shaken vigorously and centrifuged. The clear supernatant was removed and tested for activity as follows: several 0·02 ml. drops were placed on the surface of a 1 per cent fibrin plate prepared by adding thrombin to fibrinogen in a 4 inch Petri dish. Each fibrin plate also contained 2,000 u of penicillin and 1,500 u of streptomycin in order to avoid complications caused by bacterial growth. The fibrin plates were put in an incubator at 37° C and examined at intervals up to 14–22 h.

During this incubation, control fibrin plates—plates to which no material had been added—underwent complete lysis. On the other hand, on plates to which drops of mesothelial extract had been applied, button-shaped, opaque zones of unlysed fibrin remained. These zones were larger than the areas originally occupied by the drops of test material, suggesting that some substance present in the mesothelial extract had diffused into the fibrin and had in some way prevented fibrinolysis (Fig. 2). When the mesothelial extract was heated to 100° C for 2 min before testing, no zones of inhibition were formed.

E. M. J. Pugatch
J. C. F. Poole

Sir William Dunn School of Pathology,
University of Oxford.

Received August 28; revised November 14, 1968.

[1] Todd, A. S., *Nature*, 181, 495 (1958).
[2] Todd, A. S., *J. Pathol. Bact.*, 78, 281 (1959).
[3] Todd, A. S., in *Thrombosis and Anticoagulant Therapy* (edit. by Walker, W.), 30 (Livingstone, Edinburgh, 1961).
[4] Warren, B. A., *Brit. J. Exp. Path.*, 44, 365 (1963).
[5] Pugatch, E. M. J., and Saunders, A. M., *J. Atheroscler. Res.*, 8, 735 (1968).
[6] Pugatch, E. M. J., and Poole, J. C. F., *Quart. J. Exp. Physiol.* (in the press, 1969).
[7] French, J. E., Florey, H. W., and Morris, B., *Quart. J. Exp. Physiol.*, 45, 88 (1960).
[8] Casley-Smith, J. R., *Quart. J. Exp. Physiol.*, 52, 105 (1967).
[9] Cotran, R. S., and Karnovsky, M. J., *J. Cell Biol.*, 37, 123 (1968).

BARNOTHY

Abnormalities in Organs of Mice induced by a Magnetic Field

During the past few years it has been found that homogeneous magnetic fields produce morphological, functional and behavioural changes in living organisms[1]. There have been various hypotheses[2–6] to explain these observations. The second day minimum in the weight of mice exposed to strong (3,000 to 10,000 oersted) magnetic fields[7] seems to have the characteristic of stress effects—alarm reaction followed by adaptation to the stress. The questions remain whether there is a similarity between stress effects and those observed in animals exposed to magnetic fields, and whether the magnetic field is a rather weak non-specific stressor. We report experiments in which the magnetic field caused significant changes in the organs of mice.

Ten male 47 day old random bred Swiss mice each weighing 30 g were kept for 13 days in a vertical, homogeneous magnetic field of 9,000 oersted, with a gradient of less than 200 oersted/cm. (This field is about 20,000 times stronger than the geomagnetic field to which probably all organisms have adapted during the million years of evolution.) The field was produced by a 7 inch electromagnet, the upper pole of which was the north pole of the magnet. The vertical field direction prevented the direction of the field vector reversing relative to the biological coordinate system of the animal during the movement of the mice in the cage. Ten male mice of the same age and same starting weight were kept during the 13 days of magnetic exposure in "dummy magnets", in which they were, with the exception of the field, in every other respect (including the pole caps) in identical conditions as the magnet mice. The cage areas were 310 cm² and 7 cm high. The living area was therefore 70 per cent of that available in standard plastic mice cages. Water cooling of the magnet coils maintained the temperature of the magnet within ±3 C of room temperature. The cages (together with the pole caps) were enclosed in larger copper boxes, which were open on two opposite sides and thermally insulated from the electromagnet. Small electric fans maintained a light breeze through the copper boxes and the cages, keeping the temperature in the magnet and the dummy magnet cage alike within ±0·5° C. The room was ventilated by filtered and temperature controlled air. Both magnet and dummy magnet group mice had free access to tap-water and Purina Mouse Chow. It seemed of interest to discover whether the electromagnet produces a noise when it is functioning, because continual, especially high pitched frequencies, are known to have a very stressful effect on mice, sometimes resulting in audiogenic seizures. Because of the large weight of the magnet (about 2,000 pounds), high frequency vibrations are unlikely. The current fed to the magnet, rectified from 3-phase 60 cycles, is very well filtered with condensers, chokes and the high inductance of the magnet coils themselves. To make sure that there was no high pitched noise in the magnet cage, however, we have measured with an audiometer the noise level between 31·5 Hz to 31,500 Hz. The power supply when switched "on" produced a maximum increase of 14·5 dB in the noise level at 250 Hz in both magnet and dummy magnet cages. Above 2,000 Hz no difference could be observed with power supply "on" or "off". Because the sensitive microphone of the audiometer could not be placed between the magnet poles when the magnet was energized, the sound level was measured at distances of 50 and 100 cm from the centre of the magnet cage. Between 31·5 and 31,500 Hz no measurable difference could be observed with the magnet energized or not, nor with the cooling water of the coils "on" or "off".

