Chlamydomonas; EDTA at a concentration of 5×10^{-5} M and 10^{-5} M had the same effect as IAA. At higher concentrations, 10^{-4} M and above, EDTA inhibits motility of the organism. The action of EDTA was not so sharply *p*H dependent as that of IAA, being equally effective at *p*H 6·0 and 5·6 at a concentration of 5×10^{-5} M.

It is at present difficult to conjecture about the possible mode of action of IAA and EDTA in inducing phototactic responses and in reversing the inhibitory action of acetic acid. Probably IAA does not act here as a conventional hormone. Although both IAA and EDTA can act as chelates, for example, of calcium it seems unlikely that they do so here, in view of the *p*H range in which they are active and the very weak chelating capacity of IAA. However, it must be noted that IAA and EDTA may be regarded as derivatives of acetic acid. This, together with the interaction of IAA with the latter, makes it possible that they compete for some specific site within the flagella or cell. This site would determine the correct translation of the impulses sent from the light receptor into the flagella. Correct translation results in flagellar beat such as to orient the organism toward the light; prevention of translation also prevents oriented movement.

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Actions and Dosage of Chloralose

Many books recommend chloralose as an anæsthetic for laboratory animals and give a dosage rate of 40–80 mg/kg body-wt.; the drug is considered particularly useful for animals in which autonomic reflexes are to be preserved. I have recently carried out a series of experiments in the dog and rabbit, and in no case did surgical anæsthesia result from intravenous injections of 70 mg/kg or less. If chloralose was used after induction with ether slight improvement was seen, but surgery was still not possible. The heart rate was slowed by 30 per cent in these experiments and excessive secretions caused respiratory embarrassment.

The action of chloralose has not been well understood in the past: "The action is a remarkable one, for the animal does not appear to pass beyond a stage of narcosis, and paddling movements of the legs or nodding of the head may appear throughout. . . . It is suggested that the condition is possibly one of hypnosis superimposed on narcosis, rather than anæsthesia"[1]. Electrocorticographic recordings now show that it makes the Betz cells susceptible to sensory stimuli[2], and that the convulsive response to slight stimulus which readily occurs under chloralose is a cortical reaction, for "chloralose, like the convulsants, facilitates transmission in a reflex arc including the cortex"[3]. Grieg[4] described these convulsions as "strychnine-like". Shukla *et al.*[5], studying its effect on autonomic reflexes, found that it slowed the heart rate by 50 per cent, while Kochmann[6] found that "it reinforces some actions of epinephrine and acts like atropine in reducing vagal activity".

In view of the fact that chloralose does not affect the electrical activity of the brain in the same way as other drugs known to be general anæsthetics, and bearing in mind that it does not leave autonomic reflexes unaltered, it would seem that some substitute should be sought for this drug; a great variety of new substances acting on the central nervous system have been developed since the Second World War, and traditional procedures may not be justified in the light of present knowledge. The fact that chloralose is not used in human medicine casts further doubt on the validity of its use as an anæsthetic for laboratory animals.

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Some Negative Results in the Search for a Lethal Effect of Magnetic Fields on Biological Materials

During recent years there has been an increasing number of reports in the literature of the ability of magnetic fields to affect biological systems. Gerencser and Barnothy[1] noted differences from control of cultures exposed to 15,000 gauss, while Butler and Dean[2] found growth inhibition in tissue culture of *KB* cells exposed to 4,000-gauss magnetic fields. Maclean[3], using intense and 'mild' magnetic fields of unspecified strengths, reported various effects on tumour-bearing mice, but since only 12 animals were used for the entire investigation and these were divided into 3 groups, the results can have no statistical significance. Negative reports have appeared too, and in the most recent of these Halpern and Greene[4] reported that the growth rate of HeLa cells cultured in a 1,200-gauss magnetic field was not significantly different from controls. This communication extends this negative finding to much higher magnetic field strengths. Three separate experiments were performed, using HeLa cells cultured *in vitro*.

The cells were cultured by the methods described elsewhere[5]. In each experiment 200 cells were inoculated into either 50 mm 'Falcon' plastic Petri dishes or into 'Falcon' plastic culture flasks. Two containers were reserved for controls and two were subjected to a magnetic field for various lengths of time as detailed here. The cells were afterwards incubated for 10 days at 37° C in an incubator continuously flushed with a mixture of 5 per cent carbon dioxide and 95 per cent air. At the end of this period the cells were fixed with 10 per cent formalin in normal saline, stained with crystal violet, and the number of macroscopic colonies on each dish or flask counted. The following treatment conditions were used.

(*a*) A steady field of 5,000-gauss was obtained with a permanent magnet having 2-in. diameter pole pieces which were just large enough to cover the 50 mm plastic Petri dishes. The magnet was mounted inside the incubator and the treated plates were subjected to the magnetic field throughout the 10-day period of incubation. The control plates were kept in the same incubator at approximately 12 in. from the pole pieces. Approximately 150 colonies were counted in each dish, but there was no significant difference between the number of colonies counted on the treated against the control dishes (Table 1). There was no obvious difference in the size of the colonies, or their morphology.

(*b*, *c*) To obtain stronger magnetic fields, it was necessary to use electromagnets. Consequently it was not possible to mount the magnets inside the incubator and, for this and other reasons, treatment times were much shorter.

