involved ($t_{1/2} = 3$ minutes for ^{218}Po) and the lack of evidence for a secondary source of Po from α -decaying precursors in the U decay chain (7). Evidence in support of this identification is now available in the form of mass spectrometric analyses of the Po halo inclusions made with the ion microprobe, a new type of mass spectrometer with a sputtering ion source (8), which makes possible an analysis of the halo inclusions in situ.

The particular halo type analyzed possessed ring structure that correlated with the ^{218}Po α -decay sequence (Fig.

1A) which terminates with ^{206}Pb and thus it was suspected that the halo inclusion would reflect an excess of ^{206}Pb when compared to previously observed isotopic abundances of either common or radiogenic Pb. Such proved to be the case. In general, although variations were noted in the Pb isotope ratios in the U and Th halo inclusions, these ratios were within the range of previously reported values (5). In contrast, the Pb isotope patterns of the Po halos revealed ratios of ^{206}Pb to ^{208}Pb of ≈ 2.2 and of ^{206}Pb to ^{207}Pb of ≈ 19 for one inclusion and ratios of ^{206}Pb to ^{208}Pb of ≈ 6 and of ^{206}Pb to ^{207}Pb of ≈ 60 for a second inclusion. Uranium was virtually nonexistent in the first analysis and constituted only a small fraction of the Pb content in the second analysis (9). These results were confirmed when a different ion microprobe was used on a third Po halo inclusion (10), and the following ratios were observed: $^{206}\text{Pb}/^{208}\text{Pb} \approx 33$, $^{206}\text{Pb}/^{207}\text{Pb} \approx 20$, $^{206}\text{Pb}/^{204}\text{Pb} > 1000$, and Pb/Th and $\text{Pb}/\text{U} > 5000$, that is, no ^{204}Pb , U, or Th was detected.

It is difficult to associate the Pb isotope ratios and Pb/U and Pb/Th ratios in these inclusions with any previously reported sources of terrestrial, meteoritic, or lunar Pb. For example, common Pb, as generally defined, refers to Pb assumed to consist of a primordial component containing the isotopes ^{204}Pb , ^{206}Pb , ^{207}Pb , and ^{208}Pb , and a radiogenic component containing the isotopes ^{206}Pb , ^{207}Pb , and ^{208}Pb ; however, the high Pb/U and Pb/Th values characteristic of phases containing common Pb imply that the radiogenic component was not produced by in situ decay of U or Th. Clearly then, the absence of or low abundance of U or Th in the third halo inclusion cannot of itself be taken as an indication of the presence of significant quantities of common Pb, since ^{204}Pb was not detected. Likewise, this Pb could hardly be characterized as radiogenic Pb in the sense of being derived from the in situ decay of U and Th, because these elements were absent. This Pb is, however, radiogenic in the sense of being derived from Po decay.

Additional evidence for an unusual history of these leads may be seen from the relatively high $^{206}\text{Pb}/^{207}\text{Pb}$ ratios, which for common Pb is about unity in many terrestrial rock types and for radiogenic Pb [produced by the in situ concurrent decay of ^{238}U (to ^{206}Pb) and ^{235}U (to ^{207}Pb)] is in the range of ≈ 4 to 15 (5). The theoretic-

cal maximum possible radiogenic $^{206}\text{Pb}/^{207}\text{Pb}$ ratio, on the basis of an instantaneous production of Pb from U decay, would be 21.8. Thus the $^{206}\text{Pb}/^{207}\text{Pb}$ ratios in the Po halo inclusions clearly differ from similar ratios previously observed either in common or in radiogenic Pb from U decay.

Rather than attempting to ascribe these unusual ratios to the general phenomenon of differential movement of U, Th, or Pb, I consider the evidence in these specific cases to provide confirmation for the existence of ^{206}Pb from Po decay unsupported by U, especially in view of the fact that the highest value of the $^{206}\text{Pb}/^{207}\text{Pb}$ ratio (≈ 60) was recorded in the most densely colored Po halo of the three. Further studies of this type may lead to a reevaluation of the basic premise that there is a unique origin for radiogenic Pb, namely, that it originates solely from U and Th decay. Because of the small size of the halo inclusions ($\approx 2 \mu\text{m}$), such variations in Pb isotope ratios would easily have escaped prior detection with almost any other mass spectrometric techniques. Indeed, such variations may exist only in certain halo inclusions.

