Effects of estrogen and testosterone on the metabolism of mevalonate by the shunt pathway

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Abstract Mevalonate is metabolized by a sterol-forming and a non-sterol-forming, also called the "shunt", pathway. Effects of estrogen and testosterone administration on the shunt activity were examined in male and female Wistar and Sprague-Dawley rats. Shunt activity was determined in vivo from the yield of expired $^{14}CO_2$ following [5-^{14}C]mevalonate injection. Total mevalonate utilized was determined from the yield of expired $^{14}CO_2$ following [1-^{14}C]mevalonate injection. In the female, about 45% of mevalonate appears to be metabolized via the shunt, and in the male about 20%. This difference between male and female rats is dependent on both testosterone and estrogen, and apparently on testosterone to a greater extent. Thus estrogen treatment produced a 20–35% increase in shunt activity over castrated controls, while castration of males without hormonal treatment resulted in about a 50% increase in shunt activity, and testosterone administration returned castrated male and female shunt activity to that of intact males, or nearly so. Light/dark cycle had no effect in vivo on shunt activity, but may be critical in demonstrating sex differences in shunt activity in kidney slices.—**Brady, P. S., R. F. Scofield, S. Mann, and B. R. Landau.** Effects of estrogen and testosterone on the metabolism of mevalonate by the shunt pathway. *J. Lipid Res.* 1983. **24:** 1168–1175.

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Mevalonate (MVA) is an obligatory intermediate in the de novo synthesis of sterols from acetyl CoA. MVA is also metabolized by a non-sterol-forming pathway (1, 2). The first reaction in this "shunt" pathway is proposed to be the dephosphorylation of isopentenyl pyrophosphate to form dimethylallyl alcohol. The dimethylallyl alcohol is oxidized by alcohol and aldehyde dehydrogenases to β-methylcrotonic acid and the CoA derivative of this acid then fixes CO_2 to form trans-3-methylglutaconyl CoA, which is converted to HMG CoA. The HMG CoA is cleaved to form acetoacetate and acetyl CoA. The acetyl CoA can then be oxidized to form CO_2. We have reported distributions of ^{14}C in hydroxybutyrate excreted by rats given specifically ^{14}C-labeled MVA in accord with this scheme (3). Carbon 5 of MVA provides the carbonyl carbon of acetyl CoA in the scheme and, therefore, the formation of $^{14}CO_2$ from [5-^{14}C]MVA can be used as the measure of the shunt activity (4, 5).

In rats and humans, the yield of $^{14}CO_2$ from [5-^{14}C]MVA is greater in females than in males (6, 7). Estrogen administration to castrated adult male rats increases the formation of $^{14}CO_2$ from [5-^{14}C]MVA while the yields of $^{14}CO_2$ from immature female rats are the same as from immature male rats (6). Further, incorporation of ^{14}C into cholesterol is greater in the male than in the female rat (6). It has therefore been suggested that differences in cholesterol levels between the sexes may be related to shunt activity (6, 7), although in humans incorporations of ^{14}C from [5-^{14}C]MVA into serum and erythrocyte cholesterol are similar in the two sexes (7). Kidney appears to be the major site of mevalonate metabolism via the shunt (8, 9) and the organ responsible for the sex difference in shunt activity (6).

While the effect of estrogen on the shunt pathway has been examined (6), the effect, if any, of testosterone, which exerts opposite effects from estrogen in so many circumstances, has not been reported. The purpose of the present study was to assess the role of testosterone on shunt activity, comparing it with estrogen.

MATERIALS AND METHODS

Experiment 1

Six Sprague-Dawley rats of each sex were used. Data on age for weight were provided by their supplier, Zivic-Miller Laboratories. Three of the rats of each sex were between 6 and 8 weeks of age and hence, near puberty, while three were between 10 and 12 weeks of age, i.e., sexually mature (10). The rats were housed in groups of three with ad libitum access to feed (Ralston Purina Rat Chow, St. Louis, MO) and water. Lighting was on a 12-hr cycle (6 PM to 6 AM dark). The rats were fasted overnight and then each was injected via tail vein with

Abbreviation: MVA, mevalonate.

