(12) or rabbits(13) increased prolactin release *in vivo,* and injections of estrogen into rats increased prolactin release by the pituitaries when subsequently incubated *in vitro* (14).

The present results are believed to help explain the considerable variations previously observed in randomly cycling rats in prolactin release by anterior pituitary tissue incubated *in vitro,* and also the fluctuation in hypothalamic PIF content. Recently it was shown that the use of pituitaries from mature male rats show much more uniformity in prolactin release *in vitro* than pituitaries from randomly cycling female rats(15). We are currently using male rat pituitaries for assaying PIF *in vitro,* but are incubating them for 4 instead of the 2 hours used previously for pituitaries from female rats. This is because male rat pituitaries release considerably less prolactin than female rat pituitaries. Use of hypothalami from male rats or from female rats during a particular stage of the cycle should also give more uniform results in measuring PIF content than hypothalami from randomly cycling rats.

Summary. Comparisons were made of pituitary prolactin content, pituitary prolactin release *in vitro* and hypothalamic PIF content at different stages of the estrous cycle of the rat. The anterior pituitaries of proestrous and estrous rats contained significantly more prolactin than anterior pituitaries of diestrous rats; the former released significantly more prolactin when incubated *in vitro* than the latter, and proestrous and estrous rats had significantly less PIF in the hypothalamus than diestrous rats. Ovarian estrogen secreted during proestrus and estrus is believed to increase prolactin release by depressing hypothalamic PIF production and by directly stimulating the pituitary.

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Endogenous Creatinine Clearance by Rats.* (32266)

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The present study examines 3 aspects of the clearance of endogenous creatinine in rats: (1) Twenty-four hour, undisturbed creatinine clearances, (2) urinary non-creatinine chromogen content, and (3) correlation of clearance with body weight, kidney weight and surface area. Previous endogenous clearances have been performed during short term studies, usually one hour or less. In general, these studies have involved

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manipulation of the animals including anesthesia, special restraint and injection. Muntwyler and Griffin(1) are the only investigators who have reported 24-hour endogenous creatinine clearances on undisturbed animals, and their data have never been corroborated. The presence of serum non-creatinine Jaffe chromogens is well known, and most recent investigators have made appropriate corrections by determining the amount of creatinine adsorbed onto Lloyd's reagent. However, only Lippman(2) has published values for urinary non-creatinine chromogen. He found an average value of 8% in the urine of 6 males and 6% in the urine of females. He believed that the non-creatinine chromogen was sufficiently small to be ignored. Creatinine clearances have been corrected for weight, kidney weight, and surface area. Addis(3) showed that kidney weight correlated with the two-third power of body weight and thus appears to cerrelate with surface area. Since the kidney weight can be obtained directly, this method has some practical advantage. However, there are no reported data to show that either kidney weight or surface area actually correlate more closely with the creatinine clearance than does body weight.

Materials and methods. Studies were performed on 31 male albino rats, ranging from 200 to 450 g. The animals were fed Purina rat-chow *ad lib* for one week prior to the study. Each rat was then placed in a metabolic cage, and the 24-hour urine was collected on alternate days until three collections had been made. The animals were fed between collection periods. During the collection period the animals were fasted, but were allowed water *ad lib*. After the third clearance they were weighed, anesthetized, then a cannula was placed in the femoral artery, and the animals were killed by exsanguination. The kidneys were immediately dissected from the carcass, stripped free of fat and connective tissue, and weighed. The urine and plasma were analyzed for both apparent and "true" creatinine, using modifications of the methods of Brod and Sirota(5) and of Hare(6).

