zure pattern is clearly anticonvulsant (Fig. 1). The absence of a relation between intensity of maximal seizures, post-seizure depression, and brain ACh concentration and ChE activity, whereas seizure thresholds appear to be related to ChE activity, is further evidence of the independent control of these 3 measures of convulsive responses.

Summary. Marked strain differences in minimal electroshock seizure threshold, maximal electroshock seizure pattern, and duration of post-seizure depression were found in adult males and females of 6 strains of rats. Seizure thresholds in one group averaged only 65% of thresholds in another group. Duration of tonic extension during maximal seizures and duration of post-seizure depression showed 10- to 16-fold variations among strains tested. Females in general showed greater convulsive susceptibility than males of the same strain, probably because of the excitatory action of estradiol. In males, differences in seizures were compared with values for brain acetylcholine (ACh) concentrations and cholinesterase (ChE) activities reported previously for these strains. In each of the 3 pairs of strains, selectively bred from 3 foundation stocks, the strain with the lower seizure threshold had the higher ChE. On the other hand, intensity of seizures and post-seizure depression could not be related to brain ACh or ChE.

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Role of the Thymus in Recovery of the Immune Mechanism in the Irradiated Adult Mouse. (28170)

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It is now well established that the thymus is essential for the provision of cells with immunological competence during development(1,2,3,4,5). Mice thymectomized at birth characteristically show (a) the development of a fatal wasting disease between 1 and 4 months of age (actual age depending on the strain(2,3,6), (b) a marked deficiency of small lymphocytes in blood and tissues (1,2,3), (c) a diminished ability to produce serum antibodies to some antigens(2,7), and (d) a permanent failure to reject skin grafts not only from foreign strains but also from rat donors(2,3). Thymectomy after 3 weeks of age is not associated with any marked defect except for a reduction in lymphoid cell population(8). This might at first suggest that the specific function of the thymus is restricted to the period of life before it involutes. Experiments performed in this laboratory(9) have, however, clearly shown that the thymus in adult life is essential for com-

plete recovery of the immune mechanism following sublethal irradiation. The data reported here indicate that the immune mechanism fails to be reestablished after lethal irradiation in mice, thymectomized in adult life, followed by syngeneic marrow therapy.

Materials and methods. Highly inbred CBA mice were divided into 2 control and 2 experimental groups as follows:—Group I (first control group): the mice were sham-operated at 8 to 10 weeks of age, irradiated (850 r total body) one week after sham-thymectomy and given 5 million syngeneic normal marrow cells intravenously 4 to 6 hours after irradiation. Group II (second control group): the mice were thymectomized at 8 weeks of age, sham-irradiated one week after thymectomy and given 5 million normal syngeneic marrow cells 6 hours later. Group III (first experimental group): the mice were thymectomized at 10 weeks of age, irradiated (850 r) one week after thymectomy and given 5 million normal syngeneic marrow cells 6 hours after irradiation. Group IV (second experimental group): the mice were treated as in Group III except that they received marrow from adult CBA mice that had been thymectomized at birth.

Thymectomy and sham-operation were performed as described previously(10). The X-rays were generated in a 220 kv Westinghouse therapy unit operating at 15 mA without added filtration. The half value layer in copper was 0.4 mm, the target distance 100 cm and dose rate 60 r per minute. The mice were housed in perspex containers during the procedure. For sham-irradiation, the mice were placed under the tube for the same length of time as the irradiated mice with the machine switched on but the shutter kept closed. Measurements made during this exposure showed that they received 3.5 r total body irradiation.

Marrow was obtained from the femurs of 2-month-old donor CBA mice. It was suspended in 199 culture medium and each mouse received an intravenous injection of 5 million viable marrow cells in 0.4 ml of medium.

Four weeks after irradiation skin grafts were performed according to the method of Billingham and Medawar(11). The mice were challenged with 0.2 ml sheep erythrocytes in Alsever's solution 60 days after irradiation and bled from the retro-orbital venous plexus 7 days after challenge in order to titrate the sera for haemagglutinins. A second challenge was made 7 days after the first and the sera titrated 5 days later. Sera from normal unchallenged mice and from normal mice, challenged with sheep red cells in the same way as above, were also titrated.

Absolute and differential white cell counts were made from tail vein blood, the first sample being obtained 76 days after irradiation and the second 100 days. Blood smears were stained with Giemsa's stain. Differential counts were made on 1000 leucocytes for each sample.