In a related context it appears that in certain cases small quantities of ^{208}Pb [and possibly ^{207}Pb (11)] may have an origin similar to that of the excess ^{206}Pb referred to in the above analysis. I have found a halo with rings consistent with the α -decay pattern from ^{212}Po and possibly ^{212}Bi as well as the ^{218}Po decay sequence (see Fig. 1, B and C). This halo and similar halos with rings from the α decay of ^{212}Po , ^{210}Po , and ^{212}Bi occur in very low abundance in the micas from the Faraday Mine near Bancroft, Ontario. By analogy with the above results, it would be expected that these composite Po halos would exhibit a Pb isotope ratio pattern high in ^{206}Pb and ^{208}Pb without corresponding amounts of the parent nuclides ^{238}U and ^{232}Th . Indeed, at this time I cannot rule out the possibility that some of the ^{207}Pb and ^{208}Pb (as well as the ^{206}Pb) observed in the ion microprobe analyses might also have originated with Po or Bi decay unsupported by U or Th. Halo rings from the radioactive precursors of ^{207}Pb and ^{208}Pb would not necessarily have formed as indicated by the lower abundance of these isotopes. In an earlier study no support was found for a secondary source of Po originating with α -decay precursors, that is, no variation in α -recoil density near the

halo inclusions as compared to the background density (7).

Many years ago Joly (1) reported the existence of some very unusual dwarf halos with radii of approximately 5.2 and 8.5 μm in the black micas from the pegmatite quarry at Ytterby (near Stockholm). The halo with a radius of 5.2 μm was later tentatively associated with the decay of ^{147}Sm ($E_\alpha = 2.24$ Mev), whereas the larger halo has never received a satisfactory nuclide identification (12, 13). The extreme rarity of the occurrence of the dwarf halos apparently has precluded much research on the nature of their origin, and it has been only recently that I have found a few samples of mica from the old Ytterby quarry that contain any significant number of these unique halos for further study. Joly considered the radioactive origin of the halos beyond question and tentatively attributed their bleached appearance to an overexposed, radiation-damaged condition or perhaps to some other phenomena related to the meta-

morphic history of the mica. I consider the radiogenic origin to have been confirmed when Mahadevan (14) later reported equivalent dwarf halo sizes in an Indian cordierite sample, which also contained Po halos. Generally α -particles from (n, α) reactions are far too insufficient to produce a halo.

My observations on the dwarf halos are, in general, in accord with those of Joly, except that I have found several additional sizes that are somewhat difficult to account for on the basis of α -decay systematics of known radioactive nuclides. I find that the smallest dwarf halos range from only 1.5 to about 2.5 μm with associated α energies in the range of approximately 1 Mev. The half-lives of known α -radioactive nuclides are in excess of 10^{13} years for α -decay energies of 2 Mev or less and thus normally correspond to such weakly active nuclides as to almost escape detection and would hardly be expected to produce a halo at all. These considerations do not seem in accord

with the appearance of both some very small and some intermediate-sized dwarf halos, which show intense bleaching or reversal effects characteristic of a highly radiation-damaged region. The inference is that, whatever the difference in the half-lives of the nuclides responsible for the various dwarf halo sizes, the half-lives of the nuclides involved do not produce measurable differences in the degree of halo development.

Further, I have found other dwarf halos in the size range from 3 to 11 μm (see Figs. 2 and 3) corresponding to α energies ranging from approximately 1.1 to 3.4 Mev. The dwarf halos in this range do reflect some coloration differences which tentatively may be attributed to varying concentrations of parent radionuclides in the halo inclusions. Uranium and Th halos in a given hand specimen often exhibit similar effects for this reason. Although the dwarf halos which exhibit the most intense reversal effects have proved very satisfactory for radius measure-

