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Embryological changes induced by weak, extremely low frequency electromagnetic fields

JOSÉ M. R. DELGADO, JOCELYNE LEAL, JOSÉ LUIS MONTEAGUDO AND MANUEL GARCÍA GRACIA

Departamento de Investigación, Centro 'Ramón y Cajal', Madrid, Spain

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INTRODUCTION

The effect of magnetic fields on growth, which was first observed in plants by Ssawastin (1930), has been investigated during the last two decades mainly in mammals, with inconsistent results. In some cases, magnetic fields seem to accelerate growth or the index of mitosis (Mericle *et al.* 1964; Novitskii, 1966), but more often a delay in growth has been reported (Barnothy, 1963; Neurath, 1968) as well as metabolic inhibition (Cook, Fardon & Nuttini, 1969; Malinin, Gregory, Morelli & Ebert, 1979; Shternberg, 1966).

The hope that magnetic fields could induce regression or total inhibition of tumoral cells (Barnothy, 1964; D'Souza, Reno, Nuttini & Cook, 1969; Kim, 1976) has remained controversial and unsubstantiated by other investigators (Biggs, 1979; Hall, Belford & Leash, 1964; Halpern & Green, 1964; Mulay & Mulay, 1961). In these studies, the magnetic fields used were generally static and of intensities 40 to 10^6 higher than the earth's field. In early chick embryos treated with magnetostatic fields of 31 Gauss, pathological effects were observed in 15 % of the cases (Veneziano, 1965) and much higher intensities (up to 1000 Oe) were needed to influence development in the frog (Neurath, 1969). Differential effects have been described for enzymic reactions, and a field of 3200 Oe may modify the enzyme-substrate activity of DNAase without influencing the RNAase. According to several reports, intermittent electromagnetic fields are more effective than static fields (Adey, 1979; Bawin & Adey, 1976; Cook *et al.* 1969; Tabrah, Guernsey, Chou & Batkin, 1978).

The interest in possible biological effects of extremely low frequency magnetic fields (ELMF) (1–1000 Hz) has increased in recent years due to the growing problem of technological contamination of the environment and the social awareness of the need to preserve our ecology. As mentioned by Kim (1976), we live in an environment which has geomagnetic fields of about 0·5 Oe and magnetic pollution from countless sources. The use of electrical energy lines could cause detrimental biological effects, but available data indicate that lines of up to 800 kV do not constitute a danger for man (Cabanes, 1980). In a recent colloquium on risks due to electricity, it was concluded that "overhead lines conducting up to 1·5 million volts will not cause any noticeable effects" (Schaefer, 1980). The establishment of land based, 50–100 km long transmitting antenna by the United States military for extreme low frequency communication with atomic submarines represented a biological risk and was one of the first military projects evaluated by a public scientific forum, as reported by Grissett (1980).

According to available information, ELMF do not influence fertility, genetic development or growth, while their possible mitotic modifications in a slime mould

are controversial. Circadian rhythms and insect behaviour have also been considered to be unaffected by ELMF. Some behavioural, neurophysiological and neurochemical effects have been reported in mammals, but they require high intensities and present evidence indicates that no potential hazard exists at exposure levels of 0·02 mili-Tesla and 0·07 V/m (Grissett, 1980). Rhesus and squirrel monkeys have shown no behavioural effects when exposed to ELMF with frequencies from 7 to 75 Hz and intensities of 3–10 Gauss or electrical fields from 1 to 29 V/m (rms) (de Lorge, 1972, 1973*a*, *b*).

Within the central nervous system, electrical transmission is performed through the specific gap junctions, but field interactions may exist in a neural pool and ephaptic transmission of impulses is possible without synapsis between axons artificially placed in contact (Arvanitaki, 1942). In the brain, field effects may be important, depending on the orientation and electrical characteristics of the neurons involved, as well as on the properties of the surrounding tissues. In this way, excitation or inhibition of postsynaptic targets would be possible (Korn & Faber, 1980). One model used for the study of neuronal field effects is the paired Mauthner cell of the teleost medulla, which mediates the fast escape reaction of many fish (Furukawa & Furshpan, 1963).

To investigate the many parameters and mechanisms which may intervene in biological effects of ELMF, a reliable model was needed, if possible with multiplicity of systems able to react at low levels of energy which would yield results in a short period, facilitating performance of many experiments. As reported in the present paper, the model of fertilized chicken eggs in the first stages of development (48 hours) seems to be a sensitive and suitable preparation.

MATERIALS AND METHODS

Fertilized eggs from white Leghorn hens were placed in a Memmert incubator at 38 °C and 55 % humidity for 48 hours. A total of 68 eggs was used. In each experiment, two eggs were placed inside a coil 55 mm in diameter and 110 mm long, made of 0·1 mm enamelled copper wire forming an electromagnetic exposure chamber. A third egg was placed as a control in the same incubator 20 cm from the coil. Electromagnetic fields were created in the coil by currents provided by a Grass S88 stimulator, using rectangular waves of 0·5 msec pulse duration, with the intensities and frequencies indicated in Table 2. Magnetic field values in the centre of the coil were calculated as: $B_0 = i \times 8.10^{-3}$ Teslas $= i \times 80$ Gauss, where i represented the intensity of electrical current applied to the coil. Thus, an intensity of 0·015 mA provided by the Grass stimulator corresponded to 0·12 micro Teslas (μT) and therefore 0·15 mA = 1·2 μT, and 1·5 mA = 12 μT.

Two similar experiments (a total of six eggs) were conducted simultaneously, activating two coils with the same Grass stimulator coupled by a series capacitor for the zero mean field. Current parameters were continuously monitored by a Tektronix 5113 oscilloscope.

After 48 hours of incubation, the embryos were expected to have reached stages 11–12 of the Hamburger & Hamilton (1951) scale and the eggs were opened for examination, immersing the yolk in Tyrode solution and removing the chick embryo.

For morphological studies, all embryos were fixed with Carnoy's solution (absolute ethanol 60 %, chloroform 30 %, glacial acetic acid 10 %) and were observed at $\times 30$ magnification through a Nikon binocular stereomicroscope. Pictures were taken with a photographic attachment.

Table 1. *Malformations in controls and in chick embryos exposed to magnetic fields (ELMF)*

Organ	Controls (n = 26)			Exposed to ELMF (n = 42)		
	Malformed embryos	%	S.E.	Malformed embryos	%	S.E.
Cephalic nervous system	3	11·5	6·4	33	78·5	6·3
Truncal nervous system	2	7·7	5·3	26	61·9	7·5
Heart	2	7·7	5·3	23	54·8	7·7
Vessels	3	11·5	6·4	28	66·7	7·3
Somites	3	11·5	6·4	24	57·1	7·6

n = total number of samples; S.E. = standard error.

For histological studies after fixation, the embryos were dehydrated through an alcohol series and embedded in paraffin. Sections transverse to the main axis of the embryo were cut serially in slices 7 μm thick with a rotary Leitz microtome, mounted and stained with alcian blue pH 3 (Gabe, 1968) and PAS (Luna, 1968) and then contrasted with haematoxylin. The slides were later examined microscopically. The total number of control embryos was 26 and there were 42 experimental embryos exposed to ELMF.

RESULTS

(I). *Morphological classification of embryos*

After examination of the embryo for gross form, a microscopic study was performed of the prepared slides in order to analyze the development and organization of (*a*) the cephalic nervous system; (*b*) the truncal nervous system; (*c*) the heart; (*d*) vascularization; (*e*) somites.

According to these data, each embryo was classified as normal (N) or as abnormal (A) in each of the five morphological characteristics. Tabulated results are presented in Tables 1 and 2. Criteria for normality required embryological development to stages 11–12 of the Hamburger & Hamilton series, and normality of the following gross morphological features: the cephalic nervous system showing the three primary vesicles with the anterior neuropore closed; the optic vesicles constricted at the base; and the auditory pits wide open. The truncal nervous system was already closed. In the opaque area, blood island and vessels were well developed; the heart was large, bent to the right and S-shaped. The number of somites was between 13 and 16. Criteria for abnormality required retarded embryological development without reaching stage 9, plus evident deviations in any of the five above mentioned morphological characteristics (*a* to *e*).

