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Experimental Transmission of *Sarcocystis* from Icterid Birds to Sparrows and Canaries by Sporocysts from the Opossum

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EXPERIMENTAL TRANSMISSION OF *SARCOCYSTIS* FROM ICTERID BIRDS TO SPARROWS AND CANARIES BY SPOROCYSTS FROM THE OPOSSUM

Edith D. Box* and Donald W. Duszynski†

ABSTRACT: Cowbirds (*Molothrus ater*) and grackles (*Cassidix mexicanus*) infected with muscle cysts of *Sarcocystis* were fed to opposums (*Didelphis virginiana*) and fecal sporocysts from the latter were given to sparrows (*Passer domesticus*, Family Ploceidae), canaries (*Serinus canarius*, Family Fringillidae) and ducks (*Anas platyrhynchos*, Family Anatidae). Asexual parasites were found in the endothelium of sparrows and canaries but not in ducks. When birds were kept 10 weeks or more after infection, muscle cysts were found grossly and microscopically in the majority of sparrows, and in 1 canary, but not in ducks. Muscle zoites were found in digests of all sparrows and canaries but not in that of ducks. Metrocytes and forms dividing by endodyogeny also were found in the digest. Thus, avian *Sarcocystis* was transmitted experimentally from 2 genera of 1 family (Icteridae) to 2 different families of passerine intermediate hosts by sporocysts from the definitive host. This is the broadest intermediate host spectrum known for a species of *Sarcocystis*.

Transmission of *Sarcocystis* by obligatory alternation of hosts for the sexual and asexual stages has been accomplished with a number of predator-prey combinations. The most intensively studied have been the cycles between the canine and feline predators and wild and domestic meat animals (Levine, 1977). Transmission of rabbit *Sarcocystis* by cats (Fayer and Kradel, 1977; Crum and Prestwood, 1977) and murine *Sarcocystis* by cats (Ruiz and Frenkel, 1976), owls (Černá, 1976; Munday, 1977) and snakes (Rzepczyk, 1974) also has been described. We recently were able to transmit avian *Sarcocystis* from grackles and cowbirds (the intermediate hosts) to opossums, which served as definitive hosts (Duszynski and Box, 1978). Although previously *Sarcocystis* was thought to be rather host specific, parasitizing one genus of intermediate host (Levine, 1977), this report describes the successful transmission of the muscle parasite from Icteridae (cowbirds and grackles) to Ploceidae sparrows) and Fringillidae (canaries) by sporocysts from opossums.

MATERIALS AND METHODS

Experimental intermediate hosts

Adult sparrows (*Passer domesticus*) were captured in a Havahart trap on the University of Texas Medical Branch campus in March before the breeding season began so they were at least 7–8 months old when captured. They were caged in pairs indoors and fed commercial starter mash. Experimental canaries (*Serinus canarius*) included 8 canaries reared coccidia-free in the laboratory the previous year and 3 canaries reared indoors at the senior author's home. Canaries were caged in groups according to the origin of sporocysts they were given. Newly hatched ducklings (*Anas platyrhynchos*) were purchased from Sears Roebuck and infected 5 days after arrival. They were kept in groups in brooders, separated according to inoculum. As they grew, they were transferred to an inside pen and later to an outside pen until necropsied.

Inoculum

Sporocysts were collected from the feces of 2 experimentally infected opossums (*Didelphis virginiana*), one of which had been fed 7 *Sarcocystis*-infected cowbirds (*Molothrus ater*) and the other, 2 infected grackles (*Cassidix mexicanus*) (for details, see Duszynski and Box, 1978). The sporocysts in feces were refrigerated in 2% (w/v) aqueous $K_2Cr_2O_7$, then concentrated by sugar flotation, washed, counted and administered to recipient birds by stomach tube. The age of the sporocysts was from 15 to 52 days when administered. Doses given sparrows and canaries varied from 10^3 to 500×10^3 sporocysts except for birds killed 24 hr after infection which received 2×10^6 sporocysts (Table I). Ducks were given 10^6 sporocysts.