2 μCi of the sodium salt of R,S-[5-^{14}C]MVA (Research Products International, Mt. Prospect, IL) and 1 μmol total sodium R,S-mevalonate in 0.4 ml of 0.154 M NaCl. Expired CO_2 was collected in 1 N NaOH (6) over the next 6 hr. At intervals throughout this period aliquots of the CO_2 in the 1 N NaOH were dissolved in a liquid scintillation fluid (Formula 963, New England Nuclear Corp., Boston, MA) and assayed for ^{14}C activity. Results are expressed for this and succeeding experiments as nmol of MVA oxidized, calculated by dividing the dpm in $^{14}CO_2$ by the specific activity of the [5-^{14}C]MVA in dpm/nmol. While this is a commonly used method for expressing such data, it is not the measure of mevalonate actually oxidized, since the extent of dilution of the specific activity by endogenous mevalonate is uncertain. Rather, it is an expression for the quantity of the labeled carbon oxidized to CO_2 relative to that injected. Only the R isomer is assumed to be oxidized (4, 7).

Experiment 2

Ten male (267 ± 8 g, mean ± SEM), and ten female (236 ± 68 g) Sprague-Dawley rats from the same supplier were housed in groups of five and under the same conditions as in the first experiment. Based upon age-for-weight, these rats were sexually mature. Each rat was fasted overnight and injected via the tail vein with 2 μCi of the sodium salt R,S-[1-^{14}C]MVA, prepared from R,S-[1-^{14}C]mevalonolactone (Amersham Corp., Arlington Heights, IL) using 0.01 N NaOH (3), and 1 μmol total sodium R,S-mevalonate in 0.4 ml of 0.154 M NaCl. The expired CO_2 was then collected in 1 N NaOH for 180 min and counted as before.

Experiment 3

Sixteen adult male Sprague-Dawley rats weighing between 300 and 450 g were castrated under ether anesthesia. Twenty-four hours following surgery, estrogen administration was begun to one-half of the rats. These rats received either subcutaneous injection daily of 10 μg β-estradiol or twice weekly of 30 μg β-estradiol-3-benzoate. After 30 days the rats were fasted overnight and injected with [5-^{14}C]MVA as in the earlier study, and CO_2 was collected and counted.

Experiment 4

Twenty-four 6-week-old male Sprague-Dawley rats were used. Twelve rats were housed under a 6 AM–6 PM light period while the other half were housed under the 6 PM–6 AM light period (reverse phase). Nine of each group were gonadectomized under ether anesthesia. The remaining six were anesthetized and incisions were made as for the gonadectomized rats, but without manipulation of the testes. These served as intact controls. Three in each subgroup received estrogen and three in each subgroup received testosterone. This was accomplished using subcutaneous implants of Silastic (Dow-Corning Corp., Midland, MI) tubing (11–13). In brief, capsules were prepared from 3.175-mm o.d. and 0.8-mm wall thickness tubing. A 30-mm-long capsule was packed with testosterone powder (Sigma Chemical Co., St. Louis, MO) while a 5-mm capsule was packed with β-estradiol (Sigma Chemical Co.). The capsules were implanted 24 hr after the initial surgery under light ether anesthesia via a single dorsal incision. After 4 weeks the rats were fasted overnight, [5C-^{14}C]MVA was injected, and CO_2 was collected.

Experiment 5

Twenty-four male and 24 female 6-week-old Wistar rats (Charles River, Wilmington, MA) were used. Eighteen rats of each sex were gonadectomized and the remaining six of each sex were treated as described in Experiment 4 to serve as intact controls. Six of each sex were given estrogen and six of each sex were given testosterone, again by Silastic implants. After 4 weeks the rats were fasted overnight and injected with [5-^{14}C]MVA, and CO_2 was collected.

