EFFECTS OF NOREPINEPHRINE ON UPTAKE AND OXIDATION OF PLASMA FREE FATTY ACIDS IN COLD-ACCLIMATED RATS

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Abstract In order to know the mode of action of norepinephrine in enhanced utilization of plasma free fatty acids (FFA) in cold acclimation, albumin-bound ^{14}C -palmitate was infused intravenously at a constant rate into cold- and warm-acclimated rats under pentobarbital anesthesia with and without norepinephrine infusion. Rectal temperature, CO_2 output and plasma FFA, from which the rates of FFA uptake and oxidation were calculated, were determined simultaneously. Similar fractions, that is, 7 and 5% of the total uptake of plasma FFA by the tissue were immediately oxidized in both cold- and warm-acclimated rats, respectively. Norepinephrine infusion caused a significant increase in all the parameters examined. In addition, during hormone infusion the percentage of FFA uptake oxidized was elevated from 22% in control to 50% in cold-acclimated rats. This increment was accompanied with greater increases in rectal temperature and CO_2 output. Similarly, in functionally eviscerated cold-acclimated rats, norepinephrine induced a greater increase in the percentage of FFA uptake oxidized than in eviscerated controls. These results indicate that although the FFA uptake was increased simultaneously norepinephrine infusion caused a more marked acceleration of the oxidation of plasma FFA in the cold-acclimated rats than in control rats.

Enhanced nonshivering thermogenesis in cold-acclimated animals is mainly mediated by norepinephrine (HSIEH and CARLSON, 1957; HSIEH *et al.*, 1957). Norepinephrine mobilizes free fatty acids (FFA) from white adipose tissue more efficiently in cold-acclimated animals (THERRIAULT *et al.*, 1969). However, few

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observations have been made on direct analysis of norepinephrine action on the *in vivo* oxidation of plasma FFA to CO_2 in cold-acclimated animals. MASIRONI and DEPOCAS (1961) reported that the oxidation rate of plasma FFA in cold-acclimated rats was higher in an environment at 6°C than at 30°C, but these values remained similar in magnitude to those in warm-acclimated rats.

In a previous study in this laboratory (MORIYA *et al.*, 1974), it was shown that norepinephrine induced a higher uptake of plasma FFA by the tissue in cold-acclimated rats even with lower plasma FFA concentrations and a greater elevation in the rectal temperature than in warm-acclimated rats. In both groups, a significant positive correlation was obtained between the concentration and the uptake, respectively. It is known that the uptake of plasma FFA by the tissue is controlled, on the one hand, by its concentration according to the mass action effect (ARMSTRONG *et al.*, 1961a, b) and by changes in the status of energy requirement (ISSEKUTZ *et al.*, 1964; MINAIRE *et al.*, 1973) on the other. Therefore, it was assumed (MORIYA *et al.*, 1974) that cold acclimation brought about changes in the energy status so that norepinephrine might activate directly or indirectly greater FFA uptake.

The purpose of the present study was to decide whether the primary site of action of norepinephrine lies in the promotion of FFA uptake or not. To this end, the oxidation rate of ^{14}C -palmitate to $^{14}\text{CO}_2$ was determined simultaneously with its uptake measurement. The result obtained were compared with those in warm-acclimated rats and a possible site of action of the hormone was discussed.

MATERIALS AND METHODS

Male Wistar rats, weighing from 170 to 400 g (average 300 g), were used. The animals were acclimated to cold by exposing them to 5°C for 4 to 6 weeks and control rats were kept at 22°C. They were provided with water and a rat chow (Oriental MF) *ad libitum*.

After 18 hr fasting, rats were moved from rearing rooms to an experimental room at 25°C one hour prior to anesthesia with pentobarbital (70 mg/kg body weight, *i.p.*). When anesthesia of longer duration was required, urethane (1.5 g/kg body weight, *i.p.*) was used instead. The animal was placed in a chamber with minimal dead space. The chamber was connected proximally to air and distally to a respirator pump. The rate of flow of effluent air was approximately 500 ml/min. A continuous infusion of ^{14}C -palmitate-albumin complex was given throughout the experiment *via* the right femoral vein using a constant infusion pump which delivered 14 μl of solution (^{14}C : 5 m μCi) per min through an indwelling polyethylene catheter. The first blood sample (1.2 ml) was drawn from the jugular vein into heparinized syringes at 20 min after the start of continuous infusion of ^{14}C -palmitate. Immediately after the sampling infusion of norepinephrine (Sankyo Pharmaceut. Co.) was started concurrently with ^{14}C -palmitate infusion *via* another

