still apparent on the fourth day of continued drug administration. During these treatment periods there was no significant improvement in the cardiovascular symptoms arising from the tumor. Weight gain resulted only after cessation of treatment.

The difference between laboratory and clinical findings regarding diuretic activity for P-286 cannot be explained presently. However, the close chemical similarity between P-286 and P-275 and the results obtained make it tempting to speculate that these compounds act in the kidney through an antiadrenal steroid mechanism.

Summary. A renal evaluation of a series of aminoalkylureas administered orally to male rats disclosed several compounds with diuretic activity, the most potent of which (P-275) was studied in some detail. When administered intravascularly to dogs, P-275 was without effect on glomerular filtration and renal plasma flow while sodium and water excretion was either only transiently or moderately elevated. Oral administration to dogs produced Na^+ and water diuresis in males. Removing the ovaries induced responsiveness in the female while treatment of the male with estrogen produced a reversible refractoriness to the saluretic effect of this compound. Testosterone treatment failed to overcome the refractory state to P-275 in the intact female. These findings plus the results from an isolated clinical case with the chemical congener, P-286, are discussed in terms of a possible antagonism of adrenal cortical hormone.

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An Additional Effect of Yohimbine-HCl (32702)

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That yohimbine-HCl affects the endocrine organs and the reproductive cycle of the female rat has been amply demonstrated. Twenty mg of yohimbine-HCl per kg of body weight induced pseudopregnancy, caused decidual reaction of traumatized uteri, increased the weight of the ovaries and adrenal glands, enlarged corpora lutea, and produced lobuloalveolar development of the mammary glands. These effects were postulated to be due to a release of luteotropin by the action of yohimbine on the hypothalamo–hypophyseal axis (1,2).

In the present study several new aspects of yohimbine action were studied. An attempt was made to determine to what extent the endocrine effects were mediated by stress. In addition, the growth rate of young rats

treated with yohimbine was ascertained and the effect of the drug on length of gestation and litter size was investigated.

Materials and Methods. To examine the possible stress effect of yohimbine the growth rate of yohimbine-treated rats was studied. Fifty-seven female Holtzman rats were divided into 3 groups at 29 days of age and the following daily injections started: (i) saline (0.9% NaCl), (ii) yohimbine-HCl (10 mg/ml), or (iii) formaldehyde (4%). The volume of yohimbine solution was increased as the animals grew to provide a constant daily dosage of 20 mg/kg of body weight. Volumes of saline or formaldehyde equal to those of yohimbine were given in the other 2 groups. Weights were recorded at 2-day intervals for the 28-day treatment period and the animals were observed daily for opening of the vagina. Vaginal smears were taken during the last 10 days of treatment. Rats were killed the day after the last injection and the adrenals, ovaries, and pituitaries were weighed to the nearest 0.1 mg.

The influence of yohimbine on pregnancy was examined in 63 rats. Holtzman rats (250 gm) were used and the day in which sperm were observed in the smear was designated as day 0 of gestation.

A minimum of 13 rats per group received either yohimbine or saline from day 15 of gestation to either parturition or to sacrifice on day 21 of gestation. Eight rats received a single injection of yohimbine on day 20 of gestation.

Yohimbine-HCl (Mallinckrodt Chemical Works, Control RBY) was dissolved in hot distilled water at a concentration of 15 mg/ml. Treated animals received 5 mg (20 mg/kg of body weight at the time of conception) of yohimbine by daily subcutaneous injections, while control rats received an equal volume of 0.9% NaCl. This is the same amount of yohimbine used by Butcher and Fugo (2) and intermediate to the levels used by Sulman and Black (3). In groups sacrificed on day 21 of gestation, weights for the placentas, fetuses, pituitary, adrenals, and ovaries were recorded. The pituitary, adrenals, ovaries, and portions of the placentas were fixed in Bouin's fluid and stored in 70% ethanol. Paraffin sections of these organs were cut at 8 μ and stained with hematoxylin and eosin.

