light, or solution (*2*, *3*). Natural and artificial blue salt, and unpressed amber salt, were bleached by heat, light, and solution to compare coloration properties. All apparent coloration was removed from all samples when dissolved in water. Cleavages 3 mm thick were heated over bunsen burners in crucibles with thermometers attached. One and one-half minutes at a final temperature of 150°C bleached artificial blue salt and unpressed amber salt. Two and one-half minutes at a final temperature of 220°C bleached natural blue halite. In natural blue halite, before all color bleached, a change to violet was apparent. At the end of two and one-half minutes a few blue streaks remained, oriented along diagonals to the cube faces, the known slip, or glide directions (110) caused by prior natural deformation. Such slippage would induce more intense deformation along diagonals and thus cause coloration to be more intense and more slowly bleached.

One 250-watt lamp was placed 15 cm from 3 mm-thick cleavages of natural blue salt, artificial blue salt, and amber salt to investigate bleaching by illumination. In 1 hour, the amber salt was bleached. In 8 hours, the artificial blue salt was bleached, although most of the bleaching occurred by the end of the first 3 hours. With the natural blue salt, no bleaching effects were noted after 35 hours.

Halite, upon gamma irradiation, application of pressure, and exposure to light in the order given, will change from colorless to amber and finally to blue. Long-continued minor pressure even in darkness will also turn irradiated salt blue.

Spectrophotometric examination of natural blue halite and artificially produced blue salt shows that the curves for the two agree in absorption peak positions and in the intensity of the predominant blue coloration. Figure 1 illustrates absorption spectra curves for natural blue halite, artificial blue salt, and amber F-center irradiation coloration.

Colorless halite from bedded deposits or from salt domes can be turned amber yellow to brown and even to black (F-center coloration) by gamma-ray irradiation from a cobalt-60 source. The intensity of color for a given irradiation interval is increased when specimens are subjected to confined or unconfined pressures prior to irradiation (*4*). Color intensity increases with exposure time, an increase of 14 times being noted between samples irradiated from 1 hour to 1 day. However, further exposure of 4 days yields an increase on the order of only two times that of a single day. This confirms that additional coloration proportionately decreases with longer irradiation times. Optical absorption spectra obtained with the spectrophotometer indicate that pressure causes shifts of color center band maxima, removal of some color centers, and development of more complex color centers (*5*).

Application of pressure prior to irradiation enhances the F-center coloration, but pressure applied after irradiation produces a change to blue. In this change to blue all amber F-center coloration is destroyed.

Gamma irradiation of natural blue halite does not appear to produce F-center coloration since spectrophotometric curves taken before and after irradiation for 1 hour are identical.

It would appear reasonable to infer that blue halite may be caused by natural gamma irradiation followed by deformational rock pressures (*6*). This interpretation is supported by the reported occurrence of blue halite in areas of deformation, such as accompany faults, shear zones, and contorted strata (*2*). Study indicates that even minor earth pressures may be adequate to yield blue in naturally irradiated salt (*7*).

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An Inherited Male-Producing Factor in Aedes aegypti

Abstract. An inherited factor causes a predominance of males in certain strains and in progeny of single pairs of *Aedes aegypti* L. This factor appears to be transmitted only by males and is not due to differential mortality, at least in postgametic stages. Mass release of male-producing males might be used in control operations.

With few exceptions, data on the sex ratio in mosquitoes have been collected only as a part of investigations of other phenomena. For some species, conflicting reports have given figures ranging from equal numbers of the sexes to a strong preponderance of one sex. In *Culex pipiens*, Qutubuddin (*1*) found that apparent departures from a 1:1 ratio were not statistically significant. Such a ratio would be expected since Gilchrist and Haldane (*2*) have shown that maleness appears to be due to a single dominant factor (male M/m, female m/m) in this species. While no controlled studies of sex ratio have been made for *Aedes aegypti*, most reports indicate that males predominate [35 to 45 percent female (*3*)].

In a study of genetic variability in populations of *A. aegypti* (*4*), laboratory strains were found to differ with respect to sex ratio. Among 16 strains of diverse origins, there were variations from 38 to 52 percent female. However, certain other strains were 18 to 32 percent female. Replicate counts on some strains over several generations indicated that the frequency of females was constant and predictable for each strain.