femoral vein. Norepinephrine was infused at rates of 0.25, 1.0, 2.0 and 4.0 $\mu\text{g}/\text{min}$ (the volume: 28 $\mu\text{l}/\text{min}$) for 20 min. The second blood sample was drawn 20 min after the start of norepinephrine infusion. These samples were used for measurements of plasma FFA concentration and its radioactivity. The effluent air samples were directed through U-tubes containing 5 ml of 0.5 M hyamine in methanol for five min before the first and the second blood samplings, respectively. This trapping solution was used for measurement of CO_2 expiration and its radioactivity.

A plateau in specific activity (SA) of FFA was obtained within the first 10 min after ^{14}C -palmitate infusion irrespective of norepinephrine infusion and thereafter it remained almost constant during the following 40 min period (Fig. 1). Under the steady state condition, the infusion rate of ^{14}C -palmitate divided by SA of FFA represents turnover rate of plasma FFA (ARMSTRONG *et al.*, 1961a). Since this value indicates uptake rate in the steady state, we used the term "uptake" instead of

Fig. 1. Time course of changes in the plasma FFA concentration and ^{14}C -palmitate in plasma during continuous infusion of ^{14}C -palmitate-albumin complex into warm- ($\square$, $\blacksquare$) and cold-acclimated rats ($\circ$, $\bullet$) under pentobarbital anesthesia (70 mg/kg body weight). Open symbols represent values in the basal state and closed symbols values under norepinephrine infusion at a rate of 2 $\mu\text{g}/\text{min}$. Infusion of ^{14}C -palmitate-albumin complex at a rate of 1.42 m $\mu\text{Ci}/\text{min}$ started at 0 min. Each value was analyzed from 0.6 ml of blood. Total blood loss was within 3 ml per rat.

turnover rate in the following text. During the steady state with constant CO_2 output, the labeled substrate is oxidized to $^{14}\text{CO}_2$ which becomes mixed with the unlabeled bicarbonate pool. The SA of CO_2 (CO_2SA) as well as the total CO_2 output rises in a form of exponential curve approaching an asymptotic value.

Fig. 2. Time course of recovery of $^{14}\text{CO}_2$ in terms of total CO_2 , CO_2SA and $^{14}\text{CO}_2$ output in the exhaled air during the constant infusion of $\text{NaH}^{14}\text{CO}_3$ in warm- ($\square$, $\blacksquare$) and cold-acclimated rats ($\circ$, $\bullet$). Open symbols represent values under the basal state and closed symbols those under norepinephrine infusion. Infusion of $\text{NaH}^{14}\text{CO}_3$ at a rate of $24 \text{ mCi}/\text{min}$ started at 0 min. Hatched areas on the abscissa indicate the sampling periods that correspond to specific pairs of data above them. The infusion of norepinephrine ($2 \mu\text{g}/\text{min}$) intervened as described in the text. This combination of the sampling periods was exclusively used throughout the experiments in this study except this series of experiments with $\text{NaH}^{14}\text{CO}_3$ infusion. Numbers in the top figure are means $\pm$ SE of percentage recovery of $\text{NaH}^{14}\text{CO}_3$ for five warm- ($\square$, $\blacksquare$) and five cold-acclimated rats ($\circ$, $\bullet$), respectively.

It is assumed that in small animals where the fractional turnover rate of the bicarbonate pool is high (ARMSTRONG *et al.*, 1948; SKIPPER *et al.*, 1951), the measured value of CO_2SA is similar to the asymptote $\text{CO}_2\text{SA}_{t=\infty}$. To ascertain this assumption, $\text{NaH}^{14}\text{CO}_3$ (New England Nuclear Corp., SA: 50 Ci/mol) was infused intravenously at a constant rate under the same experimental conditions as used in the ^{14}C -palmitate infusion. The time course of average $\text{NaH}^{14}\text{CO}_3$ output as $^{14}\text{CO}_2$ in warm- and cold-acclimated rats is shown in Fig. 2. $^{14}\text{CO}_2$ output was less than the rate of infusion of $\text{NaH}^{14}\text{CO}_3$ in the basal state. To find the proper condition, $\text{NaH}^{14}\text{CO}_3$ infusion of longer duration (up to 140 min) was tried in rats anesthetized with urethane, but the results obtained were essentially similar to those shown in Fig. 2. Hence calculations of the asymptotic values were corrected accordingly.