(II). *Gross morphological features of the embryos*

(A) *Controls*

As shown in Tables 1 and 2A, 22 of the 26 controls displayed normal development (84·6 %). Abnormalities in the other four controls were: One untreated embryo in the ELMF series exposed to 10 Hz/1·2 μT was abnormal in all its morphological features. A second control embryo from the series of 1000 Hz/0·12 μT had deficits in its cephalic nervous system and number of somites. A third control, also from the 1000 Hz/0·12 μT series, was abnormal only in vascularization and number of

Table 2A. *Gross morphological evaluation of control chick embryos*

Frequency (Hz) ...	10			100			1000		
Int. (μT) ...	0·12	1·2	12	0·12	1·2	12	0·12	1·2	12
Cephalic nervous system	N	A	N		N	N	A	N	N
			N		N	N	N		N
			N		N	N	N		N
			N			N	N		N
						N			A
						N			
						N			
Truncal nervous system	N	A	N		N	N	N	N	N
			N		N	N	N		N
			N		N	N	N		N
			N			N	N		N
						N			A
						N			
						N			
Heart	N	A	N		N	N	N	N	N
			N		N	N	N		N
			N		N	N	N		N
			N			N	N		N
						N			A
						N			
						N			
Vessels	N	A	N		N	N	N	N	N
			N		N	N	N		N
			N		N	N	A		N
			N			N	N		N
						N			A
						N			
						N			
Somites	N	A	N		N	N	A	N	N
			N		N	N	N		N
			N		N	N	A		N
			N			N	N		N
						N			A
						N			
						N			
Totals:	6 embryos			10 embryos			10 embryos		

These embryos were *not* exposed to MF but corresponded to the exposed series as indicated in Table 2B. N = Normal. A = Abnormal. In each organ, each N and A = one embryo. Int. = Intensity in micro Teslas (μT).

somites. The fourth abnormal control belonged to the series of 1000 Hz/12 μT and had deficits in all its morphological features. It should be emphasized that all controls were normal in the 100 Hz series and only one was abnormal in the 10 Hz series.

(B) *Embryos exposed to magnetic fields*

As shown in Tables 1 and 2B, 33 of the 42 embryos exposed to ELMF of different frequencies and intensities had detectable abnormalities in their development, representing 78·5 % of the total sample. Compared with controls, these results were statistically significant ($P < 0{\cdot}001$).

Table 1 also indicates that the cephalic nervous system was the most affected by

Table 2B. *Gross morphological evaluation of chick embryos exposed to ELMF of the indicated frequencies and intensities*

Frequency (Hz) ...	10			100			1000		
Int. (μT) ...	0·12	1·2	12	0·12	1·2	12	0·12	1·2	12
Cephalic nervous system	N N A	A A N A	A N N N A	A A N N	A A A A A	A A A A A A A A A A	A N A	A A A A	A A A A
Truncal nervous system	N N A	A N N N	N N N N A	A A N N	A A A A A	A N A A N N A A A	A A A	A A A A	A A N N
Heart	N N N	A N N N	N N N N N	N A N N	A A A A A	A N A A N N A A A	A N A	A A A A	A A N A
Vessels	N N N	A N N N	A N N N A	A A A A	A A A A A	A N A A N N A N A	A N A	A A A A	A A A A
Somites	N N N	N N N N	N N N N N	A A N N	A A A A A	A N A A N N A A A	A A A	A A A A	A A N A
Totals:	12 embryos			19 embryos			11 embryos		

Abbreviations: as in Table 2A.

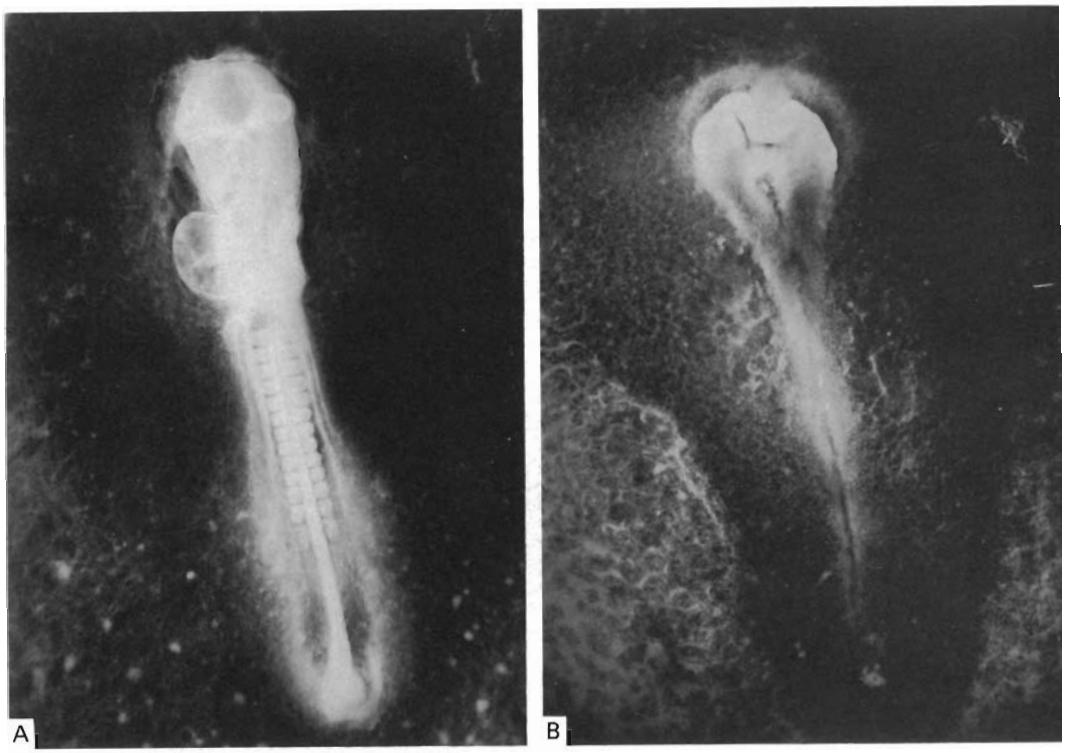

Fig. 1. (A) Unexposed chick embryo (control) after 48 hours incubation. × 25. (B) Embryo after 48 hours incubation and exposure to a magnetic field (ELMF) of 100 Hz/1·2 μT. × 35.

ELMF (78·5 %), while development of the heart was least disturbed (54·8 %), although both malformations were highly significant. The truncal nervous system was less affected (61·9 %) than its cephalic part. These general results were further analyzed with respect to different frequencies and intensities.

(1) *Exposure to* 10 *Hz*. Exposure of the incubating eggs to the lowest frequency (10 Hz) and lowest intensity (0·12 μT) did not produce any abnormality in the development of the heart, vascular system or somites, and malformations of cephalic and truncal systems were found in only one embryo (Table 2B). Although the sample was too small (n = 3) to draw conclusions, the trend toward normality was evident, especially when comparing results of different series.

In this frequency range of 10 Hz, somites were not affected even by the highest intensity used (12 μT). The heart was abnormal in only one embryo exposed to 1·2 μT and was normal in all cases of exposure to 12 μT. Both cephalic and truncal nervous systems presented cases of abnormality with the three tested intensities. The differences in nervous system malformations were not proportional to the 100-fold difference (from 0·12 to 12 μT) in the total amount of energy received by the eggs.

(2) *Exposure to* 100 *Hz*. The most marked and uniform effects on embryological development were obtained with 100 Hz/1·2 μT (Table 2B). All embryos (n = 6) so treated had malformations in all organs studied. Macroscopic examination revealed that development was considerably delayed. No embryo advanced beyond stage 6 of the Hamburger & Hamilton scale; the cephalic nervous system was

Table 3. *Percentage of abnormalities in each of the five organs studied in embryos not exposed (controls) and exposed to ELMF, summarizing the data of all intensities*

	10 Hz		100 Hz		1000 Hz	
	Control ($n = 6$)	Exposed ($n = 12$)	Control ($n = 10$)	Exposed ($n = 19$)	Control ($n = 10$)	Exposed ($n = 11$)
Cephalic nervous system	16·6±16·6	50±15	0	89±7·4	20±13·3	90±9·5
Truncal nervous system	16·6±16·6	25±13·1	0	73±10·4	10±10	81±12·4
Heart	16·6±16·6	8±8·2	0	68±11	10±10	81±12·4
Vessels	16·6±16·6	25±13·1	0	78±9·8	20±13·3	90±9·5
Somites	16·6±16·6	0	0	73±10·4	20±13·3	90±9·5

Abbreviations as in Table 1.

