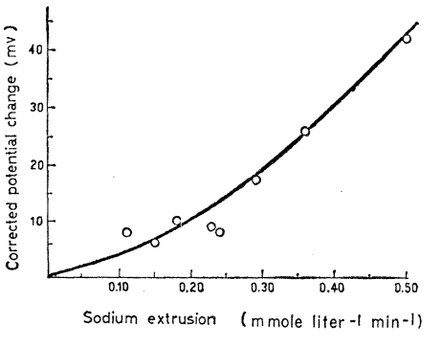

Fig. 3. Changes (corrected) in potential plotted against sodium extrusion. Circles represent values taken from Table 1.

electrogenic. The second mechanism will always lead to a change of the membrane potential (regardless of pump neutrality), but only because of changes in ionic concentrations produced by the pump. Such an alteration requires time for significant changes in ion concentrations to occur. According to this line of reasoning, the mechanism by which membrane potentials are generated may be deduced by recording the change in membrane potential with time in response to changes in the rate of ion pumping. Change in the rate of pumping can be initiated by change in temperature (*9*).

Sartorius muscles of the South American frog, *Leptodactylus ocelatus*, were dissected and kept overnight in the cold (3°C) in potassium-free Ringer's solution (per liter: Na^+, 120 mmole; Ca^{++}, 1.8 mmole; Cl^-, 123.6 mmole) to increase the internal sodium concentration of the fibers. Next morning, both muscles of the same pair were transferred to normal Ringer solution (per liter: Na^+, 120 mmole; K^+, 2.5 mmole; Ca^{++}, 1.8 mmole; Cl^-, 126.1 mmole) at 3°C, and the membrane potential was recorded by the microelectrode technique (*10*). One of the muscles (*A*) was separated in a crucible for analysis, while the other (*B*) was kept in the bathing solution. The temperature of the bath was then raised as rapidly as possible to 25°C, and the potential of the membrane was recorded for 1 hour by repeated puncturing of different muscle fibers. The muscle was then taken from the bath and both muscles were analyzed for sodium by flame photometry. From the initial concentration (of muscle *A*) and the final one (of *B*) and the time elapsed, an estimate was made of the amount of sodium extruded by the second muscle per unit time during the period at 25°C. Some examples of the curve obtained when potential is plotted against time are shown in Fig. 2, where the temperature was changed at time zero. As the temperature changed, the potential of the membrane increased. The rise was rather steep at the beginning, but after a few minutes it reached a maximum and then declined slowly. A maximum potential appeared in all the experiments, and the change in the potential ΔV was always higher than the change in potential that would be obtained by changing temperature T in the equation for the potential of a membrane (*11*)

$$V = \frac{RT}{F} \log_e \frac{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_o}{P_K[K]_o + P_{Na}[Na]_o + P_{Cl}[Cl]_i}$$

In this equation R is the gas constant, F the Faraday constant, P_K, P_{Na}, and P_{Cl} are, respectively, the permeabilities for K^+, Na^+, and Cl^- and the symbols within brackets represent concentration; subscripts o and i indicate, respectively, outside and inside. After 20 to 30 minutes the result showed considerable variation, although in all the experiments there was a tendency for the potential to decline, as shown by the dashed lines in Fig. 2. Table 1 shows the changes in sodium concentration per unit time, as well as the potentials obtained from several experiments. The last column gives the maximum corrected potential with the effect of temperature taken into account; that is, the maximum potential change observed minus the change obtained by introducing the same temperature change in the above equation. Figure 3 shows the corrected changes in potential plotted against sodium extrusion per unit time. It is possible to draw a line passing through the origin, which means that if there is no sodium extrusion, the potential does not change. Evidently, there is a direct relation between sodium extrusion and potential change, although it is not yet possible to establish a function that relates both variables.

The curves in Fig. 2 show that the maximum change in potential occurs in a time so short that internal ion concentrations have not changed. For example, in experiments E and F (see Table 1) the change in the potassium concentration in 6 minutes might be 1.5 mmole/liter at most, which is not enough to explain a potential change of about 8 mv observed within that period. With respect to chloride, if it is assumed that it is passively distributed, its concentration is a consequence rather than a cause of the changes in potential. If, on the contrary, the pump were not neutral and generated an outward current, the difference in potential across the membrane would increase until the passive currents of chloride and potassium would compensate its effects. This would occur within the first 10 minutes at the maximum height of the curves. The final decline of the curves might be explained by a decrease in the rate of the pump as the concentration of sodium decreases.

