lysin titers than did the control group, which had only received one injection. In fact, the response was lower than the normal primary response. These observations confirm Rowley's data(1) showing that the spleen is necessary for a response to a second injection of low doses of SRBC. The group splenectomized after the initial dose of antigen and not reinjected, Group C, produced the lowest level of circulating antibody, as expected. The non-splenectomized control rats given 2 injections of antigen showed a typical secondary rise in titer. These data indicate that the spleen is necessary for maintenance of normal low level antibody production and is also necessary for a response to a second i.v. injection of SRBC.

*Discussion.* In the experiments reported here, we showed that (1) splenectomy of rats after a single intravenous injection of SRBC inhibits continued production of antibody, and (2) splenectomy of rats after a first but before a second intravenous injection of small amounts of antigen results in much lower levels of antibody in response to the second injection of antigen. These data confirm Rowley's(1) data and extend his experiments as follows: Our data demonstrate that splenectomy abolishes hemolysin production completely when the antigen is given in small doses intravenously. The normal response of a rat to a single injection of SRBC is a rise in antibody titer until day 6, followed by a decrease in titer to a baseline level of antibody production. Splenectomy after the peak is reached causes the antibody titer to drop to zero rather than to maintain baseline levels. This drop is not immediate, but occurs 2-3 weeks after splenectomy. We also found that splenectomy before an initial injection of antigen results in no antibody production, while splenectomy between a first and second injection of antigen results in only small amounts of antibody in response to the second injection.

Thus it seems that splenectomy, whether after a primary injection of antigen or before a secondary injection of antigen, removes the cells capable of producing hemolytic antibody in response to intravenous injections of small amounts of SRBC antigen.

*Summary.* Splenectomy one day before antigen will inhibit completely the response to i.v. SRBC, but will depress the response below normal levels when performed 8 or 14 days after antigen. Secondary response is inhibited by splenectomy prior to the second injection of antigen. This appears to be different from results obtained with *S. typhi* flagellar antigen, in which no effect of splenectomy on secondary response can be demonstrated. This suggests that the anamnestic response to i.v. injections of SRBC depends on the presence of the spleen.

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## Cellular Respiration in Intermittent Magnetic Fields.* (31795)

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The possibility that man may be exposed to high magnetic fields has arisen from the proposed use of magnetic shielding against cosmic radiation and of magnetohydrodynamic propulsion in space travel(1). Reports on the inhibition of tumor growth *in vivo*(2) and *in vitro*(3) have conceivable clinical implications. In seeking a more

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quantitative technique for the study of biomagnetic effects and in hope of obtaining information that may ultimately help to clarify the mechanism of action we have sought to measure the effects of magnetic fields on cellular respiration. Our previous investigations(4,5) have shown that the 7300 gauss field of a permanent magnet significantly depressed the oxygen consumption of embryonic mouse kidney and sarcoma (S-37) cells. In limited experiments the respiration of baker's yeast (*Saccharomyces cerevisiae*) was stimulated. In the present investigations, these observations were not only confirmed and extended to mouse hepatic tissue, but the use of intermittent fields of an electromagnet demonstrated the ready reversibility of the effects. One problem in biomagnetic effects is the source of energy which may come either from the interaction of the field with internal components in motion in the cell or from the movement of the specimen in and out of the field. The intermittent field could provide greater total energy from the latter source because of the repeated establishment and collapse of the field. Finally, the use of the electromagnet allowed study of a series of field strengths and led to the conclusion that a relatively low critical field strength exists for these effects.

*Apparatus and method.* The experimental apparatus consisted of a Harvey Wells water cooled 9-1/2-inch electromagnet with a 2-inch pole gap and a microrespirometer mounted in a constant temperature (37 ± .01 C) water bath. The specimen chamber of the respirometer remained between the poles of the magnet. The field strengths employed in the experiments (Table I) ranged from 40 through 10000 gauss. With the power supply off, the residual field strength between the pole faces was 40 gauss, which was found to have no effect on oxygen uptake of the cells. The constant pressure differential microrespirometer was modified from a previous design(5,6). The respirometer was left for 40 minutes with its stopcock open for equilibration. After closing the stopcock the readings during the first half hour with the magnet off were disregarded in evaluating the experiments and served only to verify that