Table 4. *Percentage of abnormalities in each of the five organs studied in embryos not exposed (controls) and exposed to ELMF, summarizing the data of all frequencies*

	0·12 μT		1·2 μT		12 μT	
	Control ($n = 5$)	Exposed ($n = 10$)	Control ($n = 5$)	Exposed ($n = 14$)	Control ($n = 16$)	Exposed ($n = 18$)
Cephalic nervous system	20±20	50±16·6	20±20	92±7·5	6·2±6·2	83±9·1
Truncal nervous system	0	60±16·3	20±20	78±11·5	6·2±6·2	50±12·1
Heart	0	30±15·3	20±20	78±11·5	6·2±6·2	50±12·1
Vessels	20±20	60±16·3	20±20	78±11·5	12·5±8·5	61±11·8
Somites	40±25	50±16·6	20±20	71±12·6	12·5±8·5	44±12·0

Abbreviations as in Table 1.

reduced to a thickening of the asymmetric ectodermal tissue and the truncal nervous system was represented by only a thin plate (see Figs. 1B, 3A, B, C).

All embryos ($n = 9$) tested at 12 μT also had malformed cephalic nervous systems but the effects were less constant in other organs, and several embryos appeared normal in some respects (Table 2B). At lower intensities (0·12 μT), the teratogenic effect of the ELMF also lacked uniformity and some organs seemed normal in several cases. The vascular system, however, was abnormal in all four embryos incubated at 0·12 μT.

(3) *Exposure to* 1000 *Hz.* All organs in all the exposed embryos were malformed when 1·2 μT intensity was employed, whereas with lower (0·12 μT) or higher (12 μT) intensities, results were variable and some embryos appeared normal (Table 4).

(III) *Histological analysis*

A more detailed study of ELMF effects was made by microscopical examination of sectioned embryos.

(A) *Controls*

Normal chick embryos of 48 hours had clearly differentiated optic vesicles as well as a subdivision of the prosencephalon into telencephalon and diencephalon, with persistence of the mesencephalon and the initial subdivision of the rhombencephalon into metencephalon and myelencephalon. The auditory pits were visible. A transverse section at the level of the heart revealed a well developed neural tube (see

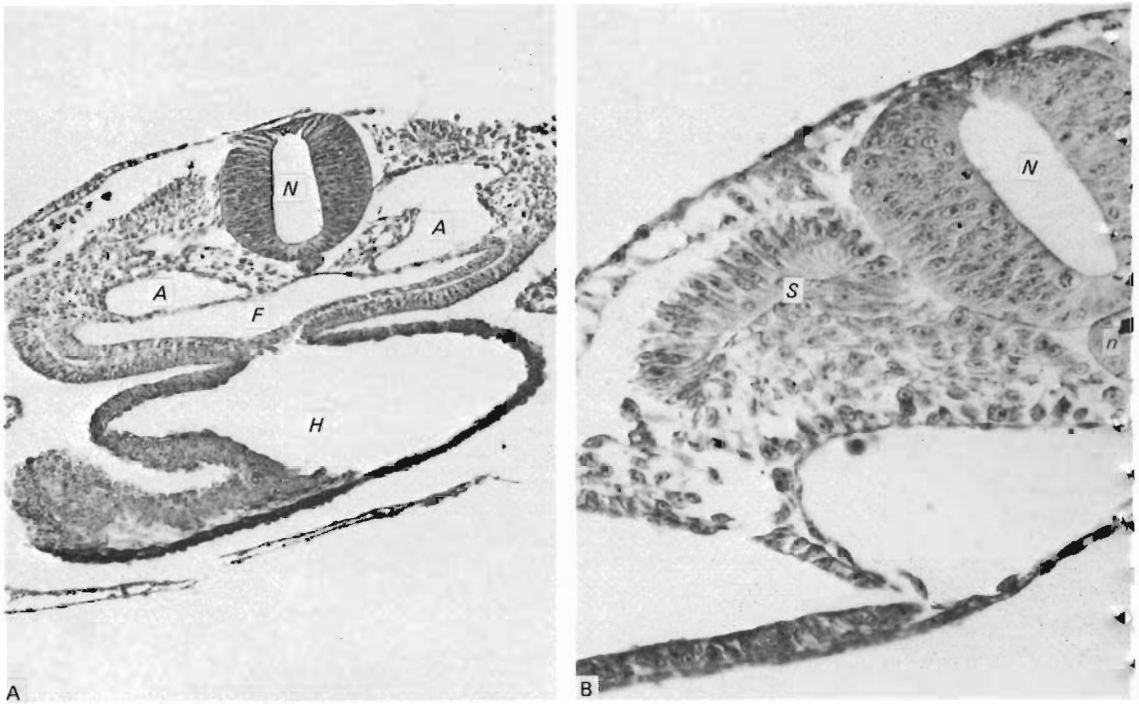

Fig. 2. (A) Control embryo: transverse section at the level of the heart. ×200. (B) Control embryo: transverse section through the 3rd-4th somite. ×500. *N* neural tube; *A*, aorta; *F*, foregut; *H*, heart; *S*, somite; *N*, notochord.

Fig. 2A, *N*) with an elliptical cavity limited by thickened lateral walls. Below and at both sides of the neural tube, the aortic arches (*A*) were evident, and below and medial to them, the primitive gut had acquired a floor and its subcephalic fold had progressed to constitute the foregut (*F*). The heart bulged, forming a still undivided loop occupying a large space in the preparation (*H*). A section at the level of the 3rd–4th somite in a normal 14 somite embryo showed (Fig. 2B) that the neural tube was closed and well organized; the somite (S) was clearly limited in its upper part, while its ventromedial face had lost its originally well defined boundary, merging with the central core of the sclerotome cells, extending toward the notochord.

(B) *Embryos exposed to magnetic fields*

Since morphological studies showed that the greatest embryological effects were produced with 100 Hz/1·2 μT, histological results from embryos exposed to this treatment will be presented first, followed by findings in others exposed to the greater intensity of 12 μT, the greater frequency of 1000 Hz, and the lowest frequency of 10 Hz.

(1) *Effects of* 100 *Hz*/1·2 μ*T*. The embryos exposed to ELMF of 100 Hz/1·2 μT showed drastic changes in their histological structure corresponding to the alterations in their gross form. They showed a lack of morphogenesis where only the three primitive layers and some thickening of neuroectoderm were apparent. There was little cellular differentiation, and a lack of organization of cells, without mutual cohesion, resulted in a fragile tissue which broke easily during microscopic preparations.