In one experiment an electromagnet was used which had tapered pole pieces, 4-in.–2-in. diameter, with a gap between the poles of 15 mm. Two culture flasks were exposed to a magnetic field of 27 kilogauss for a period of

Table 1

Exp.	Field strength (gauss)	Time of exposure	No. of colonies on control dish or flask	No. of colonies on treated dish or flask
(*a*)	5,000	10 days	150 145	149 158
(*b*)	27,000	1 h	168 180	175 165
(*c*)	77,000	15 min	143 151	161 148

1 h. In another experiment, two culture flasks were placed inside a solenoid, 2-in. diameter, and exposed to a magnetic field of 77 kilogauss for a period of 15 min. In both cases control cultures were handled in a similar manner except that the magnets were not energized. In both experiments, approximately 150 colonies were counted on both treated and control flasks, there being no significant difference between them, either in cell number or morphology (Table 1).

It would seem, therefore, that gross lethal effects on mammalian cells are not produced either by powerful magnetic fields applied for short periods, or by lower field strengths extending over many cell generation times, The work recorded here is subject to the same fundamental limitation as previous reports in the literature—namely, the necessity of using permanent magnets for long-term experiments, with a consequent limit to the field strength of 5–15 kilogauss. For fields higher than this, it is necessary to use electromagnets which consume such vast quantities of electrical power that long-term experiments are out of the question. An obvious alternative would be to use super-conducting magnets, which can provide fields of the order of 50 kilogauss for extended periods at a nominal cost.

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A Persistent Diurnal Rhythm in Photosynthetic Capacity

IN 1939, Meyer[1] discovered a daily rhythm in the rate of photosynthesis in *Ceratophyllum*, a fresh-water angiosperm. In 1957, similar rhythms were found in natural populations of marine phytoplankton[2,3]. Four years later, Hastings *et al.*[4], working on the photosynthetic-capacity rhythm in the marine dinoflagellate *Gonyaulax*, demonstrated that these rhythms would persist for a few days in the laboratory in the absence of day–night environmental cycles, that is, in continuous dim illumination and constant temperature. Negative evidence was presented by Blinks and Givan[5], who performed experiments which suggested the absence of photosynthetic rhythms in 13 species of marine littoral algae. In view of the different results, we have undertaken comparative investigations in an attempt to establish the distribution of persistent rhythms in marine flora.

Pure cultures of the marine diatom, *Phaeodactylum tricornutum*, were used in the work reported here. Cultures of this algae were grown in Guillard's medium *f* (ref. 6) and maintained in a light régime of 12 h of illumination (600 ft.-candles from 'cool white' fluorescent tubes) alternating with 12 h of darkness. When the cultures approached the stationary phase of growth, periodic measurements of photosynthesis were commenced. At the midpoints of each light and dark period, 3 or 4 10-ml. samples were withdrawn from a parent culture and incubated with 1 μc. $Na_2C^{14}O_3$ for 15 min at 600 ft.-candles. Usually, one of these samples served as a 'dark bottle' control, that is, it was incubated in the dark and used to correct for any adsorption of carbon-14 on the cells, etc. At the end of the incubation period the reaction was stopped by adding 2-ml. 1 N hydrochloric acid to the sample; the cells were then collected on a 'Millipore' filter, washed with 5-ml. 0·1 N hydrochloric acid, dried in a desiccator, and the uptake of carbon-14 measured. Concurrently, aliquots of the culture were extracted with methanol and the chlorophyll *a* content determined spectrophotometrically. Cell counts were also made at each sampling time.

This method measured the capacity, or the rate, at which the algae could maintain photosynthesis under a given set of conditions; and, in the experimental design described here, compared the rate at which photosynthesis could proceed at night (when, of course, the photosynthetic machinery is normally inactive) with the midday rate. Fig. 1 shows the results of these measurements; photosynthesis was maximal during the light period and minimal during the dark period, the difference being more than three-fold. (Using identical methods, we found a similar rhythm in the dinoflagellate, *Gymnodinium nelsoni*.) It is clear that some physiological change must have taken place in the cells, which resulted in an altered capacity of the cells to maintain photosynthesis. This change could have been induced in the cells by the different light conditions to which they were subjected (either 6 h of light or darkness) immediately prior to the time at which the rate-measurements were made; or the fluctuations could be controlled by a biological clock, the oscillations being relatively independent of light–dark cycles. By following photosynthesis in cells maintained in continuous illumination—as was done in the next experiment—this problem was resolved.

Cultures were now first grown in alternating cycles of light and darkness for 10 days and then placed in either continuous darkness or in various intensities of continuous light. On the 2 days prior to placing the cultures in static conditions, periodic photosynthetic-rate measurements were made to make sure a rhythm was present; this done, the cultures were then placed in continuous light or darkness at a constant temperature (15° C). As before, and

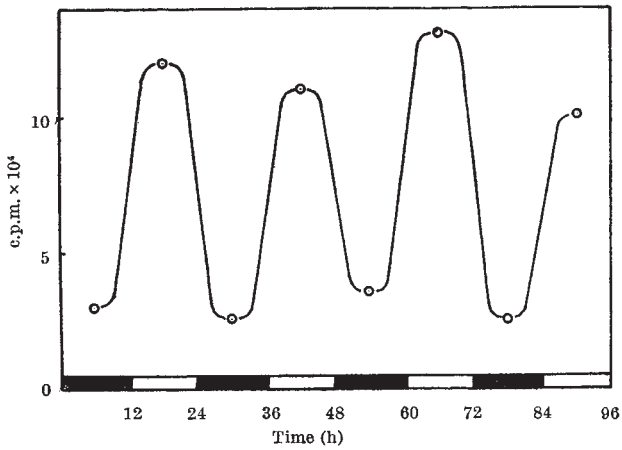

Fig. 1. Photosynthetic-capacity rhythm in *Phaeodactylum tricornutum*. Shaded blocks along abscissa represent times of darkness; open blocks represent periods of light (600 ft.-candles). Ordinate, amount of radioactive carbon assimilated