density of 10:20 (0.8 L/cm^2) all 1st instar larvae were consumed in 24 h, while at higher densities i.e. 10:40 or 20:40 (1.3 and 1.6 L/cm^2) some young larvae were still present after 24 or 48 h. In a similar study with *A. stephensi*[7] the authors concluded that all the 1st instar larvae were consumed by the 4th instar ones when present together at densities of 0.8 and 1.6 L/cm^2. They also showed that the consumption rate varied significantly with the 4th and 1st instar larval densities. The authors also added that 3-day-old larvae were less readily consumed due to their larger size and/or more vigorous escape attempts. This was not the same with *A. pharoensis* as repeating the previous experiments with the 2nd instar larvae as prey revealed almost the same degree of consumption as observed in the 1st instar, with slight differences. In the case of the 2nd instar larvae, some mortalities were observed among the 4th instar larvae which did not appear with the 1st instar larvae.

Dissection of the predaceous 4th instar larvae showed the presence of some undigested bristles, scales, food brushes and sometimes a semi-complete head capsule in the midgut. It would be interesting for further studies to clarify how these parts are eaten and later on digested, and the effect of such behaviour on the biology of the insects. It is possible that one day we might consider some mosquito larvae as carnivorous insects.

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2 A. Shoukry, Thesis, Ain Shams University, Cairo, 1965.
3 A. Shoukry, in press.
4 M.E. Mac Gregor, J. trop. Med. *18*, 193 (1915).
5 D.N. Roy, Indian J. med. Res. *19*, 635 (1931).
6 S.R. Christophers, *Aedes aegypti,* the yellow fever mosquito. Cambridge Univ. Press, 1960.
7 W.K. Reisen and R.W. Emory, Mosquito News *36*, 198 (1976).

Power frequency electric field induces biological changes in successive generations of mice[1]

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Summary. Electromagnetic fields arising from the electrical power system are pervasively present in the environment. To help evaluate their public-health risk we raised 3 successive generations of mice in a low-strength, 60-Hz electric field. We found that the field caused an increased mortality in each generation, and, altered body weights in the 3rd generation.

Electric and magnetic fields emanating from components of the electric power system - a frequency of 60 Hz in the United States and 50 Hz in Europe - are pervasively present in the environment. With the development of increasingly larger overhead high-voltage transmission lines, the public-health consequences of chronic exposure to such fields has come into sharper focus. Well over 50 groups of investigators have reported biological effects in organisms ranging from amoeba to man following laboratory exposure to an electrical environment similar to that created by a typical high-voltage transmission line[2]. A question consequently arose concerning the degree of risk experienced by individuals who come within the zone of influence of such lines[3] - up to several 100 m[4].

Until the mechanisms of interaction between electromagnetic radiation and biological systems are elucidated, it will be necessary to base human exposure standards for power-frequency fields on an assessment of risk as distilled from appropriate animal studies. This work was intended as one such study.

Methods. Initially, mature male and female Ha/ICR mice were purchased commercially and split into 4 groups - horizontal-exposure, vertical-exposure, horizontal-control, and vertical-control. Mice in the horizontal-exposure group were allowed to mate, gestate, deliver, and rear their offspring in a horizontal 60-Hz field of 3.5 kV/m. At maturity, randomly selected individuals from the 1st generation were similarly allowed to produce and rear their offspring while being continuously exposed. Randomly selected individuals from the 2nd generation then produced the 3rd generation. A parallel procedure was followed for each of the other 3 groups. The vertical-exposure group consisted of 3 generations raised in a 60-Hz field of 3.5 kV/m. The horizontal-control group was raised in the ambient electric field and the vertical-control group was raised in the complete absence of electric fields - otherwise the environment for each group was identical to that of the corresponding exposed group.

The mice were housed in 15×30 cm non-metallic cages contained in 1 of 3 specially constructed units. The horizontal unit held both the exposed and control mice of the horizontal-field study, whereas separate units were built for each of the vertical groups.

