eight times greater than that of embryonic protein. This specific activity indicates selective synthesis of ciliary protein (Fig. 4a and Table 1). Actinomycin D does not affect ciliary protein synthesis. The fact that total protein uptake is decreased by about one-third demonstrates that actinomycin D did enter the embryo. Puromycin inhibits uptake into both total embryonic and ciliary protein by 89 and 93 percent, respectively.

Similar results are obtained if C^{14}-L-glutamic acid (208.5 mc/mmole) is used as the precursor instead of leucine (Fig. 4b and Table 1). Since glutamic acid is also utilized by the embryo as a metabolite in the Krebs cycle, the specific activity of the proteins is much less when this amino acid is used than when leucine is the precursor. But the specific activity of ciliary protein is still seven to eight times greater than that of the total embryonic protein. It is thus unlikely that the greater specific activities of ciliary proteins, being nearly the same in both experimental conditions, reflect greater amounts of leucine and glutamic acid in ciliary protein than in total cellular protein.

The fact that actinomycin D does not affect the initial appearance of cilia (*9*) nor their synthesis in the regenerating system supports evidence that ciliary protein synthesis in the sea urchin embryo is controlled by a stable maternal template RNA. When exposed to puromycin, a substance which inhibits overall protein synthesis, embryos can regenerate cilia four times over a 10-hour period. This finding suggests the presence of an extensive intracellular pool of ciliary proteins.

The need for a large reserve may stem from the frequent cell divisions of the ciliated ectodermal cells, this reserve assuring the daughter cells an adequate supply of proteins for immediate cilia formation. To account for such an apparently extensive pool, one must postulate that the proteins synthesized during regeneration mix readily with the preformed pool, thus labeling the cilia. In two species of bacteria newly synthesized flagellar proteins do mix freely with the preexisting pool, and, although flagellar protein synthesis is inhibited by chloramphenicol, the regeneration of new flagella is not affected (*12*).

The process of aggregation of proteins to form cilia is not understood, but it probably is the key to the initial appearance of cilia in the blastula, since ciliary proteins are probably already available (*9*). It is possible that cilia and mitotic apparatuses have proteins in common, or have ones that are structurally similar, and they may have similar mechanisms for organizing the protein. Colchicine, at a concentration ($10^{-4}M$) that inhibits organization of the mitotic figure, immediately stops ciliary growth. Both structures may be under the organizational influence of homologous organelles (the centriole and basal body); both contain submicroscopic microtubular elements (*8, 13*), have adenosine triphosphatase activity (*14*), and incorporate amino acids during early development (*7–9*). Kane (*15*) and Stephens (*16*) have described an extensive pool of a mitotic apparatus protein making up about 10 percent of the total cell protein in unfertilized sea urchin eggs.

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Comparative Mutagenicity of Two Chemosterilants, Tepa and Hempa, in Sperm of Bracon hebetor

Abstract. *In an assessment of the mutagenic efficiency of the alkylating agent, tepa, and its nonalkylating analog, hempa, both chemicals induced a high frequency of recessive lethal mutations in the sperm of the parasitic wasp,* Bracon hebetor *Say* (Habrobracon), *although tepa was the more efficient mutagen.*

Because certain recently discovered insect chemosterilants with low mammalian toxicity were classified chemically as nonalkylating agents (*1*), some investigators may have assumed that they were also nonmutagenic. Since any chemical that produces sterility in insects without affecting the hereditary material would act quite differently from most male chemosterilants currently available, we were interested in determining whether the nonalkylating analog of tepa, known as hempa (*2*) (Fig. 1), is indeed nonmutagenic. Both tepa and hempa produce sterility in several economically important insects, although tepa is more efficient (*1*). If one of these chemicals were mutagenic and the other not, we would then have some basis for assuming that the type of sterility they produced was different. If both compounds were mutagenic, even though one were more effective than the other, the type of sterility produced by each would probably be similar. The two chemicals might differ only in their availability at critical sites.