To investigate the abnormal ratios observed, frequency of the sexes was determined in the progeny of single pairs. Virgin adults were selected at random from laboratory strains having either high or low female ratios. Single-pair matings were made both within and between these strains. Repeated blood meals were offered to insure satisfactory egg production. Crosses giving fewer than 20 offspring, an infrequent occurrence, were not included in the results. In general, about 50 to 80 eggs were deposited after each blood meal, the total number of eggs from a cross depending upon the number of blood meals taken. Progeny in subsequent batches from any particular mating showed similar sex ratios. For example, a cross with 210 offspring gave 16, 12, 10, and 7 percent females in four egg batches. Since such variations were not statistically significant, the frequency of the sexes was computed from the total progeny produced in a cross.

Special efforts were made to discount the influence of mortality in the immature stages on the sex ratio. All eggs from each pair were collected, counted, and subjected to a hatching stimulus. Unhatched eggs were checked for embryonation. No correlation was observed between frequency of unembryonated or unhatched eggs and sex ratio. Newly hatched larvae were counted and carefully reared in uncrowded containers on a diet of liver powder. Pupation was usually completed by the 7th day after hatching. Sex ratios were determined initially on pupae and were later checked on newly emerged adults. All individuals from each egg batch were counted, even though a few required more than 7 days for pupation. Mortality levels for immature stages were below 10 percent

Table 1. The frequency of females in *Aedes aegypti* as determined in the progeny of single-pair matings made within and between certain strains. Only crosses yielding more than 20 progeny counted.

Strain	No. pairs	No. individuals in progeny	Frequently of females (%) Mean and S.D.	Range
Key West*	33	4648	48.9 ± 5.0	40–59
Texas†	21	2154	37.6 ± 11.2	11–56
Texas-D‡	30	2115	31.7 ± 15.5	4–66
Texas-D-S§	24	1724	22.5 ± 15.0	0–51
♀ Texas-D-S × ♂ Key West	15	889	47.9 ± 6.6	37–62
♀ Key West × ♂ Texas-D-S	22	2678	25.5 ± 11.6	6–49
♀ Key West × ♂ F_1 (♀ Key West × ♂ Texas-D-S)	23	5987	37.7 ± 9.3	15–53

* Field collected by A. O. Lea, Key West, Fla., in November 1959.
† Received from D. W. Micks, Univ. of Texas Medical School, 1957.
‡ Selected for dark larval color by inbreeding from the Texas strain followed by subsequent mass rearing.
§ Inbred line from the Texas-D stock selected for the male-producing factor.

for most of the data reported. There was no correlation between immature mortality and abnormal sex ratios. In a typical example, one female deposited 99 eggs containing embryos. Of these, six failed to hatch and 90 reached the adult stage, only four of these being females.

Table 1 and Fig. 1 show the percentage of females in offspring from single pairs. The results obtained from the Key West strain approximate a normal probability curve with a mean of 50 percent female. This strain resembles many other "normal" strains where a 1:1 sex ratio prevails. In the Texas strain both the mean of 37.6 percent and the range of 11 to 56 percent female indicate factors which cause a departure from the "normal" 1:1 ratio. This preponderance of males has been observed in other strains (*4*).

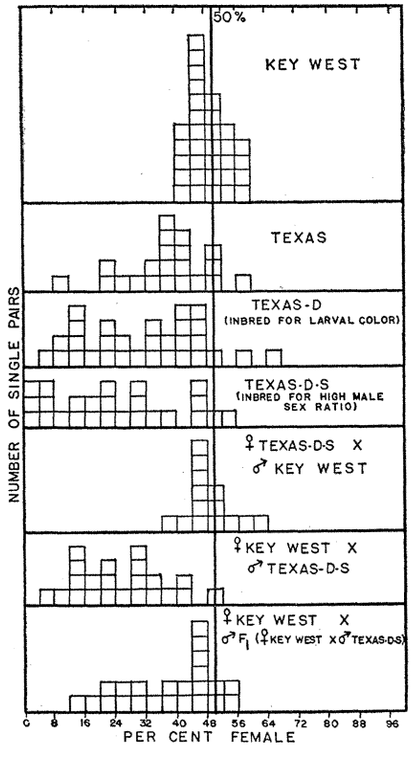

Fig. 1. Histograms showing distribution of frequency of females in the progeny of single-pair matings within and between certain strains of *Aedes aegypti*. Each box represents the progeny from one mating. Each pair had at least 20 offspring and most had 90 or more offspring.