Each vertical unit consisted of 3 pairs of shelves; each shelf was a plate of aluminum sandwiched between 2 sheets of wood. The cages were supported between each pair of plates by 2.5 cm thick closed-cell foam rubber - glued directly to the wood insulation of each bottom plate - to negate the possibility of artifacts arising from field-induced vibration. Using this technique we found previously that any interference from vibration can be eliminated, even at much stronger fields than employed here[5]. The cages were centered on the shelves - which were 2.4 m long - with their long axes directed along the 0.6 m shelf width. The metal plate extended to within 2 cm of each shelf edge. In the vertical exposure unit, 1120 V were applied to each pair of plates, thereby producing 3.5 kV/m in the intra-plate region. The plates in the vertical-control unit were grounded.

The mice in this study were housed in a single windowless room of our accredited animal care facility. In this room we measured a 60-Hz field of 2-12 V/m from the lighting and air conditioning systems as well as from other sources. To establish a well-defined baseline for the vertical exposure, we wrapped the cage-containing region with grounded aluminum screening, thereby creating Faraday conditions (zero electric field) for the vertical-control group. This in turn slightly reduced the measured ambient light levels

inside the vertical-control unit. To insure identity of environment the vertical-exposure unit was wrapped with non-metallic screening - which did not perturb the electric field - that had lighttransmission properties identical to those of the aluminum screening.
The horizontal unit consisted of 5 wooden shelves which accommodated 9 cages each. The cages were situated between vertical metal plates which formed a stall-like arrangement. By appropriately grounding or energizing the plates, we could create the exposure and control environments in adjacent stalls. On the 1st, 3rd, and 5th shelf, 3 plates were energized - with 890 V, thereby producing a field of 3.5 kV/m - and 7 were grounded, resulting in 5 exposure stalls and 4 control stalls. On the remaining 2 shelves, 2 plates were energized and 8 were grounded, resulting in 4 exposure stalls and 5 control stalls. This design was utilized to guard against the possibility of interference from some hidden microenvironmental factor. It is perhaps the ultimate practical step to insure that - on the average - the environment of the exposed and control groups is identical but for the applied electric field.
Feeding and watering were ad libitum. Food was placed on the wood bedding, and the water bottle was placed inside the cage with the mice. Because the water was at essentially the same electric potential as the mice - and since the animals' environment was totally non-metallic - no grounding microcurrents were created throughout the exposure period. In the room which housed the animals the light:dark cycle was 12:12, commencing at 06.00 h, the temperature was 22 ± 1 °C, and the humidity - which was not directly controlled - was generally less than 50%.
Breeding was accomplished by allowing 2 females and 1 male to occupy a single cage. When a pregnancy was determined - by abdominal palpation - the male and the 2nd female were destroyed. Pregnant females were placed in individual cages and remained with their offspring until

Table 1. Average body weight at various times after birth of 3 successive generations of mice exposed continuously to 3.5 kV/m at 60 Hz

1st generation		21 days	35 days	49 days	63 days
Vertical control	♂	11.4±2.9	24.6±3.6	29.2±3.1	31.6±3.4
	♀	11.4±3.2	20.7±3.2	23.7±2.3	25.3±3.1
Vertical experimental	♂	11.0±2.5	23.6±3.2	28.0±3.0	30.1±3.6
	♀	10.5±2.0*	19.9±1.9	23.2±2.2	25.6±2.8
Horizontal control	♂	10.2±1.7	24.1±2.3	28.2±2.4	30.4±2.3
	♀	10.2±2.0	21.1±1.7	23.5±1.5	24.8±2.2
Horizontal experimental	♂	10.5±2.8	23.6±3.6	28.9±2.6	31.8±4.3
	♀	10.4±2.8	20.2±3.6	23.6±3.0	25.7±2.6
2nd generation		**21 days**	**48 days**	**70 days**	**108 days**
Vertical control	♂	13.8±1.2	29.0±5.0	35.0±2.6	38.0±3.4
	♀	13.8±1.3	25.6±2.7	27.2±2.3	29.6±4.0
Vertical experimental	♂	14.1±1.5	30.3±3.0	35.5±2.9	37.3±3.9
	♀	13.9±1.7	24.8±2.4	27.2±2.4	29.0±2.4
Horizontal control	♂	14.2±1.6	31.0±2.3	34.0±4.1	35.5±4.2
	♀	14.1±1.4	24.8±2.4	26.9±2.3	29.1±3.2
Horizontal experimental	♂	13.9±2.0	31.4±2.6	31.8±3.4*	36.8±3.1
	♀	14.2±1.6	25.8±1.9*	27.9±2.4	29.6±2.4
3rd generation		**21 days**	**49 days**	**63 days**	**119 days**
Vertical control	♂	14.6±1.5	29.6±3.1	32.7±3.0	40.7±4.8
	♀	14.3±1.5	24.9±1.8	26.6±2.2	31.8±3.6
Vertical experimental	♂	15.6±2.0*	30.4±4.5*	34.5±3.1	41.4±5.5
	♀	14.7±1.4	25.9±2.3*	26.2±3.1	31.5±4.3
Horizontal control	♂	14.0±2.1	29.9±3.1	32.4±3.3	39.2±4.9
	♀	13.4±1.8	24.6±2.8	25.0±2.3	28.6±2.5
Horizontal experimental	♂	14.6±1.6*	31.1±2.5*	32.3±3.4	40.8±5.3
	♀	14.5±2.1*	25.7±2.5*	26.9±2.1*	30.9±3.6*