Ten pregnant rats were subjected to cold stress (cold room 45°F) for 12 hours per day starting on day 15 of gestation to determine if the results obtained with yohimbine could be due to general stress. Six rats were killed on day 21 of gestation and the fetuses, placentas, ovaries, adrenals, and pituitaries were weighed. The 4 remaining rats were allowed to continue to delivery.

Differences in length of gestation and in weights of fetuses and organs were analyzed by the t test of group means. This analysis of fetal and placental weights is somewhat biased against significance due to any variance resulting from differences in litter size. Differences in weight gain were analyzed by Least Significant Difference.

Results. All 8 animals given a single injection of 5 mg of yohimbine-HCl on day 20 of gestation delivered on day 22. However, when daily injections of 5 mg of yohimbine were given from day 15 of pregnancy until delivery, gestation was extended to 23 days in 12 of 14 rats. The remaining 2 rats delivered on day 22. In the saline-injected controls 10 of 13 rats delivered on day 22, while 2 litters were born on day 21 and 1 litter on day 23. Many of the newborn rats from the yohimbine treated mothers were dead when the litters were first observed. The increase in gestation length due to the yohimbine treatment was statistically significant ($p<0.01$).

Table I presents a comparison of the mean fetal and organ weights. Treatment with yohimbine from day 15 to day 21 of pregnancy resulted in a lower ($p<0.01$) average fetal weight (5.7 gm vs 3.8 gm). Placentas of the treated rats were also significantly lighter ($p<0.01$) than the controls (av 388 mg vs 567 mg). One dead fetus was found in the treated group and none in the controls, although the fetuses of the treated group were much weaker than the larger controls as determined by activity.

The weights of both the pituitary and adrenal glands were significantly increased ($p<0.01$) with yohimbine treatment. The increase in adrenal size was due to a thickening of all layers without alteration of cell types.

TABLE I. Comparison of Tissue Weights in Yohimbine Treated and Control Rats at 21 Days of Gestation.

Group	Mean weight[a] (mg ± SE)				
	Fetuses	Placentas	Ovaries[b]	Adrenals[b]	Pituitary
Control	5740 ± 35.7	567 ± 5.8	113.7 ± 3.7	56.9 ± 3.1	12.0 ± 0.5
Yohimbine	3810 ± 38.1[c]	388 ± 5.8[c]	109.5 ± 3.6	77.1 ± 4.0[c]	15.2 ± 0.5[c]

[a] Averages based on 14 rats per group with 165 and 166 fetuses in the control and yohimbine groups, respectively.

[b] Average paired weights.

[c] Significantly different from controls at $p<0.01$ by the t test.

Likewise the cell types of the hypophysis when stained with hematoxylin and eosin were unchanged. The ovarian weights and histology exhibited no alterations due to yohimbine treatment (Table I).

The 4 rats which experienced cold stress delivered on day 21 or 22. Delayed parturition as was observed with yohimbine treatment did not occur. The young, from 5 of 6 litters of cold-stressed animals killed on day 21 of gestation were of normal weight (5.5 gm av), while one litter had small fetuses (3.1 gm av). The paired adrenal weights of the stressed animals were greater than those of yohimbine treated animals (86.3 vs 77.1 mg).

Weight gains in young rats treated for 28 days with formaldehyde or yohimbine were not significantly different ($p>0.05$) from saline injected rats. The adrenal weights of the formaldehyde treated rats (58.5 mg) were significantly larger ($p<0.05$) than those of the controls (51.9 mg) while the weight of adrenals in the yohimbine group (54.0 mg) was not significantly increased. Vaginal opening at 38–40 days of age was not modified by the treatments ($p>0.05$). Vaginal smears demonstrated that yohimbine produced pseudopregnancy, as previously reported (1) while formaldehyde or saline treated rats continued to have estrous cycles. The difference in stage of estrous cycles or pseudopregnancies within groups prevented a valid analysis of ovarian weights.

