These four analogs appear to be the most effective antagonists of the antidiuretic response to ADH yet reported. If these analogs have a similar action in man they could be useful pharmacologically for studies on the contribution that ADH may make in a variety of pathological states involving water retention. They could also be effective and specific agents for treating the syndrome of inappropriate secretion of ADH (the Schwartz-Bartter syndrome or SIADH). This syndrome may complicate a number of disorders, including carcinomas, pulmonary diseases, intracranial diseases, and head injuries (*20*).

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Chemical Impurity Produces Extra Compound Eyes and Heads in Crickets

Abstract. *A chemical impurity isolated from commercially purchased acridine causes cricket embryos to develop extra compound eyes, branched antennae, extra antennae, and extra heads. Purified acridine does not produce similar duplications of cricket heads or head structures nor do the substituted acridines proflavine, acriflavine, or acridine orange. A dose-response relation exists such that the number and severity of abnormalities increase with increasing concentration of the teratogen.*

While determining toxicity of acridine, an azaarene of interest as a component of wastes from coal gasification and liquefaction, a contaminant of the commercial product (*1*) was found to be teratogenic to the cricket *Acheta domesticus* (L.) (*2*) in minute amounts. Treatment of eggs resulted in duplication of cricket head structures. This finding underscores the necessity for complete chemical characterization of mixtures subjected to toxicological assays because a minor component can assume major significance in producing a response. In a more positive light, however, this teratogen offers possibilities as a new tool for exploring cellular events in embryonic development such as determination of cell fate, pattern formation, and possibly the role of cell death in morphogenesis.

Cricket eggs were exposed to commercial samples of acridine by permitting gravid females to oviposit in sand moistened with aqueous solutions of the compound (*3*). After the embryos were exposed to acridine, a small percentage developed extra compound eyes; this percentage increased with increasing concentrations of the commercial product (Table 1). Purification of the commercial product, which was initially > 98 percent acridine, resulted in complete loss of teratogenic activity, whereas the mixture of impurities showed a positive response (*4*). These impurities were obtained in quantities too small to weigh accurately; however, ten chemicals were isolated by preparative thin-layer chromatography on silica gel G plates (E. Merck) in a mixture of chloroform, methanol, and acetic acid (90:5:5, by volume). Each compound was assayed for teratogenicity to cricket eggs; and only one (R_F = 0.74), which showed blue fluorescence under long-wave ultraviolet light, was active (Table 1).

Speculation about the chemical identity of the active impurity is difficult since the method of synthesis is not available from the manufacturer. Many substituted acridines, however, bind nucleic acids (*5*) and are active bactericides, antimalarial and antineoplastic agents, mutagens, and carcinogens (*6*). Three of these, proflavine (3,6-diaminoacridine hydrochloride), acridine orange [3,6-bis-(dimethylamino)acridine hydrochloride], and acriflavine hydrochloride (a mixture of the hydrochlorides of 3,6-diamino-10-methylacridinium chloride and 3,6-diaminoacridine), were evaluated for toxicity to eggs and ability to produce multiple-eyed crickets. None was as toxic to the eggs as pure acridine (Table 1, LC_{50} data), and none produced dupli-

Table 1. Toxicity and teratogenic activity of various chemicals to developing cricket embryos.

Treatment	LC_{50} (ppm)	Concentration (ppm)	Eggs (No.)	Embryos with abnormal number of eyes	
				No.	%
Distilled water			30,153	0	
Impure acridine		20	2,264	28	1.24
		9	2,234	10	0.44
		6	4,802	7	0.14
Purified acridine	15.1 ± 0.61*	20	3,214	0	
		9	4,337	0	
		6	5,123	0	
Acridine impurities			3,728	212	5.69
Unknown (R_F, 0.74)			2,681	25	0.93
Acriflavine	> 100	100	7,817	0	
		10	6,645	0	
Proflavine	> 100	100	5,056	0	
		10	4,343	0	
Acridine orange	> 100	100	2,627	0	
		10	2,372	0	

*From Walton (*14*).