The insect utilized for the tests was the parasitic wasp, *Bracon hebetor* Say (also known in the literature as *Habro-*

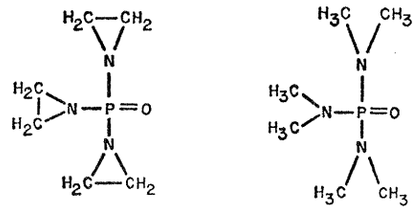

Fig. 1. Structural formulas for tepa [tris(1-aziridinyl)phosphine oxide] and hempa (hexamethylphosphoric triamide).

bracon juglandis Ashmead). It is particularly suitable for studies of recessive lethal mutations because of its arrhenotokous parthenogenetic reproduction, that is, females develop from fertilized eggs (*3*, *4*) and males from unfertilized, haploid ones. Recessive mutations on any chromosome can be detected in the haploid progeny of a heterozygous female. After a male wasp containing mature sperm is treated with a chemosterilant, genetic or physiological damage to the sperm can be expressed as sperm inactivation, dominant lethal mutations, recessive lethal mutations, or "visible" mutations. The first two effects are both components of sterility. Neither sperm inactivation nor dominant lethal mutations in the sperm permit survival of any F_1 diploid progeny derived from treated sperm. But F_1 females heterozygous for either recessive lethal or visible mutations survive, and by appropriate tests one can determine the number of recessive lethal or visible mutations present over the whole of the treated genome (*5*).

In the tests unmated adult male wasps were exposed to a residual film of chemosterilant in small vials. The film was prepared by placing 1 ml of a solution containing the chemosterilant at a concentration of 0.1 or 0.001 percent in a glass shell vial and rotating the vial while evaporating the solvent. Several dozen young adult males were held in the vials for different intervals, removed, placed in untreated vials, fed, and stored at 30°C. Twenty-four hours after treatment they were mated with untreated females from a different strain (*4*). Males were discarded and females were placed on host caterpillars to lay eggs. Some eggs hatched and developed into adults. F_1 daughters were isolated as virgins from pupal cocoons and allowed to produce eggs for 6 to 10 days. Egg hatchability was determined for each F_1 female to determine if a recessive lethal was present in the paternal genome.

In estimating the number of recessive lethals, we calculated the average hatch in the control; the percent hatch for females suspected of carrying one or more lethals (hatch value lower than 60 percent) was adjusted accordingly (*5*). Using the corrected percent hatch, we determined the standard deviation for each F_1 female suspected of carrying a lethal. If the number of eggs that hatched was not greater than twice the standard deviation obtained for that particular female, the number of recessive lethals carried by that F_1 female was accepted as the same as we had estimated. In several females the results were borderline; that is, the actual percent hatch did not fit any estimated number of lethals, possibly because of crossing-over between linked recessive lethal mutations. Data from these females were placed in the category they most nearly fit. Results are given in Table 1.

We conducted five separate tests over a 5-month period. In the preliminary tests (Table 1, Series A), males from the No. 1 white-eyed stock were treated and mass-mated to females from the No. 33+ wild-type stock. However, fecundity of the F_1 daughters from this cross was extremely low (many produced fewer than 50 eggs in 10 days and a considerable number produced none at all). Thus, a different cross was used in subsequent tests. The results (Series A) indicated that both tepa and hempa induced a fairly high frequency of recessive lethals in the sperm. For example, a 15-minute exposure to the residual film (2.12×10^{-4} mg/mm^2) of hempa resulted in 21 percent recessive lethal mutations—three times the number in the control. The same exposure to tepa completely sterilized the males, and the solution had to be diluted 100-fold to obtain daughters. A 10-minute exposure to a film (2.12×10^{-6} mg/mm^2) of tepa resulted in 21 percent recessive lethal mutations. However, the estimated number of recessive lethals in the control group was also high. We suspected that perhaps this result was due to clustering: one P_1 female may have been heterozygous for a preexisting recessive lethal, and thus half of her daughters should also carry it. In these preliminary studies we did not keep lineage records for each pair and, therefore, had no way of knowing whether the high rates of recessive lethals were due to a clustering effect.