Some mice were sacrificed for histological purposes at about 80 days after irradiation. Sections were fixed in Bouin's fluid and stained in haematoxylin and eosin.

Results. The mortality before 30 days in control and experimental groups was as follows:—Group I, 26 mice treated, 8 died; Group II, 24 mice treated, 1 died; Group III, 26 mice treated, 3 died; Group IV, 28 mice treated, 9 died. Longevity data for the survivors will be reported later.

The peripheral leucocyte levels of experimental and control mice at 76 and 100 days after irradiation or sham-irradiation are shown in Table I. These data can be summarized as follows:—(a) There is no significant difference between mice of Group I and entirely normal (untreated) mice of the same age in the absolute leucocyte levels and ratios at either 76 or 100 days. (b) All absolute leucocyte levels of Group II mice are significantly ($P<.001$) lower than values for normal or Group I mice at either 76 or 100 days. As this depression involves both polymorphonuclears and mononuclears and also both small and large lymphocytes, ratios between these values are not significantly different from normal. Values for polymorphonuclears in Group II are low on day 76 but within normal limits on day 100. (c) Values for total leucocyte, total mononuclear and small mononuclear levels in all thymectomized mice (Groups II, III and IV) are sig-

TABLE I. Peripheral Blood Leucocyte Levels of CBA Mice Thymectomized in Adult Life, Irradiated and Injected with Syngeneic Marrow.

	Group*	Total leucocyte per cu mm	Total mononuclear per cu mm	Total polymorphonuclear per cu mm	*Mononuclear* polymorphonuclear	Small‡ mononuclear per cu mm	Large mononuclear per cu mm	Small/large mononuclear
5-mo-old untreated CBA mice		9730 ± 900†	8190 ± 860	1440 ± 210	5.9 ± .6	6820 ± 670	1370 ± 200	5.6 ± .6
76-day sample	I. Sham-thymectomized; 850r; I.V.I. syngeneic marrow	10160 ± 820	8170 ± 370	1990 ± 190	4.2 ± .3	6860 ± 590	1310 ± 150	5.6 ± .6
	II. Thymectomized; sham-irradiated; I.V.I. syngeneic marrow	3710 ± 370	2790 ± 300	920 ± 90	2.8 ± .2	2280 ± 220	510 ± 60	4.6 ± .3
	III. Thymectomized; 850r; I.V.I. syngeneic marrow	2540 ± 360	1300 ± 210	1240 ± 180	1.1 ± .4	830 ± 130	470 ± 100	2.2 ± .4
	IV. Thymectomized; 850r; I.V.I. marrow from neonatally thymectomized CBA mice	5210 ± 630	2470 ± 280	2740 ± 370	.9 ± .1	1350 ± 120	1110 ± 170	1.4 ± .2
100-day sample	I. Sham-thymectomized; 850r; I.V.I. syngeneic marrow	8110 ± 970	6210 ± 790	1900 ± 270	3.5 ± .4	5170 ± 640	1040 ± 120	5.0 ± .5
	II. Thymectomized; sham-irradiated; I.V.I. syngeneic marrow	3610 ± 610	2320 ± 480	1290 ± 220	2.0 ± .3	2320 ± 240	620 ± 80	2.7 ± .4
	III. Thymectomized; 850r; I.V.I. syngeneic marrow	4620 ± 470	2130 ± 220	2490 ± 270	1.0 ± .2	1430 ± 160	710 ± 80	2.1 ± .4
	IV. Thymectomized; 850r; I.V.I. marrow from neonatally thymectomized CBA mice	4750 ± 560	1920 ± 270	2830 ± 320	.7 ± .1	970 ± 100	950 ± 90	1.0 ± .2

* 10 mice in each group. † ± standard error. ‡ Diameter $<8\mu$.