The asymptotic $\text{CO}_2\text{SA}_{t=\infty}$ was divided by the SA of FFA and multiplied by 17 to obtain the percentage of CO_2 derived from the immediate oxidation of plasma FFA (PAUL and HOLMES, 1973). The rate of plasma FFA oxidation was calculated from the percentage of CO_2 and respiratory CO_2 output (PAUL and HOLMES, 1973).

^{14}C -U-palmitate or ^{14}C -I-palmitate (New England Nuclear Corp., SA: 500 or 52 Ci/mol, respectively) was prepared for infusion by dissolving its sodium salt in distilled water and diluting with an equal volume of 10% solution of bovine serum albumin (Armour Pharmaceut. Co., Fraction V).

Plasma FFA concentration was determined by the method of DUNCOMBE (1963). Radioactivity in the FFA fraction which was isolated by the method of STEINBERG *et al.* (1964) was counted with a scintillation spectrometer. Total CO_2 output and its radioactivity were determined according to the method of FREDRICKSON and ONO (1958): One milliliter of the trapping solution of expired CO_2 was titrated with 0.1 N HCl for total CO_2 determination. One milliliter of the trapping solution was also added to the scintillator which consisted of 2,5-diphenyloxazole (PPO, 4 g/liter of toluene) and 1, 4-di-2 (5-phenyloxazolyl)-benzene (POPOP, 0.1 g/liter of toluene).

Rectal temperature was measured by inserting a thermistor to a depth of 5 cm from the anus in a room with a temperature of 25°C at 0, 10, 20, 30 and 40 min after the start of the ^{14}C -palmitate-albumin complex infusion.

Functionally eviscerated rats were prepared by ligating the rectal colon, superior mesenteric and coeliac arteries and portal vein. Before the operation, glucose was subcutaneously injected in a dose of 100 mg/kg body weight in order to maintain blood glucose at a nearly constant level. In these rats the first blood sample was drawn 15 min after ^{14}C -palmitate infusion and rectal temperature was measured at a depth of 3 cm from the anus.

Regression equations were obtained by the method of least squares. Student's *t* test was used for the comparison of two values.

RESULTS

Thermogenic responses to norepinephrine infusion

In the basal state, before the intravenous infusion of various doses of norepinephrine, the average value of rectal temperature was $34.80 \pm 0.20^\circ\text{C}$ ($n=20$) in warm- and 35.04 ± 0.22 ($n=20$) in cold-acclimated rats and it lowered by $1.21 \pm 0.95^\circ\text{C}$ and 1.15 ± 0.76 , respectively, during following 20 min of saline infusion. Norepinephrine infusion at a rate of $0.25 \mu\text{g}/\text{min}$ allowed a gradual lowering of rectal temperature in cold- as well as warm-acclimated rats, but infusions at rates of 1.0, 2.0 and $4.0 \mu\text{g}/\text{min}$ resulted in significant elevation of rectal temperature $+0.98 \pm 0.26^\circ\text{C}$ ($n=4$), $+1.53 \pm 0.44$ ($n=6$) and $+1.19 \pm 0.33$ ($n=4$), respectively, in cold-acclimated rats, but not in warm-acclimated controls.

Average value of respiratory CO_2 expiration rates before the infusion of norepinephrine was $43 \pm 2.4 \mu\text{mol}/\text{min}/100 \text{ g}$ body weight ($n=20$) in cold-acclimated rats (Table 1). Norepinephrine infusion caused an increase in respiratory CO_2 output in both groups. At doses of more than $1.0 \mu\text{g}/\text{min}$, CO_2 output was greater in cold- than in warm-acclimated groups.

Table 1. Effects of norepinephrine (NE) infusion on the respiratory CO_2 outputs in warm- and cold-acclimated rats under pentobarbital anesthesia.