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Uracil Mustard: A Potent Inducer of Lung Tumors in Mice

Abstract. *Uracil mustard, injected intraperitoneally, increased the incidence and average number of pulmonary tumors in A/J mice. In comparison to a typical alkylating agent, nitrogen mustard, or to urethan, uracil mustard (on a molar basis) was considerably more carcinogenic.*

The carcinogenic polycyclic aromatic hydrocarbons (*1*), urethan (*2*, *3*), and both sulfur mustard and nitrogen mustard (*4*) increase the incidence and average number of pulmonary tumors in inbred strains of mice. This fact is the basis of an assay of carcinogens that has been used in numerous investigations to study the effects of strain, age, diet, sex, and other factors (*5*). The system is useful because of the relatively short latent period before tumors arise. Also the assay may be quantitatively evaluated in both the percentage of the tumor-bearing mice and in the number of individual tumor nodules in the lungs. These two criteria permit accurate correlation of dosage to response and of chemical structure to activity.

Table 1. Increase in the incidence of pulmonary tumorigenesis in A/J mice. The mice were injected intraperitoneally with either nitrogen mustard, uracil mustard, or urethan, each dissolved in 0.1 ml tricaprylin, three times a week for 4 or 14 weeks as indicated. Control groups received 0.1 ml tricaprylin alone or they were untreated. The animals were killed after a total of 39 weeks. The lungs were examined for surface tumors, and individual nodules were counted on the fresh tissue.

Uracil mustard			Nitrogen mustard			Urethan		
Total dose (μmole/kg)	Mice with tumors (%)	Nodules/mouse (No.)	Total dose (μmole/kg)	Mice with tumors (%)	Nodules/mouse (No.)	Total dose (10^3 (μmole/kg)	Mice with tumors (%)	Nodules/mouse (No.)
125	100	41	66	66	1.4	118	100	62
48*	100	16	44	71	1.6	29.4	100	32
31	100	10	17*	63	1.0	7.6	100	13
12*	100	7.3	11	46	0.6	7.8	72	1.3
8.0	89	3.9	4.3*	29	0.4			
3.0*	80	1.7	3.0	48	0.6			
2.0	60	1.0	1.1*	49	0.6			
0.7*	59	0.9	0.8	34	0.4			
0†	33	0.4	0.2*	46	0.8			
‡	34	0.3						

* Dose given for 4 weeks. † Controls given vehicle alone for both 4 (120 mice) and 14 (120 mice) weeks. ‡ Untreated controls.

Because of the chemotherapeutic value of nitrogen mustard, numerous derivatives of the mustards and other alkylating agents have been synthesized, tested for carcinostatic activity, and used clinically (*6*). Since these compounds are also potentially carcinogenic (*7*), we assessed the activity of a series of chemically related alkylating agents by the pulmonary tumor induction technique. Uracil mustard markedly increased the incidence and number of pulmonary tumors in A/J mice. This drug was considerably more carcinogenic, on a molar basis, than either nitrogen mustard or urethan.

Groups of 30 A/J mice (Jackson Memorial Laboratory, Bar Harbor, Maine), 15 of each sex, 4 weeks old, each weighing approximately 14 grams, were injected intraperitoneally three times a week for either 4 or 14 weeks at 4 dosages with either uracil mustard, nitrogen mustard (*8*), or urethan, dissolved in 0.1 ml tricaprylin. The animals were killed 39 weeks from the start of the experiment and were examined for tumors. At least 25 of 30 animals in each group survived, except in the case of those given the highest total dose of uracil mustard (125 μmole/kg), when 15 of 30 animals survived. There was no appreciable sex difference in survival or tumor yield and therefore the results at any given dose were pooled (Table 1).

The highest tolerable dose of either uracil mustard or nitrogen mustard was derived from preliminary experiments on the toxicity of each compound. At all doses studied, uracil mustard increased the incidence and average number of pulmonary tumors. The lesions, examined both grossly and histologically, were the characteristic clear or pearly white nodules described as adenomas, adenocarcinomas, or both (*9*).

