CRF circulates systemically to affect ACTH secretion. It is also suggested that this neurohumor has a relatively short half-life because 4 hours after removal of the hypothalamus and thalamus, corticosterone secretion was markedly reduced. However, after complete forebrain removal none of the corticosterone measurements decreased to levels found in hypophysectomized control animals. It has been our experience that within 4 hours after acute hypophysectomy, the adrenal corticosterone secretion rate falls to minimal values similar to those observed in chronically hypophysectomized rats. Therefore, it is possible that a small portion of the ACTH secretion of heterotopic pituitaries is not brain-dependent or that CRF has a longer half-life than ACTH. It is nonetheless clear that the major portion of ACTH secretion of heterotopic pituitaries is under the stimulatory influence of some subcortical portion of the brain.

Summary. Hypophysectomized rats with multiple heterotopic pituitaries have nearly normal adrenal weight and significant, though subnormal, corticosterone secretion. Removal of the entire forebrain, but not decortication, in these animals reduced corticosterone secretion nearly to values found in hypophysectomized control animals. These results indicate that heterotopic pituitary ACTH secretion is dependent on some subcortical portion of the forebrain.

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Instrumental Acquisition in Rats After Twelve Exposures to Deep Hypothermia.* (31128)

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When a mammal is being cooled the intensity of most of its body processes decreases. However, some processes increase initially but decline when hypothermia progresses further. An example of this is oxygen consumption, which in many mammals reaches a peak at a body temperature of 30°C (8). Further lowering of the body temperature diminishes and eventually stops all physiological processes. For various processes the critical temperature of cessation is found at different body temperatures. While

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the critical temperature for locomotion in rats is around 20-21°C, the critical temperature for lung ventilation is 13°C and for cardiac activity below 8°C(1). Like other physiological processes, the activity of the central nervous system is not abolished at a moderate hypothermia. It has been shown that inexperienced rats, cooled to a body temperature of 29°C, are able to acquire a simple heat reinforcement motor response in order to obtain external heat which will permit them to rewarm to euthermia and to survive a situation to which otherwise they would succumb(6). The latency period for response acquisition of these rats was only 48 minutes, much shorter than in euthermic rats exposed to cold environment. However, if the body temperature of the rats was further decreased and fell below 25°C, the rats failed to perform (7).

This stage of hypothermia, below 25°C, at which rats fail to acquire the selected test performance, was chosen to check the ability of "hypothermia-adapted" rats to acquire the same response. It was reasoned that animals which have been previously cooled on several occasions might be in a better physiological condition and respond in deep hypothermia better than rats which are cooled for the first time. If this were true, then the hypothermia-adapted rats should be able to acquire the response at even lower body temperatures than the control rats. The present experiments were designed to test this hypothesis.

Methods. Seven young white rats (Sprague-Dawley), 3 male and 4 female, were taken at the age of 10-20 days from 3 litters and were cooled 13 times by the Giaja confinement technique(2) to a body temperature of 16 to 18°C and then left at room temperature to rewarm spontaneously to euthermia. The hypothermia was induced 3 times per week for 4 successive weeks. When the animals were cooled the thirteenth time (average body weight—199 g), their bodies were clipped of hair and the animals cooled to a body temperature of 18°C. The rats were then taken from the cooling chambers and left to rewarm to 22°C. As soon as they reached this body temperature, they were placed in a dimly illuminated heat reinforcement cage(6,10) situated in a 2°C chamber. The body temperature remained rather constant at 22 to 25°C because of prior shaving, a decreased heat production, and a cold external environment. In a few instances it was necessary to elevate the chamber temperature briefly to avoid a fall of body temperature below 22°C. A T-shaped 8 g sensitive lever protruding 2.5 cm through the wall of the chamber was the only object in the cage besides the rat. Pressing of the lever closed a microswitch actuating the lighting of a 250 W infrared heating lamp for 2 seconds. The lamp was centrally placed 23 cm above the floor of the cage. Reactivation of the lamp was possible only after the lever was released and then pressed again. While the lamp was on, pressing of the lever had no effect. Each heat reinforcement delivered to the animals was recorded by a Varian Graphic Recorder. The same recorder was used to monitor deep colonic temperatures by thermistors. The average body temperature, from the beginning of the training phase until the moment the animal began to perform the required test readily, was integrated by use of a planimeter for measuring curves and areas. Food and water were not available during the experiment. Each experiment lasted 3 hours.