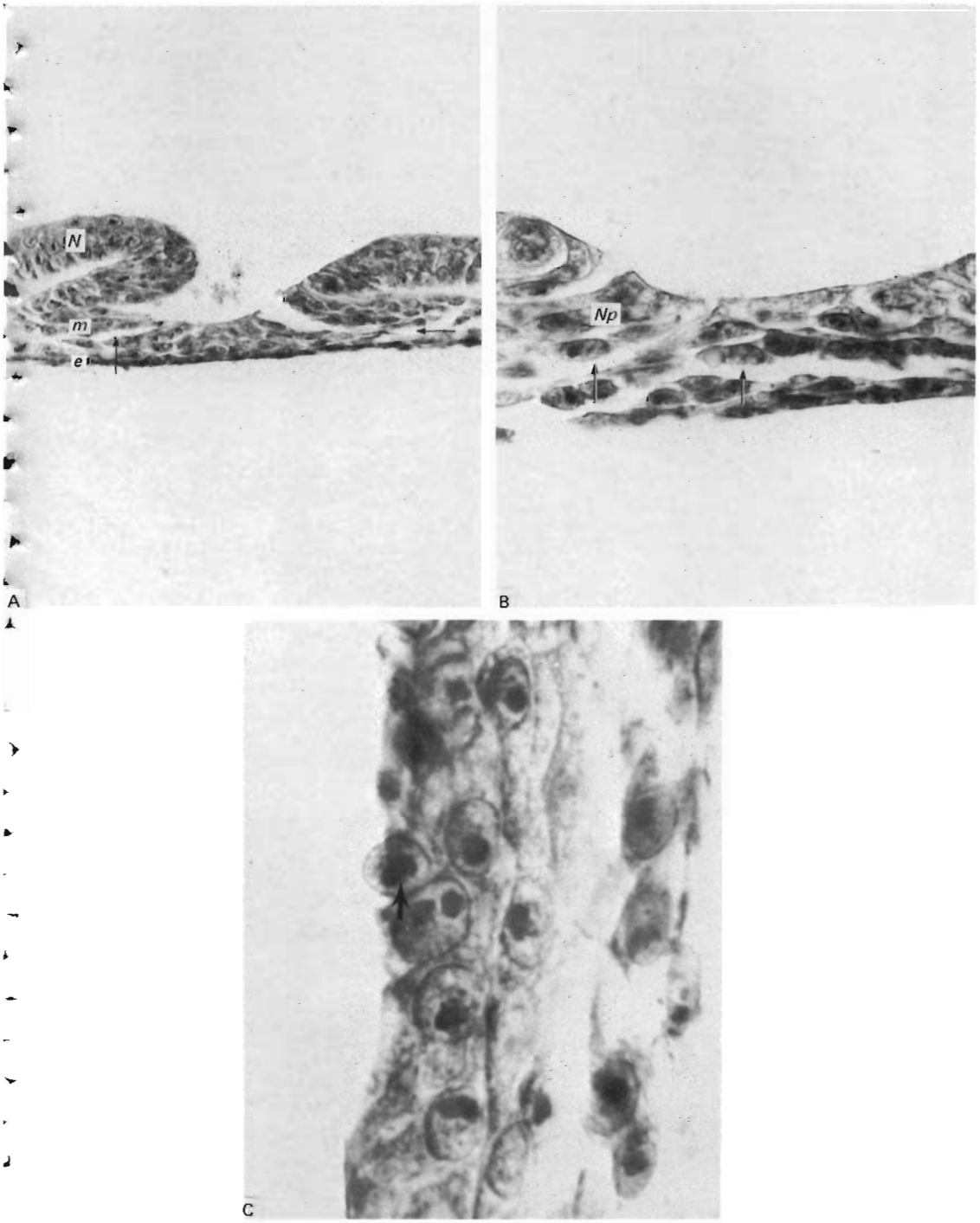

Fig. 3. Embryo exposed to ELMF 100 Hz/1·2 μT. (A) Transverse section through the cephalic region. × 500. Histological development has been confined to three levels without the formation of organs. (B) Transverse section through the truncal region. × 500. There is a very primitive development without formation of organs, and abnormal acellular spaces are indicated by arrows. (C) Neural ectoderm from the same specimen at higher magnification showing dense nucleoli, acellular spaces and abnormal appearance of the cells. × 2500. *N*, neural tube; *m*, mesenchyme; *e*, endoderm; *Np*, neural plate.

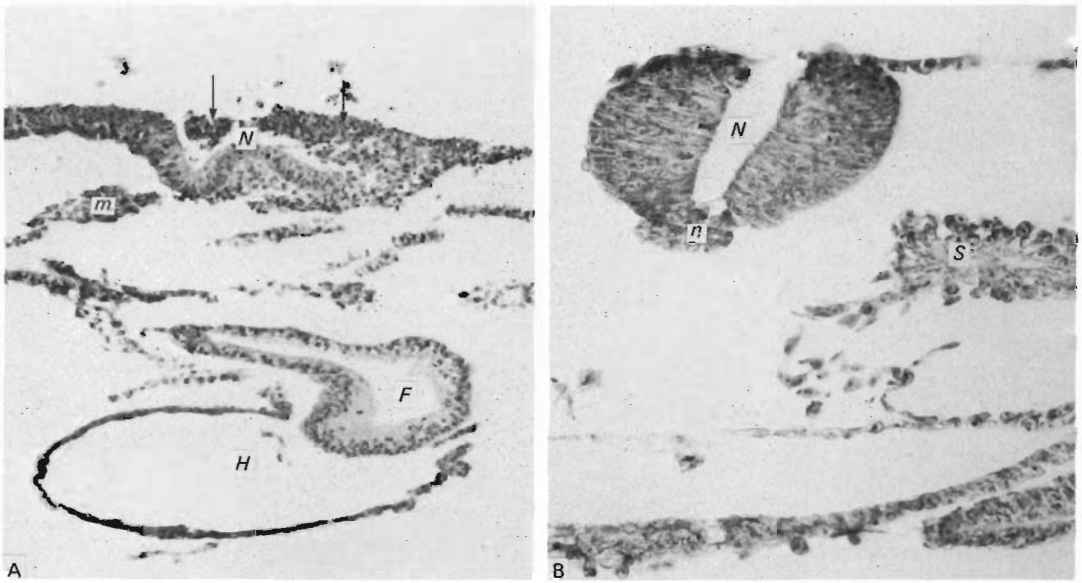

Fig. 4. (A) Embryo exposed to 100 Hz/12 μT. Transverse section at the level of the heart. $\times$200. This Figure may be compared with the control in Fig. 2(A). (B) Transverse section through the truncal region. $\times$500. This Figure may be compared with the control in Fig. 2(B).

The neuroectoderm had not developed into a nervous system. The folds of the cephalic region rose but did not join each other to form a neural tube (see Fig. 3A). The brain vesicles were therefore absent. The auditory pit did not exist. Some spaces in the mesenchyme (marked by arrows in Fig. 4A) were empty and devoid of extracellular matrix material.

Sections, cut at the level where the first somites should have been, revealed a neural plaque with only elemental characteristics, and abnormal spaces lacking cells (marked with arrows in Fig. 3B). There was no evidence of the beginning of a heart. The regional thickening of the splanchnic mesoderm, which normally starts after about 25 hours of development, was absent. There was no fusion of the mid-region of the endocardial primordia to form a single tube and blood vessels did not exist.

The primitive gut, which normally starts to acquire a cellular floor in its cephalic region and then progresses caudally, could not be detected in these embryos.

Alcian blue (pH 3) staining of the preparations revealed the absence of glycosaminoglycans which are essential for cell development.

Observation of the neural ectoderm at higher magnifications (Fig. 3C) revealed cells with nucleoli of high density, suggesting a low metabolic cellular activity. The preparations also showed dispersed areas of local necrosis, demonstrating the lethal power of the applied ELMF.

(2) *Effects of* 100 *Hz*/12 μT. Experimental results showed that gross form was less affected, and microscopic examinations confirmed that the disturbances, although evident, were less pronounced than those caused by 1·2 μT.

In the four treated embryos, the nervous system, heart, vessels and somites could be identified but their development was considerably delayed, and a variety of abnormalities were evident (Fig. 4A). The formation of a neural tube (*N*) had begun, but it had not closed and was of abnormal form, with underdeveloped organization and with its lumen invaded dorsally by disorganized neuroectodermic cells. In only