The rats were killed by decapitation 4 to 5 days later. The kidneys from each were removed and homogenized in HEPES buffer containing dithiothreitol (14). Dimethylallyl alcohol dehydrogenase activity was assayed as described by Lumeng, Bosron, and Li (14) for ethanol dehydrogenase activity. Reduction of NAD was followed at 340 nm in a cuvette containing supernatant obtained on centrifuging the homogenate at 100,000 *g* for 60 min at 4°C, 0.5 M Tris, 2.8 mM NAD, and 3.4 mM dimethylallyl alcohol (Aldrich Chemical Co., Milwaukee, WI) at pH 7.2. Activity was also determined using ethanol as substrate (14).

Experiment 6

Adult male and female Wistar and Sprague-Dawley rats were allowed 2–3 weeks to adjust to their specific light/dark cycle (either 6 AM–6 PM dark with the remaining period lighted, or vice versa). The male rats weighed from 280 to 420 g and the female rats from 230 to 320 g. Male and female rats of similar age were used in each experiment. They were decapitated and their kidneys were removed. Kidneys from each rat were sliced using a Stadie-Riggs slicer, and 1.0 g of the slices was incubated, with shaking, at 37°C for 90 min in either Krebs-Ringer bicarbonate or phosphate buffer (15), 10 ml, at pH 7.4, containing 5 μCi R,S-[5-^{14}C]MVA and 0.1 mM R,S-MVA (5, 6). The gas phases were as recorded in Table 5. The incubation was terminated by addition of 2 N sulfuric acid. The $^{14}CO_2$ evolved was trapped for counting in Scintisorb-C (Isolab, Inc., Akron, OH).

Statistical analyses

Data from experiments 1, 3, and 5 were analyzed as factorial designs (2 × 2, 2 × 2, and 2 × 4, respectively). Experiment 4 was evaluated by analysis of variance for a completely randomized design (16). Where a significant effect ($P < 0.05$) was found, means were compared to a control mean (generally of the intact males) using Dunnett's *t*-test (17). Experiments 2 and 6 were analyzed using Student's *t*-test (18).

RESULTS AND DISCUSSION

Fig. 1 depicts the time course of $^{14}CO_2$ production following the injection of 1 μmol of R,S-[5-^{14}C]MVA into Sprague-Dawley male and female near puberty and mature rats (Experiment 1). In **Table 1** are data on the body weights of the rats, the total nmol of MVA oxidized over the 6-hr period, and the amount oxidized calculated in nmol/100 g body weight. Thus, the means in the last column of Table 1 are those at 360 min in the plot in Fig. 1. There was initially a rapid formation of $^{14}CO_2$ and then a plateauing in all four groups. $^{14}CO_2$ formed over the 6-hr period, as recorded in Table 1 (and succeeding tables), is the result of an integration of the rates, rapid at first and then becoming negligible, over the 6-hr period. There is no significant difference in $^{14}CO_2$ formation when expressed per 100 g body weight for the rats near puberty of both sexes and for the female mature rats. The male mature rats expired, per 100 g body weight, about one-half as much $^{14}CO_2$ as any other of the groups.

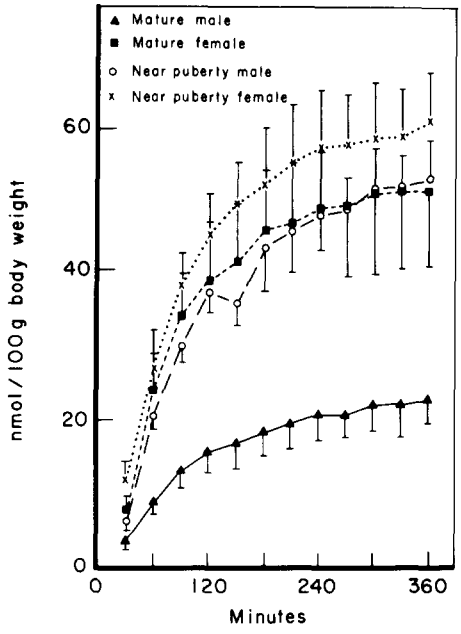

Fig. 1. [5-^{14}C]MVA oxidation to $^{14}CO_2$ by mature and near puberty male and female Sprague-Dawley rats. Either + or − the SEM is plotted for each value.