After the termination of magnetic exposure, the mice were killed with chloroform, the organs removed and embedded in paraffin. Sections 5–6 microns thick were stained with haematoxylin and eosin according to van Gieson and microscopically inspected. The liver and spleen sections were also stained with 'Azan' and with acridino-orange for fluorescence microscopic inspection. The periodic Schiff reaction according to McManus was also used. The sections were inspected in a blind experiment.

A comparison between the values obtained in the dummy magnet group with those found in mice kept in standard cages (four mice per cage) did not show significant differences, indicating that the volume of the experimental cage was sufficiently large to eliminate effects of confinement and/or crowding. To be on the safe side, however, we compared values of the averages of the magnet group mice with the corresponding averages of the dummy magnet group mice. Table 1 summarizes our results found in the adrenals, bone marrows, spleens and livers of the mice. In the last column of Table 1 these differences are expressed as a percentage of the dummy magnet values. The indicated errors are standard errors.

The most striking abnormality found in the adrenals of the magnet group mice was a narrowed zona fasciculata with normal zona glomerulosa and normal or slightly wider zona reticularis. The cells of the zona fasciculata

Table 1

	Magnet group	Dummy magnet group	Difference (per cent)
Disorganization of adrenal cortex, in per cent*	40·0 ± 5·5	None	40·0 ± 5·5 PL 1 : 5×10^{9}
Number of megakaryocytes in the bone marrow, per microscope field	5·89 ± 0·22	8·09 ± 0·63	− 27·1 ± 8·3 PL 1 : 200
Number of megakaryocytes in the spleen, per microscope field	9·42 ± 0·39	6·98 ± 0·45	+ 36·1 ± 8·5 PL 1 : 1,300
Mitotic index in the liver per 400 liver cells	11·84 ± 0·80	5·17 ± 0·35	+ 129 ± 17 PL 1 : 10^{9}

* Fraction of the area of the zona fasciculata in which cell disorganization was observed.

(15)

Bio May 813

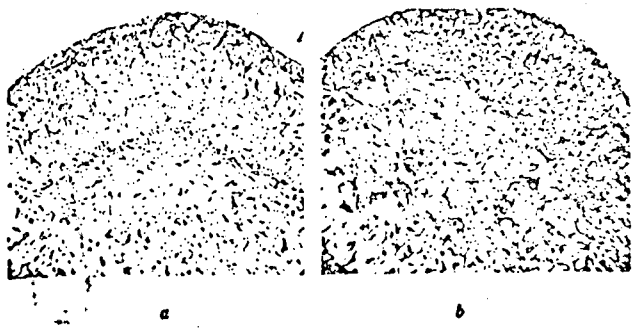

Fig. 1. Sections of the adrenal cortex. *a*, Dummy magnet group mouse; *b*, magnet group mouse.

were more or less disorganized, the usual cord-like arrangement of the cells was disturbed and the clear area between zone demarcations was absent. Fig. 1*a* shows the adrenal section of one of the dummy magnet mice. The three zones of the adrenal cortex are clearly visible. Fig. 1*b* is the adrenal section of a magnet mouse; as can be seen, the zona fasciculata is almost totally disorganized. Of the ten mice exposed to the magnetic field, only one had a normal adrenal, while the adrenals of all the dummy mice were normal. In two of the magnet group mice the abnormalities were slight; in seven 50 per cent of the zona fasciculata was disorganized and about one half of the normal width.

The cells of the zona fasciculata, being most strongly under the influence of the adrenal stimulating pituitary hormones, are the most sensitive cells of the adrenal cortex. In ordinary conditions of stress the fasciculata is widened, not narrowed. On the other hand, Akabana found that administration of alcohol widened the zona fasciculata, while acetaldehyde led to a narrowing of the zona fasciculata.

The most pronounced peculiarity seen in the bone marrows of the magnet group mice was the decrease in the number of megakaryocytes. Ten microscope fields (×350 magnification) were counted for each mouse, the fields being selected so that there was a minimum number of bone trabeculae in them. Of the ten magnet mice (with the exception of one) all had a smaller number of megakaryocytes than either of the dummy magnet mice, the range being 4·9–6·8 megakaryocytes per microscope field, compared with the range of 7·3–10·0 found in the dummy magnet group.