Certain stocks were derived from the Texas strain. The Texas-D stock was developed by inbreeding with selection for dark larval color. After five generations of single-pair sib matings, the stock was expanded by mass crossing. At this time it was observed that the mean had dropped from 37.6 to 31.7 percent female. Apparently, selection for one character led to enhancement of another. These results indicated the possibility that selection for abnormal sex ratios could further change the frequency of the sexes within a strain. To test this hypothesis, pairs from the Texas-D stock were mated and their progeny were counted for sex ratio. The progeny from pairs yielding less than than 15 percent female were inbred further. This inbred line constitutes the Texas-D-S stock. The selection process brought about a further reduction of the mean from 31.7 to 22.5 percent female. The fact that sex ratios can be changed by selection indicates that an inherited factor(s) is involved. While examples of this sort are well known in *Drosophila*, no factor causing abnormal sex ratios has been reported for mosquitoes.

Subsequent crosses between strains with high and low frequencies of females have demonstrated that the abnormal sex ratios are due to a factor here designated as the male-producing (MP) factor. In crosses between females from a male-producing line (Texas-D-S) and males from a strain in which the sex ratio is 1:1 (Key West), the F_1 showed equal numbers of males and females. This "normal" ratio is still evident when either F_1 males or females are backcrossed to Key West. Conversely, most of the crosses between "normal" females (Key West) and males from a male-producing line (Texas-D-S) showed a high male ratio in the progeny. When F_1 males from this cross are backcrossed to Key West females, the high male ratios are still evident.

Note that progeny from males carrying the male-producing factor are usually, but not always, MP themselves. A few sons from these males had 1:1 sex ratios in their progeny. However, the factor does not appear to be present in males of these families. In a typical case, a group of male sibs from an MP father gave 14, 22, 48, and 66 percent females. The males from the 14 percent family, upon inbreeding, gave 3, 9, and 23 percent females, whereas the males from the 48 percent family gave 33, 35, 36, 43, 45, 51, and 54 percent females. Experiments currently under way should make it possible to determine whether the male-producing factor can be recovered from these "normal" lines.

Certain conclusions can be drawn concerning the male-producing factor. First, it is inherited. Its frequency in a strain can be increased by selection. Moreover, the sex ratio in progeny from any particular cross is constant, as shown by data from subsequent egg batches. Second, data at present suggest that the factor is transmitted only by males. Third, it does not act through selective mortality of the sexes, at least at the postgametic level. Fourth, it is probably scattered through many strains and populations of *A. aegypti* (*4*). This may account for the conflicting data in the literature concerning sex ratio in this species.

Numerous problems concerning the male-producing factor remain to be solved. Paramount among these are the questions of its mechanism of action and its nature of inheritance and, for that matter, the sex determination mechanism for the species. The male-producing factor may act through abnormal spermatogenesis, resulting in selective production of male-determining sperm, or it may act through competition of sperm during fertilization. Furthermore, results with the factor are not entirely predictable, since males from MP-selected lines give batches of progeny varying from 0 to 50 percent female. Lines are being inbred in an attempt to stabilize the factor. This is difficult because of the paucity of females and the accumulation of sterility or lethal factors in these lines.

It is of interest to speculate on possible utilization of the male-producing factor in mosquito control programs. Perhaps breeding methods could be developed for mass rearing of male-producing males. Periodic releases of MP males in urban areas might reduce the number of females below the level required for efficient disease transmission.

In areas where resistance to chlorinated hydrocarbon insecticides is widespread, such a program would be especially effective if the males released also carried genes for susceptibility. Naturally, these hypotheses need to be tested in population cage experiments in the laboratory as well as in field populations (*5*).

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Mechanism of Fibrillation Potentials in Denervated Mammalian Skeletal Muscle

Abstract. This report concerns the origin and mechanism of fibrillation potentials. It is proposed that these potentials do not necessarily arise from degenerated end-plate organs. The precursor is the rhythmic oscillations of the membrane potential. This mechanism may also occur in the case of epileptic discharges from epileptic cells in the cerebral cortex.

An excitable element, such as a nerve cell or a muscle fiber, is said to possess the property of spontaneous activity, if it is capable of discharge at a time during which no electrical energy is supplied from outside (*1*). In denervated skeletal muscle fiber, spontaneous rhythmic fibrillation potentials can be detected. The clinical significance of fibrillation potentials has been repeatedly discussed. This report deals with the underlying mechanism by which these fibrillation potentials are produced. The conclusions presented here stem from three series of experiments previously published.

Two questions may be asked: (i) Do the spontaneous rhythmic fibrillation potentials originate from a specific focal area in the muscle fiber? (ii) Is there any difference in the membrane properties of intact and denervated muscle fibers? The following experiments were performed in order to answer these questions.