Group	NE doses in $\mu\text{g}/\text{min}$ (No. of rats)	Before	After	Paired differences	$p^\dagger$
Warm-acclimated rats	0.25 (6)	30 ± 5.2	41 ± 5.1	11 ± 3.4	<0.05
	1.0 (4)	28 ± 3.7	50 ± 2.7	25 ± 2.8	<0.01
	2.0 (6)	34 ± 3.6	58 ± 3.5	24 ± 4.8	<0.01
	4.0 (4)	31 ± 3.8	56 ± 3.8	26 ± 6.3	<0.05
Cold-acclimated rats	0.25 (6)	41 ± 2.6	55 ± 6.0	13 ± 5.0	<0.05
	1.0 (4)	$48 \pm 7.0^*$	$90 \pm 4.2^{***}$	$42 \pm 3.4^{**}$	<0.01
	2.0 (6)	42 ± 5.2	$114 \pm 8.3^{***}$	$72 \pm 10.4^{**}$	<0.001
	4.0 (4)	41 ± 5.9	$104 \pm 3.7^{***}$	$63 \pm 8.9^{**}$	<0.01

Means $\pm$ SE. All values are expressed as $\mu\text{mol}/\text{min}/100 \text{ g}$ body weight. $^\dagger p$ values for the paired differences. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ vs. warm-acclimated controls.

Significant positive correlations between CO_2 expiration (X) and rectal temperature (Y) were observed in warm- and cold-acclimated groups. In these cases, corrections were made by adding the average change of rectal temperature (as detailed above) during saline infusion to each rectal temperature measured at the end (20 min) of norepinephrine infusion. In warm-acclimated rats, the relation was given by $Y = 0.029X + 34.0$ ($n=40$), $r = 0.359$ ($p < 0.05$), in cold-acclimated rats by $Y = 0.032X + 33.9$ ($n=40$), $r = 0.698$ ($p < 0.01$). The regression coefficient and ordinate intercept of the line were not significantly different between these groups.

Changes in the metabolism of plasma FFA during norepinephrine infusion

Changes in plasma FFA concentration and its uptake induced by norepinephrine infusion are illustrated in Fig. 3. The average basal values of plasma FFA concentration were 695 ± 34 $\mu\text{Eq/liter}$ ($n=20$) for warm- and 712 ± 50 ($n=20$) for the cold-acclimated group. As seen in this figure, increases in plasma FFA concentration in response to norepinephrine were less in cold- than in warm-acclimated rats. The average value of uptake of plasma FFA for warm-acclimated controls was 2.65 ± 0.15 $\mu\text{Eq/min/100 g}$ body weight ($n=20$) and 3.13 ± 0.19 ($n=20$) for cold-acclimated rats, both values being not significantly different. Norepinephrine infusion in increasing doses caused increases in concentration and uptake of plasma FFA in both groups.

Fig. 3. Changes in plasma FFA concentration and its uptake following norepinephrine infusion. Norepinephrine doses are shown at the bottom along the abscissa. W stands for warm-acclimated, and C for cold-acclimated rats, accompanied by numbers of paired determinations. Bars on the top of columns represent SE of the means.

Between plasma FFA concentration (X) and its uptake (Y), significant positive correlation was obtained in both groups. The relations were given by $Y=0.031X+1.05$ ($n=40$), $r=0.713$ ($p<0.01$) in cold-acclimated rats and by $Y=0.019X+1.36$ ($n=40$), $r=0.588$ ($p<0.01$) in controls. The FFA uptake increased linearly with the increasing FFA concentration in both groups, but the regression coefficient for cold-acclimated rats was significantly greater ($p<0.01$) than that for controls.

Oxidation rates of plasma FFA are shown in Table 2. An average basal value of oxidation rates of plasma FFA in cold-acclimated rats was 0.22 ± 0.04 $\mu\text{Eq}/\text{min}/100$ g body weight ($n=20$) which was significantly larger ($p < 0.05$) than 0.12 ± 0.01 ($n=20$) in warm-acclimated rats. Increasing doses of norepinephrine, up to 2.0 $\mu\text{g}/\text{min}$, caused increased oxidation of plasma FFA in both groups, and the magnitude of change was greater in the former group than in the latter.

Table 2. Effects of norepinephrine (NE) infusion on the oxidation rates of plasma free fatty acids (FFA) in warm- and cold-acclimated rats under pentobarbital anesthesia.

Group	NE doses in $\mu\text{g}/\text{min}$ (No. of rats)	Before	After	Paired differences	$p^\dagger$
Warm-acclimated rats	0.25 (6)	0.10 ± 0.02	0.36 ± 0.07	0.26 ± 0.06	< 0.01
	1.0 (4)	0.13 ± 0.04	0.67 ± 0.04	0.54 ± 0.03	< 0.001
	2.0 (6)	0.14 ± 0.02	0.96 ± 0.12	0.82 ± 0.12	< 0.01
	4.0 (4)	0.11 ± 0.02	0.79 ± 0.21	0.68 ± 0.20	< 0.05
Cold-acclimated rats	0.25 (6)	0.19 ± 0.03	0.53 ± 0.06	0.34 ± 0.06	< 0.01
	1.0 (4)	0.22 ± 0.05	$1.74 \pm 0.41^*$	$1.53 \pm 0.32^*$	< 0.05
	2.0 (6)	0.19 ± 0.05	$2.44 \pm 0.32^{**}$	$2.25 \pm 0.32^{**}$	< 0.001
	4.0 (4)	0.32 ± 0.17	$1.90 \pm 0.08^{**}$	$1.58 \pm 0.21^*$	< 0.01