TABLE 1. Body weight and MVA shunt activity in near puberty and mature Sprague-Dawley rats injected with [5-^{14}C]mevalonate[a]

	Body Weight	MVA Shunt Activity[b]	
	g	*nmol*	*nmol / 100 g*
Male			
Near puberty	202 ± 6*	108 ± 11	53 ± 4*
Mature	312 ± 26	74 ± 17	23 ± 3
Female			
Near puberty	202 ± 9*	124 ± 17	61 ± 7*
Mature	255 ± 10*	134 ± 27	52 ± 11*

[a] Each value is the mean of three animals ± SEM. Asterisk designates means significantly different from mature male value.
[b] MVA shunt activity: $^{14}CO_2$ from [5-^{14}C]mevalonate over a 6-hr period.

The conditions of Experiment 1 are identical to those detailed by Wiley, Howton, and Siperstein (6), except that their rats were maintained on a reverse 12-hr light cycle, their young male and female rats were less than 5 weeks of age, and they weighed less than 100 g. Our results appear to be very similar to theirs. After 6 hr their mature male rats expired a mean of 58 nmol of $^{14}CO_2$, and since they had a mean weight of 251 g this is about 23 nmol/100 g body weight, the same as we observed (Table 1). Their mature female rats with a mean weight of 235 g expired a mean of 105 nmol, calculating to 45 nmol/100 g body weight. Assuming the mean weight of their immature rats was about 100 g, their immature male rats expired 45 nmol and the immature female rats expired 55 nmol/100 g body weight.

Wiley et al. (6) did not express their results per unit body weight, but kidneys from a 250-g rat weigh twice as much as kidneys from a 100-g rat (**Fig. 2**), and the kidneys, as already noted, appear to be the major site

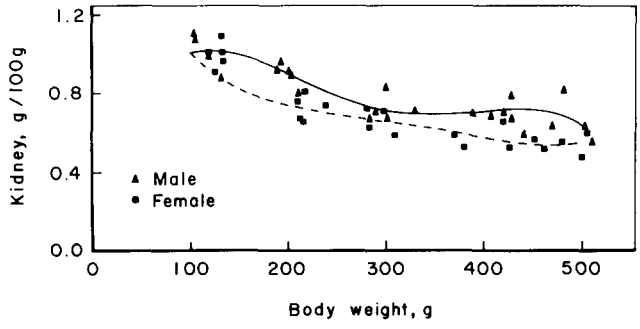

Fig. 2. Kidney weight per 100 g body weight of male and female Sprague-Dawley rats after the rats were fasted overnight. Curves were derived as fourth degree polynomials. The multiple correlation coefficients (R^2) for kidney weight as a function of body weight were 0.82 for male rats and 0.89 for female rats (18).

of shunt activity. That their mature female rats expired about twice as much $^{14}CO_2$ as their male or immature rats led Wiley et al. (6) to examine the effect of estrogen on the metabolism of [5-^{14}C]MVA. They found that estrogen increased mevalonate oxidation. The fact that mature female rats per unit body weight appear to have produced no more $^{14}CO_2$ than immature female rats in the study of Wiley et al. (6) and the observations in Experiment 1 that rats near puberty produced no more $^{14}CO_2$ than mature female rats, and that male mature rats produced half as much $^{14}CO_2$ per unit body weight, suggested to us that testosterone might be more important in depressing shunt activity than estrogen in stimulating it.

Before pursuing the possible effect of testosterone, it was necessary to establish that the lower $^{14}CO_2$ expiration from [5-^{14}C]MVA by the mature male compared to female rats was not due to less overall utilization of MVA by the males. Wiley et al. (6) provided indirect evidence that total utilization was the same in their rats, since the yield of ^{14}C in CO_2 plus sterols from the female rats was not different from that from the male rats.