In g, with SD. * $p<0.05$.

Table 2. Mortality in each generation. Statistical significance determined by χ^2 test. Mortalities not shown did not differ significantly

	Number born	Number alive at 21 days	108 days	119 days	Number of deaths due to field	Percent deaths due to field	Significance
1st generation							
Vertical control	284	236			38	14	$p \cong 0.01$
Vertical experimental	263	181					
Horizontal control	235	205			25	11	$p<0.001$
Horizontal experimental	234	179					
2nd generation							
Vertical control		114	110		6	6	$p<0.05$
Vertical experimental		107	97				
3rd generation							
Vertical control		126		119	11	10	$p<0.001$
Vertical experimental		101		84			

weaning at 21 days after birth. In the 1st generation, following weaning and weighing at 21 days after birth, the number of animals in each group was approximately halved to facilitate long-term exposure of the remaining animals. In the latter 2 generations, however, the necessary reduction in numbers was accomplished at 1 day after birth, at which time each litter was reduced to 6 animals. Because of this, the lactation index was identical for each litter in the latter 2 generations.
On the 119th day after the birth of the 3rd generation, 17 major organs were recovered for microscopic examination from each of 10 males and 10 females of each of the 4 groups. These results will be reported later. The totality of the procedures and precautions which we followed were based on the experience we gained from our prior similar study[6], and accommodated all methodological considerations brought to our attention[7].

Results. The results are presented in tables 1 and 2. In the 1st generation the 4 groups delivered 21-24 litters with an average of 11.1-12.5 pups per litter. No consistent effect on body weight attributable to the electric field was seen throughout the 63-day term of observation (table 1). But in both exposed groups infant mortality was increased (table 2). In the vertical-control group, 48 animals - about 17% - died between birth and weaning. In the vertical-exposure group, if the electric field wasn't a causative factor, a 17% mortality should also have been seen. But that group exhibited a 31% mortality - 82 animals died and not the expected 44. Thus 38 animals, about 16% of those born, failed to live to weaning because of the electric field. A similar result was obtained in the horizontal-exposure group - about 11% of the animals born failed to live to weaning because of the electric field.
In the 2nd generation the groups delivered 19-22 litters, each of which was reduced to 6 pups within 24 h after birth. Despite this, no consistent effect on body weight attributable to the field was seen throughout the 108-day observation period (table 1). The vertical-exposure group, however, exhibited a higher mortality; about 6% of the animals alive at weaning failed to live to the final day of observation because of the electric field.
In the 3rd generation each group delivered 21 litters, except the vertical-control group which delivered 23. The litters averaged 10.1-11.3 pups, and each was reduced to 6 within 24 h of birth. The exposed animals had higher body weights, particularly in the horizontal-exposure group. At 49 days after birth the males and females in each exposed group were significantly heavier than their respective controls. At 119 days after birth only the females in the horizontal-exposure group were significantly heavier - but this was part of a consistent trend for that group. Again we saw an increased mortality in the vertical-exposure group - 10% of the weaned animals failed to survive to the end because of the electric field (table 2).

