gle doses. Even this hypothetical amount of 5 μg DMBA is insufficient to cause malignant lymphoma, as was shown in our previous experiments(7).

Consideration should be given to the fact, that in the present instance a malignant lymphoma of the stem cell type was filtered and injection of this filtrate resulted in more differentiated types of lymphoma, either of the lymphocytic or the histiocytic types. Whether or not this phenomenon represents a biologically different course is not clear. However, in our experience virtually all malignant lymphomas occurring in carcinogen treated or untreated Swiss mice above the age of 35 weeks were of the lymphocytic or histiocytic types(7). Except for one instance the animals injected with cell-free filtrate were above this age when they died or were sacrificed (Table I).

It is tempting to speculate about the interpretation of the present finding, as to whether DMBA activated the latent lymphoma virus, if it exists at all in our untreated mouse colony, or a carcinogen-altered nucleic acid is responsible for transmission of this neoplasm. Further experiments are in progress.

Summary. A cell-free filtrate was prepared by the Gross technique from a thymic tumor induced by a single subcutaneous injection of 100 μg DMBA in a Swiss mouse and diagnosed cytologically as a malignant lymphoma, stem cell type. Out of 7 survivors injected with the filtrate at birth, 6 developed malignant lymphomas, while no such tumor occurred in 10 mice treated similarly at the age of 8 weeks. Cytologically they represented the more differentiated malignant lymphomas, namely the lymphocytic type in 5 cases and in one instance the histiocytic type.

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Effect of Early Postnatal Steroid Treatment on Ovarian Function in Prepuberal Rats.* (28196)

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Pfeiffer(1) implanted testis into very young female rats and observed that at puberty their vaginal smears consisted of predominantly cornified cells. Other investigators by injecting androgens(2,5) or estrogens(3,4) were able to induce a similar phenomenon. Wilson(3), on the basis of impaired mating behavior, suggested these compounds had an effect upon the central nervous system (CNS) and Barraclough(5) presented more direct evidence that the steroids modified the CNS area(s) concerned with ovulation. Everett and coworkers(12,13) concluded that the pituitary and CNS were involved in the ovulatory process in adult cycling female rats.

Because of interest in this induction phenomena and the consequences of such treatment on the differentiating reproductive system a more adaptable system of analysis was desired. The prepuberal period was there-

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fore investigated because of the general uniformity of the reproductive system and because prepuberal rats can exhibit many of the capacities of mature animals if properly manipulated.

Parabiosis of immature female rats to castrate partners(6) and cessation of antigonadotrophin serum injections into immature female rats(7) both result in precocious formation of corpora lutea. Subsequent experiments in which the intact female of the parabiotic pair had been hypophysectomized(8,9) indicated that the pituitary was necessary for formation of corpora lutea. Cole(10,11) observed that a single injection of pregnant mare serum (PMS) would induce ovulation and corpora lutea formation as well as mating, fertilization, implantation and maintenance of pregnancy in prepuberal rats. Strauss and Meyer(14) extending the observations of Cole also concluded that the pituitary and the CNS were involved in PMS induced ovulation in immature female rats.

It was therefore of interest to determine if early postnatal treatment with androgens and estrogens would induce abnormalities in gonadal reactivity in immature rats treated with PMS as well as in animals put in parabiosis.

Materials and methods. Female rats approximately 20 days pregnant, obtained from the Holtzman Rat Co., were kept in a room in 14 hours of light and 10 hours of darkness. Temperature was maintained between 74 and 76°F. The presence of pups was checked each day between 5:00 and 6:00 P. M. and the day on which young were found was considered day zero. The young were left with their mothers until day 5 at which time the number and sex of the pups was determined. All of the males and females born on any one day were combined and randomly divided into the various experimental groups, treated and returned to the lactating females. Males were added to each litter until each litter consisted of 8 young. On day 24 all of the young were weaned. On day 26 all of the animals were weighed and only those weighing 60 g or more and in good health were used in subsequent experiments. Parabiosis of the experimental females to castrate males was performed at 26 or 36 days of age according to the method of Bunster and Meyer(15). The castrate male partners were either littermates of the females or were purchased from the Holtzman Rat Co. The pregnant mares' serum (PMS) (Gonadogen)† was dissolved in physiological saline and injected subcutaneously between 10:00 and 11:00 A. M. on day 30. These animals were autopsied during the evening of day 33, unless stated otherwise, and the presence of corpora lutea, ovulated follicles and oviducal eggs was recorded.

Animals treated with steroids on day 5 and not treated with PMS or put in parabiosis were autopsied at various times and the condition of the reproductive tract noted. These are designated as "terminal" controls.