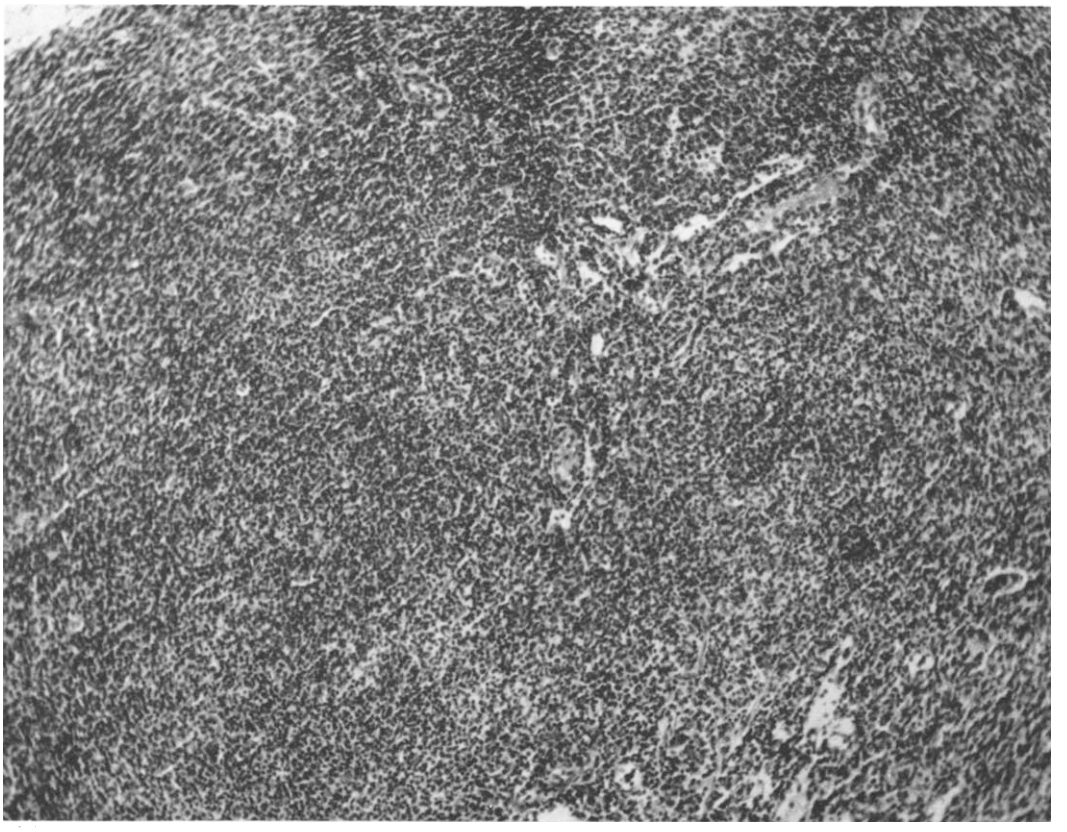

FIG. 1. Lymph node of sham-thymectomized irradiated and marrow protected mouse 80 days after irradiation (×160).

nificantly ($P<.001$) lower at either 76 or 100 days than similar values in mice of Group I. (d) There is no significant difference between mice of Groups II and III in the levels of total leucocytes at either 76 or 100 days, nor in the levels of the total polymorphonuclears and large mononuclears. Values for the total mononuclears and small mononuclears in mice of Group III at 76 days are significantly ($P<.001$) lower than those of Group II mice. Hence the ratios between mononuclears and polymorphonuclears and between small and large mononuclears are also significantly lower. The difference between the total mononuclears in Groups II and III mice is no longer significant at 100 days. (e) Total leucocyte and mononuclear counts at 76 or 100 days in mice of Group IV are not significantly different from those of Group II. However, the levels of small mononuclears and the ratio between small and large mononuclears are significantly lower in mice of Group IV. There is a relative polymorphonuclear leucocytosis in Group IV mice as compared with mice of other groups.

The white pulp of the spleen and lymph nodes of sham-thymectomized irradiated mice was well populated with small lymphocytes at time of sacrifice (Fig. 1). In sham-irradiated thymectomized mice, there was evidence of some deficiency of these cells (Fig. 2) and in thymectomized, irradiated mice, this deficiency was extreme (Fig. 3). Pyroninophilic cells were present in the lymphoid tissues in mice of all 4 groups.