Discussion. These results demonstrate that daily injections of 20 mg of yohimbine per kg of body weight from day 15 of gestation until term produce a consistent 1 day increase in length of pregnancy. This would be consistent with the theory of an increased luteotropin release by yohimbine (2) and with the increasing gestation length in rats produced by prolactin injection (4). It is postulated that yohimbine blocks prolactin inhibitor factor (PIF) and therefore increases luteotropin release, which in turn stimulates continued progesterone production. The reason for the decreased size of the fetuses and placentas have not been determined.

Parturition at the expected time and the presence of normal-sized fetuses and placentas at day 21 of gestation in cold-stressed rats suggests that the effects of yohimbine treatment are not due to a general stress effect. The failure of injected yohimbine to significantly change the growth rate would also indicate that this material does not have a marked toxic action at the dosage used.

Yonkman (5) has studied the toxicity of yohimbine-HCl in various concentrations given orally over a period of 3 months. Growth rates and autopsy findings (liver and kidney) were essentially negative for concentrations of 1:5000 or less. In the present study the effect could be a direct toxic action on the fetuses without markedly affecting the mother. However, the action could also result from changes in hormonal balance or other changes in intrauterine environment.

Ovarian weight was not increased in the pregnant rats as was found by Butcher and Fugo (2) in pseudopregnant rats. This might be expected since the corpora lutea of pregnancy may have already reached their maximum size.

Yohimbine induced pseudopregnancy in nonmated rats, but the stressing effect of

formaldehyde did not interrupt the normal estrous cycle. There was no change in the onset of puberty as demonstrated by the age at opening of the vagina. On the basis of these results and of earlier work in cycling rats (2), it is suggestive that the increased gestation length is due to prolonged progesterone secretion as a result of the hypothalamic blockage of PIF. Causes of decreased fetal and placental weights were not determined.

Summary. Daily subcutaneous injections of 20 mg of yohimbine-HCl per kg of body weight from the 15th day of gestation until parturition increases the length of pregnancy in the rat by 1 day. A single injection of yohimbine on day 20 of pregnancy was without effect on gestation length. Rats which were treated with a daily dose of 20 mg of yohimbine per kg (started on day 15 and sacrified on day 21 of gestation) had significant increased pituitary and adrenal weights as well as smaller fetuses and placentas. Cold stress did not affect the weight of the fetuses or placentas. No difference in growth rate was produced by either yohimbine-HCl or formalin injections.

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Free and Protein-Bound Lysine Flux in Developing Rabbit Brain*† (32703)

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Protein concentration increases with cerebral maturation (1,2). *In vitro* studies of separated cells (3) reveal greater capability of immature brain cells to incorporate amino acid into protein. Other *in vitro* studies show that pH 5 enzyme activity of rat brain is greater in the immature animal and also that there is a greater rate of ribosomal protein synthesis in the developing brain than in mature brain (4). Few *in vivo* studies of rate of protein synthesis in immature brain are available. Comparison of flux of lysine into cerebral protein of intact mice reveal that 10-day-old mice manifest a higher rate of flux of lysine into brain protein than mature mice (5). Recent studies have pointed out that rate of incorporation of phenylalanine-^{14}C into piglet brain protein is primarily a function of postnatal age and not of gestational age (6). Although *in vitro* studies demonstrate the potentiality for greater lysine incorporation into protein in neonatal brain, factors present in the intact animals may significantly modify the actual rate of synthesis. The present studies were undertaken to demonstrate *in vivo* changes in rate of lysine flux into rabbit brain protein during the first 12 days of life. This span coincides with a period of profound neurophysiological, behavioral, and neurochemical change.

Materials and Methods. The general scheme of the experiments and methods of calculation are essentially those used by Lajtha *et al.* (5). Rabbits of ages 1, 4, 8, and 12 days were injected intraperitoneally with U-^{14}C-L-lysine[1] (198 mC/mmole), 80 μC/kg. The brains of the animals were removed at 10,20,40, and 60 min after injection and processed as discussed below. Two rabbits of each age were studied for each elapsed time period. Hepa-

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[1] Purchased from Volk Radiochemical Company, Burbank, California.