15. This work was supported in part by PHS grant GM07804. We thank B. D. Hilton for preparing the H^3-parathion, A. M. Phillips for providing fish, and F. Matsumura for helpful discussions.

23 December 1963

Insect Chemosterilants with Low Toxicity for Mammals

Abstract. *Hexamethylphosphoramide and hexamethylmelamine are effective as male house fly chemosterilants. Both compounds are structurally similar to the two highly active sterilants tepa and tretamine, but they differ from the aziridinyl compounds in their low toxicity for mammals and in their lack of alkylating properties. This discovery of nonalkylating male sterilants of low toxicity should substantially increase the scope and practicality of the sterile-male control method.*

The most effective male insect chemosterilants so far discovered are biological alkylating agents (*1*). More specifically tepa (*2*), tretamine, and apholate, all derivatives of aziridine (ethylenimine), when applied to a great variety of insects prevent the hatching of eggs laid by females mated to the treated males. Because of the possible mutagenic and carcinogenic properties of aziridinyl compounds (*3*) the practical application of chemosterilants to natural insect populations is restricted. Therefore, the development of nonmutagenic sterilants with low mammalian toxicity will have far-reaching consequences.

Table 1. Male-sterilizing properties of HMPA administered by different methods; at each concentration ten treated males were mated with five virgin females.

Dosage (μg per fly)	Percentage in food	Total No. of eggs	Sterility (%)
Injection method			
0.5		604	1
1.0		300	3
2.5		535	19
5.0		630	38
10.0		352	86
20.0		161	93
40.0		432	100
Topical application			
5.0		119	31
10.0		844	13
25.0		1123	48
50.0		663	91
100.0		617	99.8
200.0		375	100
Oral application			
	0.01	708	3
	0.05	249	86
	0.25	300	100
	0.50	172	100
	1.00	514	99.9

The work in our laboratory (*4*) has centered around the aziridines, and an accurate and highly sensitive method for determining their sterilizing activity in male house flies (*Musca domestica* L.) has recently been reported (*5*). Although it was supposed that the alkylating property of tepa and other aziridine chemosterilants was indispensable for their biological (sterilizing) activity, other phosphoramides which were either related to the decomposition products of tepa or were structurally similar to it were investigated. Outstanding activity was exhibited by hexamethylphosphoramide (HMPA). As can be seen from the structures in Fig. 1, the compounds are sterically and structurally quite similar. Both are soluble in water and in most organic solvents. Aqueous solutions of HMPA were injected into male house flies which were subsequently allowed to mate with virgin females. The extent to which the compound affected the fertility of the flies was determined on the basis of the hatchability of eggs laid by the inseminated females (*5*). The results of the injection experiments are given in Table 1. The results were subjected to probit analysis at the computing laboratory of the Biometrical Services, U.S. Department of Agriculture. The dosage-activity regression equation was found to be $y = 2.7x + 0.32$ and the calculated ED_{50} (*6*) was 5.42 μg for each male fly.

The activity of HMPA was also tested by topical application and by addition to the fly diet. Neither of these methods allows for a quantitative determination of the activity; nevertheless, from a practical standpoint both topical and oral activities are of utmost importance. The results of the experiments are summarized in Table 1.

In the topical application, 0.85 μl of an acetone solution of HMPA was applied to the dorsal side of the fly's thorax. The effects of the treatment were evaluated in the same way as in the injection method (*5*). In the feeding experiments HMPA was added to the fly food, which consisted of nonfat dry milk (50 percent), sugar (1 percent), and water. Flies were allowed to feed on the treated food for 24 hours, and then untreated food was substituted. Evaluation was carried out as in the injection method. Female flies treated with the compound by any of the three methods and mated to untreated males often produced an appreciable number of eggs that failed to hatch. However, the results were erratic and it was concluded that the effects on females were far less pronounced than on males.

Table 2. Sterilizing activity of hexamethylmelamine applied topically to male house flies.