A total dose of 125 μmole of uracil mustard per kilogram of body weight produced an incidence of 100 percent with an average of 41 tumors per mouse, whereas a dose approximately 1/200 the size caused tumors in 59 percent of the mice, with 0.9 tumors per mouse. A comparison of uracil mustard and nitrogen mustard at similar dose ranges (48 to 44 and 12 to 11 μmole/kg) demonstrated that approximately ten times as many nodules were induced in the animals treated with uracil mustard. In the groups of control animals given vehicle alone, the tumor incidence was 33 percent with an average of 0.4 tumors per mouse. Similar results were obtained in experiments with at least four other dosages of uracil mustard and nitrogen mustard in which these agents were dissolved or suspended in 1-percent solution of acacia or in water rather than in tricaprylin. Furthermore, the difference in carcinogenic potency cannot be explained on the basis of stability because the compounds have approximately the same rates of hydrolysis in distilled water.

Urethan has been used classically as a standard for the induction of pulmonary tumors. Our results show that uracil mustard yielded approximately the same number of tumors as urethan at a molar dose only 1/200 as large.

Possible explanations for the marked carcinogenic activity of uracil mustard are as follows: (i) Uracil mustard, an analog of uracil, may be incorporated into RNA during its synthesis and consequently alters protein function (*10*). Busch *et al.* (*11*) have shown that uracil mustard affects the incorporation of uracil-C^{14} into RNA of rat liver and uniformly labels L-arginine-C^{14} and L-lysine-C^{14} into nuclear proteins in the Walker 256 carcinosarcoma. (ii) Uracil mustard may be incorporated into RNA in a unique position to alkylate a base or bases in a specific and stereochemically favored nucleotide sequence in DNA. (iii) A direct alkylation of specific receptor sites may occur. Although the alkylation of nucleic acids (*12*) and protein (*13*) has been extensively studied, a correlation between chemical reactivity and the specific biological effect of alkylating agents has not been elucidated. Thus, after a single injection in A/J mice, sulfur mustard was extensively bound to the nucleic acids and to the proteins isolated not only from the lungs but also from the liver and kidneys, sites at which tumors are not induced (*14*). (iv) Reactions of alkylating agents with components other than the genetic material of the cell may result in a heritable change (*15*).

Uracil mustard, because of its marked carcinogenic activity and structural analogy to a naturally occurring base, may represent a useful new tool for the elucidation of the mechanism of carcinogenesis.

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Polymorphic Spermatozoa in the Hymenopterous Wasp Dahlbominus

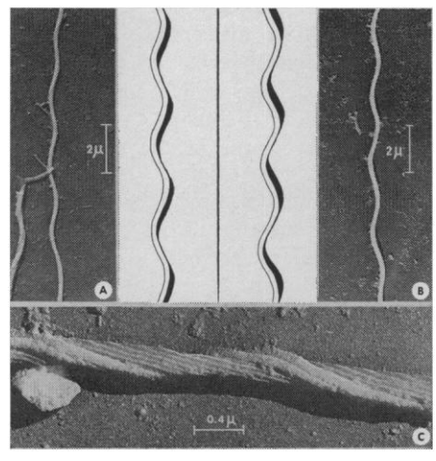
Abstract. *Studies with the light and electron microscope reveal that at least five different types of spermatozoa are produced during spermatogenesis in the arrhenotokous hymenopteran* Dahlbominus fusciupennis *(Zett. Eulophidae). Two of the types differ from the others in length, one type lacks a continuous spiral helix in the head piece, and two differ from the others in the direction of the helical coil. Few of the first three types reach the female sperm-storage organ. All the spermatozoa of the last two types that do reach the storage organ have heads with either dextral or sinistral helices extending from the apex of the head to the beginning of the tail piece. The mitochondrial filaments of the tail are also spirally coiled around the central axial filament, but only in a dextral direction. It is suggested that the coiling dimorphism may be related to fertilization of the egg and may thus affect the sex ratio.*

