mately $^1/_3$ of a donor spleen was transplanted to a bed prepared in the renal cortex by the method of WHEELER, CORSON and DAMMIN[8]. ALS or NRS was administered 3 times during the 1st post-operative week, twice in the 2nd week, and once during the 3rd week.

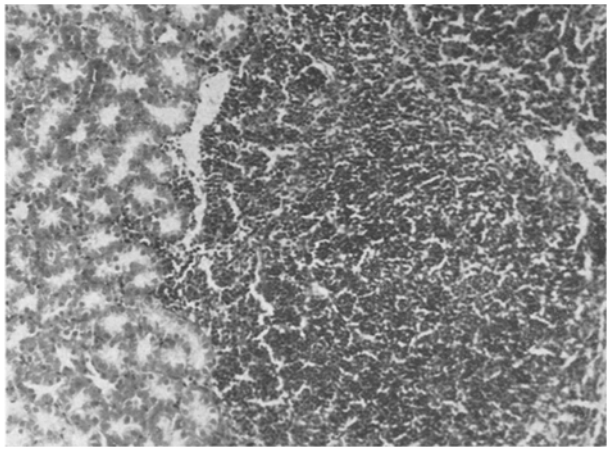
Red blood cell counts made by routine haemacytometry, and electrophoresis of the haemoglobins from red cell lysates[9] were performed on day 0, before transplantation, and on days 40, 80, 120 and 200 after grafting. After day 200, chromosome studies of various tissues were made using the air drying method of FORD[10]. In some cases the spleen graft was examined cytologically and in others its histology was studied after sectioning and staining with haematoxylin and eosin.

Nine of the 14 mice (64%) treated with ALS and an allogeneic solid tissue spleen graft showed an increased red blood cell count (Table I) together with the presence of donor haemoglobin. It was first apparent on day 40 and was maintained through to day 200 when the experiment was terminated. None of the control NRS treated mice showed any increase in red cell numbers or the presence of donor type haemoglobin.

Table II. Mean percentage of donor cells in the tissues of W^vW^v anaemic mice 200 days after being transplanted with a solid tissue graft of haematologically normal spleen

% Donor cells found in				
Bone marrow	Spleen	Thymus	Lymph nodes	Spleen graft[a]
99	98	96	74	95

[a] 50 or 100 metaphase plates scored in each tissue.



Histological appearance of part of a healthy spleen graft from a haematologically normal mouse 200 days after transplantation to the renal cortex of a W^vW^v anaemic mouse. Kidney on the left, part of the spleen graft on the right. ×187.

Chromosome studies made after day 200 showed that donor cells comprised almost the whole of the dividing population of the W^vW^v bone marrow and also the spleen and thymus and much of the lymph nodes (Table II). Interestingly, the spleen graft itself did not consist entirely of its original CBA cells but in all cases it contained some W cells, emphasizing the free circulation of haemopoietic stem cells between the components of the lympho-myeloid complex.

Macroscopically, the successful spleen grafts resembled an intact spleen. They had not increased in size since grafting. Microscopically, they showed the normal splenic architecture of trabeculae, splenic nodules and red pulp (Figure). There was no obvious sign of erythropoiesis taking place. In all the control mice there was no spleen apparent to the naked eye, but a whitish layer covered the graft bed. There was no regeneration of the kidney, the bed remaining as an indentation in the cortex. Microscopically, there was a thin eosinophilic layer next to the kidney tissue, acellular except for a few mononuclear cells. On the outer side of this there was a fairly thick layer of connective tissue.

Although the adult spleen is regarded mainly as a lymphopoietic organ, HELFRE et al.[11] found that there is an erythroblastic line amounting to about 5% of the total cellular output. However, it is shown by the identification of the donor cells in some of the recipient organs that the W^vW^v mice become cured of their anaemia not by the grafted spleen becoming an erythropoietic organ, but by the migration of stem cells from the graft to the bone marrow. Some implant there, multiply and replace the defective W^vW^v cells and institute normoblastic erythropoiesis[12].

Résumé. Une portion d'une rate allogénique normale été transplantée sur le rein de souris anémiques, génotype W^vW^v, immunosuppressent avec le sérum antilymphocyte; 64% ont guéri. Les cellules hématopoïétiques émigrent hors de la greffe dans la moelle osseuse, s'y fixent, prolifèrent et remplacent les cellules défectueuses de l'hôte.