Results of additional tests are given in Table 1, Series B. In these studies males from the lemon stock were

Table 1. Recessive lethal mutations induced by two chemosterilants in sperm of *Bracon hebetor* (males treated by tarsal contact with residual film and crossed with untreated virgin females; untreated F_1 daughters tested).

Time treated (min)	Number of F_1 females tested	Number of F_1 females with indicated number of lethals					Total number of lethal mutations	Percent lethal mutations
		0	1	2	3	4		
Series A*								
0†	99	94	3	2			7	7.1
(2.12×10^{-4} mg hempa/mm^2)								
5	112	108	4				4	3.6
15	66	56	6	4			14	21.2
30	45	40	4	1			6	13.3
45	24	21	2			1	6	25.0
(2.12×10^{-6} mg tepa/mm^2)								
3	56	55	1				1	1.8
5	90	84	4	1		1	10	11.1
10	52	46	3	2		1	11	21.2
15	31	27	3		1		6	19.4
Series B‡								
(2.12×10^{-4} mg hempa/mm^2)								
0†	177	176	1				1	0.5
5	161	151	9	1			11	6.8
15	159	140	14	4	1		25	15.7
30	42	36	4	2			8	19.0
45	47	38	6	1	1	1	15	30.4
(2.12×10^{-6} mg tepa/mm^2)								
0†	147	146		1			2	1.4
3	140	137	3				3	2.1
5	123	120	3				3	2.4
10	43	35	5	3			11	25.6
15	65	58	7				7	10.8

* Cross used: No. 1 white-eyed males × 33+ females. † Control. ‡ Cross used: lemon males × wild-type Raleigh females, corrected for clusters.

treated and mated to virgin females from the Raleigh stock (*4*). Fecundity of the resulting F_1 (virgin) females was very high. Lineage of each daughter tested was recorded to detect lethal clusters. The data in Table 1, Series B, have been corrected for the pair in which clustering was found. The data from these tests again indicate that both tepa and its nonalkylating analog, hempa, are mutagenic. Lethal frequencies of 31 and 61 times the control value resulted from 15- and 45-minute exposures, respectively, of males to hempa. Ten-minute exposures to a film of tepa induced 21 times more lethals than were present in the control. However, the 15-minute treatment yielded a lower frequency of recessive lethals. The longer treatments produce higher levels of dominant lethal mutations and significant amounts of sperm inactivation (*6*). Data from the tests support the following conclusion: both tepa and its nonalkylating analog, hempa, are mutagenic.

Tepa is about 100 times more effective than hempa in inducing recessive lethal mutations in *Bracon hebetor*. Other studies (unpublished), utilizing males treated simultaneously with the males used in the present experiments, showed that tepa is similarly more effective than hempa in producing sterility (dominant lethal mutations or sperm inactivation, or both).

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1. S. C. Chang, P. H. Terry, A. B. Borkovec, *Science* **144**, 57 (1964).
2. Tepa is tris(1-aziridinyl)phosphine oxide. Hempa is hexamethylphosphoric triamide.
3. A. R. Whiting, in *Advances in Genetics*, E. W. Caspari and J. M. Thoday, Eds. (Academic Press, New York, 1961), vol. 10, p. 295.
4. Fertilized eggs usually give rise to diploid females only, providing the parents come from unrelated stocks with different sex alleles. In crosses of closely related individuals, some fertilized eggs give rise to diploid males that are homozygous for the sex alleles. These males are highly inviable. For this reason we utilized males from a mutant stock (either No. 1 white-eyed or lemon body) crossed to wild-type females from stocks with different sex alleles (No. 33+ or Raleigh). These *Bracon* stocks are known by this nomenclature to geneticists familiar with this organism.
5. To determine the frequency of recessive lethal mutations, one collects virgin F_1 daughters from treated males crossed to untreated females. These virgin daughters are then allowed to lay eggs. If a female is free of recessive lethal factors (which are expressed in the egg stage), all her eggs should hatch. If she is heterozygous for one recessive lethal, 50 percent of her eggs should hatch; if she is heterozygous for two unlinked lethals, 25 percent should hatch; and so forth. Although the method does not exclude chromosomal rearrangements, this technique allows the investigator to detect recessive lethal factors on any of the 10 chromosomes contained in the sperm. Thus he is not limited to detecting only those recessive lethals located on the X chromosome or a single autosome.
6. L. E. LaChance *et al.*, unpublished.