It is now generally recognized that not all products of spermatogenesis are alike or regularly functional. Among insects, aberrant spermatozoa are produced regularly in a number of families, classical examples being those reported by Schrader and others in the Pentatomidae (*1*). In species of this family, spermatozoa larger and smaller than normal, and with variable numbers of chromosomes, are produced by the "harlequin" lobe of the testis. Giant spermatozoa have also been found regularly among normal-sized spermatozoa in certain stick insects (*2*) and the American cockroach (*3*). Most of the polymorphism reported so far has been related to size, and it is doubtful whether the aberrant spermatozoa produce viable offspring. Thus they would appear to have little direct influence on the heredity of the species. Aberrant spermatozoa have not been reported in the Hymenoptera where, in most species, femaleness is dependent on fertilization of the egg. In these species, nonfunctional spermatozoa could have direct effect on the sex ratio and on subsequent inheritance, provided that polyspermy was infrequent.

Recent studies (*4*) of the chalcid wasp *Dahlbominus fuscipennis* (Zett.), in which dispermy of the egg is rarely greater than 2 percent, revealed that males of this arrhenotokous species regularly produce spermatozoa of several aberrant types, at least two of which do not relate to size. Under the light microscope, the normal spermatozoa appear thread-like, with distinctive corkscrew-like heads and long, attenuated tail-pieces. The mean overall length of 194 spermatozoa removed from the spermathecae of several females was 189.05 ± 1.98 μ; of the spiral head piece, 30.82 ± 0.23 μ, the sharply pointed, spirally convoluted head being consistently about one-sixth of the total length. In the seminal vesicle of the male, however, variability in total length, and coiling of the head of the spermatozoa, were very pronounced. Some were much shorter and others longer than normal; some lacked the usual spiral coiling of the head (the heads gave a positive reaction with Feulgen's stain); such spermatozoa constituted about 7, 8, and 26 percent, respectively, of the spermatozoa in the seminal vesicle. However, none of the long or short spermatozoa and very few of the noncoiled spermatozoa (20 of 2754 examined) were found in the female storage organ or spermathecal capsule. Inseminated females invariably contain in their spermathecae spermatozoa uniform in size and degree of coiling.

Studies with the phase-contrast or light microscope revealed that the helical coils of the head pieces of spermatozoa from the seminal vesicle of the male and the spermatheca of the female are of two types. In some, the coil is directed clockwise; in others, counterclockwise. When carefully focused under a light microscope at × 1600, on either the upper or lower surface of the spermatozoa, the lines of the helices in focus can be seen to run in opposite directions. The proportion of spermatozoa with dextral and sinistral coils has thus been determined and the findings will be reported elsewhere (*5*).

To confirm the presence and precise nature of the dimorphic coiling, we have made electron micrographs of spermatozoa from the seminal vesicle of mature adult males; the vesicles were dissected from a number of freshly killed insects and the sperma-



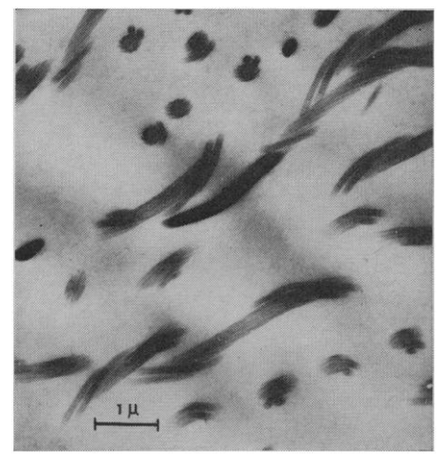


Fig. 1 (left). Electron micrographs of spermatozoa shadow-cast with palladium. (*A*) Head dextrally coiled, as indicated by the prominent diagonal shadows from left to right and as emphasized in the accompanying diagram. (*B*) Head sinistrally coiled, with the diagonal shadows from right to left. Sinistral coil illustrated in diagram at left of micrograph. (*C*) Part of a tail piece showing the dextral direction of the helix formed by the coiling of the double mitochondrial filaments around the central axial filament complex. Fig. 2 (right). Electron micrographs of sections of sperm in the male seminal vesicle. Sections of the head are electron-opaque, and ridged double strands of the mitochondrial filaments surround the axial filament complex.