Discussion. In the 1st generation about 14% of the vertically-exposed newborn mice and 11% of the horizontally-exposed newborn mice died within 3 weeks as a result of field exposure. In the 2nd and 3rd generations, 6% and 10% respectively of the vertically-exposed mice alive at 21 days of age failed to survive the approximately 4-month observation period because of the electric field. We found no signs of morbidity, nor evidence for any common, specific cause of death. Detailed histological studies of tissues from the 3rd generation are incomplete, and may shed light on the issue. Pending that data, the increased mortality must be ascribed to a nonspecific action of the electric field - the field produced a biological stress which exceeded the capacity of a susceptible subgroup of the population resulting in death - because that is the simplest explanation.
The ability to produce a systemic response - death is 1 example - is the hallmark of a biological stressor[8]. The theory of biological stress is quite general and is distinct from sub-cellular or molecular theories of the interaction between the stressor and the organism. But now, while low-frequency field studies are in their infancy, stress is a useful framework within which to attempt an understanding of the great variety of biological effects such fields have produced[2,9-11].
In the 3rd generation, males and females in both exposed groups were heavier than the corresponding control groups, but the body weights of the animals in the 1st and 2nd generations were not altered by the electric field. We showed previously that at 10-15 kV/m the 1st and 2nd generations of exposed animals were lighter than the control animals, and that the 3rd generation reflected this pattern[5]. The 2 multigeneration studies clearly show that the simple physical model of a linear dose-response relationship for low-frequency field bioeffects[12,13] is incorrect.
The tendency towards heavier body weights in animals chronically exposed to relatively low-strength fields - reported here and found by others[10] - is not understood. If a generalized stress response is involved, the excess body weight may be due to adrenal corticosterone secretion and its stimulation of renal sodium resorption[14] with concommitant water retention.

1 This work was supported by the National Institute of Environmental Health Sciences, Department of Health, Education and Welfare, the Environmental Protection Agency, and the Veterans Administration.
2 A.A. Marino and R.O. Becker, Physiol. Chem. Phys. *9,* 131 (1977).
3 A.A. Marino and R.O. Becker, Environment *20,* No.9, 6-15, 40 (1978).
4 F.X. Hart and A.A. Marino, IEEE Trans. Biomed. Eng. *BME-24,* 493 (1977).
5 A.A. Marino, T.J. Berger, B.P. Austin, R.O. Becker and F.X. Hart, Physiol. Chem. Phys. *9,* 433 (1977).
6 A.A. Marino, R.O. Becker and B. Ullrich, Experientia *32,* 565 (1976).
7 Testimony of Solomon Michaelson, Morton Miller, Edwin Carstensen, and Herman Schwan on behalf of the Power Authority of New York, the Niagara Mohawk Power Corporation, and the Rochester Gas and Electric Corporation before the Public Service Commission of New York in the Common Record Hearings on Health and Safety of 765 kV Transmission Lines, 1974-1978.
8 H. Selye, Stress. ACTA, Montreal 1950.
9 A.A. Marino, J.M. Cullen, M. Reichmanis and R.O. Becker, in: Proceedings of the 18th Annual Hanford Life Sciences Symposium: Biological Effects of Extremely-Low-Frequency Electromagnetic Fields. U.S. Department of Energy, Washington, in press (1979).
10 J.D. Grissett, J.L. Kupper, M.J. Kessler, R.J. Brown, G.D. Prettyman, L.L. Cook and T.A. Griner, Exposure of Primates for One Year to Electric and Magnetic Fields Associated with ELF Communications Systems, NAMRL-1240. Naval Aerospace Medical Research Laboratory, Pensacola, Florida 1977.
11 Yul.D. Dumanskiy, V.M. Popovich and G. Prokhvatilo, Ind. Hyg. *8,* 19 (1976).
12 N.S. Mathewson, G.M. Oosta, S.G. Levin, M.E. Ekstrom and S.S. Diamond, Extremely Low Frequency Vertical Electric Field Exposure of Rats: A Search for Growth, Food Consumption and Blood Metabolite, Alterations. U.S. Defense Nuclear Agency, Bethesda, Maryland 1977.
13 R.D. Phillips, W.T. Kaune, J.R. Decker and D.L. Hjeresen, Biological Effects of High Strength Electric Fields on Small Laboratory Animals. Battelle Pacific Northwest Labs, Richland, Washington 1976.
14 J. Tepperman, Metabolic and Endocrine Physiology. Year Book Medical Publ., Chicago 1974.