Necropsy

Birds were necropsied at various intervals postinoculation (PI). They were killed with chloroform, skinned, and muscles were inspected grossly under a magnifying lamp. A sample of thin abdominal muscle about 10 mm^2 was pressed between 2 slides and examined microscopically

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TABLE I. *Size and origin of inoculum given experimental birds.*

Recipient bird	No. sporocysts in inoculum $\times 10^3$	Sporocyst origin				
		Cassidix		*Molothrus*		
		No. birds	Mortality*	No. birds	Mortality*	Total
Sparrow	1	2		–		2
	100	3		3	1D	6
	500	3	2D	3	1D	6
	2,000	1	1K	1	1K	2
Canary	1	1		–		1
	200	3	1KM	3	2KM	6
	2,000	1	1K	1	1K	2
	0	–		–		2
Duck	1,000	5	2K	5	2K	10

* Mortality by 16 days PI; D = died; KM = moribund when killed; K = killed, not sick.

for cysts. The head, feet, wing tips, and viscera were removed from sparrows and canaries and the remainder of the carcass digested (Box and McGuinness, 1978). A 50 g sample of combined breast and thigh muscle was taken from each duck for digestion. The material to be digested was ground for 30 sec at high speed in a commercial Waring Blendor with enough pepsin solution (0.75% w/v pepsin, 0.75% w/v NaCl and 1% v/v HCl in water) to cover it. Pepsin solution was added to the blend to equal 10 × the weight of the material to be digested. Digestion was for 1 hr at 37 C while agitating with a magnetic stirrer. The digest was then strained through double layers of gauze, centrifuged for 10 min at 700 g, and a drop of sediment was examined for zoites at 400 × using phase optics on a Zeiss photomicroscope.

Slides

Impression smears were made of liver, spleen, lung, brain, and duodenal loop of sparrows and canaries. They were wet-fixed in Bouin's and stained in 4% Giemsa's in buffered water (pH 7.2) overnight. Zoites from muscle cyst digests were stained by smearing the sediment on a slide, air-drying, fixing with absolute methanol and staining with 2% aqueous Giemsa's (pH 7.2) for 45 min. For sections, tissues were fixed in buffered formalin, cut 4–5 μm thick and stained with hematoxylin and eosin (HE).

Measurements were by ocular micrometer at 1,000 × of Giemsa-stained parasites and at 100 × of cysts in muscle compressed between slides. All measurements are in μm unless otherwise specified.

