TABLE I. Eosinophils Per cu mm of Tail Blood at 2 Different Periods of the Day in 5 Stocks of Inbred Mice.

Stock	N	Morning level Mean ± st. error	N	Midnight level Mean ± st. error
B_4 (Subline 4 of C_{57} black)	15	700 ± 59	7	369 ± 42
D_8 (Subline 8 of dilute brown)	14	297 ± 42	7	30 ± 10
Zb (Fostered line of C_3H)	14	198 ± 19	7	30 ± 6
Ax (Fostered line of A)	14	168 ± 15	7	55 ± 8
ZD_8F_1 Hybrids, Z° x $D_8{}^\circ$)	14	130 ± 14	5	40 ± 15

TABLE II. Comparison of Differences Between Eosinophil Levels at 2 Periods of the Day in 5 Stocks of Inbred Mice.

Stock	Diff. ± S.E.*	t†	P_t‡
B_4	431 ± 92	4.68	<.001
D_8	223 ± 60	3.72	.002
Zb	168 ± 27	6.23	<.001
Ax	113 ± 22	5.23	<.001
ZD_8	90 ± 26	3.48	.003

* Differences expressed as morning minus night means of eosinophils per cu mm tail blood (see Table I) with their standard errors.

† Assuming homogenous variance within samples.

‡ Probability that a difference as large as that observed would be expected by chance alone.

of difference between the values obtained at the respective periods of the day is presented in Table II. The values in column P_t, calculated by means of Student's t value, are indicative of significance below the one percent level.

This difference between the midnight low and the morning high level in all the strains investigated and in the absence of any exogenous stress suggests that eosinopenia may occur physiologically in mice which have not been artificially stressed. Since the mouse is a nocturnal animal the greatest activity, as noted above, occurs at night. The question may legitimately be raised whether normal activity constitutes a physiological stress capable of exciting the same structures and mechanisms as are activated by exogenous stresses(3-5).

It may be noted that a similar difference has been recently reported for the lipid content of the adrenal cortex in white mice killed in midsummer at two different times of the day(2). In animals killed at 9:00 A.M. the zona fasciculata was filled *in toto* with lipid. In mice killed at midnight the lipid did not extend beyond the outer third of the fasciculata.

Summary. In 4 inbred strains and in one hybrid group of mice a regular diurnal variation of large magnitude in the circulating eosinophil count has been demonstrated. On the average, in these strains, the midnight count was less than one third of the morning level.

3. Dalton, A. J. and Selye, H., *Folia Hematologica*, 1939, v62, 397.

4. Spiers, R. S., and Meyer, R. K., *Endocrinology*, 1949, v45, 403.

5. Forsham, P. H., Thorn, G. W., Prunty, F. T. G., and Hills, A. G., *J. Clin. Endocrinol.*, 1948, v8, 15.

Received November 21, 1950. P.S.E.B.M., 1950, v75.

Transplantation of the Adrenal Gland in the Spleen of Rats. (18366)

DONALD EARL BERNSTEIN. (Introduced by G. R. Biskind.)

From the Department of Pathology, Mount Zion Hospital, San Francisco, Calif.

Previous communications(1,2) have reported the successful autotransplantation of the adrenal gland to the spleen of adrenalectomized animals. The present paper enhances the series previously reported(2), and includes data which suggest that reproduction is not inhibited when secretions from the adrenal

1. Butcher, E. O., *Endocrinology*, 1948, v43, 30.

2. Bernstein, D. E., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 175.

gland pass through the portal circulation. In addition, this investigation describes the successful homotransplantation of adrenals of newborn rats into the spleen of adult castrated and non-castrated, adrenalectomized animals.

Method. (A) *Autotransplantation.* Forty-five immature Long-Evans rats were bilaterally adrenalectomized and one adrenal was placed in the spleen at the same operation. Postoperatively the animals were maintained on a regular diet and saline for 12 to 30 days, after which time the saline was replaced by tap water. The rats were surgically explored at various intervals from 30 to 200 days. At laparotomy, careful search was made for adhesions from the transplant or spleen that could circumvent the portal circulation. Two male and 5 female rats that had large adrenal transplants without adhesions, were later mated with each other and with normal animals.

(B) *Homotransplantation.* Twenty-five male and 31 female immature rats of the Long-Evans strain were bilaterally adrenalectomized, and at the same operation a single adrenal gland from newborn rats 1 to 4 days old, was transplanted to the spleen. In every case the donor and recipient of the homotransplant were of the same sex. Postoperatively the rats were given 2 injections of 5000 units each of penicillin and maintained on a regular diet and saline for 30 days, after which time the saline was replaced by tap water. The animals were weighed initially and at 45 days after operation, at which time autopsy was performed.

Results. (A) *Autotransplantation.* When the spleen was removed, 33 of the 45 animals showed viable transplants, which averaged 3 mm in diameter and on microscopic examination revealed adrenal cortical cells, with no medullary tissue present. Of the 12 animals in which no adrenal transplants were found, 5 died within 9 days after operation and were too autolysed for adequate necropsy. In 5 of the animals adrenal cortical tissue in the form of accessories was found, and in the remaining 2, neither transplants nor accessories were found. In no instance where successful transplantation occurred, were adrenal accessories or remnants discovered. When the rats with successful transplants were bred, it was found that all of the animals were sexually potent. The 5 females gave birth to normal litters which consisted of 3, 6, 8, 11 and 14 puppies respectively. Although 2 babies died within 10 days after birth, the mother that had 14 puppies nursed all, and the entire 14 survived to weaning with only one runt in the litter. Autopsy of the mothers revealed 3 to 5 mm adrenal transplants completely free of adhesions.