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Brain Norepinephrine Levels and Turnover Rates in Castrated Mice Isolated for 13 Months[1]

Environmental isolation is known to induce behavioral changes in both animals[2,3] and man[4,5]. One such change is the increased aggressiveness observed in a number of strains of adult male mice following isolation[6–8]. The presence of testosterone, the male sex hormone, appears necessary for the occurrence of this phenomenon in mice. For example, isolation-induced aggression can be prevented if mice are castrated prior to isolation[9]. Steroid replace-

ment, in isolated animals which have been castrated, results in an increased aggressive behavior which is directly related to the androgenic properties of the steroid employed[10]. Furthermore, it has been suggested that the failure to obtain increased aggression in mice isolated for extremely long periods of time, i.e. a year or more, may be due to a decreased amount of testosterone in these aged animals[11].

Research on the biochemistry of aggresssion has focussed on the role of the brain biogenic amines[12]. Since alteration in testosterone levels produce significant changes in catecholamine metabolism[13] the possibility exists that the effect of testosterone in isolation-induced aggression may be mediated by changes in the dynamics of brain norepinephrine (NE). In the same study in which aggression was measured, SIGG[9] also investigated the effects of castration and short-term isolation (3–6 weeks) on brain NE levels. No changes in NE levels were observed with isolation alone while castration plus isolation resulted in increased levels.

The purpose of the present study was to investigate the interaction of the effects of castration and long-term isolation (13 months) on whole brain NE activity. In addition to measuring levels, possible changes in NE rate constants and turnover times were also measured in order to better assess the functional dynamics of this system.

Fifty male mice of the C57/Br/6J strain, 35–40 days old (Jackson Laboratories, Bar Harbor, Maine), were either castrated or sham-castrated. All animals were then placed in individual isolation compartments formed by placing a masonite board diagonally across a 25.4 cm × 19.05 cm × 12.7 cm metal cage. Animals were maintained undisturbed in these compartments except for their gentle removal every 2 weeks for cage cleaning. Free access to food and water was provided throughout the 13 months-period of isolation.

Norepinephrine turnover rates were assessed by inhibiting catecholamine synthesis with α methyl-p-tyrosine (AMT)[14,15] and then measuring the rate of disappearance of NE from brain tissue. This was done by injecting one-half of the animals on both the castrate and sham castrate groups with 80 mg/kg i. p. of AMT suspended is 5% Tween 80. The remaining subjects received an equivalent volume of the suspension fluid. All animals were decapitated 4 h later. Whole brains (anterior to the obex) were removed within 30 sec following decapitation, weighed to the nearest 2 mg and homogenized in 15 volumes of 0.85% acid-butanol (0.85 ml conc HCl in 1 l butanol). NE content of the homogenates was assayed using a modification of the spectrophotofluormetric method previously described by ANSELL and BEESON[16]. The data were analyzed in a manner similar to that described by BRODIE et al.[14], and the results are presented in the Table. Norepinephrine levels in the castrated animals were significantly lower than the sham-castrated controls as was the percent decrease produced by AMT (43% versus 52%). NE levels obtained 4 hours following AMT did not differ significantly. When the data of the 2 groups were plotted as log brain [NE] versus time following AMT, neither the resulting instantaneous rate constant (k) not the turnover time TT were significantly different. The utilization rate K, i.e. the product of $[NE]_0$ and k (sometimes referred to as the turnover rate), was significantly decreased in the castrate subjects as compared to the controls.

It is interesting to note that the decrease in NE levels and percent depletion obtained in the present experiment, employing castrated mice were isolated for 13 months, are the same as those seen in mice isolated for short durations of 8–14 weeks[9,17] even through the level of aggressive behavior is apparently markedly different at

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Brain norepinephrine levels and turnover dynamics in castrated and sham-castrated mice isolated for 13 months

	Sham-castrated (± S.E.)	Castrated (± S.E.)	[a] $P <$
Number	11	18	
Body wt. (g)	32.8 ± 0.58	33.9 ± 0.97	N.S.
Brain norepinephrine			
Saline control (ng/g)	367 ± 5.7	319 ± 13.0	0.01
α methyl-*p*-tyrosine (ng/g)	177 ± 12.0	182 ± 13.0	N.S.
Instantaneous rate constant (h^{-1})	0.185 ± 0.018	0.156 ± 0.017	N.S.
Turnover time (h)	5.4 ± 0.52	6.4 ± 0.70	N.S.
Utilization rate (μg/g/h)	0.068 ± 0.0039	0.050 ± 0.0049	0.01

[a] Analyzed using Student's *t*-test, two tailed.

these 2 intervals[11]. In addition, the present study indicates that the actual NE utilization rate has been significantly decreased by castration.