RESULTS

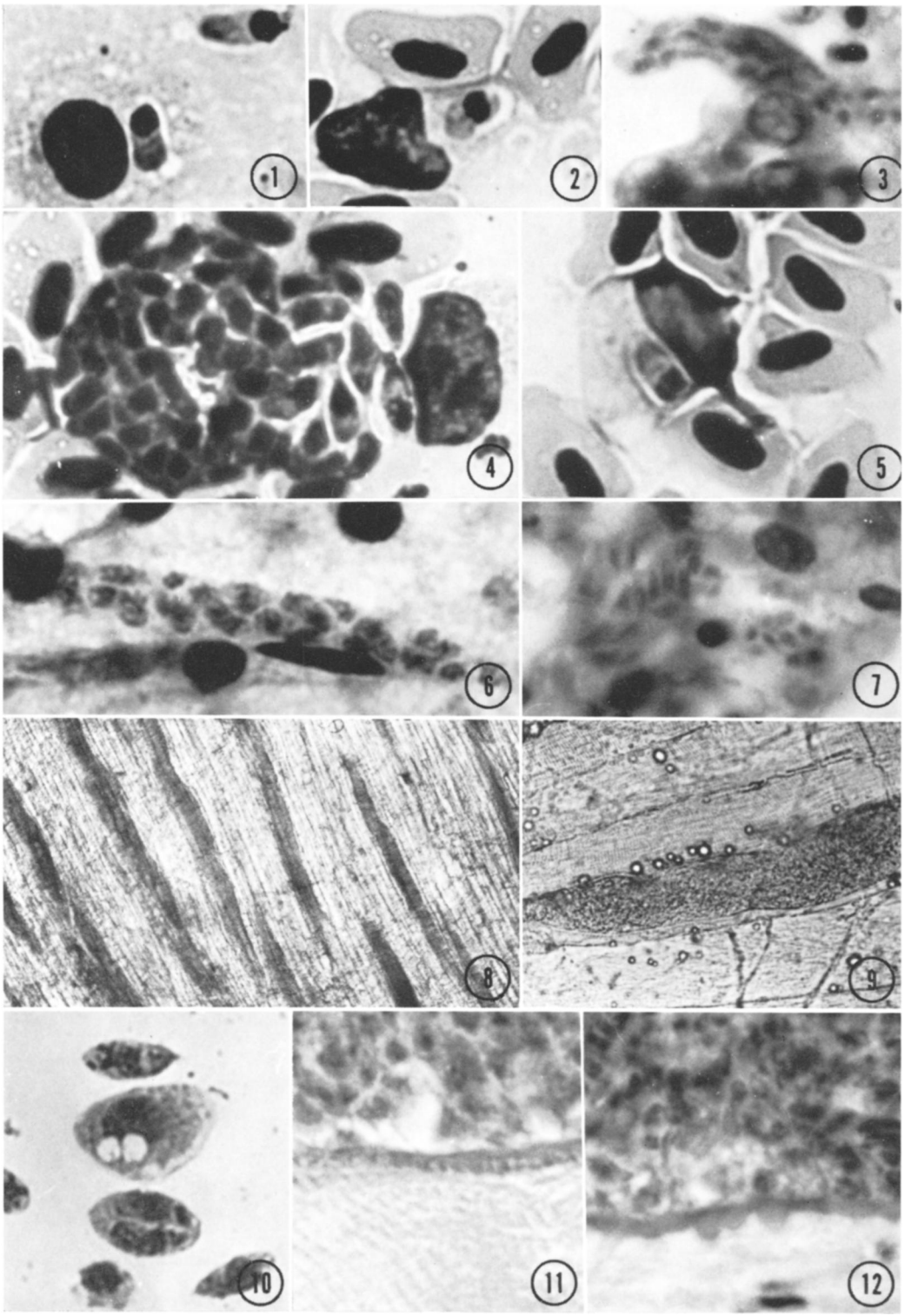
Early asexual stages

For information on early phases of infection, e.g. excystation, two canaries were each given two doses of 10^6 sporocysts; one was given sporocysts of cowbird origin and the other sporocysts of grackle origin. The first inoculum was given about 24 hr and the second about 2 hr before necropsy. In both birds, many extracellular sporozoites were found in the small intestine and a few were in mononuclear phagocytes (Fig. 1). They were most numerous about 6 cm from the duodenal loop. Of the internal organs sampled, the lungs were the most heavily parasitized. Here, the parasites were commonly inside mononuclear phagocytes (Fig. 2). An occasional sporozoite was found in liver and spleen smears. Sporozoites were 2.35 × 5.65 (2–3.5 × 5–7, n = 10); one end was pointed, the other rounded and often stained red with Giemsa's. These parasites inside mononuclear phagocytes resembled stages of *Isospora serini* (Box, 1977) but the nucleus was more compact and cytoplasmic granules were less abundant than in *I. serini*.

A similar experiment with sparrows was difficult to interpret because they were naturally infected with *Isospora*. Stages of the latter in mononuclear phagocytes were difficult to distinguish from intracellular sporozoites of *Sarcocystis* but some extracellular sporozoites could be identified in intestinal smears by their similarity to those seen in canaries.

One of the ducklings given 10^6 sporocysts of cowbird origin and one given 10^6 sporocysts of grackle origin were killed 20 hr PI. A single sporozoite was seen after examination of smears taken from five equally spaced segments of the small intestine from the ducklings.