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Release of Coordinated Behavior in Crayfish by Single Central Neurons

Abstract. *By stimulating and recording from the same interneuron at two separate points, we have shown that coordinated output to the postural abdominal muscles of crayfish can be produced by electrical stimulation of a single cell. Several central neurons can individually initiate one type of movement (for example, flexion), each producing a unique abdominal geometry.*

Complex, coordinated behavior can be evoked by electrical stimulation of certain brain regions in vertebrates (*1*) and invertebrates (*2*). Since these effects are produced with relatively gross electrodes, they undoubtedly depend upon the activation of large numbers of neurons by the stimulating current. In the nervous systems of some arthropods, however, activation of a very few cells may release similarly stereotyped motor patterns. For example, the abdominal appendages of the crayfish, the swimmerets, normally beat in a metachronous fashion (*3*); Wiersma and Ikeda (*4*) generated patterns of motor-nerve discharge appropriate for this behavior by stimulating fine bundles of fibers isolated consistently from the same region of the central nervous system.

Such experiments strongly suggest that ordered output can be released by activity in single interneurons. Working with the system controlling antagonistic postural muscles in the crayfish abdomen, we have tried to demonstrate rigorously that single-cell stimulation can produce such patterns, to find the sensory inputs for these cells, and to discover how several such elements producing similar actions differ from one another. On one side of an abdominal segment, the slow extensor and flexor muscles are each supplied with six efferent neurons; five of these are motor, and one is a peripheral inhibitor. These neurons have characteristic sizes and activity patterns and, therefore, can be individually identified in an electrical record from the appropriate nerve (*5*). There are a limited number of central elements (*6*) which, when stimulated, cause a coordinated motor output—that is, a set of central effects appropriate to either flexion or extension. The flexion command involves excitation of the five flexor motoneurons, inhibition of the peripheral inhibitor to the flexors, excitation of the extensor inhibitor, and inhibition of the five extensor motoneurons; extension command fibers mediate precisely the opposite actions. These effects are normally seen in several segments at once. If single cells are indeed responsible for the entire output, then it must be concluded that one interneuron can influence the discharge of at least 120 efferents.

The procedure used was similar to one developed for analyzing the point of impulse initiation in interneurons (*7*). Crayfish (*Procambarus clarkii*, collected locally) were pinned ventral side up in a chamber containing van Harreveld's solution (*8*). The nerve cord was exposed, and its sheath was removed between ganglia 1 and 2 (rostral site) and between 5 and 6 (caudal site). Bipolar recording electrodes were placed under the superficial third roots supplying the slow flexor muscles on the right side of segments 2, 3, and 4. The saline level was then lowered, oil was layered on top, and the recording electrodes carrying the nerves were lifted into the oil layer. Signals from these three recording sites, each monitoring the discharge of six efferent neurons, were amplified and displayed on a multichannel oscilloscope. Bundles of nerve fibers were stripped from the rostral site by fine dissection; they were left connected caudally and drawn over a pair of platinum wires in the oil layer for stimulation with brief (0.1 msec) pulses of current. Most bundles had no effect upon flexor motor discharge when stimulated, but some produced coordinated flexor or extensor output at a sharp and reproducible threshold intensity. If necessary, active bundles were further dissected until a strand was obtained that produced a pure effect. The pair of electrodes carrying the strand was then switched to an amplifier connected to a fourth oscilloscope trace. At this point, a similar dissection was begun in the homologous region of the caudal site, these bundles being left attached rostrally. In successful experiments, electrical stimulation of a series of such