Dosage (μg per fly)	Total No. of eggs	Sterility (%)
10	140	20
20	130	38
40	256	85
60	192	100
80	203	100

One of the important characteristics of an effective chemosterilant is the margin between the sterilizing and lethal doses of the material. Because a sterile male (*7*) which is sexually competitive with normal males is of cardinal importance in the sterile-male control method (*8*) whereas a dead male is useless, any chemosterilant treatment must not cause undue mortality among the treated insects. In practice it is impossible to apply to a natural population just the minimal quantity of a compound required for 100 percent sterilization, and a severalfold overdosage to some individuals is inevitable. In this respect HMPA rates very well because its LD_{50} (as determined by the injection method) is about 95 μg for a fly of either sex. This value corresponds to a safety margin (LD_{50}/ED_{50}) of about 20.

In comparison to HMPA, tepa is

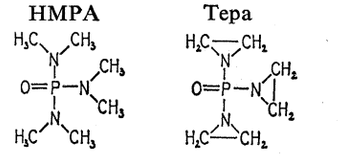

Fig. 1. Comparison of structures of hexamethylphosphoramide and tepa.

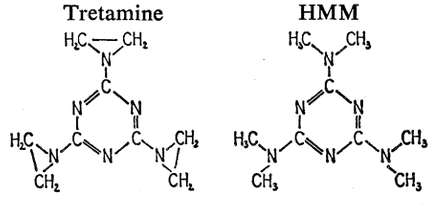

Fig. 2. Comparison of structures of tretamine and hexamethylmelamine.

about 50 times as effective (based on the ED_{50} values in the injection treatment), but this advantage of tepa is outweighed by the low toxicity of HMPA, whose minimum lethal dose, determined by oral feeding to rats, is 2640 mg per kilogram of body weight (*9*). The undiluted compound killed rats when administered orally at a dosage of 6400 mg/kg (*10*). The minimum lethal dose for domestic rabbits (administered through a stomach tube) is reported to be 1500 mg/kg (*11*).

Because of the structural similarity of HMPA and tepa, the sterilizing activity of hexamethylmelamine, an analog of tretamine, was investigated. The structures of the two compounds are presented in Fig. 2.

Because of the very low solubility of hexamethylmelamine in water, the injection technique did not give reliable results, but the topical application of an acetone solution was satisfactory. The results are given in Table 2.

The acute toxicity (LD_{50}) of hexamethylmelamine is reported (*12*) as 220 mg/kg for mice and 265 mg/kg for rats. According to the same report the compound does not produce any cytological alterations in the bone marrow of rats similar to those produced by nitrogen mustards.

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References and Notes

1. A. B. Bořkovec, *Science* **137**, 1034 (1962).
2. Tepa is tris-(1-aziridinyl)-phosphine oxide. Tretamine is 2,4,6-tris-(1-aziridinyl)-*s*-triazine. Apholate is 2,2,4,4,6,6-hexahydro-2,2,4,4,6,6-hexakis-(1-aziridynyl)-1,3,5,2,4,6-triazatriphosphorine.
3. C. Auerbach, *Ann. N.Y. Acad. Sci.* **68**, 731 (1958); A. L. Walpole, *ibid.*, p. 750.
4. A. B. Bořkovec and C. W. Woods, *Advan. Chem. Ser.* **41**, 47 (1963).
5. S. C. Chang and A. B. Bořkovec, *J. Econ. Entomol.*, in press; presented at the St. Louis meeting of the Entomological Society of America, 2–5 December 1963.
6. ED_{50} refers to the dosage required to reduce the hatch of eggs 50 percent as compared to that of the control.
7. The term "sterile male" as used in this report implies only that crosses between a sterile male and a normal female produce no progeny.
8. E. F. Knipling, *Science* **130**, 902 (1959).
9. Monsanto Chemical Co., Technical Data Sheets, Hexamethylphosphoramide, p. 2, 24 August 1956.
10. Eastman Chemical Products, Inc., Technical Data, Eastman Inhibitor HPT, p. 5, August 1961 (rev.).
11. T. R. Adkins, Jr., W. L. Sowell, F. S. Arant, *J. Econ. Entomol.* **48**, 139 (1955).
12. F. S. Philips and J. B. Thiersch, *J. Pharmacol. Exptl. Therap.* **100**, 398 (1950).

