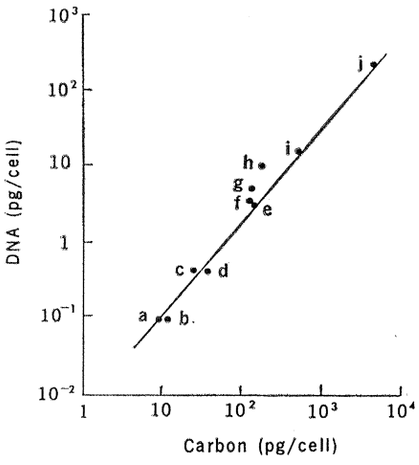

Fig. 1. Content of DNA unicellular algae as a function of the total organic carbon. *(a) Navicula pelliculosa; (b) Monochrysis lutheri; (c) Skeletonema costatum; (d) Dunaliella tertiolecta; (e) Amphidinium carteri; (f) Syracosphaera elongata; (g) Thalassiosira fluviatilis; (h) Cachonina niei; (i) Ditylum brightwellii; (j) Gonyaulax polyedra.*

Navicula pelliculosa and Cachonina niei indicates that the volume of the nucleus also increases proportionally with cell size (*6, 12*).

The observed correlation between cell size and DNA content in algae (Fig. 1) apparently holds for a variety of diverse plant and animal cells. Thus, data for bacteria (*13*), yeasts (*14*), protozoans (*15*), flagellates (*16*), sponge cells, and vertebrate cells (*1*) show that DNA accounts for about 0.2 to 5.0 percent of the cellular dry weight, or about 0.4 to 10 percent of the cellular organic carbon. Each cell of the amoeba *Chaos chaos* (approximately 400,000 pg of carbon per cell) contains 1400 pg of DNA, whereas somatic cells of man contain only about 6 pg of DNA (*2, 15*).

The amount of DNA in a cell cannot be equated either with phylogenetic position or biochemical and physiological complexity of the organism. There is, however, a parallelism between DNA content and cell size. It seems likely therefore that the amount of DNA required per cell does not directly reflect the amount of template information required by the cell, but rather it reflects rate-limiting reactions between the nucleus and the cytoplasm. A similar conclusion (*1*) has been suggested previously by comparison of DNA contents of bacteria and vertebrate cells. Thus, Mirsky and Ris (*1*) have stated that "the variations in DNA content per cell in vertebrates would hardly seem to be due simply to differences in the number of genes," while Brawerman and Shapiro (*3*) have written "a larger cytoplasm would require a larger number of metabolic units, such as enzymes, to perform a certain function, and if the genetic factors that control the formation of these units operate at a relatively slow rate, more of these factors would be required." Such a redundancy of certain DNA segments has been described by Britten and Kohne, who report that the genomes of some organisms contain hundreds of thousands of copies of certain DNA sequences (*17*). A correlation between DNA content and metabolic activity is afforded by Brown and others who have shown by specific hybridization techniques that the oocyte of the amphibian *Xenopus laevis* contains an enormously increased number of genes for 28*S* and 18*S* RNA (*18*). This increase in ribosomal genes correlates with the period of massive ribosome synthesis during oogenesis, after which the extra ribosomal DNA copies are somehow destroyed. It has been postulated (*4*) that DNA has two main roles: (i) It conveys genetic information through its action as a template for protein synthesis and (ii) it regulates cellular metabolism through the control of concentrations of free nucleotides by sequestration reactions (*4*).

The proportionality between DNA and cell carbon can be used for biomass determinations in ecological studies. Some estimate of the total living crop of organisms, both autotrophic and heterotrophic forms, is often desired, and analyses for total organic carbon or protein are not feasible because of organic detrital material. The usefulness of DNA measurements for estimation of biomass may be limited to some extent by the rate of hydrolysis of cellular nucleic acids upon death of the cell. However, DNA has been used as a biomass indicator in ocean samples (*8*).