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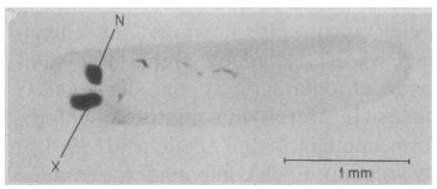
cation of cricket eyes, antennae, or heads.

The observations made of eggs treated with the mixture of acridine impurities are summarized below. The compound eyes of *A. domesticus* embryos are readily visible at low magnification (×30) as bright red spots (*7*). Treated eggs develop two, three, or four compound eyes, which are seen in a variety of sizes and locations at the anterior end of the egg (Fig. 1). Nymphs emerging from abnormal eggs have visible abnormalities of the head. Extra antennae, branched antennae (Fig. 2), and extra head sclerites are sometimes present in addition to extra compound eyes (Fig. 3). Two-headed nymphs arise from eggs that exhibit four red eye spots. These nymphs typically have one normal head and a smaller, poorly developed head (with prominent compound eyes) arising dorsally near the cervix of the normal head capsule (Fig. 4). The proportion of four-eyed embryos produced relative to three-eyed embryos increases with increasing concentration of the teratogen. No morphological abnormalities of the thorax, legs, abdomen, abdominal appendages, or mouthparts were seen in nymphs from treated eggs; however, duplicated mouthparts were visible in some two-headed embryos that died without emerging.



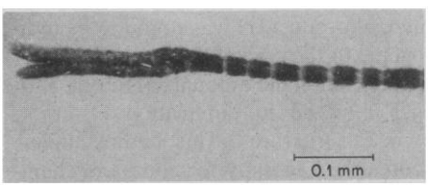


Fig. 1 (left). Teratogen-treated cricket egg showing one normal compound eye (*N*) and a larger, extra compound eye (X) not present on untreated eggs (lateral view). Fig. 2 (right). Tip of an antenna from a three-eyed cricket nymph showing duplication of the distal segments. A normal antenna consists of a single strand of segments.

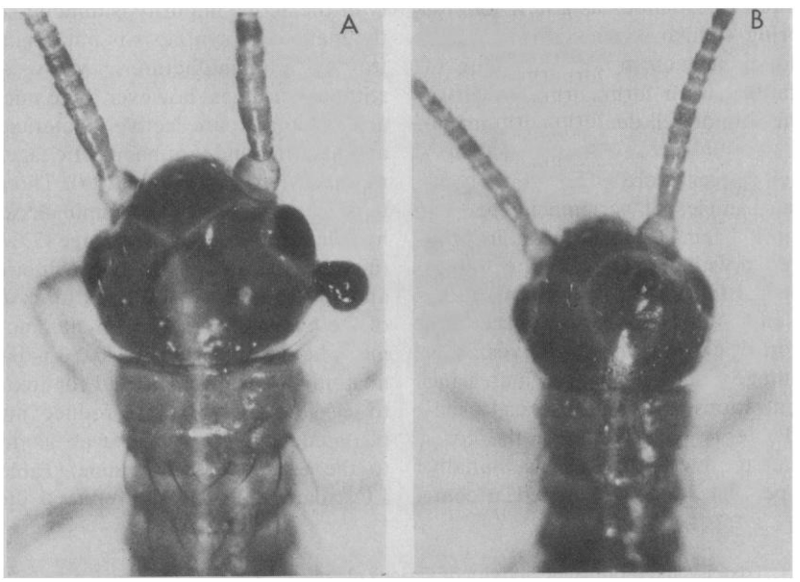


Fig. 3. (A) Head of a first-instar nymph with an extra compound eye on the right side of the head. The insect was treated during the egg stage with a teratogen. (B) Head of a first-instar nymph with an extra compound eye in the center of the head capsule. Treatment was the same as in (A).

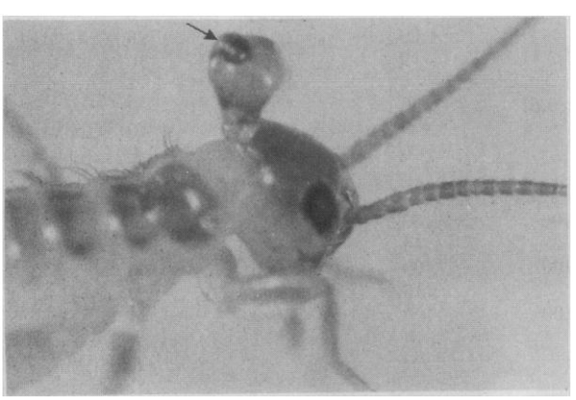


Fig. 4. Two-headed nymph 6 days after emerging from a teratogen-treated egg with four visible eye spots. Location of the eyes on the diminutive head is shown with an arrow.

