Brownian movement was arrested. We believe that Brownian movement may not have been photographed previously in color.

A xenon flash-tube with flash duration of 65 μsec (above half-peak) from a 5- by 5-mm source, operated from a 100-μf capacitor charged to about 2 kv, was positioned under the substage condenser lens of a microscope and fired with the camera shutter held open manually. Acceptable transparencies were obtained on 35-mm high-speed daylight Ektachrome film at magnifications up to 800. Our microscope has a built-in camera with interchangeable cassettes; a second cassette, loaded with Kodachrome II type A, was used to obtain a paired time exposure of each field. By comparison, the crystals in Brownian movement were later readily identified. Slides were prepared by simply trapping a drop of suspension under a cover slip.

Similarly prepared slides of cows' milk containing stained fat globules in Brownian movement have also yielded acceptable transparencies. But the main purpose of this technique is to study the distribution of fine color markers in suspensions.

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Note

1. We thank Dr. R. F. Burbidge of Bristol University Electrical Engineering Department for devising and testing the electronic circuits.

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Mosquitoes: Comparative Serology of Four Species of Aedes (Ochlerotatus)

Abstract. *Antigens of female adult* Aedes communis *(DeG.),* A. punctor *(Kby.),* A. trichurus *(Dyar), and* A. excrucians *(Walk.) were compared by precipitin tests. There is a wider divergence among species than is indicated by external comparative morphology.*

Certain intrageneric groups or "complexes" of mosquitoes show marked morphological similarities although physiological and behavioral differences often exist. The phylogenetic relationships among members of such groups are difficult to ascertain and often must be postulated from the subjective interpretations of morphological or behavioral variations (*1*). Recent applications of serological techniques to insect taxonomy (*2*) showed that precipitin reactions with insect antigens differentiate species and indicate their relative taxonomic positions.

Extracts from adult females of *Aedes communis* (DeG.), *A. punctor* (Kby.), *A. trichurus* (Dyar), and *A. excrucians* (Walk.) were compared by precipitin tests. The mosquitoes were collected in the vicinity of Ottawa, Ontario, in the third- and fourth-instar larval stages. Adults that emerged were maintained in the laboratory for 72 hours and fed 10 percent sucrose solution but not blood.

The antigens for the preliminary experiments were prepared from whole, freshly-killed mosquitoes by extraction with buffered physiological saline, *p*H 7 (*3*). Subsequent experiments were made with the antigenic protein-containing fraction separated from extracts of whole mosquito by continuous filter-paper electrophoresis in veronal buffer, *p*H 8.6, ionic strength 0.02, 30 ma (*4*). The fraction so obtained was identified by its mobility and its characteristic pattern in single-diffusion antiserum-agar tests. A comparable fraction was obtained from all species tested, additional studies (*5*) indicated that it was present in pupal and adult mosquitoes but not in larvae. In gel-diffusion tests the fraction which showed three precipitate zones appeared less antigenically complex than whole-mosquito extracts which showed 8 to 12 precipitate zones. The antisera were prepared by injecting rabbits with three subcutaneous doses (1, 1½, and 2 ml) of mosquito antigen on alternate days.

The Libby photronreflectometer, a photoelectric instrument designed to measure the turbidities of antigen-antibody reactions was used to compare the mosquito antigens (*6*). Serial dilutions of antigen were made in buffered saline, and 0.7 ml of each dilution was mixed with 0.3 ml of undiluted antiserum. The resulting turbidity of each mixture was recorded in galvanometer units from the photronreflectometer and the sum of the turbidity values (ST) for each series of mixtures was determined. For all tests the concentration of antigen was adjusted so that the turbidity reading of the inital antigen dilution was zero. This was done so that the entire range of reaction from antigen excess to antibody excess could be measured. The *homologous* reaction was that which occurred between the antigen from one species of mosquito and the antiserum produced when this antigen was injected into a rabbit. *Heterologous* reactions were those which occurred between the same antiserum and antigens from other species of mosquito. Percentage relationship values were estimated by the calculation of (ST heterologous/ST homologous) 100 (*7*).

Table 1. Percentage-relationship values (and ST values) determined by turbidity of precipitin reactions of antigens from adult, female *Aedes* mosquitoes.

Source of antigen	Antisera to			
	A. communis	*A. punctor*	*A. trichurus*	*A. excrucians*
	Saline extract from whole mosquitoes			
A. communis	100 (140)	84 (143)	62 (172)	20 (45)
A. punctor	82 (114)	100 (170)	76 (210)	25 (55)
A. trichurus	31 (43)	48 (82)	100 (276)	47 (104)
A. excrucians	23 (82)	21 (36)	18 (49)	100 (219)
	Electrophoresis fraction from whole extracts			
A. communis	100 (162)	69 (98)	43 (97)	
A. punctor	59 (72)	100 (158)	52 (106)	(Not
A. trichurus	28 (45)	31 (49)	100 (204)	obtained)
A. excrucians	10 (17)	18 (28)	9 (19)	

The results of turbidity tests (Table 1) indicated that mosquitoes were readily distinguishable by precipitin reactions; *A. communis* and *A. punctor* were most antigenically similar and *A. trichurus* appeared more closely related to these species than did *A. excrucians*. Comparisons with antigens obtained by electrophoresis showed an order of species relationships similar to that obtained in tests with extracts of whole mosquitoes. The tests with the isolated fractions showed greater distinction between species, suggesting that the antigens in the reactions were highly specific. Unfortunately, a sufficiently potent antiserum against the fraction from *A. excrucians* was not obtained and the limited supply of this fraction

was exhausted in tests with other antisera.