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Intellectual Deficit Associated with Transplacentally Induced Microcephaly in the Rat

Abstract. *Fischer rats injected with methylazoxymethanol late in pregnancy produce young with considerably reduced cerebral hemispheres. They appear normal otherwise. As adults these animals make many more errors in the Hebb-Williams maze than do control animals.*

Microcephaly can be chemically induced in the rat by injection of pregnant females with methylazoxymethanol, a potent carcinogen which is the aglycone of a naturally occurring glucoside (cycasin). The effect is remarkably uniform, and has been replicated in more than one strain of rats. Such microcephalic animals have been maintained for over a year and showed no gross deficiencies in physiology or behavior (*1*).

It would appear, however, that a significant reduction of brain mass should affect adaptive capacity. Therefore we sought to establish whether such transplacentally induced microcephaly is associated with impairment of intellectual function in the adult animal.

The experimental subjects (12 male and 12 female rats) were offspring of six Fischer females that had each been injected intraperitoneally with a total of

20 mg of methylazoxymethanol per kilogram of body weight on the 14th, 15th, and 16th days of gestation. The control rats (10 male, 2 female) were the young of two females injected with physiological saline. The young, all born within a week, were 4 months old at the start of behavioral testing and were coded so that their identity was not apparent during testing. They were housed individually, maintained on a 23½-hour water deprivation schedule, and had free access to food.

The rats were tested in a Hebb-Williams maze, an instrument devised for testing intellectual function in the rat (*2*). Two sets of problems (1 to 12 and 13 to 24) were used, the problems of the second set being rotated mirror images of the first. Each rat was pretrained on practice problems until it reached a criterion of nine successive runs in 60 seconds. Two experimental males did not reach this criterion within 3 weeks and were not used further. Maze testing extended over 24 consecutive days, one for each of the 24 problems, with seven massed daily trials on each problem. A trial terminated in the goal box with a 15-second access to water. Errors were recorded on each trial.

During the maze experiment the rats were weighed three times a week. Weights of experimental and control animals of the same sex were not significantly different. Water consumption measures, made on the last three experimental days during the daily 30-minute access to water, also showed no group differences.

All animals were finally given an overdose of pentobarbital and were perfused intracardially with physiological saline followed by 10 percent formalin. The viscera and brains were examined for gross abnormalities and neoplasms. None were found. After paraffin embedding, serial sections were prepared for five of the experimental and four of the control brains. Succeeding sections were stained in rotation with a variety of procedures (hematoxylin and eosin, Nissl, Bodian, luxol fast blue, phosphotungstic acid hematoxylin, and periodic acid Schiff). The remaining brains were sectioned at representative levels.

All the experimental animals were microcephalic (Fig. 1). They all had lower brain weights than the control animals (Table 1). The reduction in size was mostly in the neo- and paleocortex, without a proportionate reduction in brain stem structures and cerebellum. Microscopic inspection revealed no demyelination, gliosis, or inflammatory infiltrates. However, there appeared to be some decrease in the number of neurons, particularly in the neocortex and hippocampus. Such a finding in the adult animal would agree with subsequent observations that methylazoxymethanol has a cytotoxic effect on neuroblasts in the rat fetus (*1*).

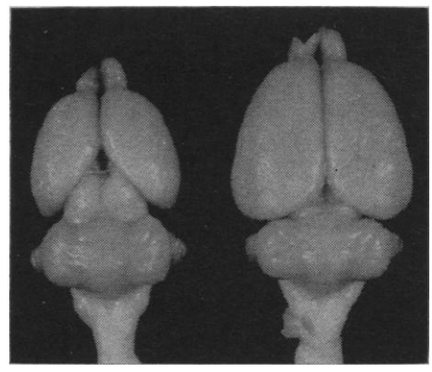

Fig. 1. Typical microcephalic (on the left) and normal brains of Fischer rats.

The maze performance of the microcephalic rats was substantially and significantly inferior to that of normal rats (Table 1). While poorer learning after a considerable reduction in telencephalic mass is not extraordinarily surprising, the finding that microcephalic females made significantly more errors than microcephalic males ($P < .025$) was completely unexpected, and is as yet unexplained. There was no such sex difference in errors for the normal animals ($P > .05$) although the brain weights of the female animals were less ($P < .025$) than those of the males for both the normal and the microcephalic groups.