Means $\pm$ SE of the mean, expressed as $\mu\text{Eq}/\text{min}/100$ g body weight. $^\dagger p$ values for the paired differences. $^*p < 0.05$, $^{**}p < 0.01$ vs. warm-acclimated controls.

Percentages of oxidation to uptake of plasma FFA were estimated in both groups (Table 3). The percentages were increased by various doses of norepinephrine in both groups, and the percentage increases were greater in cold- than in warm-acclimated groups. Percentages of CO_2 derived from plasma FFA were

Table 3. Effects of norepinephrine (NE) on the ratio of oxidation rate to uptake rate of plasma free fatty acids (FFA) in warm- and cold-acclimated rats under pentobarbital anesthesia.

Group	NE doses in $\mu\text{g}/\text{min}$ (No. of rats)	Before (%)	After (%)	Paired differences (%)	$p^\dagger$
Warm-acclimated rats	0.25 (6)	3.3 ± 0.6	11.0 ± 1.7	7.7 ± 1.7	< 0.01
	1.0 (4)	5.6 ± 1.8	22.1 ± 2.6	16.3 ± 3.0	< 0.05
	2.0 (6)	6.4 ± 0.8	26.2 ± 2.1	19.8 ± 2.6	< 0.001
	4.0 (4)	3.7 ± 0.5	18.1 ± 3.0	14.4 ± 2.9	< 0.05
Cold-acclimated rats	0.25 (6)	5.4 ± 0.9	13.5 ± 2.3	8.1 ± 2.0	< 0.01
	1.0 (4)	7.7 ± 1.7	$49.5 \pm 7.9^*$	$41.9 \pm 6.7^*$	< 0.01
	2.0 (6)	5.9 ± 1.4	$54.0 \pm 12.8^*$	$48.1 \pm 11.6^*$	< 0.01
	4.0 (4)	11.8 ± 4.9	43.9 ± 7.4	$31.7 \pm 5.3^*$	< 0.01

Means $\pm$ SE. $^\dagger p$ values for the paired differences. $^*p < 0.05$ vs. warm-acclimated controls.

elevated by various doses of norepinephrine in both groups. The change at each dose in cold-acclimated rats was not significantly different from that in controls. As a whole, they were increased from $6.6 \pm 0.5\%$ to 22.3 ± 7.9 in warm-acclimated rats ($n=20$) and from 8.7 ± 1.2 to 29.2 ± 3.2 in cold-acclimated rats ($n=20$).

The amount of FFA oxidized and percentage of CO_2 derived from plasma FFA increased linearly with increasing FFA concentration in both warm- and cold-acclimated groups, but at any equivalent concentrations, the values in cold-acclimated rats were considerably higher than those in the controls. The amount of FFA oxidized also increased linearly with the increasing FFA uptake in both groups. The correlations between uptake (X , in $\mu\text{Eq}/\text{min}/100\text{ g}$) and oxidation rates (Y , in $\mu\text{Eq}/\text{min}/100\text{ g}$) of plasma FFA were given by $Y=0.404X-0.588$ ($n=40$), $r=0.468$ ($p<0.01$) in cold-acclimated rats and by $Y=0.268X-0.425$ ($n=40$), $r=0.690$ ($p<0.01$) in controls. The regression coefficient for cold-acclimated rats was significantly greater ($p<0.01$) than that for the controls.

Relationship between thermogenesis and FFA metabolism

CO_2 expiration, as an indicator of thermogenesis, increased linearly with increases in oxidation rate of plasma FFA (Fig. 4A). The regression coefficient for cold-acclimated rats was essentially similar to that for the controls. On the other hand, the regression coefficient between CO_2 output and uptake of plasma FFA for cold-acclimated rats was greater ($p<0.01$) than that for the controls, although correlations were significant in either group (Fig. 4B).