Of the mosquitoes studied, *A. communis, A. punctor,* and *A. trichurus* are morphologically closely similar and all lack bands of white scales on the tarsi. Edwards (*8*), in a classification of the subgenus *Ochlerotatus*, placed *A. communis* and *A. punctor* in the same group (Group "G") and *A. trichurus* in an adjacent group (Group "H"). *Aedes excrucians*, although in the same subgenus, has bands of white scales on the tarsi and is readily separated from the other species.

The relationships shown by serological methods are in general agreement with those indicated by comparative morphology. The ease with which the species were distinguished by precipitin tests suggests that there is a wider separation between species than morphological comparisons would indicate. There can be little doubt that certain biochemical or physiological characters are more sensitive indicators of divergence than are morphological structures. Observations on the physiological and behavioral variations in other closely-related groups of mosquitoes support this view (*1*). Serological techniques should prove of value in assessing the interrelationships among various species-complexes in the Culicidae. Studies on groups comprised of autogenous and anautogenous forms would be particularly useful (*9*).

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21 December 1962

Yttrium-88 on High-Activity Zirconium-95 Fallout Particles

Abstract. *Yttrium 88 has been identified, by gamma spectroscopy, in residues of grass samples gathered in the neighborhood of the Euratom Research Center, Ispra, Italy. The yttrium-88 is associated with zirconium-95.*

The gamma spectra of samples of grass from the neighborhood of the Euratom Nuclear Center at Ispra showed a photopeak at 1.85 Mev. This peak was observed for the first time when samples were collected for examination during the last week of July and the first week of August 1962.

Because this peak was associated with another at 0.90 Mev, the radiation could be attributed to yttrium-88. This hypothesis was fully confirmed when yttrium (Y) was isolated by chemical methods.

The activity accompanied the yttrium carrier during the various steps of the analysis, which included oxalate precipitations of the rare earths and solvent extraction with tributyl phosphate. The spectrum, measured on 24 August 1962, of hay and the spectrum of the separated Y^{88} are shown in Figs. 1 and 2 respectively.

Because of the difficulties encountered in dissolving the active component, we believed that the activity was concentrated on single particles, the bulk of which might be zirconium oxide. Fusion in mixtures of potassium and sodium carbonate were unsuccessful. The active component was dissolved finally with hydrofluoric and nitric acid.

Other samples were fractionated before chemical treatment with the hope of isolating a single particle that contained all the activity.

With the aid of the gamma spectrometer we separated such a residue, of which the dimensions were less than 0.1 by 0.5 mm, from each of the samples treated. Our work was greatly facilitated by the presence of a combined activity of (Zr^{95} + Nb^{95}) which was 10 to 20 times greater than the usual Y^{88} activity encountered.

Figure 3 shows a spectrum obtained with a particle. Other gamma emitters commonly found in fission products are absent. On one occasion only, there was some activity at 0.14 Mev, probably attributable to the isotopes cerium-141 and 144.

The ratio of the activities of (Zr^{95} + Nb^{95}) to Y^{88} varies in the rather narrow range of (1:10 to 1:20). On 1 October the activity of the "hottest" particle was 2000 pc (Zr^{95} + Nb^{95}), whereas the other particles all showed half this activity. Although we were not able to identify the particles by microscopy, we succeeded in isolating an active fragment of inorganic material, the diameter of which did not exceed 10 μ.

That local contamination is the source of Y^{88} is not likely for several reasons. There is no experimental work on Y^{88} here, nor does any work at the Center result in production of Y^{88}; none of the devices run for routine control of environmental radioactivity (air monitors, pot samples for fallout and so forth) showed Y^{88} activity; two samples taken at a distance of 75 km from here and in a direction where fallout of airborne

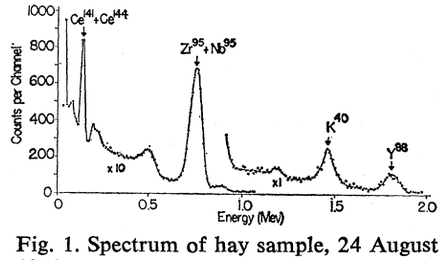

Fig. 1. Spectrum of hay sample, 24 August 1962.

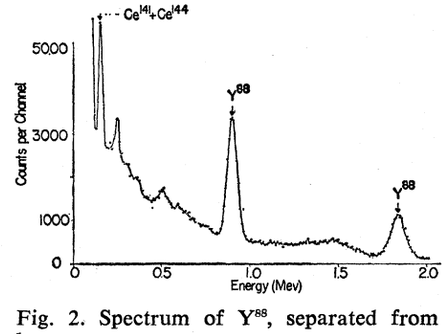

Fig. 2. Spectrum of Y^{88}, separated from hay.

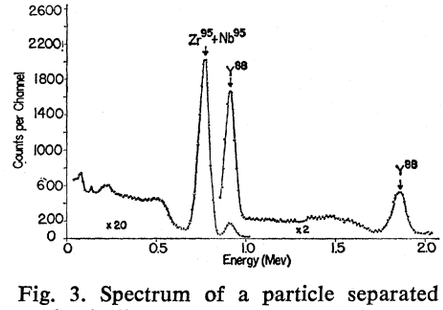

Fig. 3. Spectrum of a particle separated mechanically.