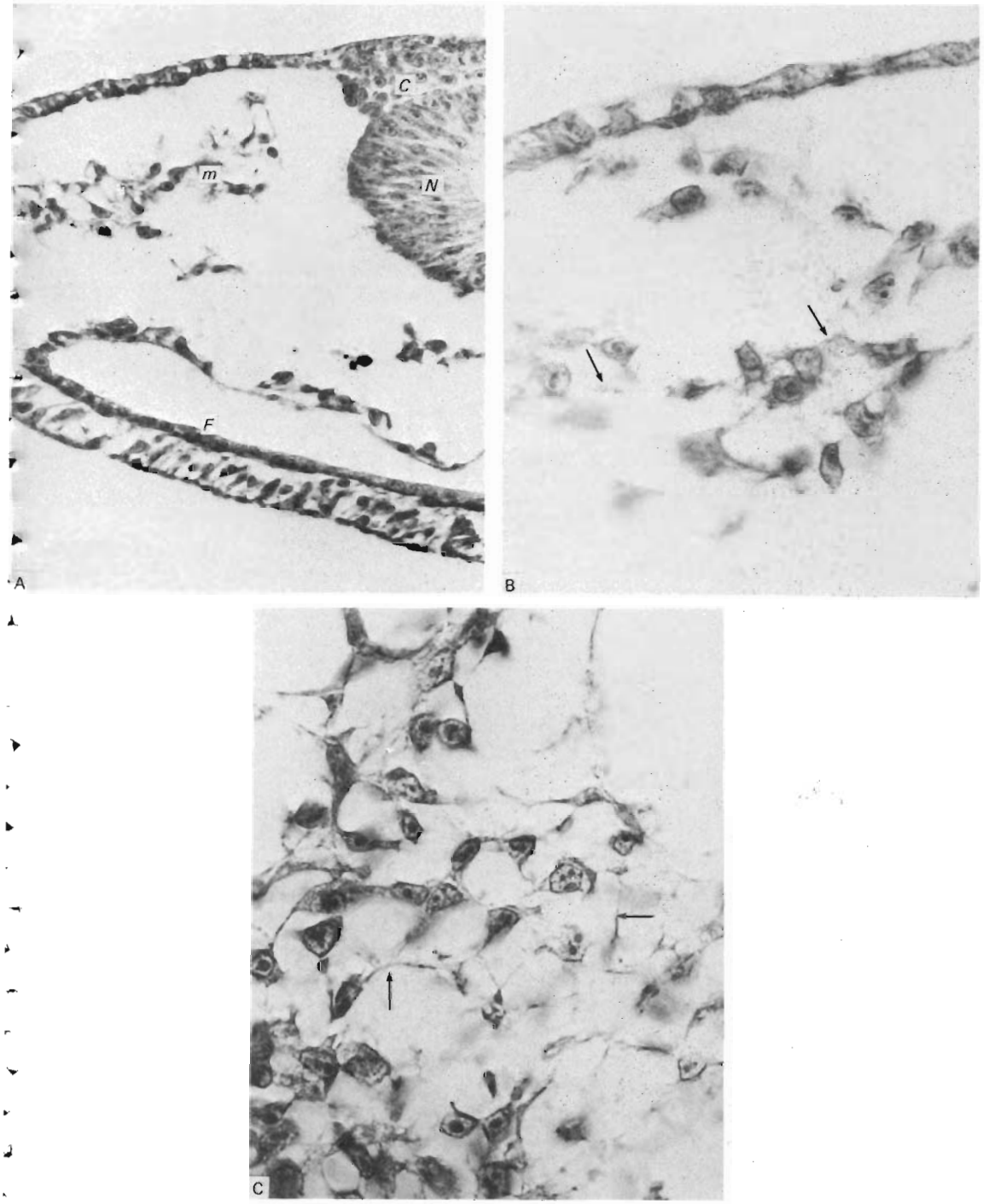

Fig. 5. (A) Embryo exposed to 1000 Hz/12 μT. Transverse section through the foregut. C, neural crest. $\times$500. (B) Embryo exposed to 1000 Hz/12 μT. High magnification of the cephalic mesenchyme. $\times$1250. Arrows indicate disorganized glycosaminoglycans. There are cells of abnormal shape and acellular spaces. (C) Control embryo. Similar region and magnification as in (B), demonstrating the normal appearance of cells and glycosaminoglycans.

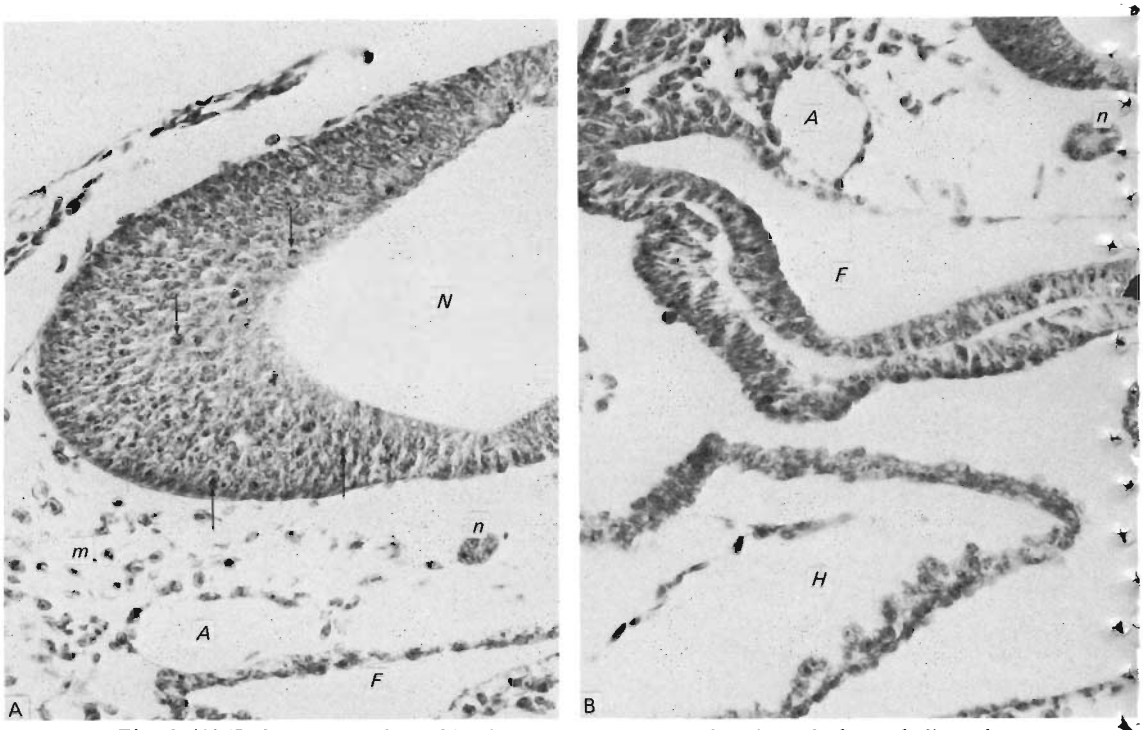

Fig. 6. (A) Embryo exposed to 10 Hz/12 μT. Transverse section through the cephalic region. × 500. Arrows indicate dividing cells. (B) Embryo exposed to 10 Hz/12 μT. Transverse section through the heart. × 500.

one embryo was the anterior neuropore (which normally closes by the 34th–35th hour) already closed, but it presented asymmetries and dislocated neural walls. The primitive pharynx tissue also showed signs of disorganization. The foregut was asymmetrical and had abnormal walls. In the vascular system, there was no aortic differentiation and the heart was only an empty chamber with thin and primitive walls.

Other results of the typical delay in development were disorganization, empty spaces, necrotic areas and a few mesenchymal cells which tended to group together (Fig. 4A). Sections cut at lower somitic levels showed a relatively well developed (although not closed) neural tube. The notochord was present although less organized and developed than normal and lacking its outer sheath. There was no somitic differentiation and only a mesoblastic segmentation could be identified (Fig. 4B).

In the whole embryo a lack of cohesion was evident among the cells and different organs. This fact may be related, in part, to the absence of basal membranes in the tissues, and also to the lack of glycosaminoglycans in the acellular spaces.

(3) *Effects of* 1000 *Hz*/12 μT. These embryos presented widespread delays in development. Malformations were especially evident in organs of mesodermal origin, where cells multiplied very little, did not migrate, and, when they did organize, produced malformations. The embryos contained a nervous system, notochord, vascular system and somites.

At the anterior foregut level, a relatively well established neural tube with a normal histological appearance was present (Fig. 5A), although its inner part and limiting walls were poorly organized. In most cases in this series, the neural tube was

still open along its entire length. Mucopolysaccharides were present within the lumen and basal membrane although they were rather depolymerized. The neural crest was of nearly normal appearance but occupied too medial a position.

The notochord was rather primitive, being represented by a group of cells lacking the sheath which normally contains them. The pharynx had poorly organized walls. The mesenchyme, as shown in Figure 5A, was represented by a few cells grouped mainly in the cephalic and pharyngeal zones. Observations at higher magnifications (Fig. 5B) revealed scanty mesenchymal cells with few mitoses and dense nucleoli, indicating slow metabolic activity. The arrows in the Figure mark patches of glycosaminoglycans which had a depolymerized appearance, possibly related to the lack of migration of neural crest cells.