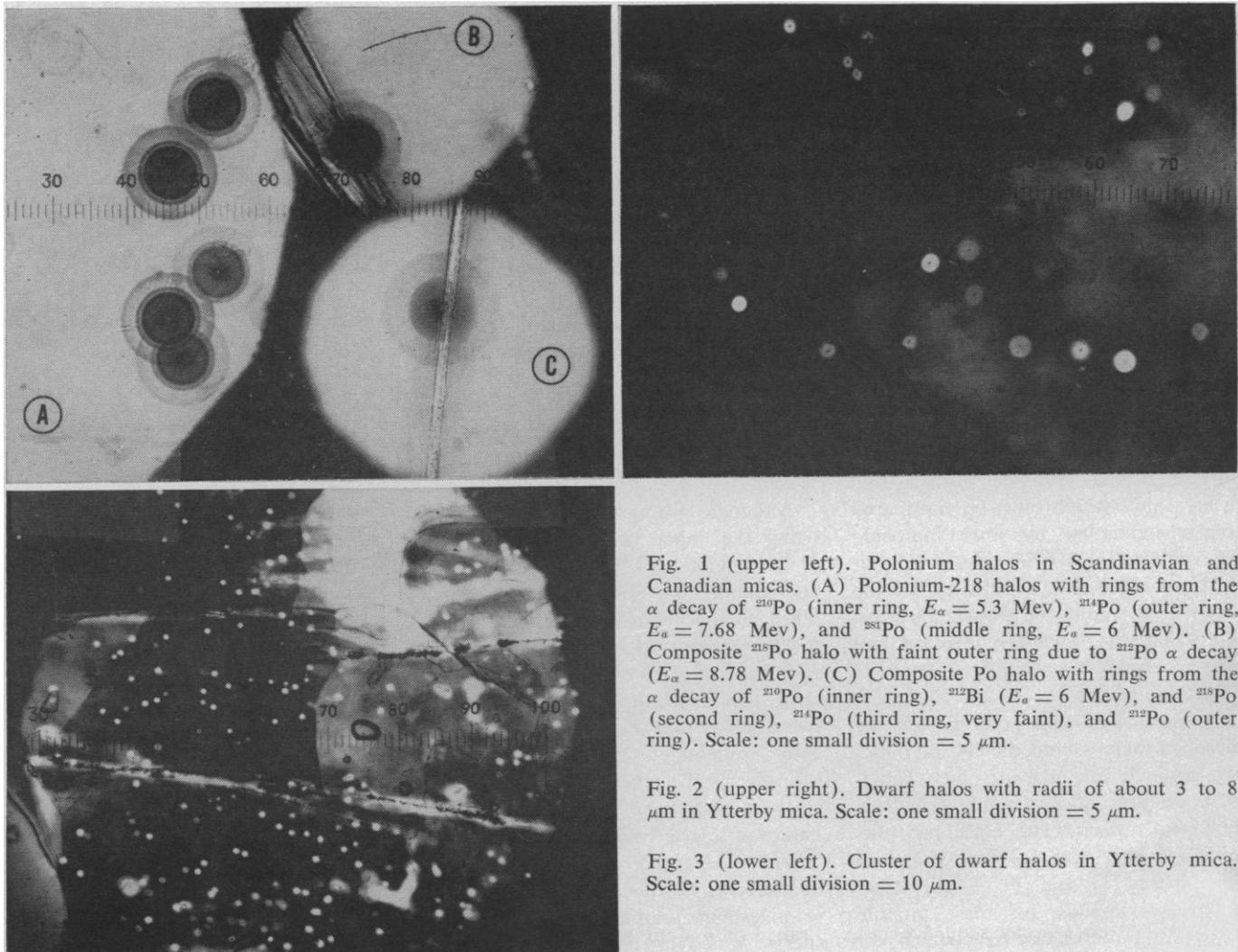


Fig. 1 (upper left). Polonium halos in Scandinavian and Canadian micas. (A) Polonium-218 halos with rings from the α decay of ^{210}Po (inner ring, $E_\alpha = 5.3$ Mev), ^{214}Po (outer ring, $E_\alpha = 7.68$ Mev), and ^{281}Po (middle ring, $E_\alpha = 6$ Mev). (B) Composite ^{218}Po halo with faint outer ring due to ^{212}Po α decay ($E_\alpha = 8.78$ Mev). (C) Composite Po halo with rings from the α decay of ^{210}Po (inner ring), ^{212}Bi ($E_\alpha = 6$ Mev), and ^{218}Po (second ring), ^{214}Po (third ring, very faint), and ^{212}Po (outer ring). Scale: one small division = 5 μm .

Fig. 2 (upper right). Dwarf halos with radii of about 3 to 8 μm in Ytterby mica. Scale: one small division = 5 μm .