We assessed utilization of the injected MVA from the yields of $^{14}CO_2$ from [1-^{14}C]MVA (Experiment 2), since every molecule of [1-^{14}C]MVA, whether converted to sterols or oxidized via the shunt, must first be decarboxylated to form one molecule of $^{14}CO_2$ (1). Under the same conditions as in Experiment 1, mature male and female rats expired similar amounts of $^{14}CO_2$ from [1-^{14}C]MVA (**Fig. 3**). Thus, the expiring of less $^{14}CO_2$ from [5-^{14}C]MVA by mature male than female rats does

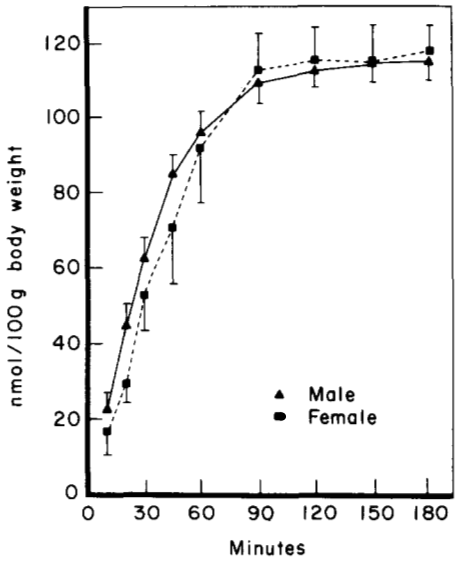

Fig. 3. [1-^{14}C]MVA oxidation to $^{14}CO_2$ by male and female adult Sprague-Dawley rats. For each value ±SEM is plotted.

TABLE 2. Body weight and MVA shunt activity among castrated male Sprague-Dawley rats with and without estrogen supplementation[a]

Treatment	Body Weight	MVA Shunt Activity	
	g	*nmol*	*nmol/100 g*
Castrated	496 ± 37	55 ± 3	11 ± 1
+ β-Estradiol (daily)	404 ± 22*	54 ± 5	14 ± 1*
Castrated	516 ± 32	63 ± 6	12 ± 1
+ β-Estradiol-3-benzoate (biweekly)	422 ± 21*	65 ± 6	15 ± 1*

[a] Each value is the mean ± SEM of four rats. Asterisk designates values significantly different from the castrated control.

indicate a lesser fraction of utilized MVA was metabolized via the shunt in the male rats.

We next examined the effect of estrogen administration on castrated male Sprague-Dawley rats (Experiment 3) as Wiley et al. (6) had done. Intact rats were not used because Wiley et al. reported that intact and castrated males showed the same shunt activity. They found that estrogen treatment of castrated male rats increased the oxidation of [5-^{14}C]MVA to the level in normal female rats. We observed a small—about 25%—but significant increase produced by estrogen, but only when yields were expressed per unit body weight (**Table 2**). The shunt activity values recorded in Table 2 are again the total yields of $^{14}CO_2$ expired over the 6-hr period following the injection of [5-^{14}C]MVA. The curves of $^{14}CO_2$ expired over this period parallel those depicted in Fig. 1.

Experiment 4 was similar in design to Experiment 3 except that there were intact rats as controls, estrogen was given continuously, and testosterone was also administered. While estrogen and testosterone were not measured in the present study, previous studies provided evidence that capsules of the dimensions used provide physiologic levels of estrogen (12) and testosterone (13), and without the fluctuations inherent in daily injections. Since Wiley et al. (6) had used the reverse light cycle, both light cycles were examined. There was no difference due to the light cycle and therefore the results in **Table 3** were obtained by summing data for both cycles. Again, only the total yields of expired $^{14}CO_2$ over the 6-hr period are recorded, but the curves of expired $^{14}CO_2$ parallel those in Fig. 1.