In a similar region in a control embryo (Fig. 5C) the glycosaminoglycans had a typical polymerized appearance, with more abundant strands. The cells were also more numerous with frequent mitosis, clear boundaries, star-like shape and nitid prolongations.

In two of the four treated embryos, there was no detectable heart and there were only undeveloped vessels, limited to an incipient dorsal aorta. The foregut consisted of only thin, discontinuous walls. Somites were very primitive, lacking their lax basal membrane.

(4) *Effects of* 10 *Hz*/12 μT. In this series, most embryos appeared normal except for two which had disturbed nervous systems in which the cephalic vesicles were evident but the thickness of their walls, including the telencephalic wall, was variable and presented empty spaces. In the germinal layer, the metaphasic plaques of the mitotic cells were oriented obliquely or parallel to the dorsoventral axis of the neural tube, and the density of their chromatin seemed higher than normal. Many of these mitotic cells underwent a metaphase within the tissue, even in zones far from the optic and otic vesicles. The notochord was greatly reduced in size (Fig. 6A). The neural tube was closed and appeared normal in four of the five treated embryos. The foregut, aorta and heart appeared to be normal (Fig. 6B). In three of the five embryos, blood was present and the vessels had well organized walls corresponding to stage 11 of development. Vessels in the cephalic region, however, seemed abnormally fragile. The somites were normal in number and development.

DISCUSSION

Literature on the biological effects of magnetic fields is abundant and controversial, as indicated in the Introduction. The lack of uniformity in experimental results may be due to the wide range of parameters used, the variety of animal species tested, and perhaps also to the use of high intensities. Very little information is available concerning the possible effects of pulsating fields of extremely low frequencies and very low intensities which were the focus of the present research.

The present findings demonstrate that fields of 100 Hz/1·2 μT have a powerful effect on chicken embryogenesis, delaying or arresting it at a very early stage and limiting development to the formation of the three primitive layers, without signs of neural tube, brain vesicles, auditory pit, foregut, heart, vessels, or somites. This remarkable uniformity of results occurred only in the 100 Hz/1·2 μT series. Exposure to magnetic fields does not produce a sudden effect because all embryos showed some development, reaching at least a three layer stage, with the ectoderm rising up and cells multiplying. The appearance of variable necrotic points suggests the

existence of secondary effects. The mechanism of action of ELMF seems to be slow and cumulative.

The present experiments thus demonstrate a 'window effect', the intermediate parameters of 100 Hz/1·2 μT being more effective than any other values used in our studies. Table 4, which summarizes all frequencies used, clearly shows that in all organs, exposure to 1·2 μT was more effective than 12 μT. Table 3, a summary of results with different intensities, indicates that 1000 Hz frequency evoked the highest percentage of changes in different organs.

According to Table 2B, the intensity of 1·2 μT produced equivalent results in gross form with 100 and 1000 Hz, but histological examination showed conclusively that 100 Hz had greater teratogenic effects than 1000 Hz.

The window effects of electromagnetic fields have already been reported in the literature and according to Bawin & Adey (1976), in the chicken brain under magnetic fields there is a major decrease in the efflux of calcium at frequencies of 6–16 Hz (fields of 10 and 56 V/m) but only a small decrease with fields of 5 V/m and 100 V/m. These windows were also evident in studies with radio frequency signals producing tissue fields of 10^{-3}–10^{-2} V/cm which are comparable to the 50 mV/cm EEG signal. Using radio frequency signals with amplitude modulation at low biological frequencies, an increase in efflux of calcium may be produced as a function of frequency modulation, as well as a sharp increase in calcium efflux using 6–20 Hz, which disappears at 35 Hz (Bawin, Kaczmarek & Adey, 1975). There is a power window in addition to the frequency window, because the calcium efflux only increases at incident power densities of 0·1–1 mW/cm^2. Below and above these amounts, the efflux is negative or shows only an insignificant increase (Bawin, Shappard & Adey, 1978). These findings have been confirmed by Blackman *et al.* (1977). For a discussion of the possible mechanisms of these window effects, see Adey (1979).

The embryonic organs reacted with different sensitivity according to the ELMF parameters used, indicating the existence of different window effects on specific organs. As shown in Table 1, which summarizes the effects obtained with all frequencies and intensities used, the cephalic nervous system exhibited the highest sensitivity (78·5 %) while the heart had the lowest (54·8 %). As seen in Table 2A, with relatively high levels of ELMF (100 Hz/1·2 μT and also 1000 Hz/1·2 μT and 12 μT), all embryos had abnormal cephalic nervous systems, while the other organs remained normal in several embryos. Although the cephalic nervous system had the greatest ELMF sensitivity when exposed to 100 Hz/1·2 μT, it was the only system which developed a primitive organization, while indications of all other organs were absent.

At the lower frequencies of 10 Hz and the three intensities used (0·12, 1·2, 12 μT), the somites were normal in all embryos, while in several cases the cephalic nervous system was abnormal. The greatest histological effect on vessel development was obtained with 1000 Hz/12 μT, which completely blocked the formation of blood and vessels although traces of other organs were present.

As shown in Table 2A, four of the controls presented embryological abnormalities. Statistical analysis demonstrated the high significance of our results, and the normality of eggs exposed to the lowest ELMF (10 Hz/0·12 μT) increased the significance of these findings. The control egg of the series incubated under 10 Hz/1·2 μT showed a mild, uniform abnormality in all organs and could have been spontaneously defective. With respect to the abnormal control in the 1000 Hz/12 μT series, the possible effects of very weak ELMF cannot be dismissed because the egg

was only 20 cm from the coil. However, since the other controls were normal, this possibility is considered remote.

Our results are difficult to compare with other available reports because of methodological differences. Veneziano (1965) has described disturbances in chick embryos exposed to ELMF, but he used static fields, found alterations in only 15 % of the exposed embryos, and used much higher intensities (31 Gauss) than ours (1 μT = 0·001 Gauss). Kim (1976) has reviewed the literature on the effects of static magnetic fields on growth rates of bacteria, drosophila, frog embryos, rabbits, cats and other animals. Intensities used were up to 120000 Oe and results were variable and not comparable to our findings except for the study by Kiyushkin *et al.* (1966) who described retarded development in the pigeon embryo using intensities of 4–7 Oe.

The main characteristics of the present experiments were the effectiveness of very low intensities and the use of pulsatile instead of static magnetic fields. Thermal effects were therefore minimal and may be disregarded. The use of pulses permitted analysis of frequency effects. Teratogenic results did not seem to be related to the amount of applied energy because levels were very low and also because equivalent total amounts of energy such as 10 Hz/12 μT and 100 Hz/1·2 μT produced very different effects. A parametric biological sensitivity and not an unspecific energetic disturbance therefore seemed to be involved.

Our results were homogeneous when parameters of 100 Hz/1·2 μT and also 1000 Hz/1·2 μT were used (see Table 2B), but even in these series, histological abnormalities varied, indicating that some embryos were more sensitive to ELMF than others. Heterogeneity of results was greater when other parameters were used: for example, in the 100 Hz/12 μT series, three embryos were normal whereas six were abnormal in their truncal nervous system, heart, vessels and somites, and five had abnormal vessels. This finding indicates a varied sensitivity of the embryos to ELMF, possibly related to their lack of genetic homogeneity. Evidence indicates that there may be two types of embryological sensitivity to ELMF: (*a*) *Individual*, meaning that the whole embryo may or may not be affected by exposure to ELMF; and (*b*) *organ*, related to differential sensitivities within the same embryo; for example, the greater reaction of the cephalic nervous system in comparison with that of the embryonic heart.

Explanations of the ELMF mechanisms of action on embryogenesis are rather difficult to formulate. The possible cooperativity at membrane surfaces in the sensing of weak fields is an attractive theory. In the model proposed by Grodsky (1976) and evaluated by Adey (1979), the membranes could have lines of electric strain coming from the lipid bilayer, with glycoproteins playing an essential role and with dipoles arranged basically in an antiferromagnetic state. Energy added in a coherent fashion could cause the dipoles scattered about on the surface glycoproteins to change their orientation from the antistate to the flop phase.