TABLE I. Tabulation of Results of Attempted Homotransplantation in Adrenalectomized Rats.

	Initial No. rats	Survivors after 45 days			
		Total No.	Transplants	Accessories	No adrenal tissue
Males	15	9	3	3	3
♂ castrates	10	6	4	2	0
♀	15	7	3	2	2
♀ castrates	16	12	4	8	0
Total No. rats	56	34	14	15	5

(B) *Homotransplantation.* At the time of autopsy 34 animals were still alive (Table I). In only 14 were viable transplants found. These averaged 3 mm in diameter and were composed of adult adrenal cortical cells with no medullary tissue present. In 15 of the remaining 20 animals, adrenal cortical accessories were found. In the other 5 rats neither transplants nor accessory adrenal tissue were discovered. In every case the animals that survived showed a good weight gain. The 22 rats that did not survive the experimental period were accounted for as follows: 19 died while still on saline, and 3 died within 5 days after being taken off saline. Autopsy was performed in 13 animals and revealed neither transplants nor accessory adrenal tissue; the remaining 9 animals were too autolysed for adequate examination.

Discussion. Butcher(1) attained 12 viable autotransplants from 14 experimental animals. No mention was made of adhesions from the transplant or spleen that could circumvent the portal circulation. Two of his rats from the above mentioned series were mated and bore litters of 7 and 8 puppies

respectively, only 2 of which survived until weaning. The present report corroborates the finding that reproduction was not inhibited when the adrenal gland was transplanted to the spleen, but there is insufficient evidence to suppose that these females show inadequate lactation, as proposed by Butcher(1). The role of the adrenal gland in spermatogenesis, ovogenesis and pregnancy is still not clearly defined. However, several authors present evidence that at least for the rat, adrenalectomy inhibits the estrous cycle(3), and usually prevents pregnancy(4). The total lack of prevention of sexual activity in both sexes in the present investigation strongly suggests that the liver does not inhibit those factors in the adrenal gland responsible for adequate gonadal function in the rat.

In this report it is seen that autotransplants occurred in 35 of 45 animals. The number of surviving homotransplants obtained in the present investigation was 14 in 56 rats; 6 in 30 noncastrated and 8 in 26 castrated rats. Adrenal cortical accessories were found in 15 animals; 5 noncastrated and 10 castrated rats. The presence of accessory adrenal tissue would inhibit successful transplantation (2). Omitting the group in which accessory adrenal tissue was found, 14 of 41 animals had successful homotransplants. It is seen that takes in autotransplantation occur at least twice as frequently as in homotransplantation. From Table I, neither the sex, nor the presence of gonads significantly influenced the number of successful homotransplants. At the time of the author's previous report on autotransplantation(2), no definitive statement could be made about an increase in size of the transplants. However, since newborn adrenal glands are about 1 mm in diameter, it is obvious that surviving adrenal transplants to the spleen may increase in size (Fig. 1). Moreover, like autotransplants, the homotransplants maintained life and weight gain of the recipients.

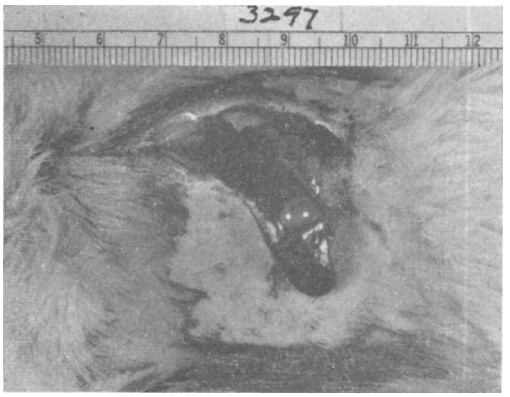

FIG. 1.
Homotransplant of newborn adrenal gland to spleen of an adult adrenalectomized rat, after 73 days.

Summary. 1. Thirty-three of 45 autotransplants of the adrenal gland to the spleen of adrenalectomized rats were successful. 2. Evidence is presented that the liver does not destroy those factors in the adrenal secretions responsible for the maintenance of spermatogenesis in the male, and pregnancy and parturition in the female rat. 3. Homotransplantation of the newborn adrenal gland to the spleen of adrenalectomized rats was successful in 14 of 56 animals, and is not significantly influenced by the sex of the recipient, or the presence or absence of gonads. 4. Homotransplants of the adrenal gland grew in castrated and noncastrated adrenalectomized rats, and maintained life and maturation of the recipients.

3. Corey, E. L., and Britton, S. W., *Am. J. Physiol.*, 1934, v107, 207.

4. Wyman, L. C., *Am. J. Physiol.*, 1928, v86, 528.

Received October 23, 1950. P.S.E.B.M., 1950, v75.