Castration of the male rats increased the oxidation of [5-^{14}C]MVA by about 60% and there was an additional increase of about 35% when estrogen was given (13.0 ± 1.4 is significantly different than 17.6 ± 1.6, $P < 0.05$, by Student's *t*-test). While Wiley et al. (6) observed no effect of castration, they did not give the number of rats studied nor the variations observed. We

TABLE 3. Body weight and MVA shunt activity among adult intact, castrated, and sex hormone-treated male Sprague-Dawley rats[a]

Treatment	Body Weight	MVA Shunt Activity
	g	*nmol/100 g*
Intact	529 ± 15	8.2 ± 0.6
Castrated	490 ± 19	13.0 ± 1.4*
+ Estrogen	389 ± 26*	17.6 ± 1.6*
+ Testosterone	496 ± 28	9.0 ± 1.2

[a] Each value is the mean ± SEM of six animals. Asterisk designates means significantly different from intact control.

found testosterone administration to the castrated males returned oxidation to the level in the intact males. Thus, while estrogen increased shunt activity, testosterone decreased it.

These first four experiments were done using rats of the Sprague-Dawley strain, the strain used by Wiley et al. (6). Dembić and Sabolić (19) reported that castration of female rats of the Wistar strain decreased ethanol dehydrogenase activity in the kidneys and estradiol administration increased it. We therefore proceeded to use Wistar rats (Experiment 5). Wistar male rats, gonadectomized and treated with estrogen and testosterone, oxidized [5-^{14}C]MVA (**Table 4**) similarly to male Sprague-Dawley rats (Table 3). That is, removal of the testes produced about a 50% increase in shunt activity. A slight—about 20%—but not statistically significant (by Student's *t*-test) further increase accompanied estrogen administration. Testosterone supplementation to the castrated males resulted in a return to the intact level. In the female Wistar rats, in accord with estrogen enhancing shunt activity, gonadectomy resulted in a decrease in $^{14}CO_2$ yield of about 25%, but the variations were such that the decrease was at borderline significance. Estrogen administration to the gonadectomized females resulted in a value not different from that in the intact female, but the variations were sufficiently great to preclude an effect of estrogen on the gonadectomized female rat from just these data. Testosterone administration to the gonadectomized female rat decreased MVA oxidation to a level near that of the intact male rat.

Hormonal treatment had a considerable effect on body weight. Yet, kidney as a percent of body weight was not affected by treatment (third column of Table 4). Therefore, these data are also expressed per 100 g body weight. Such expression allows evaluation of the effect of hormonal treatment on shunt metabolism independent of the effect of hormonal treatment on body weight. If adjustments are not made for weight, the patterns remain essentially the same, although differences are less pronounced. Thus, we conclude that estrogen also enhances MVA oxidation and that testosterone has a greater effect in decreasing than estrogen has in increasing shunt activity in the Wistar strain of rat.

Since dimethylallyl alcohol compares favorably with ethanol as a substrate for liver alcohol dehydrogenase (20), alcohol dehydrogenase is postulated to catalyze the dehydrogenation of dimethylallyl alcohol in the operation of the shunt pathway (1, 2), and the kidney appears to be the major site of shunt activity (8, 9), we measured dimethylallyl alcohol dehydrogenase activity in the kidneys of these Wistar rats. In keeping with the report of Dembić and Sabolić (19) for ethanol, and paralleling the changes in MVA oxidation, estrogen increased the

TABLE 4. Effect of various hormonal treatments on body weight, kidney to body weight, in vivo MVA shunt activity, and kidney dimethylallyl alcohol dehydrogenase activity among 10-week-old Wistar rats[a]

	Body Weight	Kidney Weight	MVA Shunt Activity	Dimethylallyl Alcohol Dehydrogenase
	g	*g/100 g body weight*	*nmol/100 g*	*nmol/min per g kidney*[b]
Male				
Intact	428 ± 25	0.73 ± 0.03	13.7 ± 1.4	21 ± 7
Gonadectomized	372 ± 9*	0.67 ± 0.03	20.8 ± 2.0*	19 ± 4
+ Estrogen	290 ± 11*	0.77 ± 0.04	24.5 ± 3.3*	104 ± 10*
+ Testosterone	342 ± 14*	0.76 ± 0.03	14.4 ± 0.7	24 ± 7
Female				
Intact	256 ± 11	0.70 ± 0.04	31.9 ± 4.1	82 ± 8
Gonadectomized	308 ± 13*	0.65 ± 0.04	23.8 ± 2.4*	43 ± 8*
+ Estrogen	218 ± 9*	0.75 ± 0.05	27.2 ± 2.0	116 ± 10*
+ Testosterone	253 ± 12	0.75 ± 0.06	17.8 ± 0.9*	25 ± 9*