All hormones were injected in cottonseed oil subcutaneously in a volume of 0.1 ml. All doses of steroids used had in preliminary experiments been shown to induce anovulatory animals with predominantly cornified smears when examined postpuberally. The steroids were testosterone propionate (Δ^4-Androstene-17(β)- propionate-3-one) or estradiol dipropionate(1,3,5, estratriene-3, 17β dipropionate).

Results. Parabiosis experiments. Regardless of the amount of steroid injected on day 5 corpora lutea were not formed after 10 days of parabiosis in the intact partner. Control rats, however, had greatly enlarged ovaries filled with numerous corpora lutea and the vaginal smears mostly consisted of leucocytes (Table I). Ovaries of the steroid treated females were ischemic and uteri were filled with fluid, in marked contrast to the hyperemic ovaries and uteri of the controls. Uteri of the controls contained no fluid. All of the steroid treated females responded to the castrate serum in a uniform manner although terminal controls autopsied at the same time as the parabionts showed reduced ovarian weights, especially in animals treated with 1.0 mg of testosterone propionate or 10 μg estradiol dipropionate (Table I).

Injection of 10 or 100 μg of testosterone

† "Gonadogen" was supplied through the courtesy of the Upjohn Co. According to the Company, one Cartland-Nelson unit is equivalent to approximately 20 International Units.

TABLE I. Response of Intact Female Rats Treated with Steroids on Day 5 of Life to Castrate Male Gonadotrophins After 10 Days of Parabiosis.*

Treatment	Amount (μg)	No. of pairs	Avg organ weights at autopsy: Body wt (g)	Uterine (mg)	Ovarian (mg)	Range	Terminal control, ovarian wt (mg)
Test. prop.	1000	2	104.0	133	66	(57 - 75.5)	17.0
" "	100	2	88.0	204	69	(65 - 72.5)	26.0
" "	10	2	92.5	234	70	(63 - 77)	25.5
Estrad. diprop.	10	2	95.5	264	70	(69.5- 71)	14.5
Cottonseed oil		2	96.5	156	198	(178 -218)	27.0

* All animals represented in Tables I and III were born on 2 consecutive days. Each group in each table contains animals born on both days.

propionate on day 5, 7 or 9 of life prevented formation of corpora lutea in the intact parabiont (Table II). When these quantities were injected on day 13 luteinization of the ovaries occurred except in one animal injected with 100 μg of testosterone propionate. Injection of 1.0 mg of testosterone propionate on day 5 and 7 also prevented formation of corpora lutea in 5 of 6 parabionts. The possibility exists that the absence of corpora lutea in the animals treated with doses of 1 mg of the androgen may be due to a prolonged inhibitory effect on gonadotrophin secretion. This hypothesis was tested by injecting 8 pups with 1 mg of testosterone propionate on day 13. Four of these were put in parabiosis at 26 days of age and 4 at 36 days of age. All females united at 36 days of age had luteinized ovaries. No ovaries were luteinized in those united at 26 days.

PMS-treated animals. Animals treated with steroids on day 5 and injected with PMS on day 30 (Table III) did not ovulate, except for one animal treated with 100 μg of testosterone propionate. These rats, like steroid treated rats put in parabiosis, had ischemic ovaries and uteri. The uteri were fluid filled. Typical hyperemic ovaries and uteri were observed in the animals which ovulated.

Animals injected with PMS but not autopsied at 33 days of age gave essentially the same results (Table IV). All were injected with 16 I.U. of PMS on day 30 and vaginal smears were taken after day 33. On day 34 all were unilaterally ovariectomized and presence or absence of corpora lutea recorded. The vaginal smears of animals injected with steroids on day 5 remained cornified for 3-5 days after unilateral castration. This was followed by a short period of leucocytic smears after which cornified cells reappeared and persisted in all of the animals prior to the second laparotomy. Two of 5 animals treated with 100 μg of testosterone propionate on day 13 did not have corpora lutea at the time of unilateral castration but did so at

TABLE II. Effect of Day of Injection and Amount of Testosterone Propionate on Capacity of Treated Intact Females to Form Luteinized Ovaries After 10 Days of Parabiosis.