The sheet arrangement of the membrane may be interrupted by protruding strands of glycoproteins or of glycosaminoglycans. The latter have characteristics as polyanions with acidic sulphate and/or carboxyl groups, possessing a polyelectrolyte nature and forming proteoglycans in which several chains are covalently linked to polysaccharide units (Lindahl & Höök, 1978). The electrostatic properties of these polysaccharides are essential for their interactions and form the basis of their many functions, including ionic distributions, osmotic pressure and electrical fields in cellular membranes, and cooperative electrostatic binding to a varied number of

macromolecules, for example collagen and lipoproteins. Exposure to ELMF could change the electrostatic properties of glycosaminoglycans, altering their embryogenetic role. Their histological staining properties could also be modified. Especially interesting is the accumulation of hyaluronate in the tissues at a stage characterized by extensive cell migration and the subsequent enzymatic removal of hyaluronate. The role of glycosaminoglycans seems well established in organogenesis (Bernfield, Cohn & Banerjee, 1973; Fisher & Solursh, 1977; Kosher & Lash, 1975; Strudel, 1973). Glycosaminoglycans are components of the extracellular matrix and are associated with cellular membranes in embryonic and also in adult tissues (Toole, 1976). In chick embryos, hyaluronic acid is present in the cellular matrix of the migrating sclerotome of neural crest and developing heart and limbs (see bibliography in Fisher & Solursh, 1977). It is a constituent of the extracellular matrix of the chick primary mesenchyme and is necessary for maintenance of the extensive intercellular spaces which are characteristic of the mesenchyme in the head region of the embryo, and for cellular migration.

It is also known that glycosaminoglycans promote certain types of morphogenesis and cell differentiation (Hay & Meier, 1974, 1976) and that, in the absence of glycosaminoglycans, there is no embryonic development (Trillo, Cuevas & Leal, in preparation).

The mechanism of delays and malformations of embryogenesis could be related to the disruption or absence of glycosaminoglycans in embryos treated with 100 Hz/1·2 μT, as revealed by alcian blue stain. Granular and poorly organized patches of glycosaminoglycans are very clear in Figure 5B and contrast with the neat fibrillar organization in the normal embryo shown in Figure 5C. In the most affected embryos (exposed to 100 Hz/1·2 μT), glycosaminoglycans were absent; in less affected embryos (exposed to 10 Hz at different intensities) where morphogenesis occurred (although with some delay) they were present (although modified) in the acellular spaces. Embryos exposed to 100 Hz/12 μT had traces of glycosaminoglycans in their cellular surfaces and contained indications of incipient development of nervous system and other organs. Because this growth requires some synthesis and excretion of glycosaminoglycans, cellular metabolism was evidently less disturbed with 12 μT than with 1·2 μT.

In the series exposed to 1000 Hz/12 μT, glycosaminoglycans were more abundant than in embryos exposed to 100 Hz, but their appearance was abnormally granular. The fact that, in these embryos, the neural crest cells were grouped together, sticking to the neural tube (Fig. 5A), indicates an intrinsic lack of mobility possibly related to alterations of glycosaminoglycans (Pratt, Larsen & Johnston, 1975).

The teratogenic effects of 12 Hz/12 μT were milder, and malformations were limited to the cephalic nervous system and notochord. The abnormal thickening of the cephalic nervous system was probably due to an increase of mitotic cells and the disorderly disposition of metaphasic plaques. These disturbances may also be associated with alterations of glycosaminoglycans, modifying their role in cell proliferation (Ohnishi, Oshima & Ohtsuka, 1975).

The greater ELMF sensitivity of the cephalic nervous system could not be attributed to neuronal electrical activity because, at these early stages of development, the neuroblasts have not yet acquired the polarizing and depolarizing capability necessary for neural transmission existing in the more mature neural tissue.

Organogenesis of each tissue is closely related to the local environment and arrest in development may be caused by ELMF influences on the functional properties of

the environment or, alternatively, on effects upon inductors and programmed mechanisms. For example, delays in heart formation may be due to alterations in the floor of the primitive pharynx which is the inductor of cardiac development. Exposure to ELMF seems to be an excellent method for investigation of these embryogenetic mechanisms.

As shown by Jaffe & Stern (1979), in the early stages of chick development, the primitive streak has a steady current, with exit density of approximately 100 μA/cm^2, which pours out of the whole streak and returns through the epiblast. The epicentre of these currents lies near Hensen's node and some of them may react in ways that affect embryonic development (Robinson & Cone, 1980). Application of external magnetic fields to embryos probably influences the steady currents of the primitive streak, and this could be a mechanism for its disturbing effect in embryogenesis shown in this paper. This working hypothesis is at present being tested experimentally.

SUMMARY

Fertilized chicken eggs were incubated for 48 hours while exposed to extremely low frequency magnetic fields (ELMF) of 10 Hz, 100 Hz and 1000 Hz with intensities of 0·12, 1·2 and 12 μT.

Gross morphological and histological analysis of the exposed embryos revealed the following effects:

(1) ELMF of 100 Hz/1·2 μT had the most consistent and powerful inhibitory effect on embryogenesis. Development of embryos was reduced to the formation of the three primitive layers. Brain vesicles, auditory pit, neural tube, foregut, heart, vessels, and somites were not developed. Glycosaminoglycans were almost absent.

(2) The above results demonstrate a window effect because embryos exposed to 100 Hz/1·2 μT were less developed than embryos exposed at lower and higher intensities and frequencies.

(3) Developing organs reacted with different sensitivity to ELMF of specific frequencies and intensities. The cephalic nervous system was the most and the heart the least sensitive. Somites were not disturbed by exposure to 10 Hz with any of the intensities used. Formation of blood vessels was completely blocked by ELMF of 1000 Hz/12 μT while traces of other organs were present.

(4) The drastic embryological disturbances described were obtained with much lower intensities (1 μT = 0·01 Gauss) than those used in studies by other investigators.

(5) Embryological alterations induced by ELMF may depend on disturbances in the presence and structure of glycosaminoglycans which are essential elements in cellular activities, including cell migration.

(6) The use of ELMF of low intensity may be a powerful method to investigate embryogenetic mechanisms and may also be a useful technique for investigation of other biological systems.

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ADDENDUM

After preparation of this paper we had the opportunity to contact Professor C.

Andrew L. Bassett and his group at Columbia University, New York City, and reference must be made to this excellent work. See, for example: Pulsing electromagnetic fields: A new approach to surgical problems (C. A. L. Bassett). In *Metabolic Surgery* (ed. H. Buchwald & R. L. Varco), pp. 255–305. New York: Grune & Stratton. 1978.

REFERENCES

ADEY, W. R. (1979). Long-range electromagnetic field interactions at brain cell surfaces. In *Magnetic Field Effects on Biological Systems* (ed. T. S. Tenforde), pp. 57–80. New York: Plenum Press.

ARVANITAKI, A. (1942). Effects evoked in an axon by the electric activity of a contiguous one. *Journal of Neurophysiology* **5**, 89–108.

BARNOTHY, M. F. (1963). Biological effects of magnetic fields on small mammals. *Biomedical Scientific Instrumentation* **1**, 127.

BARNOTHY, M. F. (1964). Rejection of transplanted tumors in mice. In *Biological Effects of Magnetic Fields*, vol. 1 (ed. M. R. Barnothy). New York: Plenum Press.

BAWIN, S. M. & ADEY, W. R. (1976). Sensitivity of calcium binding in cerebral tissue to weak environmental electric fields oscillating at low frequency. *Proceedings of the National Academy of Sciences of the USA* **73**, 1999.

BAWIN, S. M., KACZMAREK, L. K. & ADEY, W. R. (1975). Effects of modulated VHF field on the central nervous system. *Annals of the New York Academy of Sciences* **247**, 74–81.

BAWIN, S. M., SHAPPARD, A. R. & ADEY, W. R. (1978). Possible mechanisms of weak electromagnetic field coupling in brain tissue. *Bioelectrochemistry and Bioenergetics* **5**, 67–76.

BERNFIELD, M. R., COHN, R. H. & BANERJEE, S. D. (1973). Glycosaminoglycans and epithelial organ formation. *American Zoology* **13**, 1067–1083.