The procedure was developed so that: a) Rats would be placed in a situation with strong motivation for performing. The animals would not survive if they failed to respond by steady lever pressing. b) By accidentally pressing the lever, rats would be exposed to heat which reinforced the lever pressing activity. Moreover, these random heat reinforcements helped to maintain body temperatures of the animals at a constant level of 22 to 25°C during the whole experiment, thereby providing a lengthy exposure time.

The criteria used in deciding that the animals had acquired the heat reinforcement response were: A) a 3-5-fold increase in the rate of reinforcement and B) a consistent use of the heat reinforcement lever. All judgments were confirmed by visual observations through a small window of the cold chamber.

In the control group 7 adult rats (3 male and 4 female) were used. Each rat had the

TABLE I. Number of Heat Reinforcements and Time in Experimental Chamber Prior to Steady Performance in Hypothermia-Adapted Rats Maintained at a Body Temperature of 22 to 25°C.

Hypothermia-adapted rats

		Colonic temperature, °C		Performance latency	
Animal No.	Body wt	Avg before performance	At moment of performance	Minutes	Prior reinforcements
131	150	22.9	24.5	34	53
121	151	24.2	24.8	40	26
124	173	22.6	24.4	84	23
117	192	22.8	24.3	116	96
141	215	23.5	24.6	78	104
125	236	23.8	24.9	104	60
115A	261	23.6	22.2	64	47
Range	150–261	22.6–24.2	22.2–24.9	34–116	23–104
Avg	199	23.3	24.2	74	58

same body weight as the matching rat in the experimental group and was taken from the same litter. After being cooled for the first time the control animals were rewarmed to a body temperature of 22.0°C and then placed in the same experimental situation as the rats in the first group. Colonic temperatures and time and rate of heat reinforcements delivered prior to the onset of steady performance were measured by using the same procedures as with the hypothermia-adapted group.

Results. The body temperature of the hypothermia-adapted rats was maintained between 22 and 25°C. During apparently random movements in the cage, heat reinforcements averaged 0.8 per minute. After an average of 58 (23-104) lever presses, and after spending an average of 74 (34-116) minutes in the experimental chambers, all 7 experimental rats abruptly began to use the heat reinforcement lever much more often, an average of 5.2 (4.6-6.0) times per minute instead of 0.8 and continued to do so until their body temperature reached normal level. The visual observations also indicated a strikingly increased interest in the lever. At this point it was concluded that the rats had reached the level of a consistent performance which indicated a trained response. At this moment the average body temperature of the animals was 24.2°C (22.2 to 24.9°C). The mean body temperature of the rats during the whole period of training session was 23.3°C (Table I). Several minutes after the rats began to perform, their body temperature began to rise suddenly. The body temperature continued afterwards to rise regularly. The increase was approximately one degree for every 17 reinforcements or one degree for every 3.3 minutes, until euthermia was reached. The average peak temperature was 37.0°C (36.5-37.9°C). After reaching this body temperature, the reinforcement rate decreased by one-third.

The body temperature of the rats in the control group was kept, as far as possible, at the same level as the body temperatures of the rats in the hypothermia-adapted group. The average body temperature of control rats was maintained between 22.3 and 24.7°C. Nevertheless 5 control rats failed to perform even though, being left in the chamber for the full 3-hour experimental period, they were exposed to more accidental heat reinforcements than were the hypothermia-adapted rats (Table II). Only 2 control rats eventually began to perform for heat reinforcement. They did this later than any of the hypothermia-adapted rats (Table I).

Discussion. Although hypothermia has been studied extensively for many years, only recent experimental work revealed that the physiological state of cooled animals is improved when the animals have been previously exposed several times to the lowering of their body temperature. It was shown that while previously uncooled rats survive only 5 hours when cooled to a body temperature of 15°C, the rats cooled to the same body temperature survive up to 8 hours if they have been previously chilled 5 or 6 times(8). It appears, therefore, that mammals can be

TABLE II. Number of Accidental Heat Reinforcements and Time in Experimental Chamber Prior to Steady Performance in Rats Cooled First Time to a Body Temperature of 22 to 25°C. Non-adapted rats

		Colonic temperature, °C		Performance latency	
Animal No.	Body wt	Avg before performance or during 3-hr exp	At beginning of performance	Minutes	Prior reinforcements
144	150	22.3	24.5	132	109
146	153	22.9	(no performance)	(180)	(132)
145	176	24.7	(" ")	(180)	(181)
149	188	22.3	(" ")	(180)	(65)
147	202	22.7	23.8	175	178
98	245	23.0	(no performance)	(180)	(76)
94	253	24.0	(" ")	(180)	(61)
Range	150–253	22.3–24.7	23.8–24.5 (2 animals)	132–175 (2 animals)	109–178 (2 animals)
Avg	195	23.1	24.2 (2 animals)	154 (2 animals)	144 (2 animals)
			(no performance) (5 animals)	(180) (5 animals)	(103) (5 animals)

Parentheses ( ) indicate that experiment was terminated after 180 min. During this time the animal did not meet our criteria for performance.

adapted to a low body temperature similar to what is experienced when the animals are kept at a low external temperature for several weeks.