The data in Table II indicate that mice in the control groups (I and II) had completely recovered the capacity to produce both a primary and a secondary immune response to sheep erythrocytes. The majority of the mice in both experimental groups (III and IV) failed completely to produce detectable levels of haemagglutinins after both a pri-

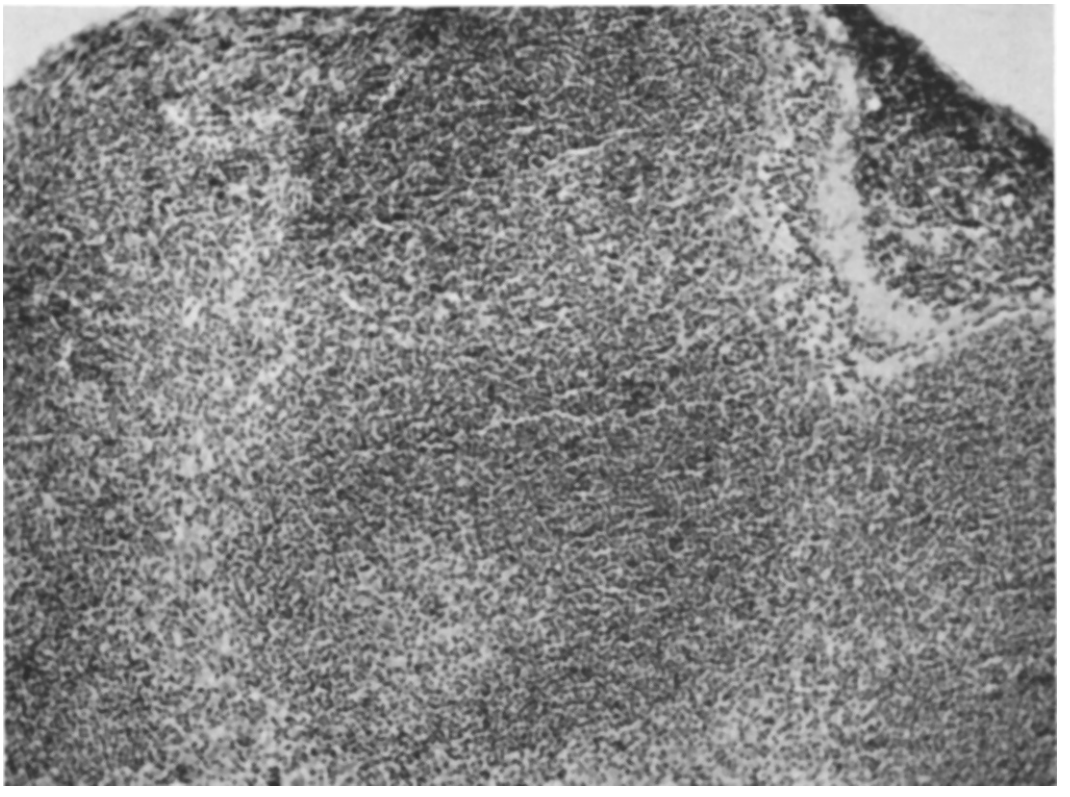

FIG. 2. Lymph node of sham-irradiated thymectomized mouse 80 days after sham-irradiation (×160). Note partial depletion of small mononuclear cells.

TABLE II. Immune Response of CBA Mice, Thymectomized in Adult Life, to Sheep Erythrocytes.

Treatment	No. of mice in group	No. of mice showing following $\log_2$ haemagglutinin titer: 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Untreated and not challenged with sheep erythrocytes	10	10																		
Not thymectomized; not irradiated; I.P.I. sheep erythrocytes	10*									2	8									
	9†											2		4		2				1
I. Sham-thymectomized; 850r; I.V.I. syngeneic marrow; I.P.I. sheep erythrocytes	14*									2	12									
	15†											14		1						
II. Thymectomized; sham-irradiated; I.V.I. syngeneic marrow; I.P.I. sheep erythrocytes	12*									3	9									
	15†											6		7		2				
III. Thymectomized; 850r; I.V.I. syngeneic marrow; I.P.I. sheep erythrocytes	14*	10	2	2																
	13†	10	2	1																
IV. Thymectomized; 850r; I.V.I. marrow from neonatally thymectomized CBA mice; I.P.I. sheep erythrocytes	14*	12	1					1												
	14†	12		1	1															

* Primary response. † Secondary response.