The majority of the birds were scheduled to be held for 10–20 weeks at which time it was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anticipated that muscle stages would be present if development time was similar to that of other *Sarcocystis* species. However, three of six canaries given 200 × 10^3 sporocysts became moribund and were killed on days 7–12 PI. One of the canaries had been inoculated with sporocysts of grackle origin and two had been inoculated with sporocysts of cowbird origin (Table I). Breathing was labored, the lungs were grey and consolidated and the spleens were enlarged. In sections, lung capillaries were found outlined by the schizonts (Fig. 3) and extracellular merozoites and schizonts were found in lung smears (Fig. 4). Merozoites in smears were 1.9 × 5.5 (1.5–2 × 4–7, n = 11). Occasionally, single merozoites were in mononuclear phagocytes in the lung (Fig. 5).

Four of 12 sparrows given 100–500 × 10^3 sporocysts died on days 11–16 PI. Two sparrows had received sporocysts originating from grackles and the other two had received cowbird origin sporocysts (Table I). They were not observed to be sick, but a certain proportion of these wild birds often die of stress, coccidiosis, etc. after being confined in cages. However, asexual stages, similar to those found in canaries, were seen in smears and sections. Most schizonts and merozoites were found in the lungs; an occasional extracellular merozoite was found in spleen and liver. In addition, several schizonts were found in endothelial cells in the brain smear from a sparrow which died 11 days PI (Fig. 6), and two schizonts with apparently different-sized merozoites were seen in the lung of a sparrow necropsied 14 days PI (Fig. 7). Sizes of merozoites in smears were 2 × 4–8 (mean = 2 × 6, n = 14). No parasites were found in smears of lungs, liver, and spleen of two ducks killed 2 weeks PI.

TABLE II. *Muscle parasites in passerines given* Sarcocystis *sporocysts collected from opossums previously fed either cowbirds or grackles.*

Opossum sporocyst origin	Recipient bird	Days after infection	Muscles at necropsy: Positive/total no. birds		
			Gross	Abdominal	Digest
Grackle	Sparrow	84–136	4/6	5/6	6/6
	Canary	95–142	0/3	0/3*	3/3
	Duckling	58–122	0/3	0/3	0/3
Cowbird	Sparrow	105–127	4/4	4/4	4/4
	Canary	120	0/1	0/1	1/1
	Duckling	58–75	0/3	0/3	0/3
Control†	Canary	0	0/2	0/2	0/2

* Muscle cysts found in leg muscle of 1 bird.
† Six sparrows died prior to 3 mo after infection; all were grossly negative for muscle cysts.

Muscle stages

Sparrows dying before 10 weeks PI were examined grossly for muscle cysts; all were negative. Surviving canaries and sparrows were necropsied from 12 to 20 weeks PI and all were found by the digestion method to be infected (Table II). Most of the sparrows had heavy infections; cysts were visible on gross inspection and numerous cysts were seen in each microscopic field of the abdominal musculature preparations (Fig. 8). Canaries, on the other hand, would have been evaluated as negative without the use of the digestion technique. No cysts could be seen grossly and none was found in the abdominal muscle. After finding canaries positive by digestion, an extra effort was made to find muscle cysts and one cyst was discovered microscopically by teasing apart leg muscles of a canary necropsied on day 119 (Fig. 9). Sections of leg muscle from this same bird contained two additional cysts. No muscle parasites were

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FIGURES 1–7. Precystic stages of avian *Sarcocystis*. × 2,250. Figures 1, 2, 4, 5, 6, Giemsa-stained, Figs. 3, 7, HE-stained. **1.** Sporozoites in canary intestine smear, 24 hr postinoculation (PI). **2.** Sporozoite in mononuclear phagocyte of canary lung smear, 24 hr PI. **3.** Schizont in canary lung section, 11 days PI. **4.** Schizont in canary lung smear, 11 days PI. **5.** Merozoite in mononuclear phagocyte, canary lung smear, 11 days PI. **6.** Sparrow brain smear showing schizont in capillary, 11 days PI. **7.** Schizonts with two sizes of merozoites in sparrow lung section, 14 days PI.