Extra compound eyes appear to be fully differentiated at the time of nymphal emergence, although the total number of corneal facets present is usually less than in normal eyes. Histological examination of sectioned eyes revealed the presence of a corneal lens, crystalline cone, retinula cells, and rhabdom in the individual ommatidia; however, extra eyes do not continue to grow at each nymphal molt as do normal eyes. With successive molts, the extra eyes become elongate and protrude from the head capsule forming a small peg. In one case, the eye fell off during the adult stage more than 7 weeks after emergence. Extra branches of the antennae are also lost during nymphal molts as are extra heads; but extra heads grow for the first few molts, whereas extra antennal branches are usually lost with the first molt.

The abnormal embryos produced by treating *A. domesticus* eggs with a teratogen isolated from acridine are remarkably similar to the malformed embryos observed by Seidel after he cauterized the anterior tip of damselfly (*Platycnemis pennipes*) eggs (*8*). Depending on the timing and severity of the wound, Seidel was able to produce embryos with either an extra dorsal eye, or two extra eyes and an extra head capsule. The similarity of these results suggests that the isolated teratogen might act by causing cell death prior to germ band formation, as in Seidel's experiments, and that the apparent specificity of the teratogen is a result of the controlled timing of chemical entry. This entry presumably occurs primarily during the initial period of water imbibition in *A. domesticus* eggs (*9*). Unlike the defective damselfly embryos developing after cauterization, however, many of the chemically treated cricket embryos emerged successfully and lived to the adult stage (approximately 6 weeks), providing an opportunity to observe insect repair of teratogenic defects. In addition, a small percentage of the offspring from these malformed adults were morphologically abnormal.

Duplication of normal insect structures has been achieved by various experimental embryological techniques in addition to microcautery. These include centrifugation (*10*) and ultraviolet irradiation (*11*) of insect eggs, as well as

heating eggs of temperature-sensitive mutant flies (*12*). While these experiments are of considerable value to embryology and developmental biology, the techniques employed do not represent realistic means for inducing developmental abnormalities in natural insect populations. In my studies, however, morphological abnormalities resulted after cricket eggs were oviposited in sand contaminated with trace amounts of a teratogen. This finding indicates that a potential hazard exists for inducing morphological deviates in insects as well as other terrestrial invertebrates that undergo embryonic development in chemically contaminated substrates (*13*).

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1. Acridine (2,3,5,6-dibenzopyridine) was purchased from Aldrich Chemical Company, Inc., Lot Nos. 12267 PB and 082987 TC.
2. Orthoptera: Gryllidae.
3. In brief, female crickets oviposit in contaminated sand, and eggs develop in the sand for 5 days. The eggs are then separated by flotation in a 0.5*M* sucrose solution, collected, rinsed, and transferred to moist filter paper for incubation in a covered petri dish. Typically, nymphs begin to emerge on day 12 when incubated at 31°C [B. T. Walton, *Environ. Entomol.* **9**, 18 (1980)].
4. Acridine was purified by first dissolving it in hexane and washing several times with an aqueous solution of NaOH at *p*H 12.2 followed by two washings with distilled water. The hexane solution was then extracted with distilled water adjusted to *p*H 2.2 with H_3PO_4, which brought the acridine into the water phase and left the teratogenic fraction in the hexane. The *p*H of the water was returned to 12.2 with NaOH, and the acridine was back-extracted into clean hexane. The hexane was evaporated at room temperature to obtain acridine crystals.
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14. ———, *Environ. Entomol.* **9**, 18 (1980).
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Specific Antigen in Serum of Patients with Colon Carcinoma