Day of treatment	μg of test. prop.	No. of pairs	Age at parabiosis	Avg wts at autopsy: Body wt (g)	Ovarian wt (mg)	Range	No. of pairs luteinized ovaries
5	10	4	26	116	70.6	(54- 90)	0
7	"	3	"	96	64.7	(54- 70)	0
9	"	2	"	97	70.0	(67- 72)	0
13	"	4	"	108	226.8	(150-297)	4
5	100	2	"	95	78.5	(67- 90)	0
7	"	2	"	82	55.0	(50- 60)	0
9	"	1	"	80	54.0	(54-)	0
13	"	3	"	101	232.0	(52-338)	2
5	1000	3	"	103	48.3	(25- 72)	0
7	"	3	"	97	159.0	(60-332)	1
13	"	4	"	99	67.5	(60- 90)	0
13	"	4	36	137	195.0	(120-285)	4

TABLE III. Response of Immature Female Rats to 16 I.U. PMS After Treatment with Steroids on Day 5.

Treatment day 5	μg dose	No. of animals	Avg wt at autopsy: Ovarian (mg)	Avg wt at autopsy: Body wt (g)	Animals demonstrating response/total No. of animals: Ovulation points	Eggs*	Mating
Test. prop.	1000	3	31.8	106	0/3	0/3	2/3
" "	100	3	44.0	103	1/3	1/3	2/3
" "	10	3	34.8	109	0/3	0/3	2/3
Estradiol dipropionate	10	3	17.0	107	0/3	0/3	2/3
Cottonseed oil		3	66.2	95	3/3	3/3	3/3

* Any animal in which 1 egg or more was found in the oviducts was considered a positive animal.

the time of the second laparotomy. All 5 of these rats showed normal cyclic changes in vaginal smears before the second laparotomy.

Terminal controls. From a total of 12 terminal controls treated with 10 or 100 μg of testosterone propionate on day 5 and autopsied 3-8 months later only one animal injected with 100 μg had ovaries containing corpora lutea.

Discussion. Wilson *et al.*(2,3) was the first to observe that age at time of initial steroid treatment is important in determining the postpuberal reproductive condition of the female rat. After approximately 10 days of age steroids were incapable of inducing anovulatory rats.

We interpret our data to mean that the same type of phenomenon is present when animals treated at various days of life are put in parabiosis or injected with PMS prepuberally. That the absence of luteinization or ovulation in animals injected with steroids prior to day 10 is not due only to gonadotrophin inhibition is demonstrated by the presence of luteinized ovaries in animals given 10 or 100 μg of testosterone propionate on day 13 and tested prepuberally (Table II). We conclude, therefore, that early postnatal injection of steroids induces a defect in the hypothalamic-pituitary-gonad axis which can be detected prior to sexual maturity.

It is evident from our data and that of others(2,3) that a marked decrease in sensitivity to testosterone propionate or estradiol dipropionate occurs at about day 10. In animals treated with 1 mg of testosterone propionate on day 15 no difficulty in conceiving, parturating or lactating was observed. It is reasonable to assume that a marked change occurs in the metabolism or maturation of the animals by approximately 10 days of age. This maturation appears to involve the hypothalamus and to be a prerequisite for the normal differentiation and functioning of the ovulatory mechanism(s).

It is unknown whether these 2 gonadotrophins (PMS and castrate gonadotrophin) are incapable of inducing ovulation for similar reason(s) in steroid treated rats. Adequate knowledge of the sequence of events required for ovulation and the degree of replacement of these by the 2 gonadotrophins is not available. Data obtained from adult cycling female rats(13) and in prepuberal PMS treated rats(14) indicate that there is a "critical" period on the afternoon of proestrus during which the ovulating material is released from the pituitary. This period in which the pituitary is required and which appears to be unexpressed in steroid induced anovulatory rats appears to be the last of the sequence of events needed for ovulation to occur. The

TABLE IV. Response of Animals Treated with 16 I.U. of PMS on Day 30 and Their Subsequent Postpuberal Reproductive Condition.

Treatment	Day of treatment	No. of animals 60 g or more at 26 days of age	No. of animals with corpora lutea at: 34 days	58 days
10 μg test. prop.	5	17	0	0
100 " " "	13	5	3	5
Cottonseed oil	5	5	5	5

parameter(s) of this "critical" period affected by steroids is unknown. Rising levels of estrogen have been implicated in induction of ovulating hormone release and subsequent corpora lutea formation in the parabiosis preparation(8). Everett and Sawyer, however(13,16), in a study of adult cycling rats found that both estrogen and progesterone were capable of advancing or retarding ovulation and we have found both steroids capable of inhibiting PMS induced ovulation in normal rats. Different secretion rates or ratios of these 2 steroids stimulated by either gonadotrophin may therefore be possible in inhibiting or facilitating ovulation(17).