Despite their inferior performance,

Table 1. Brain weights and maze errors of microcephalic and normal rats.

Sex	Microcephalic rats		Normal rats		P*
	Median	Range	Median	Range	
		Brain weights (g)			
Male	1.34	1.22–1.54	1.86	1.81–1.97	$< .001$
Female	1.26	1.19–1.43	1.72	1.69–1.75	$< .025$
Male and female	1.30	1.19–1.54	1.86	1.69–1.97	$< .000003$
		Maze errors			
Male	290.5	145–360	171.0	138–221	$< .01$
Female	347.0	265–450	191.0	179–202	$< .025$
Male and female	314.5	145–450	177.5	138–221	$< .00003$

* The probabilities given in this table as well as elsewhere in this report are based on Mann-Whitney U tests and are one-tailed (*3*).

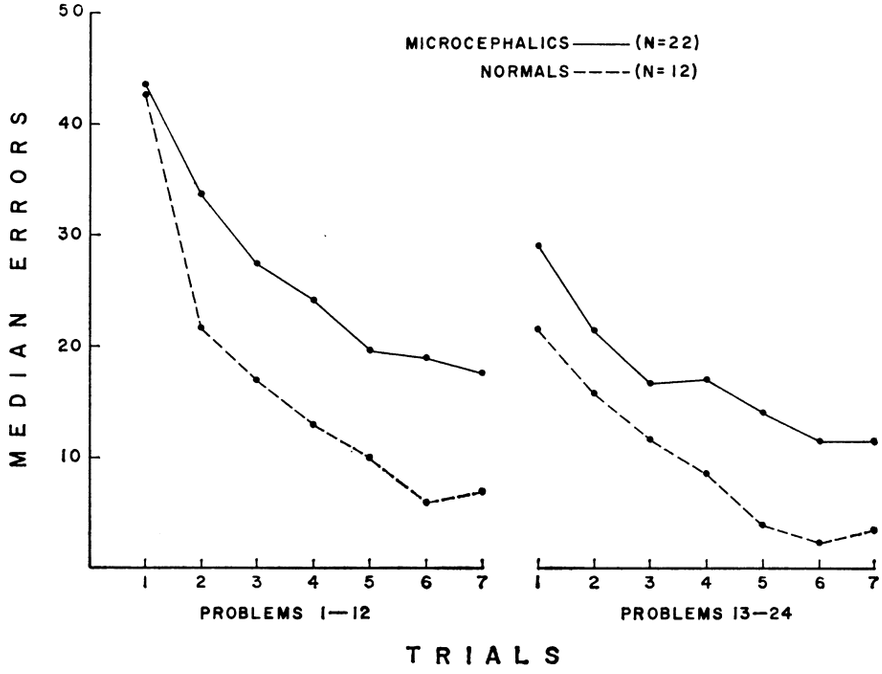

Fig. 2. Performance on the Hebb-Williams maze. Errors on seven daily trials, summed across problems 1 to 12 and problems 13 to 24.

the microcephalic rats nevertheless decreased their error rate in a manner quite similar to that of the controls (Fig. 2). Both groups did significantly better ($P < .005$) on the second set of problems (13 to 24) than on the first (1 to 12). This suggests that both microcephalic and control animals were forming learning sets (learning how to learn). Within the seven daily trials both microcephalics and controls improved their performance significantly: the average decrease in errors from trial 1 to trial 7 was significant ($P < .005$) for both groups on both sets of problems.

After completion of this experiment, a cytotoxic effect of methlazoxymethanol on retinal neuroblasts of the rat fetus was detected (*1*). This finding indicates the need for further controls for a possible visual deficit. However, there was no difference in error scores for the first trial on the first set of problems, which suggests that there was little or no impairment of sensory-motor function in these microcephalic rats.