24 December 1963

Humoral Factor from the Brain Which Activates Gastric Motility

Abstract. *When the vagus nerves in the neck of the dog are cut and the ends toward the brain are stimulated, gastric contractions follow. These contractions are not abolished by section of the cervical spinal cord, section of anterior and posterior thoracic and lumbar spinal nerves, section of the splanchnic nerves, or paralysis or excision of the celiac plexus. Evidence for the existence of a humoral blood-borne substance originating in the brain was obtained by experiments with an isolated perfused head, by perfusion of isolated stomachs by donor dogs, by cross-perfusion between two dogs, and by plasmapheresis.*

We have observed that when both vagus nerves in the neck of a dog are severed, stimulation of the cut ends toward the brain is followed by a considerable degree of gastric motility. In order to analyze this phenomenon, we used various procedures; for example, cutting the splanchnic nerves, cutting anterior and posterior roots of the spinal cord, cutting the spinal cord at various levels in the neck and chest of the dog, and excising or paralyzing the celiac ganglia. In a few animals, none of these procedures abolished the contractions of the stomach which followed central vagus stimulation in the neck (*1*). For this reason, we began to suspect that a humoral substance might be liberated in the brain or medulla and then be carried by the blood to the stomach where it activates gastric motility. With the exception of a suggestion by Semba *et al.* (*2*) that such a substance might exist, we have been unable to find reports of previous work on such a substance.

To obtain evidence for the existence of this substance we used four different methods. In all experiments, the dogs were anesthetized with pentobarbital sodium. Blood pressure was recorded from a femoral artery with a Statham transducer on a Dynograph. Stomach motility was recorded by three fine plastic tubes inserted at various levels of the stomach and connected to individual Statham transducers arranged to record on the Dynograph.

In our first method, the head was isolated completely from the body except for both carotid arteries and both external jugular veins. The blood vessels were painted with phenol or absolute alcohol in order to destroy the nerve fibers. The cut ends (toward the head) of the vagus nerves were stimulated and, within a few seconds, distinct stomach motility occurred.

In the second method, the stomach was isolated. The celiac axis and the portal vein of dogs were intubated. Hemostasis was accomplished by ligation of all bleeding vessels, and the stomach was removed from the body. In the neck of a heparinized dog to be used as a donor, both vagi were severed and the ends of the nerves toward the head were attached to electrodes. Both internal jugular veins were ligated. Both external jugular veins were ligated and cut above the ligature, and the ends toward the head were intubated with both ends of a Y tube, the straight end of which passed the blood through an oxygenator; the oxygenated blood was circulated by a Sigma pump through the isolated stomach by way of the celiac axis, and returned from the portal vein to a femoral vein of the donor.

When the ends of the vagi of the donor were stimulated, the isolated stomach exhibited multiple contractions, often vigorous, equivalent to a height of 2 to 50 cm of water in 60 percent of these experiments. There was a short latent period of a few minutes and the contractions persisted for 2 to 30 minutes.

In the third method, blood was assayed. In a dog used as donor, both internal jugular veins and one external jugular vein were ligated in the neck. The other external jugular vein was severed, the distal end ligated, and the end toward the head intubated. Blood from the head was thus collected with heparin in 150-ml portions, was centrifuged, and the red cells were injected back into the donor in a suspension of saline or dextran. This procedure was performed twice before, twice during, and twice after stimulation of the ends of the vagus nerves which had been severed in the neck. The plasma was immediately frozen in a thin layer and lyophilized. A dog to be used for assay was prepared so that gastric motility could be recorded from three levels of the stom-