Abstract. *The binding of monoclonal antibody specific for colon carcinoma was inhibited by serum from patients with adenocarcinoma of the colon but not by serum from patients with other bowel diseases or from healthy volunteers. Of other malignancies studied, serum from two patients with gastric carcinoma and two patients with pancreatic carcinoma also inhibited the specific binding of monoclonal antibody. The levels of carcinoembryonic antigen in these serum samples were not correlated with their levels of binding inhibition. Such monoclonal antibodies may prove useful for the detection of colorectal carcinoma.*

We have developed a binding inhibition assay for the detection of antigens unrelated to carcinoembryonic antigen (CEA) and present in the serum of patients with colon (colorectal) carcinoma (CRC). For this assay we use a monoclonal antibody that binds to CRC intact cells, cell membrane extracts, and antigen present in serum-free tissue culture media (SFTCM) from CRC cells.

Examination of the possibility of using CEA as a diagnostic tool for CRC revealed that, although the serum of most CRC patients contains CEA, the serum of many patients with ulcerative colitis, alcoholic cirrhosis, pulmonary emphysema, and other primary site carcinomas also binds antibody to CEA in radioimmunoassays (RIA) (*1*). Furthermore, the serum of approximately one-fifth of all healthy subjects who smoke cigarettes contains CEA (*2*).

These results led us to search for an assay that would distinguish between antigens that are specific for CRC and those that are shared with other tumors. The availability of monoclonal antibodies that recognize antigenic determinants associated only with CRC (*3, 4*) made possible a reinvestigation of the specificity of antigens circulating in the blood of patients with CRC.

Serum samples were obtained from 33 patients who had advanced adenocarcinoma of the colon and rectum with documented metastases in their liver or lungs; six patients with other bowel diseases, including two with multiple polyps; and 38 patients with forms of cancer other than CRC. All of the patients were hospitalized at the Oncologic Hospital, Fox Chase Medical Center, Philadelphia. Each serum sample was divided into small portions, coded, and frozen at −70°C. Preoperative patients and those with suspected but unproved recurrent cancer were not included in this study group. Serum samples were also obtained from 39 healthy volunteers: 15 females, 20 to 40 years of age; 10 females, 41 to 65 years of age; 9 males, 20 to 40 years of age; and 5 males, 41 to 65 years of age. Fifteen of these 39 healthy subjects were smokers.

Cells of CRC line SW 1116 (*5*) were used as a source of target antigen in the binding assay. This tumor is maintained in tissue culture and secretes its antigenic component (or components) into the tissue culture medium. Extracts (3*M* KCl) of cell membranes or cell-free SFTCM were used either as the target in RIA or as the inhibitor in preliminary inhibition assays. Hybridomas secreting antibody to CRC into the medium were grown according to a method described previously (*4*). The hybridoma medium contained approximately 10 μg of antibody per milliliter. The indirect RIA for the binding of antibody to its target has been described previously (*4*).

In the inhibition assay, dilutions of hybridoma antibodies were used that exhibited 40 to 50 percent maximum reactivity against a given concentration of target antigen in the form of either membrane extracts or SFTCM of cell line SW 1116. The percentage of specific inhibition of binding of hybridoma antibodies was calculated from the mean of triplicate wells according to the formula: percentage inhibition = $[100 - (R_T - R_C/R_{max} - R_C)] \times 100$, when R_T and R_C are, respectively, the radioactivity (measured in counts per minute) of the test and control cultures, and R_{max} is the maximum binding determined by incubating hybridoma antibodies with buffer solution instead of serum samples. The background radioactivity of the controls was determined by incubating test samples with nonspecific mouse immunoglobulin, a supernatant from myeloma P3 × 63Ag8 (*3, 4*). Statistically significant differences in inhibition of binding between test and control samples were calculated for each experiment by Student's *t*-test.

Of the large number of monoclonal antibodies that bind to CRC cells (*4*), an immunoglobulin G1 (IgG1) antibody was selected for the inhibition assay because of its wide range of reactivity with CRC cells of different origins. In preliminary assays we were able to inhibit effectively the binding of this antibody by the use of either cell membrane extracts of CRC, at concentrations of 5 to 20 μg of protein per unit assay, or SFTCM of CRC cultures at concentrations of 10 to 20 μg of

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