FIGURES 8–12. Cystic stages of avian *Sarcocystis*. **8.** Abdominal wall of sparrow, 110 days PI. × 53. **9.** Canary leg dissection, 119 days PI. × 211. **10.** Metrocyte and zoites in endodyogeny from digest of sparrow, 84 days PI. HE × 2,250. **11.** Cyst wall showing villi, canary, 119 days PI. × 2,250. **12.** Cyst wall showing villi, cowbird, natural infection. HE × 2,250.

TABLE III. *Comparative size of* Sarcocystis *in muscle of sparrows and canaries infected with sporocysts from opossums.*

	Cowbird origin Mean size (range) μm	Grackle origin Mean size (range) μm
Muscle cyst:		
Sparrow*	102 × 716 (50–210 × 330–1,350) n = 40	84 × 1,037 (50–120 × 750–1,680) n = 24
Canary	none found	50 × 500, n = 1
Zoites:		
Sparrow	2.1 × 6.4 (2–3 × 5–7) n = 40	2.1 × 6.5 (1.5–3 × 5.5–8) n = 60
Canary	2.2 × 6.3 (1.5–3 × 5–7.5) n = 10	2.1 × 6.6 (1.5–3 × 5–8) n = 30

* Age of infection = 105–127 days for sparrows infected with sporocysts of cowbird origin and 84–136 days for sparrows infected with sporocysts of grackle origin.

found in six ducks given sporocysts of the same origin and necropsied 8–17 weeks PI or in two control uninfected canaries.

No difference was observed between the infections initiated by sporocysts of cowbird or grackle origin. Abdominal muscle cysts were measured and the sizes are recorded in Table III. Although the muscle cysts originating from sporocysts of cowbird origin were somewhat wider and shorter on the average than those resulting from the grackle origin sporocysts, the size ranges overlap. Size of zoites from Giemsa-stained digest was almost identical from either source of infecting sporocysts and from either the canary or the sparrow serving as intermediate host (Table III). Zoite size also was comparable to those recorded from the donor intermediate hosts (Box and Duszynski, 1977; Duszynski and Box, 1978). Metrocytes and forms undergoing endodyogeny were found in digest smears with zoites (Fig. 10). Cyst walls were similar in donor and recipient intermediate hosts. In HE stained sections, they were from 1–2 μm thick with short villi (Fig. 11). Cysts from donor birds usually were larger, presumably because these cysts were older, and had thinner walls, stretched by the multiplying zoites (Fig. 12). Compartments formed by septa were present in the cysts in both donor and recipient hosts.

DISCUSSION

Although a few attempts have been made to transmit *Sarcocystis* from one species of intermediate host to another by sporocysts from a definitive host, none has been reported to infect more than a single genus. Examples of some unsuccessful cross-transmission attempts include cattle to sheep and sheep to cattle via dogs and cats (Gestrich et al., 1975; Rickard and Munday, 1976), mule deer to cattle and sheep via dogs and coyotes (Hudkins and Kistner, 1977), mouse to rat, guinea pig and hamster via the cat (Ruiz and Frenkel, 1976), mouse to rat via the owl (Munday, 1977) and cottontail to domestic rabbit via the cat (Fayer and Kradel, 1977). A broader intermediate host range is suggested by reports of muscle cysts in humans because it seems unlikely that humans serve as food for predators often enough to perpetuate the cycle.

Our experiments clearly show that sporocysts orginating from one family of avian hosts (Icteridae) excyst in the gut, undergo asexual schizogony in lung endothelium and eventually develop into muscle cysts in experimental hosts of two different avian families (Fringillidae and Ploceidae) within the Order Passeriformes. However, although sporocysts originating from icterid intermediate hosts excysted in the intestines of ducks (Order Anseriformes), the parasite did not develop to the muscle stage in these hosts.