The first series of experiments (*2*) was made under hypothermia on rats in which the electrical activity was recorded from the anterior gracilis muscle with intact motor nerve supplies. The second series of experiments (*3*) was made on rats in which the sciatic nerves had been severed 3 to 21 days prior to experimentation. The third series of experiments (*4*) was made on tissue cultures in which embryonic skeletal muscle fibers of the chick were isolated. Glass micropipette electrodes, recording intracellularly from single muscle fibers, were used in all of the experiments. The recording electrode, with a tip measuring approximately 0.2 μ in external diameter, was filled with electrolyte solution and connected by a platinum wire to a cathode follower, then to a direct-coupled amplifier, and finally to an oscilloscope.

In the first series of experiments, in which the motor nerve supplies to the rat muscle fibers were intact, the resting membrane potentials were found to be extremely stable. When the intracellular electrode impaled a muscle fiber near the motor end-plates, miniature end-plate potentials (*5*) were recorded. They were thought to represent asynchronous depolarization of small and restricted areas of the muscle membrane by a release of packages of acetylcholine molecules from the motor nerve endings (*6*). The discharge of these miniature end-plate potentials, presumably arising from different areas, could be synchronously evoked by applying a stimulus to the motor nerve. The synchronous discharge of many miniature end-plate potentials gave rise to an end-plate potential; if the end-plate potential exceeded a certain depolarization level, a spike discharge resulted. The evoked spike discharge seldom repeated itself; thus the response to the stimulus was almost always one-to-one. After the subsidence of the spike discharge, the resting membrane potential resumed its previous level and remained stable. No spontaneous rhythmic spike activity was recorded from the muscle.

In the second series of experiments with denervated skeletal muscle fibers, no miniature end-plate potentials were recorded. However, oscillation of the muscle membrane was often found. The oscillating potentials differed from the miniature end-plate potentials in that they were not all-or-none and random but were graded and rhythmic. If an oscillating potential exceeded a certain magnitude, a spike discharge or a series of rhythmic spike discharges was set off. The frequency of the rhythmic spike discharges was fairly constant in recordings from any given fiber; but occasionally a spike was missing and was replaced by an oscillation of potential. Furthermore, when the membrane potential of a muscle fiber was relatively stable, and when an electrical stimulus was applied to the fiber, the stimulus frequently precipitated a series of rhythmic spike discharges or oscillations. This is different from the responses of muscle fibers with intact motor nerve supplies.

In the tissue culture experiments, series after series of rhythmic activity synchronous with the fibrillary movements of the muscle fiber observed through the microscope were recorded by the intracellular electrode. This spontaneous activity was similar to that recorded from denervated skeletal muscle fibers and consisted of oscillating and spike potentials. The rhythmic oscillations occurred independently but gave rise to the rhythmic spike discharges. Therefore, they were the precursor of these spike discharges. Upon stimulation the cultured muscle fiber responded with a series of rhythmic spike discharges similar to the response of denervated muscle fibers. The response was sometimes a series of rhythmic oscillating potentials superimposed on a sustained depolarization following an initial spike discharge.

Earlier experiments with axons of the squid or lobster showed that oscillating potentials could be produced by sustained membrane depolarization (*7*, *8*). An increase in the oscillation of potential in axons of the squid and cerebral cortical neurons of the cat could be produced readily by veratrine and strychnine (*9*, *10*). These observations were obtained even when no electrical energy was supplied from outside the system. The spontaneous rhythmic spike discharges followed a decrease in the membrane potential and an increase in the oscillating potential.

In denervated mammalian skeletal muscle fibers, Ware, Bennett, and McIntyre also found a decrease in the resting membrane potential (*11*). They reported that the resting membrane potential dropped from a mean value of about −100 to −77 mv 22 days after denervation. Although their findings need to be confirmed (*3*), they are in keeping with the observations that sustained depolarization was accompanied by membrane oscillations or rhythmic spike discharges in nerves of the squid (*7*), lobster and crayfish (*8*, *12*), visual cells of the limulus crab (*13*), and cortical neurons of the cat (*10*).

It is known that a tropic change occurs in muscles deprived of motor nerve supplies. After denervation, an increase in hexokinase and a decrease in cytochrome oxidase activity were observed (*14*). Other metabolic changes remain to be found.

This study of denervated skeletal muscles in vivo and embryonic muscles in tissue culture explants indicates that no presynaptic activity is required for the production of rhythmic fibrillation