Our experiments have shown that response acquisition at a decreased body temperature is improved when prior to the experiment animals have been cooled twelve times. The hypothermia-adapted rats began to perform at a lower body temperature and much sooner than the control animals. While all of the hypothermia-adapted rats satisfied our criteria for response acquisition, 70 percent of the non-adapted rats at the same low body temperature failed to do so. This is in agreement with findings on 250-300 gram rats which consistently failed to perform when their body temperature was below 25°C(7). The two non-adapted rats which performed adequately did so only after longer exposure than any of the hypothermia-adapted rats.

In interpreting our results, the effect of motivation should be considered. We believe, however, that in our experiments this factor has been kept constant, prior to and at the moment at which performance begins. For this reason the body temperature of experimental animals throughout the training session was kept as constant as possible. Of course the need and motivation for heat will change as the animal begins to rewarm itself to euthermia. Age and body size are other factors which remain to be studied in respect to response acquisition in hypothermia because younger animals stand hypothermia better than adults(3,4). In the present experiments we tried to eliminate this factor by matching animals in order to obtain more easily interpretable results. The great complexity of interdisciplinary problems such as the one we undertook was recently discussed by Mrosovsky(5). He suggested that increased physical activity of animals at a decreased body temperature might be a factor in initiation of an earlier lever-pressing in hypothermia. This would suggest that earlier performance in hypothermia-adapted animals might really be an indication of increased physical activity of the hypothermic animals rather than of an earlier response acquisition. However, it has been shown that physical activity of hypothermic rats stays approximately the same in the wide ranges of body temperature. The animals which were trained to intracranial self-stimulation do not use the lever during rewarming until their body temperature reaches 36°C(9). Table III shows furthermore that oxygen consumption at a body temperature of 24-25°C is approximately the same in hypothermia-adapted animals as in animals which were cooled to this body temperature for the first time.

TABLE III. Oxygen Consumption, ml/kg/min, in Rats at a Body Temperature of 24-25°C.

Animal No.	Non-adapted rats	Animal No.	Hypothermia-adapted rats
1	15.8	5	14.3
2	17.7	6	18.5
3	22.3	7	20.0
4	23.0	8	21.4
Avg	19.7 ± 1.7	Avg	18.6 ± 1.7

t = .46 (non-significant)

The improved performance of the hypothermia-adapted rats probably reflects the "better condition" of the central nervous system in hypothermic animals which have previously been exposed several times to lowering of their body temperature. How much the physiological state of hypothermia-adapted animals is changed becomes evident when it is recalled that the body temperature at which adapted rats respond excellently is the body temperature barely higher than the one at which locomotion stops and a complete physical anesthesia by cold is induced.

We believe that our work shows a new type of adaptation—adaptation to hypothermia—which is measurable in terms of operant behavior at a low body temperature. After several prior exposures to hypothermia, rats cooled to a low body temperature acquire a simple technique for obtaining external heat faster and at a lower body temperature than unadapted hypothermic animals.

Summary. Hypothermic rats acquire a simple technique for obtaining external heat more quickly at a body temperature of 29-30°C than at other decreased body temperatures. When the body temperature is decreased below 25°C the latency period for acquisition of the new behavior is much extended and the performance is rarely observed during a period of 180 minutes. However, when rats are exposed to hypothermia several times prior to the response acquisition experiment they are able to respond steadily not only at a body temperature of 25°C but even at temperatures between 22° and 25°C. While all hypothermia-adapted animals thus responded at these body temperatures (22.2-24.7°C), 5 out of 7 non-adapted animals failed to do so. The performance latency time of the 2 non-adapted rats which eventually performed was longer than that of any hypothermia-adapted rat. It is concluded that previous exposure to hypothermia ("adaptation to hypothermia") enhances the response acquisition ability of rats at low body temperatures.

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The Manometer Factor in Measurement of Tissue Pressure.* (31129)

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Tissue pressure has been reported on occasion to be negative with respect to atmospheric pressure(1-3). Guyton(2,3) implanted small perforated plastic balls in the subcutaneous connective tissue; after subsidence of the acute inflammatory process, subatmos-

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