The evidence that canaries were infected experimentally with *Sarcocystis* originating from icterid birds appears to be valid. The canaries had been reared indoors with no predator contact. Furthermore, successive stages of developing *Sarcocystis* were found in the experimentally infected birds, first in the lung endothelium and later in characteristic muscle cysts. Although early stages of *Sarcocystis* resemble asexual stages of *I. serini*, the latter species does not form schizonts in endothelium as seen in sections of *Sarcocystis*-infected birds. The possibility that coccidia

other than *Sarcocystis* were in the digests was considered but ruled out for several reasons: (1) stages of *Isospora* were not found in touch smears of tissues likely to contain them such as duodenal loop mucosa and lung; (2) eight of the nine infected canaries had been reared coccidia-free in the laboratory and had been repeatedly negative for fecal oocysts; (3) no zoites were found in digests of two canaries not given sporocysts; and (4) fully formed cysts were found in the leg muscles of one experimentally infected canary.

It is possible that some of the sparrows were infected before capture, but the incidence of *Sarcocystis* in sparrows in this area is extremely low. In a 1967 survey (unpublished data by EDB) only one of 120 adult sparrows trapped on the Medical Branch campus was found infected. In addition, none of the six sparrows that died before 12 weeks PI had visible muscle cysts, whereas eight of the ten experimentally infected birds necropsied 12 weeks or more PI had visible muscle cysts and all had zoites in muscle digest. These findings support our contention that such muscle cysts resulted from our experimental inoculations with sporocysts produced in opossums that ate cowbirds or grackles.

Our transmission studies suggest that *Sarcocystis* of cowbirds and grackles and probably *Sarcocystis* found in many passerines are the same species. In our earlier paper (Duszynski and Box, 1978) we used the species name *S. dubonei* for the parasite from icterid birds of the genera *Molothrus*, *Cassidix*, and *Quiscalus* on the basis of a description by Vogelsang in 1929 of a *Sarcocystis* from another member of the cowbird genus, *M. bonarensis*. However, the size of zoites of *S. corderoi* from *Passer domesticus*, given in the same paper fits the size of zoites in our experimental infections better. Zoites of *S. corderoi* were 3–6 × 1–2 in size compared with 8–9 × 3–4 of *S. dubonei* from *M. bonarensis*. (It is assumed that Vogelsang misplaced the decimal point in describing the zoites as 0.03–0.06 × 0.01–0.02 mm for *S. corderoi* and as 0.08–0.09 × 0.03–0.04 mm for *S. dubonei*.) The size range of *S. corderoi* zoites fits that of zoites from both natural and experimental hosts in our transmission studies; furthermore, sparrows were susceptible to sporocysts from an opossum which had eaten a cowbird. Future transmission experiments may show that *S. falcatula* (Stiles 1893) from *Pheucticus ludovicianus* (rose-breasted grosbeak) is the same species as that in our experiments and thus has priority. Zoites of *S. falcatula* are described as 2 × 5–6; this bird is a fringillid, migratory in Texas and thus could be exposed to infections common to the cowbirds and grackles used to infect opossums in our experiments.

Canaries were less susceptible than sparrows to muscle infection with *Sarcocystis*. They also seemed more susceptible to pathology resulting from early asexual schizogony than the sparrows. Possibly a host reaction to early schizogony destroyed many of the forms which would have developed into muscle cysts in canaries. One wonders if species of *Sarcocystis* may evolve in abnormal hosts by light infections such as this which gradually adapt to a given predator-prey relationship when it is sustained over a period of time. This would help explain the apparently numerous species of *Sarcocystis* which seem to be quite specific for a given intermediate host.

The digestion technique proved to be very useful in finding light infections. Although cysts could be found in one canary by extensive examination (microdissection and histologic sections), other canaries subjected to the same type of examination were negative except by digestion. Staining of the digest concentrate with Giemsa's provides a permanent record of stages in the muscle cysts. Contrary to our expectations, cyst stages apparently do not have to be infectious to be resistant to digestion since metrocytes were found in these preparations.

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ANNOUNCEMENT . . .

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Ernest Bueding Honored

On 12 April 1978, Dr. Ernest Bueding received the first Theodore Weicker Memorial Award in Pharmacology and Experimental Therapeutics at the annual meeting of the American Society for Pharmacology and Experimental Therapeutics in Atlantic City, New Jersey.

The honor, $10,000 and a certificate, is given to an active investigator who has made sustained distinguished contributions to pharmacology.