Lorber[8] found an increase in the number of megakaryocytes in the bone marrow after he applied the stress of a variety of surgical procedures.

The number of megakaryocytes in the spleens was counted in a similar manner as in the bone marrows. While on the average the number of megakaryocytes in the spleens of the dummy magnet mice was insignificantly lower than in their bone marrows (the difference being, with the exception of one instance, always negative), in the magnet group mice the number of megakaryocytes was larger in the spleen than in the bone marrow and larger than either of the values found in the spleens of the dummy magnet mice.

A large increase in the number of megakaryocytes in the spleens of animals stressed with Walker tumour transplants was found by Selye[9]. Such increases, however, are usually associated with erythropoiesis and myelopoiesis in the red pulp of the spleen, symptoms not seen in the magnet group mice. Megakaryocyte proliferation is characteristic of the reaction to neoplastic tissue and treatment with certain tissue extracts, but not seen with ordinary non-specific stress (restrain, cold, traumatic injuries and the like).

In the liver sections of the magnet group mice an increase in the number of direct mitoses, a large number of cells with large nuclei, or multinucleated cells were seen, and pyknotic cells, necrobiotic changes and cells with poor nuclear staining were often seen. The mitotic index was used as parameter to characterize the lesion in the liver tissues, whereby the mitotic index was taken as the number of mitoses per 400 liver cells, 4,000 liver cells per mouse being investigated. In all ten mice of the magnet group, the mitotic index was larger by a factor of 2 to 3 than in the dummy magnet mice; only in one magnet mouse was the increase a mere 40 per cent. The mitotic index ranged from 4·5 to 6·7 in the dummy magnet group, while in the magnet group it was from 7·2 to 15·1.

The last column of Table 1 lists the probability level (PL), computed by using Student's *t* test, that the observed effect is caused by chance alone. The high significance of the probability levels found for each of the four investigated parameters establishes the existence of the changes in organs of mice exposed for 13 days to a 9,000 oersted static magnetic field described here. Although several of the observed abnormalities in the organs seem to suggest that the effect of magnetic fields resembles the effects of non-specific stress, at least two of the symptoms —the narrowing of the zona fasciculata and the decrease in the number of megakaryocytes in the bone marrow— are the opposite of the usual effects of non-specific stress conditions. The observed symptoms show that, during exposure to magnetic fields, some hormone imbalance occurs in the hypothalamus-hypophyseal axis. It will be interesting to repeat these experiments on adrenalectomized mice and on mice which are supplied with extracts containing the hormones involved.

We wish to thank Professor H. Selye for helpful discussions. This research was supported by a graduate research grant of the University of Illinois and by the Biomagnetic Research Foundation.

M. F. Barnothy

University of Illinois,
College of Pharmacy,
Chicago, Illinois.

I. Sumegi

Dermatological Clinic,
Department of Pathology,
Karolinska Sjukhuset,
Stockholm, Sweden.

Received October 30; revised December 5, 1968.

1 *Biological Effects of Magnetic Fields*, 1 (edit. by Barnothy, M. F.) (Plenum Press, New York, 1964).
2 Valentinuzzi, M., in *Biological Effects of Magnetic Fields*, 1 (edit. by Barnothy, M. F.), 63 (Plenum Press, New York, 1964).
3 Gross, L., in *Biological Effects of Magnetic Fields*, 1 (edit. by Barnothy, M. F.), 74 (Plenum Press, New York, 1964).
4 Barnothy, M. F., in *Biological Effects of Magnetic Fields*, 1 (edit. by Barnothy, M. F.), 80 (Plenum Press, New York, 1964).
5 Becker, R. O., *Biological Effects of Magnetic Fields*, 2 (Plenum Press, New York, in the press).
6 Amer, N. M., thesis, Univ. California (1965).
7 Barnothy, J. M., and Barnothy, M. F., *Nature*, 200, 189 (1963).
8 Lorber, M., *Acta Haematol.*, 21, 252 (1959).
9 Selye, H., *Acta Haematol.*, 21, 378 (1959).

Morphology of Motor Nerve Terminals subjected to Polarizing Currents

The electric charge carried by synaptic vesicles is of considerable physiological interest. Strong support has recently been provided for the "vesicle hypothesis", which equates these vesicles with the quantal units of synaptic transmission[1,2]. The proposal that vesicles are charged has been used to explain excitation-release coupling (ref. 3 and unpublished results of Bass and Moore) and the effects of presynaptic polarization when transmitter is released by nerve impulses[4]. Electrophoretic behaviour

(16)