Fig. 3 (lower left). Cluster of dwarf halos in Ytterby mica. Scale: one small division = 10 μm .

ments ($\Delta r \approx 0.25 \mu\text{m}$), the variety of sizes has thus far precluded the construction of a discrete radius distribution. (Some overlap in the distribution may result from the appearance of the same type of halo in later stages of development.) Generally speaking, the radius of an ordinary halo in the initial stages of development may vary a micrometer or two from that in later stages, possibly as a result of an increased ionization effect near the terminal range of the α -particle in the mineral, and a reduced effect of this nature may be operating in the dwarf halos. Interestingly, there is a close correspondence between some of these halo sizes and some very low-energy α -emitters ($E_\alpha = 1.1$ to 1.3 Mev, 1.8 to 2.0 Mev, 2.5 to 2.7 Mev, and 3.1 to 3.3 Mev) of unknown origin observed over the last several decades by Schintlmeister (15), Brukl *et al.* (16), and Gysae (17). Whether there is a causal relation between the dwarf halos and these previously reported α activities is presently open to question. The significant result is that α activities in the range of a few million electron volts apparently exist apart from the naturally occurring, rare-earth α -emitters with low decay energy (16, 17).

Further observations on the dwarf halos have shown some with an elliptical cross section as opposed to the ordinary circular cross section seen in thin section under the microscope. In certain cases this ellipticity arises from the fact that the long ($> 10 \mu\text{m}$) filament-like halo inclusion is inclined somewhat with respect to the cleavage plane. In other cases a shearing-type deformation of the crystal may have produced this effect, although it is not certain that either of these observations is the full explanation of this effect. Another observation I consider important is the presence of dual-ring dwarf halos, which must be considered similar in nature to other radiohalo types which exhibit ring structure, that is, U or Th halos. I believe that the rarity of the dual-ring dwarf halos implies that the nuclides involved are not necessarily genetically related in a decay sequence.

A most significant point is that the bleached regions immediately surrounding the inclusion etch extremely rapidly, a characteristic of highly radiation-damaged regions. The bleaching phenomenon exists in the same micas in overexposed U and Po halos. After etching thousands of these minute halos, I have found a few which ex-

hibit fission tracks with a range of only about one-half to three-fourths the normal fission-track length. I was at first inclined to consider these tracks the result of lower-energy fission events but am as yet unable to rule out the possibility that they are U fission tracks that have been annealed somewhat through a metamorphic episode in the rocks. Since, however, fission-track measurements on these dwarf halos generally reveal a low content of U in the inclusions, an attempt was made to resolve their origin by searching for additional short-range tracks in unetched samples by means of high-voltage electron microscopy. Results have thus far been negative (18).

Equally as rare as the dwarf halos are the X halos reported by Joly (1) and later referred to by other investigators (5, 9, 12). I include a summary of Joly's measurements here. The inside ring may be somewhat diffuse and measures about 8.5 to $9.8 \mu\text{m}$ in radius. The bleached rings extend out to a radius of approximately 14 to $15 \mu\text{m}$ with an adjacent dark ring at about $17 \mu\text{m}$, whereas the outer wide band extends to approximately $28 \mu\text{m}$. Actually, two different types of X halos exist, one with and one without a bleached band. This particular halo is due neither to U nor to Th decay in spite of some close similarities with certain portions of the Th decay sequence. Rings in the X halo may be attributed to α energies of approximately 2.9 to 3.2 Mev (inner ring), 4.5 to 5.2 Mev (middle rings), and 6.8 to 7.0 Mev (outer ring). The chart of the nuclides reveals no genetically related α sequence corresponding to these energies, and it is extremely difficult to concoct a mixture of α -emitters that would fit the above pattern without additional rings also being present.

Although an earlier suggestion relating the inner ring of the X halo to the spontaneous fission of U (19) is not tenable, the possibility of a combined fissiogenic and α -derived origin for the inner ring involving other nuclides must not be overlooked. It seems clear that both the X halos and the dwarf halos may be evidence of presently unidentified α activity. Detailed ion microprobe studies of the halo inclusions may very well elucidate the nature of this activity.

Note added in proof: I recently visited several European research centers to investigate earlier reports of low-energy α activity cited in (15–17). F.

Hernegger, Radium Institute, University of Vienna, carefully reviewed his collaborative efforts with Schintlmeister. Several of their unpublished reports written during World War II (20) give additional evidence of genetically related α energies of about 3.1 and 4.5 Mev—an interesting pattern when compared with the X-halo ring structure. G. Herrmann, University of Mainz, called my attention to the work of R. Coppens, University of Nancy, France, whom I subsequently visited. The low-energy α activity found by Coppens (21) and other earlier investigators (15–17) may be related to the radioactive origin of the dwarf halos.

ROBERT V. GENTRY

Chemistry Division,
Oak Ridge National Laboratory,
Oak Ridge, Tennessee 37830

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classified according to its site of termination on the postsynaptic element as well as by the presence or absence of degeneration of the presynaptic terminal. In general, the samples consisted of about 20 grid squares (700 synapses) taken from a single section, although in a few instances the same procedure was repeated at successive levels about 100 μm apart to ensure that there was no systematic variation. In these cases the ratios given in Table 2 are the means of the ratios at each level.