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3. S. Siegel, *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956). The significance levels for the differences in both the brain weights and maze errors between all microcephalics and all controls may be somewhat inflated, since a considerably larger number of more severely affected animals (females) was added to the microcephalic than to the control group. However, considering the males and females as two groups replicating the same results, multiplication of the probabilities obtained with these groups separately gives levels only slightly lower than those presented for the combined groups in Table 1.
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Escape from Self-Produced Rates of Brain Stimulation

Abstract. *Rats were allowed to self-stimulate while their responses were being recorded on tape. Subsequently, prerecorded patterns of their brain stimulation were "played back" to them. All subjects learned to escape brain stimulation delivered in exactly the same manner as they had previously elected to receive it.*

Bower and Miller (*1*) were the first to demonstrate that rats would work to terminate a self-initiated train of intracranial stimulation (ICS). Beer and Steiner (*2*) showed that the rate of stimulation is an important variable in determining the reinforcing properties of ICS. The subject's opportunity to control its rate of brain stimulation may conceivably be another important factor in determining the reinforcing properties of ICS.

In our experiment we attempted to separate the effects of rate of stimulation from the subject's control of presentation of a stimulus in order to determine (i) whether animals escape from recordings of rates of their own stimulation (that is, whether rats learn to escape from ICS delivered in exactly the same manner as they had elected to receive it on a previous occasion) and, (ii) whether alterations in self-selected rates of stimulation change the escape behavior.

Five male albino rats (about 300 g) from the Walter Reed colony were the subjects. Rats were anesthetized with sodium pentobarbital, and then each had a bipolar, insulated, stainless steel electrode, bared at the tips, implanted in its brain with the use of a stereotaxic device. The electrodes were aimed at various hypothalamic structures that are known to yield effects of self-stimulation. After the experiment the animals were killed and perfused. Histological sections, stained either by the Weil or the cresyl violet method, were made to identify the sites of the electrodes. Electrode tips were found in the anterior hypothalamic area, the lateral hypothalamic area, and the ventromedial hypothalamic nucleus.

Rats were stimulated with pairs of biphasic rectangular pulses. Each pair of pulses consisted of two 0.2-msec waves opposite in polarity and separated from each other by an interval of 0.2 msec. Pairs of pulses were delivered at a frequency of 100 per second, and stimulus-train duration was held constant at 0.25 second. Current intensity, rate, and temporal pattern of stimulus trains were varied according to the demands of the experiment. Current, voltage, and wave form were monitored continuously on a twin-beam oscilloscope.

At least 10 days after surgery, subjects were trained to self-stimulate on one of two retractable levers in a sound-deadened experimental chamber. The range of current intensities that supported responding was explored. After stable rate-intensity functions were generated, a current intensity which maintained reliable and rapid responding was selected. The subjects were allowed to self-stimulate for 1 hour at the selected current intensity on a continuous reinforcement schedule. During this session, the exact temporal patterning of their responses was recorded on tape so that it could be precisely reproduced during a future session.

After a rest period in the home cage, the subject was returned to the experimental chamber where, for the first time, both levers were present. The lever on which the subject had previously self-stimulated (lever S) no longer affected reinforcement contingencies, but responses on that lever were counted. The animals' brains were stimulated by the previously recorded tape at the same intensity at which the subject had been self-stimulating. A response on the second level (lever E) terminated the brain stimulation for 20 seconds. The number of responses on both levers was recorded as was the latency from the onset of stimulation to the subject's first response on the escape lever (lever E).

All subjects learned to respond on lever E, which terminated their own taped rate, within the 1st hour. Escape latencies initially decreased as a function of the number of trials and then reached asymptote. During the escape condition, response rates declined on the self-stimulation lever (lever S). The initial response rate on lever S was high, but dropped to almost zero after 1 hour. Response rates on the new lever (lever E) were initially low but increased rapidly within 1 hour and then reached asymptote and remained there for the duration of the 6-hour session (Fig. 1).

For the next 19 days, subjects were allowed to escape their own prerecorded self-stimulation rates for 1 hour each day. After the initial decrease in escape latency, which occurred during the first