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Tissue Isotope Clearance. IV. Effects of Castration and Diethylstilbestrol on Subcutaneous Iodoantipyrine Clearance of Rats (39066)

LOUIS P. GANGAROSA, CHING T. HUNG AND P. KENNETH MORSE

Department of Oral Biology (Pharmacology), Medical College of Georgia, Augusta, Georgia 30902

Recently, we reported that the clearance of ^{131}I-labeled IAP from subcutaneous tissue of male rats is more rapid than that of females especially at the age of maximal sexual development (1). This led us to postulate that male sex hormones are stimulators of the subcutaneous blood flow, while female hormones have an inhibitory effect. Since this postulate could be tested by castration of male rats and administration of diethylstilbestrol (DES), we decided to pursue this line of experimentation.

Methods. Twenty-eight sexually mature Sprague–Dawley rats (strain CD-1, Charles River) were divided into four groups of seven as follows: Group I, females with no treatment; Group II, males treated with nine daily subcutaneous injections[1] of 0.2 ml placebo (vegetable oil vehicle) over a 2-week period before the clearances; Group III, castrated males treated with placebo as in Group II; and, Group IV, castrated males treated with nine daily subcutaneous injections of 25 mg/kg DES[2] over a 2-week period before the clearances.

The methods of isotope clearance determination were described previously in earlier publications of this series (1–3). Briefly, in the present experiment, two subcutaneous injections were given at the dorsal lumbosacral area of each anesthetized rat. Each injection contained 0.1 ml of ^{131}IAP in saline-phosphate buffer (pH 7.4) at physiologic osmolarity. The disappearance rate of IAP from the injection site was monitored and was used as an indicator of the capillary blood flow status as described by Kety (4). The mean initial clearance rate (as determined from the straight line portion of the clearance curves) of the two injections for each rat was used in the data analysis. The rectal temperature was maintained between 98.0 and 101.2°F.[3] Three experimental hypotheses were tested, using separate one-way analysis of variance tests: (a) Clearance rates in male rats (Group II) would be more rapid than in female rats (Group I). This represents a replication of sex effects on clearance found previously (1). (b) Clearance rates in male controls (Group II) would be more rapid than in castrated males (Group III). (c) Clearance rates in castrated males (Group III) would be more rapid than in castrated males which were also given DES (Group IV).

Results. As indicated in Table I, each experimental hypothesis was confirmed ($P < 0.001$). Control males (II) showed more rapid clearance rates than either control females (I) or castrated males (III). Castration plus DES (IV) resulted in the lowest clearance rates. For the 21 male rats, the strength of the association between treatment and clearance rate was determined by correlating the 21 individual clearance rates with the mean clearance rate for the treatment groups to which they belonged. The correlation coefficient was 0.894, indicating that treatments accounted for 80% of the total variability in clearance rates ($P < 0.001$). The individual clearance rates, as plotted in Fig. 1, show almost complete separation of the three male groups.

An interesting secondary observation was

[1] Injections were given Monday, Tuesday, Wednesday, Thursday, and Friday of week 1 and M, T, W, Th of week 2. All clearances were performed on Friday of week 2.

[2] Nine injections of DES were used because Group IV (DES treated rats) showed a significant decrease in weight compared to control (Group III) after the ninth injection; this weight decrease was our end point.

[3] This range of temperature difference was shown not to decrease or increase clearance rates (1).

TABLE IA. SUBCUTANEOUS ISOTOPE CLEARANCE RATES AS A FUNCTION OF SEX AND FEMINIZING TREATMENTS.

Group	N	Sex	Castrated	Injected with		Clearance	
				Vehicle	DES	Mean	SEM
I	7	F	No	No	No	4.85	0.29
II	7	M	No	Yes	No	6.82	0.39
III	7	M	Yes	Yes	No	4.44	0.23
IV	7	M	Yes	No	Yes	3.16	0.29

TABLE IB. RESULTS OF ANALYSIS OF VARIANCE TESTS OF HYPOTHESES.

Hypothesis	F	P (one-tail)
II > I	16.34	<.001
II > III	27.58	<.001
III > IV	12.17	<.001

the effect of the feminizing procedures on body weight of males. The body weight of the rats, measured at the time that the clearances were performed, is shown in Fig. 2. The male and female control rats had grown at an approximately normal rate as determined from standard growth curves (from Charles River). The feminizing procedures diminished body weights of male rats. There was a highly significant difference in group means for the three male groups {F (2/18 df) = 14.75, $P < 0.001$}, chiefly attributable to Group IV whose mean (239.9 g) was close to that of the females (213.0 g).