The maximum number of recognizably degenerating terminals is reached at about 2 days after section of their parent fibers, whereas the process of astrocytic phagocytosis is not sufficiently advanced to cause a major reduction in the number of terminals still in contact with their postsynaptic sites. Terminal degeneration is indicated by collapse and increased electron opacity of the axonal endings, and may be used as a reliable and quantitatively consistent marker of those terminals

Sexual Dimorphism in the Preoptic Area of the Rat

Abstract. *A quantitative evaluation of the relative distribution of synapses on dendritic shafts and spines serves to differentiate the neuropil of the preoptic area from that of the ventromedial hypothalamic nucleus; it also shows that the neuropil of the preoptic area is sexually dimorphic.*

There is considerable evidence that the tuberal part of the hypothalamus is involved in the maintenance of the basal level of output of gonadotrophins, and that the preoptic area is essential for initiation of the preovulatory surge of gonadotrophins which is typical of the adult female but which does not occur in the male (1, 2). The amygdala and the hippocampus together constitute the major source of fibers from the limbic forebrain to the hypothalamus (3). The amygdala has two principal efferent tracts; these are the stria terminalis, which among other areas projects to the preoptic area and to the ventromedial nuclei of the tuberal hypothalamus, and the ventral amygdalofugal pathway (4). There is evidence to suggest that the stria terminalis may be of special importance as a route for the effects of amygdaloid stimulation on ovulation (5, 6) and for the

effects of the amygdala on the timing of puberty in the female rat (see 7). Anatomically, therefore, the stria terminalis links the amygdala with the preoptic area and the tuberal hypothalamus, all areas that have been implicated in the control of gonadotrophin release. The aim of our investigations was to examine the synapses in the neuropil of those parts of the preoptic area and tuberal hypothalamus which receive strial projections and to identify the terminals of amygdaloid origin by taking advantage of the reaction of orthograde degeneration.

Lesions were made in the stria terminalis in the rat by use of a stereotaxically guided knife blade entering from the dorsal aspect of the brain, at a level 2.2 mm behind and to a depth of 5.5 mm below the bregma with the rat in a nose-down position. This lesion also destroys the fimbria and damages some adjacent structures. A previous study of the efferent projection of the hippocampus has established that these incidental parts of the lesion do not give rise to degeneration in either of the two specific areas examined in this study (8). Two days later the animals were killed, and the brains were fixed and processed for electron microscopy. Ultrathin sections from the levels of the preoptic area and the ventromedial nucleus were mounted on uncoated grids, whose mesh served to divide the region up into convenient sampling units of about 1800 μm^2 . All the grid squares from the regions containing degenerating amygdaloid fibers were counted, and for each square every synapse was

Table 2. Ratio of the number of nonamygdaloid synapses on dendritic shafts to the number on dendritic spines. Mann-Whitney *U* test for significance of difference between male and female: for the preoptic area, $P < .001$; for the ventromedial nucleus, difference is not significant ($P = .5$).

Rat number	Preoptic area		Ventromedial nucleus	
	Male	Female	Male	Female
M1	32.3			
M4	27.2			
M27	23.8			
M26	23.4			
M53	22.6			
M54	20.9			
M28	18.2			
M49	14.6			
F11		13.9		
F42		13.5		
M51	13.4			
M50	12.9			
F44		11.7		
F12		10.7		
F45		10.5		
F46		9.7		
F43		9.1		
F6		6.2		6.2
M4			6.1	
EF6				5.1
M54			4.8	
M27			4.7	
F46				4.5
F45				4.0
M50			3.9	
F26				3.8
M28			3.7	
M26			3.4	
F12				3.3

Table 1. Total numbers of synapses of amygdaloid and nonamygdaloid origin on dendritic shafts (SH) and spines (SP) in samples taken from the preoptic area and the ventromedial nucleus of male (M) and female (F) rats. The total number of synapses counted was 28,184. The numbers of animals used were M = 10 and F = 8 for the preoptic area, and M = 6 and F = 6 for the ventromedial nucleus.

Sex	Amygdaloid		Nonamygdaloid	
	SH	SP	SH	SP
<i>Preoptic area</i>				
M	258	170	10,802	574
F	254	227	8,779	954
<i>Ventromedial nucleus</i>				
M	71	233	2,102	498
F	87	371	2,235	569