BIGGS, M. W. (1979). Studies on biomagnetic effects in mice. In *Magnetic Field Effects on Biological Systems* (ed. T. S. Tenforde), p. 40. New York: Plenum Press.

BLACKMAN, C. S., ELDER, J. A., BANNANE, S. G., WEIL, C. M. & EICHINGER, D. C. (1977). Two factors affecting the radiation-induced calcium efflux from brain tissue. *Symposium on the Biological Effects of Electromagnetic Waves*, Airlie, Va., Oct. 30–Nov. 4.

CABANES, J. (1980). Les champs électriques et magnétiques ont-ils un effet sur l'homme. *Revue générale de l'électricité* **89**, 16–22.

COOK, E. S., FARDON, J. C. & NUTTINI, L. G. (1969). Effects of magnetic fields on cellular respiration. In *Biological Effects of Magnetic Fields*, vol. 2 (ed. M. F. Barnothy). New York: Plenum Press.

DE LORGE, J. (1972). Operant behavior of rhesus monkeys in the presence of extremely low frequency–low intensity magnetic and electric fields: Experiment 1. Naval Aerospace Medical Research Lab., Pensacola, Fla., Rept. NAMRL-1155. (Available from NTIS as AD 754058.)

DE LORGE, J. (1973*a*). Operant behavior of rhesus monkeys in the presence of extremely low frequency–low intensity magnetic and electric fields: Experiment 2. Naval Aerospace Medical Research Lab., Pensacola, Fla., Rept. NAMRL-1179. (Available from NTIS as AD 764532.)

DE LORGE, J. (1973*b*). Operant behavior of rhesus monkeys in the presence of extremely low frequency–low intensity magnetic and electric fields: Experiment 3. Naval Aerospace Medical Research Lab., Pensacola, Fla., Rept. NAMRL-1196. (Available from NTIS as AS 000078.)

D'SOUZA, L., RENO, V. R., NUTTINI, L. G. & COOK, E. S. (1969). The effects of a magnetic field on DNA synthesis by ascites sarcoma. In *Biological Effects of Magnetic Fields*, vol. 2 (ed. M. F. Barnothy). New York: Plenum Press.

FISHER, M. & SOLURSH, M. (1977). Glycosaminoglycan localization and role in maintenance of tissue spaces in the early chick embryo. *Journal of Embryology and Experimental Morphology* **42**, 195–207.

FURUKAWA, T. & FURSHPAN, E. J. (1963). Two inhibitory mechanisms in the Mauthner neurons of goldfish. *Journal of Neurophysiology* **26**, 141–176.

GABE, M. (1968). *Techniques Histologiques*. Paris: Masson.

GRISSETT, J. D. (1980). Biological effects of electric and magnetic fields associated with ELF communications systems. *Proceedings of the Institute of Electrical and Electronic Engineers* **68**, 98–104.

GRODSKY, I. I. (1976). Neuronal membrane: A physical synthesis. *Mathematical Biosciences* **28**, 191–220.

HALL, E. J., BELFORD, J. S. & LEASH, M. J. (1964). Some negative results in the search for lethal effect of magnetic fields on biological materials. *Nature* **203**, 1086.

HALPERN, M. H. & GREEN, A. E. (1964). Effects of magnetic fields on growth of Hela cells in tissue culture. *Nature* **202**, 717.

HAMBURGER, V. & HAMILTON, H. L. (1951). A series of normal stages in the development of the chick embryo. *Journal of Morphology* **88**, 49–92.

HAY, E. D. & MEIER, S. (1974). Glycosaminoglycan synthesis by embryonic inductors: neural tube, notcchord, and lens. *Journal of Cell Biology* **62**, 889–898.

HAY, E. D. & MEIER, S. (1976). Stimulation of corneal differentiation by interaction between cell surface and extracellular matrix. *Developmental Biology* **52**, 141–157.

JAFFE, L. F. & STERN, C. D. (1979). Strong electrical currents leave the primitive streak of chick embryos. *Science* **206**, 569–571.

KIYUSHKIN ET AL. (1966): cited in KIM (1976).

KIM, Y. S. (1976). Some possible effects of static magnetic fields on cancer. *Tower International Technomedical Institute Journal of Life Sciences* **6**, 11–28.

KORN, H. & FABER, D. S. (1980). Electrical field effect interactions in the vertebrate brain. *Trends in Neuroscience Jan.*, 6–8.

KOSHER, R. A. & LASH, J. W. (1975). Notochordal stimulation of '*in vitro*' chondrogenesis before and after enzymatic removal of perinotochordal materials. *Developmental Biology* **42**, 362–378.

LINDAHL, U. & HÖÖK, M. (1978). Glycosaminoglycans and their binding to biological macromolecules. *Annual Review of Biochemistry* **47**, 385–417.

LUNA, L. G. (ed.) (1968). *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. New York: Blakeston Division, McGraw-Hill.

MALININ, G. I., GREGORY, W. D., MORELLI, L. & EBERT, P. S. (1979). Effects on cell function resulting from exposure to strong magnetic fields at 4 °K. In *Magnetic Field Effects on Biological Systems* (ed. T. S. Tenforde), pp. 50–51. New York: Plenum Press.

MERICLE, R. P., MERICLE, L. W., SMITH, A. F., CAMPBELL, W. F. & MONTGOMERY, D. J. (1964). Plant growth responses. In *Biological Effects of Magnetic Fields*, vol. 1 (ed. M. F. Barnothy). New York: Plenum Press.

MULAY, I. L. & MULAY, L. N. (1961). Effects of a magnetic field on sarcoma 37 ascites tumor cells. *Nature* **190**, 1019.

NEURATH, P. W. (1968). High gradient magnetic fields inhibit embryonic development of frogs. *Nature* **219**, 1358.

NEURATH, P. W. (1969). The effect of high gradient high strength magnetic fields on the early embryonic development of frogs. In *Biological Effects of Magnetic Fields*, vol. 2 (ed. M. F. Barnothy). New York: Plenum Press.

NOVITSKII, YU I. (1966). Effects of a magnetic field on the dry seeds of some cereals. In *Proceedings of the Conference on Effects of Magnetic Fields on Biological Objects*. Moscow.

OHNISHI, T., OSHIMA, E. & OHTSUKA, M. (1975). Effect of liver cell coat acid mycopolysaccharide on the appearance of density dependent inhibition in hepatoma cell growth. *Experimental Cell Research* **93**, 136–142.

PRATT, R. M., LARSEN, M. A. & JOHNSTON, M. C. (1975). Migration of cranial neural crest cells in a cell-free hyaluronate rich matrix. *Developmental Biology* **44**, 298–305.

ROBINSON, K. R. & CONE, R. (1980). Polarization of fucoid eggs by a calcium ionophore gradient. *Science* **207**, 77–78.

SCHAEFER, H. (1980). The effects of electric alternation fields of high field intensities on man. *Abstracts of the Reports, 6th International Colloquium on The Prevention of Occupational Risks Due to Electricity*, pp. 1–3. Vienna, Sept. 30–Oct. 2.

SHTERNBERG, I. B. (1966). The effect of a constant magnetic field on the production of specified antibodies. In *Proceedings of the Conference on Effects of Magnetic Fields on Biological Objects*. Moscow.

SSAWASTIN, P. W. (1930). Magnetic growth reactions in plants. *Planta* **12**, 327.

STRUDEL, G. (1973). Matériel extracellulaire périaxial et chondrogenèse vertébrale. *Anneé biologie* **12**, 401–416.

TABRAH, F. L., GUERNSEY, D. L., CHOU, S. C. & BATKIN, S. (1978). Effect of alternating magnetic fields (60–100 gauss, 60 Hz) on *Tetrahymena pyriformis*. *Tower International Technomedical Institute Journal of Life Sciences* **8**, 73–77.

TOOLE, B. P. (1976). Morphogenetic role of glycosaminoglycans (acid mucopolysaccharides) in brain and other tissues. In *Neuronal Recognition* (ed. S. H. Barondes), pp. 275–329. New York: Plenum Press.

VENEZIANO, P. P. (1965). The effect of low intensity magnetostatic fields on the growth and orientation of the early embryo of *Gallus domesticus*. *Zoology* **25**, 4319.