The finding that a) NE levels for the sham-control group were the same as those previously reported for short term isolation and b) significant differences in levels were seen between the sham-control and castrate groups, are taken to indicate the presence of a sufficient amount of testosterone, in normal animals isolated for 13 months, to maintain normal NE levels in the brain. Nevertheless, the relationship of actual level of testosterone to aggression and NE brain activity awaits further experimentation. The present experiment demonstrates the absence of a compensatory reaction in the brain NE system in response to prolonged lack of testosterone, that is, even 13, months following castration, the noradrenergic system has not reestablished the equilibrium which was altered by the absence of testosterone.

Zusammenfassung. Funktionsdynamik von Gehirn-Norepinephrin (NE) in kastrierten und scheinkastrierten männlichen, geschlechtsreifen (C57Br/6J)-Mäusen wurde nach 13 Monaten Isolierung untersucht. Kastrierung bewirkt starken Abfall des ursprünglichen Niveaus und der Stoffwechselgeschwindigkeit von NE.

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Basal Forebrain Heating and ADH Release in Dogs

There are data indicating that the activity of the hypothalamo-hypophysial antidiuretic system may be increased at high ambient temperatures[1]. A raise of the osmotic pressure of the body fluids, as well as a displacement of the blood within the cardiovascular system producing a decrease of the inhibitory influences from the volume receptors[2,3], have been suggested[1] as the factors responsbile for increase of ADH release under these conditions. The present study was performed on conscious dogs to check whether local heating of the anterior hypothalamic-preoptic (AH/PO) region could also influence ADH release.

Material and methods. Experiments were carried out on 5 mongrel dogs. They were implanted stereotaxically each with 4 thermodes and 2 thermocouples (2 thermodes on each side 6 mm apart with 1 copper-constantan thermocouple bracketed between them) under hexobarbital anaesthesia. The heater of the thermode consisted of a miniature carbon resistor[4] placed at the end of a 0.8 mm stainless steel tube. Inside the tube there was a copper wire insulated except for a tip making contact with the resistor. All thermodes were connected to pins of a plug fastened in a Plexiglas socket which was screwed into the parietal bone and fixed with acrylic cement. A period of two weeks was allowed for recovery. The influence was examined of the heating of some sites in basal forebrain on plasma ADH level, thermoregulatory functions (respiratory rate, rectal and skin temperatures) plasma osmolality and in some cases on urine output. The dogs were fasted for 18 h before the experiment but had free access to water. On the day of the experiment the dog's bladder was catheterized and emptied by air flushing. The polyethylene catheter was introduced into the saphenous vein for blood sampling. Urine output was measured every 10 min. After 30 min from start the AH/PO region was heated for 10 min by connecting the thermode to the battery so that the power delivered was 100 mW. This produced a 0.5 °C rise of the temperature of the brain tissue at a distance of 3 mm from the heater. Blood samples were taken just before the heating, at 10 min in the course and 30 min after termination of the heating. Hypothalamic, rectal and skin temperatures were continuously registered throughout the whole experiment and respiratory rate was continuously registered for a period 5 min before, in the course, and 5 min following the heating. Ambient temperature varied between 20–25 °C.

Plasma ADH level was measured by a modification[5] of the technique described by Czaczkes et al.[6]. Respiratory rate was determined using a resistance transducer placed around the chest. Skin and rectal temperatures were measured by means of the copper constantan thermocouples. After the termination of the experiments the animals were sacrifed, the brains were fixed in formalin, sectioned and stained after Weil.

Results and discussion. A clear cut increase of the plasma ADH level ranging from 6.0 to 58.4 μU/ml was observed during heating of 12 out of 18 examined sites and was accompanied by a decrease of urine output. In all cases the plasma ADH level returned to control values within 30 min after termination of the heating. On the basis of histological examination, it was established that these effects were produced by heating the following areas: the region of the nucleus commissurae anterioris – 4 cases, the lateral preoptic area – 3 cases, the region of the nucleus accumbens septi – 3 cases, the ventrolateral part of the septum – 2 cases. Plasma ADH level did not increase with tips of the thermodes found in: the internal capsule, lateral hypothalamus; fasciculus mamillothalamicus; columna fornicis and dorsomedial part of the hypothalamus. In all but two cases heating of the brain areas which produced an increase of plasma ADH level was accompanied by polypnoe. However, there was no correlation between the intensity of the thermoregulatory responses and ADH release. On the contrary, heating of the two sites, which was accompanied by a particularly high increase of respiratory rate (by 400 and 600%) and a decrease of rectal temperature, produced only a small increase of the plama ADH level. As in each experiment heating of the brain was restricted to an area surrounding a single thermode, it is possible that these strong thermoregulatory responses also produced a lowering of the temperature of the contralateral thermosensitive region

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