Results. The daily variations in urinary creatinine were not found to be significant. Therefore, the mean value of the 3 collections was used. The mean apparent serum creatinine was 0.758 mg% (st.d. 0.077), "true" serum creatinine was 0.399 mg% (st.d. 0.069), and non-creatinine chromogen 0.359 mg% (st.d. 0.070). The average serum non-creatinine chromogen was 47.3% of the total chromogen. The average urinary non-creatinine chromogen was 7.3% of the total (st.d. 2.3%) (Table I). Clearances (ml/min) were calculated on the basis of apparent plasma and urine creatinine (C_{Cr}), true plasma and apparent urine creatinine (C_{CrT}) and true plasma and urine creatinine (C_{CrTT}). Each of these clearances was then corrected to 100 g body weight, 1.0 g kidney weight and 100 square centimeters body surface area (calculated by the formula of Benedict(7): $SA = 9.2BW^{0.67}$, where SA is surface area in square centimeters and BW is body weight in grams). Table II shows the means and standard deviations for the clearances when calculated in this manner. Table III indicates the coefficients of correlation. It is apparent that for the sex and weight range of the animals used

TABLE I. Plasma and Urine Creatinine Values.

	Plasma		Urine	
	mg %	st.d.		
Apparent creatinine, mg %	.758	.077		
True creatinine, mg %	.399	.069		
Non-creatinine chromogen (NCC), mg %	.359	.070		
NCC as % of apparent creatinine	47.4%	7.6%	7.3%	2.3%

TABLE II. Creatinine Clearance.

	Per 100 g BW	Per 1.0 g KW	Per 100 cm^2 SA§
C_{Cr}*	.368 (.051)‖	.527 (.100)	.282 (.054)
C_{Cr_T}†	.709 (.118)	1.006 (.159)	.537 (.084)
$C_{Cr_{TT}}$‡	.656 (.108)	.936 (.154)	.498 (.080)

* Calculated from apparent serum and urine creatinine values.

† Calculated from "true" serum and apparent urine creatinine values.

‡ Calculated from "true" serum and urine creatinine values.

§ Surface area calculated from the formula $SA = 9.2BW^{0.67}$, where SA is in cm^2 and BW in grams.

‖ Standard deviation in parentheses.

TABLE III. Correlations.

	Body wt	Kidney wt	Surface area
C_{Cr}	.909	.875	.911
C_{Cr_T}	.844	.822	.847
$C_{Cr_{TT}}$	.851	.827	.854

the correlations are essentially the same with each method of calculation.

Discussion. Muntwyler and Griffin(1) present the only previous study in which creatinine clearance was determined on the basis of 24-hour urine collections. In their study the serum creatinine was corrected for non-creatinine chromogens, but the urinary creatinine was not. Their value of 0.73 ml/min/100 g body weight compares very closely with our value of 0.709 ml/min/100 g body weight calculated in the same manner. This value is slightly higher than those obtained by others who have manipulated their animals during the procedure and is slightly higher than values of 0.600 ml/min/100 g body weight (st.d. 0.099) which we obtained in similar acute, short-period clearances. It seems quite probable that vascular changes associated with the experimental procedures could result in a suppression of endogenous creatinine clearance of this magnitude. The agreement between the two studies of the clearances per gram kidney weight (1.01 and 1.006 ml/min/1.0 g KW) is also remarkably close. Since surface area calculations depend on the weight and the formula used, and the actual clearances per unit weight in the two studies are essentially the same, the results per unit surface area should be related when the same formulae are used.

The mean value of 7.3% non-creatinine chromogen in the urines of 31 male albino rats is of the same order of magnitude as found by Lippman(2). Although this value is small there are circumstances in which it is too large to ignore. Failure to discount the urinary non-creatinine chromogen results in a significantly higher clearance and when compared with inulin clearances indicates a level of tubular secretion in excess of that actually manifested.