[a] Each value is mean ± SEM of six animals. Asterisk designates means significantly different from intact male or intact female.

[b] Initial rates calculated from the linear portions of the light-extinction curves.

dehydrogenation of dimethylallyl alcohol in the castrated rats, while testosterone decreased it in castrated female rats (Table 4). However, there is no consistently good correlation between the extent of MVA oxidation and dehydrogenase activity. Thus, while in the intact male, gonadectomy increased MVA oxidation (13.7 to 20.8 nmol/min per g from Table 4), dehydrogenase activity did not change (21 compared to 19 nmol/min per g). Since dephosphorylation is the initial step in the shunt pathway, it might more likely be the rate-limiting step in the pathway than the dehydrogenation.

Ethanol dehydrogenase activity in these kidneys was 2.7-times greater than dimethylallyl alcohol dehydrogenase activity. This ratio was independent of sex or hormonal treatment. Christophe and Popják (20) reported a ratio of 1.3 for horse liver alcohol dehydrogenase activity and this is also the case in our hands.

Lastly, we wanted to examine how sex differences directly alter MVA oxidation in the kidney. Wiley et al. (6) reported that kidney slices from Sprague-Dawley rats oxidized [5-^{14}C]MVA to $^{14}CO_2$ at one-half the rate of slices from male rats. Kidney slices from rats housed under conditions of reversed phase lighting were incubated in phosphate buffer with air as the gas phase. Using phosphate buffer under an air phase, with a reversed phase light cycle, we did observe small, but significantly greater oxidation of [5-^{14}C]MVA by slices from female than male Wistar rats. We did not observe a difference when the light cycle was not reversed (**Table 5**). Recall that we observed no difference in $^{14}CO_2$ formation from [5-^{14}C]MVA by Sprague-Dawley rats in vivo as a function of the light cycle (Experiment 4). We also did not observe an effect of sex on $^{14}CO_2$ yields when slices from Wistar rats were incubated in bicarbonate buffer with a gas phase of 95% O_2–5% CO_2 (Table 5).

Similarly, we observed no difference in $^{14}CO_2$ yields from [5-^{14}C]MVA on incubating slices from female and male rats of the Sprague-Dawley strain (lower portion of Table 5). Feingold et al. (21), in a more recent paper, reported incubations of kidney slices from Sprague-Dawley rats with [5-^{14}C]MVA in phosphate buffer with a gas phase of 95% O_2–5% CO_2. Their yields of $^{14}CO_2$ were also the same for slices from male and female rats (Table V of reference 21). Thus, the significance of the report of Wiley et al. (6) of a sex effect on the oxidation of MVA by kidney in vitro, remains in doubt. Yields are greater in bicarbonate than in phosphate buffer and this is also evident from the papers of Siperstein and his associates (5, 6, 22). The concentration of bicarbonate achieved in phosphate buffer may not be sufficient for the effective carboxylation of β-methylcrotonyl CoA (23), one of the reactions, as already noted, that is in the shunt pathway (1).