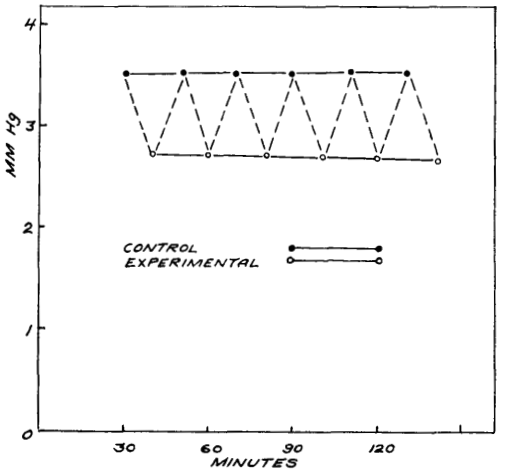


FIG. 1. Effects of intermittent magnetic fields of 80 to 1000 gauss on the respiration of ascites sarcoma 37 cells. Average of 25 experiments. The dotted line connecting individual 10-minute respiration values shows reversibility of the field effect. Average depression, 28.3 ± 2.6%.

the cells were respiring properly. The magnet was then switched on for 10 minutes (experimental period) after which the power was cut for the following 10-minute interval (control period). This procedure was repeated for a total of 2 hours, giving 6 control and 6 experimental readings in each experiment. In this way the tissue cells were exposed to intermittent magnetic fields under identical control and experimental conditions. Because the same tissue sample served as both control and experimental, results were not calculated as $Q_{O_2}$, and the average cumulative 10-minute readings of oxygen consumption in mm Hg during the experimental periods were compared with similar readings for control periods and the relation expressed in percentage of the control. Since tissue samples of 1 to 2 mg were employed, weighing was avoided as a source of error. In earlier experiments with separate control and experimental respirometers(4,5) it was necessary to calculate $Q_{O_2}$ values. Changes of ± 0.1 mm Hg were measurable with the respirometer so that differences of the order of 1 mm between experimental and control readings (as in Fig. 1) were significant and reproducible. The effects observed in the 10-minute respiration periods with S-37 were verified by experiments employing longer con-

TABLE I. Change in Respiration in Intermittent Magnetic Fields.

| Field strength, gauss | % Change (D = depression, S = stimulation) Ascites sarcoma 37 | Embryo kidney | Adult kidney | Embryo and neonatal (2-7 days) liver | Adult and neonatal (8-11 days) liver | Yeast |
|---|---|---|---|---|---|---|
| 40 | 0 | | | | | |
| | 0 | | | | | |
| 50–60 | 0 | | | | | |
| 70 | 0 | 0 | | 0 | | |
| | 0 | | | 0 | | |
| 75 | 0 | | | 0 | | 0 |
| 80 | D 31.5* | 0 | | D 19.8* | | 0 |
| | D 33.3 | D 12.0 | | D 24.0 | | S 10.2 |
| | | 0 | | | | D 6.8 |
| | | | | | | S 9.3 |
| 85 | | D 34.6* | | | | S 30.4* |
| | | | | | | S 42.1 |
| 90 | | D 29.3 | | | | S 37.5 |
| | | D 29.3 | | | | S 27.7 |
| 100 | D 16.2 | D 20.7 | 0 | D 14.8 | 0 | S 36.0 |
| | D 31.5 | D 45.4 | 0 | D 20.7 | 0 | |
| | D 42.9 | D 20.7 | | D 23.7 | 0 | |
| | D 30.0 | | | D 19.8 | | |
| | | | | D 21.4 | | |
| 200 | D 18.7 | | | | | |
| 500 | D 53.2 | | | | | |
| | D 21.5 | | | | | |
| | D 29.9 | | | | | |
| | D 31.5 | | | | | |
| 3000 | D 34.8 | | | | | |
| | D 29.5 | | | | | |
| 5000 | D 11.1 | D 25.1 | 0 | D 19.2 | 0 | S 63.2 |
| | D 24.9 | | 0 | | | S 55.7 |
| 7300 | D 40.0 | | | | | |
| | D 33.3 | | | | | |
| | D 27.2 | | | | | |
| | D 5.4 | | | | | |
| | S 5.0 | | | | | |
| 10000 | D 53.2 | | 0 | D 21.8 | 0 | S 27.3 |
| | D 22.5 | | | | | |
| | D 33.3 | | | | | |
| | D 25.7 | | | | | |
| | D 32.1 | | | | | |
| Mean change at and above critical field strength† | D 28.3 ±2.6 | D 29.3 ±3.5 | 0 | D 20.6 ±1.0 | 0 | S 40.0 ±5.0 |

* Approximate critical field strength.