Walter and Addis(4) demonstrated that the kidney weight was related to the body weight of rats according to the following formulae:

$$KW \text{ (male)} = 30.0\ (BW)^{0.717} + 20.6$$

$$KW \text{ (female)} = 40.4\ (BW)^{0.648} + 12.2$$

These formulae are of the general form $KW = a(BW)^n + b$, where weight (KW) is in milligrams and body weight (BW) is in grams. The form $a(BW)^n + b$ represents surface area if n is approximately 2/3 and $b = 0$, and represents a direct relation to body weight if $n = 1$. Thus, the formulae resemble surface area more closely than body weight since the resemblance depends mainly on the value of n. Addis also demonstrated that kidney weight and function varied in proportion to protein intake. In our animals the ratio of kidney weight to body weight was about 25% higher than reported by Addis and was comparable to the ratios reported by Muntwyler and Griffin. The reason for this difference is not apparent, but does indicate that kidney weight may vary independently of body weight. For theoretical reasons Addis chose to relate the filtration rate to the kidney weight(3). No actual correlations of kidney weight with the glomerular filtration rate were presented. On the basis of these data the creatinine clearance should correlate better with the kidney weight and the surface area than with the body weight. It is apparent from Table III that the correlation between clearances and body weight, kidney weight and surface area are all comparable. It is probable that in the weight range examined the variability of clearance is quite wide and that the corrections provided by adjustment to kidney weight and surface area are quite small. Since body weight is easily obtained and requires no further mathematical manipulation it would seem to be a satisfactory parameter for adjusting clearance values in animals weighing between 200 and 450 grams.

Conclusions. Thirty-one white male albino rats of weights ranging from 200 to 450 g were used to study 24-hour, undisturbed creatinine clearances. Determinations were made using apparent creatinine, true serum and apparent urinary creatinine and true serum and urinary creatinine. The resting creatinine clearance was 0.709 ml/min/100 g body weight, using true serum creatinine and apparent urinary creatinine, and compared closely with the values obtained by Muntwyler

and Griffin of 0.73 ml/min/100 g body weight. The urinary non-creatinine chromogen averaged 7.3% of the total chromogen; under some circumstances this value is sufficiently large to be significant. Correlations of creatinine clearance with body weight, kidney weight, and surface area were approximately the same. Therefore, in this weight range it seems most practical to use body weight as the unit for relating clearances.

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Production of Congenital Malformations Using Tissue Antisera III. Placental Antiserum.* (32267)

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The teratogenicity of rat kidney antiserum has been reported(1) and corroborated(2). Rat placental antiserum has also been reported to be teratogenic(3,4) and it is the purpose of this paper to deal with the quantitative and qualitative aspects of the production of congenital malformation in rats utilizing placental antiserum.

Materials and methods. Rat placentae were obtained from rats that were pregnant 21 days. The maternal rats were anesthetized with sodium pentobarbital and heparinized. A laparotomy was performed and a catheter was placed in the abdominal aorta. Immediately following the rapid removal of 8-12 ml of blood, the rat and its pregnant uterus were perfused with aproximately 200 ml of buffered saline. The placentae were then removed from the uterus and a catheter was placed in the umbilical vein. The placentae were perfused with buffered saline until they were a pale tan and the material decidual tissue was white in color. These tissues were lyophilized, ground into a fine powder and stored in a deep freeze.

Adult rabbits were immunized with one of 3 preparations of placentae: whole placenta, fetal placenta or maternal decidual tissue. The rabbits were injected in the lumbosacral muscles weekly for 4 weeks with an emulsion containing 100 mg of dried tissue powder, 1 ml of distilled water and 1 ml of Freund's adjuvant. Forty milliliters of blood were removed by cardiac puncture biweekly and the sera pooled. Three separate pools of rabbit placental antisera were prepared. The preparation of rat kidney antiserum has been described(1,4).

Control groups consisted of litters that were anesthetized and laparotomized after 8 days of pregnancy and another group that underwent the operative procedure and received an injection of pre-immunization normal rabbit serum (Table I). Rats 7, 8, 9, or 10 days pregnant were injected with one of the 3 placental antisera at doses of from 0.25 to 1.5 ml/100 g of pregnant rat. The intravenous or intraperitoneal routes were utilized. Just prior to injection of the antiserum, the pregnant rat was anesthetized and laparotomized. The number, conditions and positions of the embryonic sites were recorded and the incision was then closed with silk sutures and metal wound clips. The fetuses and placentae were removed after 21

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