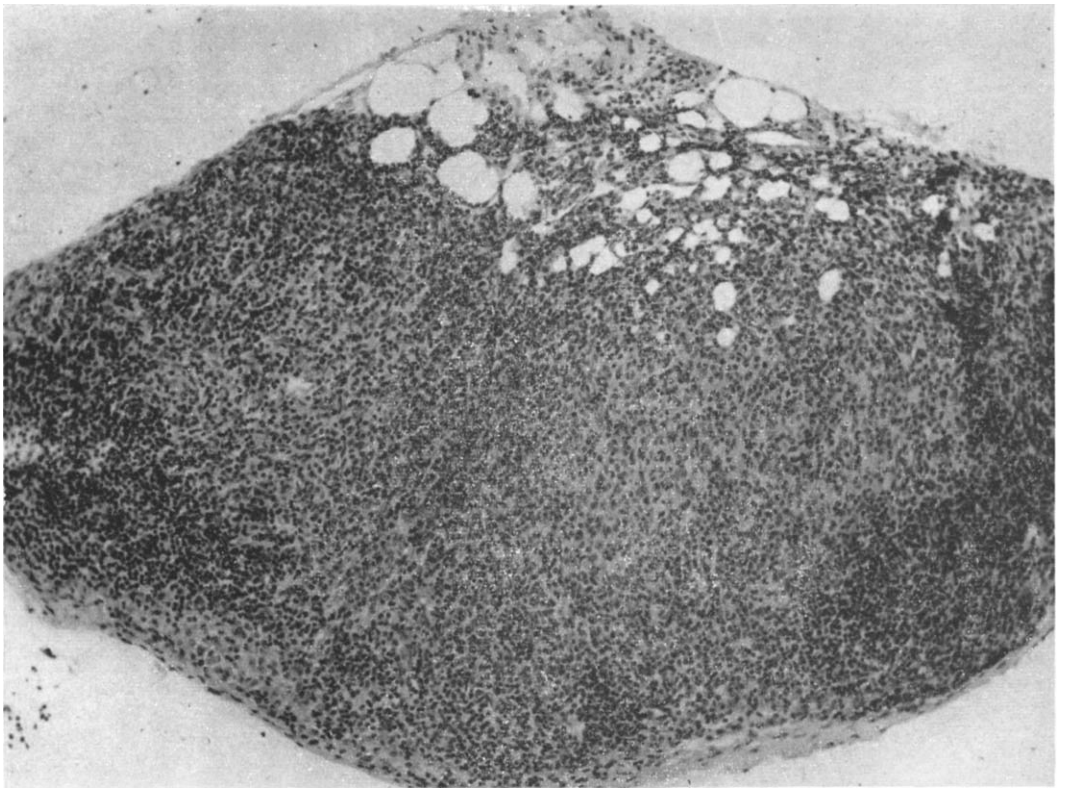

FIG. 3. Lymph node of mouse thymectomized in adult life, irradiated and marrow-protected, killed 80 days after irradiation (×160). Note severe depletion of small mononuclear cells.

mary and a secondary challenge with sheep red cells.

All the mice in both control groups were capable of rejecting skin grafts from foreign strains in less than 20 days. On the other hand, the great majority of mice in the experimental groups failed to reject grafts even from donors which differed from them at the H-2 locus (Table III). Most of these mice retained grafts permanently.

Discussion. The experiments described above clearly show that recovery of lymphopoietic and immune functions following lethal doses of total body irradiation and syngeneic marrow therapy takes place by means of a thymus-dependent mechanism. Non-thymectomized, irradiated mice had normal lymphocyte populations and normal immune func-

TABLE III. Effect of Thymectomy in Adult CBA Mice on Recovery of Immune Response to Skin Homografts Following Lethal Irradiation (850r) and Marrow Protection.

Group	No. of mice in group	Donor skin	No. of mice showing skin graft survival for: <20 days	20-70 days	>70 days
I. Sham-thymectomized; 850r; I.V.I. syngeneic marrow.	18 18	Ak C57BL	18 18	0 0	0 0
II. Thymectomized; sham-irradiated; I.V.I. syngeneic marrow.	23 23	Ak C57BL	23 23	0 0	0 0
III. Thymectomized; 850r; I.V.I. syngeneic marrow.	19 19	Ak C57BL	1 1	1 1	17 17
IV. Thymectomized; 850r; I.V.I. marrow from neonatally thymectomized CBA mice.	20 20	Ak C57BL	1 0	0 3	19 17

tions when tested 4 to 10 weeks after irradiation. In contrast, thymectomized, irradiated mice characteristically showed a marked diminution of small lymphocytes in blood and tissues, neither a primary nor a secondary response to sheep red cells and a permanent impairment of homograft immunity. This strongly suggests that the thymus in the adult mouse may be essential for reestablishing immunological function under any circumstances in which the body's immunological potential has been largely destroyed or depleted.