† ± S.E.

trol and experimental periods (20, 30, and 60 minutes) with correspondingly larger volumes of oxygen consumption and decrease in error of measurement. In these experiments depressions of 25 to 32% were obtained, in good agreement with the 10-minute readings summarized in Table I.

*Materials.* Female Boontucky (BT) mice were the source of animal tissues. Ascites sarcoma 37 (S-37) cells were used 3 to 9 days following serial intraperitoneal implantation. Two drops of tumor suspension in homologous ascites fluid were used for each experiment. Slices of embryo kidney and liver, adult kidney and liver of 14-21 weeks old mice, and neonatal liver (of mice 2-3, 3-4, 6-7, and 10-11 days old of the same litter) were suspended in Hanks' Basal Salt Solution (BSS). Baker's yeast *(Saccharomyces cerevisiae)* from commercial Fleischmann's cake yeast was also suspended in BSS. Intermittent magnetic fields of from 70 to 10000 gauss were used as shown in Table I. Four experiments were performed with HeLa cells in Eagle's medium.

*Results and discussion.* The respiration of S-37 cells was depressed at field strengths of 80 gauss or higher. Lower field strengths pro-

duced no effect (Table I). A critical field strength lying between 75 and 80 gauss appears to be necessary to obtain effects. The average of 25 experiments on S-37 at 80 to 10000 gauss is shown in Fig. 1. The average depression of respiration was 28.3 ± 2.6% ($p = .02$).

Intermittent magnetic fields of 70 and 80 gauss had no significant influence on the respiration of mouse embryo kidneys. In 7 experiments fields of 85 through 5000 gauss produced an average depression of respiration of 29.3 ± 3.5% ($p < .05$). With this tissue the critical field strength was between 80 and 85 gauss.

No influence of intermittent magnetic fields of 100, 5000 and 10000 gauss could be detected in 5 experiments on the respiration of adult mouse renal tissue cells.

In 6 experiments at 80 to 10000 gauss, respiration of mouse embryo liver was depressed. No change in respiration resulted from exposure of embryo liver to fields of 70 and 75 gauss in 3 experiments. The respiration of young neonatal mouse liver (ages 2-3, 3-4 and 6-7 days) exposed to intermittent fields of 100 gauss was depressed while the respiration of livers of mice 8-9 and 10-11 days old was not altered and thus resembled the respiration of adult livers which was unaffected by exposures to fields of 100, 5000 and 10000 gauss. The respiration data for young neonatal livers were evaluated together with those for embryonic liver and gave an average depression of 20.6 ± 1.0% ($p = .01$) in 9 experiments. The data for the respiration of older neonatal tissue were grouped with those for the adult liver.

Field strengths of 75 and 80 gauss did not affect the respiration of yeast cells, but fields of 85 gauss and higher produced an average stimulation of 40.0 ± 5.0 ($p = .03$) in 8 experiments. The critical field strength, therefore, lies between 80 and 85 gauss.

In 3 experiments on HeLa cells, field strengths of 80, 100 and 1000 gauss caused respiratory depressions of 44.7, 40.1 and 42.9%, respectively. A 70 gauss field produced no effect.

*Summary.* Table I details the individual data on response of the different tissues (except the HeLa cells) to the magnetic fields. The application of fields of or greater than a critical value of approximately 80-85 gauss to ascites sarcoma 37, mouse embryo kidney and liver, young neonatal mouse liver, and (in limited experiments) HeLa cells, produced a significant lowering of respiration. Increase of field strength to 10000 gauss (125 times the critical value) resulted in no greater effect. The oxygen consumption of adult mouse kidney and liver and older neonatal mouse liver tissue cells was not affected by any intermittent magnetic fields. The respiration of yeast cells was significantly stimulated by fields of 85 gauss or above. The response to the fields was prompt and reversible for all affected cells. The overall results suggest that more actively proliferating cells are most responsive to the fields. At this time no explanation is advanced for the effects.

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