These data also raise the question of whether the defect seen in the animals tested prepuberally is the same as that seen postpuberally. That there are differences in reactivity of the gonads to gonadotrophins, which is age dependent, has been recognized(10,18). Interestingly, PMS injection before day 10 appears incapable of inducing follicular maturation(10). Cole(11) was also impressed with the ease of induction of superovulation and superfetation in prepuberal animals with PMS as contrasted to the adult animal. Prepuberally detected anovulatory rats have large fluid filled uteri, which is the only grossly observable contrast with the normal postpuberal anovulatory rat. Postpuberal parabiosis of testosterone propionate induced anovulatory rats respond in much the same manner as prepuberal animals(20). It is interesting that animals which are anovulatory and exhibit constant vaginal cornification after early postnatal treatment with estrogens(4) respond to postpuberal parabiosis with marked luteinization of the ovary. Whether these differences in response to postpuberal parabiosis are due to a difference in the dose or type of steroid or are a reflection of a different site of action of the steroid molecule is not clear. This dichotomy in response to postpuberal parabiosis is of interest when compared to data obtained in anovulatory rats produced by electrolytic lesions to the hypothalamus(19). A proportion of lesioned animals with constant vaginal estrus ovulated in response to subsequent injections of progesterone.

Preliminary data obtained on mating behavior in PMS injected prepuberal animals treated with 10, 100 or 1,000 μg of testosterone propionate on day 5 indicate that mating can occur in all of these groups. Wilson and co-workers(2,3), however, observed impaired mating behavior in steroid treated animals castrated postpuberally and injected with estrogen and progesterone. Estrogen followed by progesterone is considered to be more effective than estrogen alone in reproducing normal mating behavior when injected into adult castrated rats(22). Wilson and Young (23) found that estrogen and progesterone were capable of inducing a lordosis response in castrate prepuberal rats as early as 20 days of age. Injected PMS in the immature female rat may cause secretion of progesterone as well as estrogen prior to ovulation.

Attempts to induce pregnancy have been unrewarding; one pregnancy has been induced which terminated near mid-pregnancy. Pfeiffer(21) noted that animals made anovulatory by early testis implants, ovulated, mated and implanted eggs after a single injection of pituitary gonadotrophin but were unable to maintain pregnancy beyond mid-term. It is interesting that hypothalamic lesions made during pregnancy can unfavorably affect the duration of pregnancy(24).

Other altered responses have been observed in steroid treated animals. Wilson and coworkers(2,3) observed impaired responsiveness of the uterus to estrogen. Noumura(4) found that corpora lutea formed after postpuberal parabiosis were maintained for long periods of time in animals made anovulatory with estrogen injections. He was able to produce vaginal anestrus females with longer periods of estrogen injection.

It would appear that the use of prepuberal animals would be of value in helping to delineate the mode and site(s) of action of hormonal and non-hormonal substances on the differentiating hypothalamic-pituitary-gonad axis and within a reasonably short time. Their use would be of value because various hormones, as well as variations in their dose and duration of injection into animals of different ages, appear to play an important role in determining the level of functional response

of various tissues and behavioral aspects of reproduction. The ability for full reproductive capacity to be realized with PMS primed immature rats allows for sequential analysis of deficits or defects and their interrelationships.

Summary. Subcutaneous injection of 10, 100 or 1000 μg of testosterone prop. in a single dose into female rats on the fifth day of life prevented luteinization of the ovaries after 10 days of parabiosis with castrated male rats. Injection of 10 or 100 μg of testosterone propionate on days 5, 7 or 9 of life also prevented luteinization of the ovaries, but animals injected with the same amount of hormone on day 13 had luteinized ovaries. Animals injected with 1 mg of testosterone propionate on day 13 formed no corpora lutea when put in parabiosis at 26 days of age. Animals joined at 36 days of age, however, did form corpora lutea. Animals treated with hormones in the early postpartum period and injected with PMS at 30 days of age did not ovulate. Animals treated with 10 μg of testosterone propionate on day 5 and injected with 16 I.U. of PMS on day 30 did not have corpora lutea at 34 days of age or at 58 days of age.

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Effects of N-Ethyl Maleimide and Insulin upon Adipose Tissue. (28197)

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If the action of insulin is dependent upon the integrity of disulfide and/or sulfhydryl groups on the cell surface, alterations of these groups should interfere with the response of the cell to insulin. There have been conflicting reports referable to this question, particularly in the case of rat diaphragm(1) and heart muscle(2) as well as adipose tissue(3, 4). The present report concerns the effects of N-ethyl maleimide (NEM), a sulfhydryl blocking agent, the response of the rat epididymal fat pad to insulin.

Methods. Modifications of the rat epididymal fat pad assay for insulin-like activity

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