The relationship between body weight and clearance rate was determined for each group separately and for the three male groups combined (Table II). Within a given treatment group, there was no significant relationship between body weight and clearance rate. However, when the three male groups were combined, this relationship was significant ($r = 0.654$, $P < 0.005$). Thus, this significant relationship was produced by the feminizing treatments which both reduced body weight and slowed clearance rates.

Discussion. In a previous report (1), we described sex and age related changes in isotope clearances from subcutaneous injection sites in rats. Immature male and female rats showed similar clearance rates. In puberal rats, the males showed increased

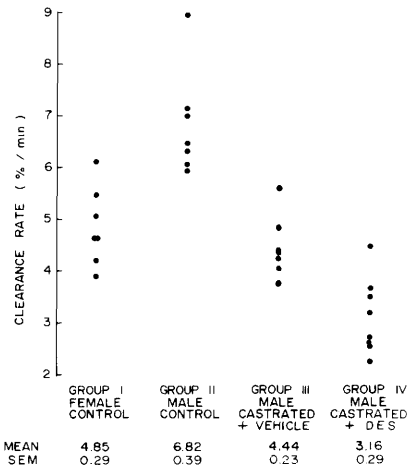

FIG. 1. Subcutaneous tissue isotope clearance rates by experimental treatments.

clearances while the females' decreased. The maximum sex differences were found at maturity; male clearances were faster than female clearances. For aged rats, the differences between males and females were not as pronounced. This age-sex interaction appeared to be related to sexual maturation and we postulated that changes in sex hormone levels caused the difference in subcutaneous clearance rates between male and female rats.

In order to test this hypothesis, we performed some feminizing procedures on male rats and compared their subcutaneous

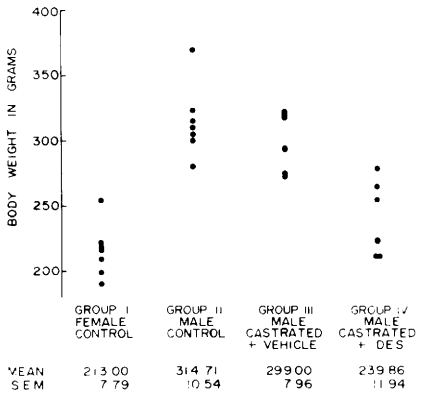

FIG. 2. Body weight of rats prior to clearance rate tests.

TABLE II. RELATIONSHIP BETWEEN SUBCUTANEOUS ISOTOPE CLEARANCE RATE AND BODY WEIGHT FOR FOUR GROUPS OF RATS.

Group(s)	*N*	Correlation between clearance rate and body weight	*P*
I	7	0.169	$>.1$
II	7	0.237	$>.1$
III	7	−0.279	$>.1$
IV	7	0.202	$>.1$
II, III, IV	21	0.654	$<.005$

IAP clearances to control male and female rats. Castrated males showed a statistically significant decrease in clearance rate (about 35% compared to control males) while castrated males treated with DES showed an even lower clearance rate (54% reduction from control males and 29% lower than the castrated control rats). The control females also showed lower clearance rate than control males verifying our previous report (1). Castration of males resulted in clearance rates approximately equal to those of female controls, while the treatment of castrated males with DES gave a clearance rate below the control female level. Thus, the hypotheses we tested concerning sex-linked influences on subcutaneous IAP clearances were verified.

The significant correlation ($r = .654$, $P < 0.005$) between body weight and clearance rate for the three male groups combined should not be interpreted as a causal relationship between body weight and clearance rate. Within each of the three groups, the correlations are low (.237, −.279, .202), nonsignificant, and vary in direction. The most reasonable conclusion is that the feminizing procedures resulted both in slower clearance rates and reduced body weights.

The biological interpretation of clearance of rapidly-diffusible, isotopically-labelled substances, for example IAP, is that it is directly related to blood flow. Kety (4) indicated that such clearance was related to "capillary efficiency" and many experiments since his original observations in 1949 have verified this relationship (5). Thus, the sex hormones appear to have inhibitory and stimulatory effects upon blood flow in subcutaneous tissues of rats. The mechanism by which the hormones exert effects in blood flow is unknown, although it has been well documented for primary and secondary sexual tissues (6).

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