The quantity of $^{14}CO_2$ from [5-^{14}C]MVA expired by the adult female rat is about 50 nmol/100 g body weight and of the adult male 25 nmol/100 g (Table 1 and Fig. 1); while the quantity of $^{14}CO_2$ from [1-^{14}C]MVA for both is about 110 nmol/100 g body weight. If the yield from [1-^{14}C]MVA is accepted as the measure of total mevalonate utilized and the yield from [5-^{14}C]MVA as that via the shunt, then about 45% of mevalonate metabolism is via the shunt in the female and 20% in the male rat. The latter estimate is in good agreement with the estimate of Wiley, Howton, and Siperstein (9) that the shunt pathway is responsible for

TABLE 5. Oxidation of [5-^{14}C]MVA to CO_2 by kidney slices from adult male and female Wistar and Sprague-Dawley rats

Strain, Sex	Light Cycle[a]	Buffer	Gas Phase	Kidney Wt.	MVA Oxidized[b]
				g/rat	*nmol/90 min per kidney*
Wistar					
Female	6 PM–6 AM	Phosphate	Air	1.8 ± 0.1	8.6 ± 0.5* (5)
Male				2.3 ± 0.1	6.4 ± 0.5 (5)
Female	6 AM–6 PM	Phosphate	Air	1.6 ± 0.1	12.6 ± 1.6 (3)
Male				2.0 ± 0.4	12.4 ± 2.3 (3)
Female	6 PM–6 AM	Bicarbonate	95% O_2:5% CO_2	1.8 ± 0.1	16.8 ± 1.7 (2)
Male				2.7 ± 0.1	28.3 ± 7.4 (2)
Sprague-Dawley					
Female	6 AM–6 PM	Bicarbonate	95% O_2:5% CO_2	1.7 ± 0.1	30.2 ± 6.2 (6)
Male				2.0 ± 0.2	39.7 ± 8.3 (6)
Female	6 AM–6 PM	Phosphate	Air	1.8 ± 0.1	5.5 ± 2.1 (6)
Male				2.2 ± 0.2	6.2 ± 2.2 (6)

[a] Times indicate period of light; the remainder of the 24-hr period was dark.
[b] Values marked with asterisk indicate female significantly greater than male ($P < 0.05$) determined by t-test. Each value is mean ± SEM for the number of experiments in parentheses.

26% of mevalonate metabolized in male rats. Their estimate is based upon the yield of $^{14}CO_2$ from [5-^{14}C]MVA and the incorporation of ^{14}C from it into sterols, studied under essentially the same conditions as we have used.

We found the quantities of $^{14}CO_2$ formed from [5-^{14}C]MVA by slices of kidney and liver from fasted and diabetic rats to be many fold less than the quantities of $^{14}CO_2$ formed from [1-^{14}C]MVA by the slices (24). Therefore, we have concluded that the yield of $^{14}CO_2$ from [5-^{14}C]MVA in such experiments likely underestimates the quantitative contribution of the shunt to overall metabolism. Siperstein and his coworkers (5, 6, 9, 25) have presented their reasons for believing that several hours after the in vivo administration of MVA, the yield of $^{14}CO_2$ from [5-^{14}C]MVA is a reliable measure of shunt activity. The lower ratio of yields of $^{14}CO_2$ from [5-^{14}C]MVA to that from [1-^{14}C]MVA in vitro than in vivo may then reflect the differences in the durations of $^{14}CO_2$ collection, i.e., 90 min compared to several hours.

In interpreting our results we have made two assumptions of note. The yield of $^{14}CO_2$ from [5-^{14}C]MVA has been taken as the measure of the shunt pathway. If a smaller fraction of the [1-^{14}C]acetyl CoA formed from [5-^{14}C]MVA via the shunt in the male than the female is oxidized to $^{14}CO_2$, presumably via the Krebs cycle, the results that Wiley et al. (6) and we have obtained could be due to this and not to decreased shunt activity (5, 24). Therefore, we have assumed that the fraction of [1-^{14}C]acetyl CoA oxidized is not sufficiently different between the sexes to alter our conclusions. Also, the $^{14}CO_2$ expired by a rat or collected on incubation of the kidney slices is the difference between $^{14}CO_2$ produced and fixed. If different quantities of $^{14}CO_2$ are fixed in the male than female rats or their kidneys, the $^{14}CO_2$ collected could also be a misleading measure of shunt activity (26). We have therefore also assumed that $^{14}CO_2$ fixation is not sufficiently different in the sexes to negate the conclusions we have made.

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