Restoration of immune reactivity after total body irradiation could presumably take place either (a) by means of a process of peripheralization of cells arising in the regenerating host thymus: these cells might either themselves be responsible for immune reactions or might, by some mechanism, reactivate neighbouring cells in the lymphoid tissues; (b) by means of a thymus-specific humoral factor inducing lymphopoiesis and lymphoid maturation in lymphoid tissues, or (c) by allowing "lymphoid differentiation" of stem cells migrating to the thymus; such cells would then recolonize the depleted lymphoid tissues.

It has been clearly established that after doses of irradiation, such as those used in the present experiments, the haematopoietic sites of the host are repopulated by injected donor cells(12). Further, evidence has accumulated to show that the lymphatic tissues, including the thymus, are also repopulated by donor cells. In rat-mouse chimaeras, it was noted that the initial regeneration of thymus cells was of mouse host type but by 21 days only thymocytes of rat type could be identified(13). In mice injected with syngeneic, chromosomally-marked marrow cells plus marked lymphoid cells, the lymphoid cell marker initially appeared only in the lymph nodes, not in the thymus nor in the bone marrow. The marked marrow cells were present in the bone marrow and thymus. After 2 months, the marked lymphoid cells disappeared and the marrow marker appeared throughout. This suggested that cells in the marrow that could differentiate towards lymphoid cells had a temporary period of residence in the thymus before passing on to repopulate the lymphoid tissues(14). One may, therefore, presume that there exists in the marrow inoculum cells with immunological potential that can express this potential only after differentiating in the thymus environment. Once differentiated, they may no longer be dependent on the thymus for their immunological role. Preliminary experiments have indicated that lymph node cells can restore immune functions in a thymectomized animal, at least for a time(15).

Thymectomy of the mouse at birth, when presumably immunological potential has not fully differentiated, has been associated with failure of the maturation of immunological faculty(1,2,3). In marked contrast is the negligible effect of thymectomy in the adult animal(2,3) in which immunological potential has presumably fully developed. Such animals, if not irradiated, are still capable of rejecting foreign skin grafts and of producing primary and secondary responses to sheep erythrocytes. Yet the population of small mononuclears in their peripheral blood steadily drops and approaches the low levels characteristic of thymectomized, irradiated animals or of neonatally-thymectomized animals. It may be, therefore, that after a long period the animals thymectomized in adult life would become depleted of cells with immunological potential, owing to the limited life span of these cells, and that eventually immunological defects would become evident.

In conclusion it may be stated that the results presented here add further evidence to the idea that, throughout life, the thymus acts as an essential factor in the function of the immune mechanism(9).

Summary. 1. Inbred mice were thymectomized at 2 months of age, given lethal doses of irradiation and syngeneic marrow therapy. Controls were either sham-thymectomized and irradiated or sham-irradiated and thymectomized. 2. Thymectomized irradiated mice failed to reject allogeneic skin grafts and to produce either a primary or a secondary immune response to sheep erythrocytes when tested 4-10 weeks after irradiation. At that time, control mice had normal immune functions. 3. Lymphocytes were markedly

diminished in the experimental and the thymectomized control groups. They were present and within normal limits in sham-thymectomized irradiated controls. The depression in the thymectomized irradiated mice characteristically involved the small mononuclear cells. 4. It is concluded that recovery of the immune mechanism following total body irradiation takes place by means of a thymus-dependent mechanism.

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Effect of Removal of Various Parts of the Brain on ACTH Secretion in Dogs.* (28171)

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The increase in adrenal glucocorticoid secretion produced by surgical trauma in dogs is blocked by lesions of the median eminence of the hypothalamus(1). However, Egdahl has reported(2) that glucocorticoid secretion is elevated after removal of the entire hypothalamus and cerebrum. He has postulated that a circulating factor from the hind brain which stimulates the pituitary to release ACTH is responsible for this elevated level of adrenocortical activity. If this hypothesis is correct, removal of the hind brain in addition to the hypothalamus should lower the level of adrenocortical secretion to that seen in animals with lesions of the median eminence. In the present studies, the effect on adrenocortical secretion of removal of the entire brain in dogs has been determined, and compared to the effects of removal of parts of the brain.

Methods. Brain operations were performed under pentobarbital anesthesia in male mongrel dogs weighing 7.5 to 16.8 kg. The operations were as follows: *Group 1.* Bilateral craniotomy with opening of the dura only (4 dogs); *Group 2.* Removal of all brain tissue

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