Thermogenesis and FFA metabolism in functionally eviscerated rats

The evisceration caused the lowering of rectal temperature, of respiratory CO_2 expiration and plasma FFA metabolism in terms of oxidation rate, percentage of FFA uptake oxidized (Table 4) and percent CO_2 from plasma FFA. In eviscerated rats, as in intact animals, norepinephrine-induced elevation in plasma FFA concentration, its oxidation rate, percent of FFA uptake oxidized and percent CO_2 from plasma FFA was in parallel with the elevation of rectal temperature and CO_2 output. The magnitude of elevation of each parameter, except FFA concentration, was greater in eviscerate cold-acclimated rats than in eviscerate controls.

When $\text{NaH}^{14}\text{CO}_3$ was infused intravenously at a constant rate, recovery of ^{14}C as $^{14}\text{CO}_2$ in the basal state was 38% in eviscerate warm- and 33% in eviscerate cold-acclimated rats. It increased under norepinephrine infusion ($2\text{ }\mu\text{g}/\text{min}$ for 20 min) to 76% in the former and 86% in the latter. Accordingly, $\text{CO}_2\text{SA}_{t=\infty}$ was corrected in the eviscerate groups.

DISCUSSION

Enhanced thermogenesis is one of the characteristics of cold acclimation. In a previous study in this laboratory (MORIYA *et al.*, 1974), changes in rectal temperature were used as a measure of enhanced heat production under norepinephrine

Fig. 4

Table 4. The metabolism of plasma free fatty acids (FFA) in eviscerated preparations in the basal state and during norepinephrine infusion (2 μ g/min) for 20 min under pentobarbital anesthesia.

Group	Body weight (g)	V_{CO_2} (μ mol/min/100 g body weight)	Plasma FFA			% of FFA uptake oxidized
			concentration (μ Eq/liter)	uptake (μ Eq/min/ 100 g body weight)	oxidation	
Warm-acclimated	288					
Before (1)		22 $\pm$ 5	1,180 $\pm$ 74	1.8 $\pm$ 0.1	0.1 $\pm$ 0.01	5.3 $\pm$ 0.4
After (2)		37 $\pm$ 7	1,940 $\pm$ 149	1.7 $\pm$ 0.05	0.3 $\pm$ 0.05	16.1 $\pm$ 2.9
Paired difference (3) (n=4)		15 $\pm$ 8	760 $\pm$ 92**	-0.1 $\pm$ 0.3	0.2 $\pm$ 0.05*	
Cold-acclimated	286					
Before (4)		23 $\pm$ 5	1,119 $\pm$ 132	1.8 $\pm$ 0.3	0.1 $\pm$ 0.02	3.6 $\pm$ 0.7
<i>p</i> vs. (1)		NS	NS	NS	NS	NS
After (5) (n=6)		76 $\pm$ 4	1,738 $\pm$ 95	2.4 $\pm$ 0.3	0.7 $\pm$ 0.09	30.0 $\pm$ 1.7
<i>p</i> vs. (2)		<0.001	NS	NS	<0.01	<0.001
Paired difference (6)		53 $\pm$ 6***	619 $\pm$ 70***	0.6 $\pm$ 0.3	0.6 $\pm$ 0.10**	
<i>p</i> vs. (3)		<0.01	NS	NS	<0.01	

Means $\pm$ SE. **p*<0.05, ***p*<0.01, ****p*<0.001.

load. Based on the correlation analysis between rectal temperature and logarithm concentration or uptake of plasma FFA, it was inferred that an increase in the capacity of uptake rather than oxidation of plasma FFA occurs in cold-acclimated rats. Since the rectal temperature is a balance between heat production and loss, it is not a direct indicator of heat production.

Using CO_2 expiration as an indicator of calorigenesis, it was confirmed in this study that norepinephrine enhanced heat production and its effect was greater in cold- than in warm-acclimated rats. It was also shown that the regression coefficient between CO_2 output and plasma FFA oxidation rate was essentially similar in magnitude in both groups (Fig. 4A). The result indicates that the CO_2 expiration rate is a direct reflection of FFA oxidation irrespective of animal's energy status such as acquisition of cold acclimation. It should be noted, however, that although the rate of CO_2 expiration was correlated well with the uptake, the CO_2 expiration rate was evidently greater in the cold- than in the warm-acclimated rats when compared at the same uptake rate (Fig. 4B). The result indicates that enhanced CO_2 production (*i.e.*, enhanced heat production) in cold-acclimated rats cannot be explained by simple enhancement in the uptake of FFA, the conclusion being in discord with the previous one (MORIYA *et al.*, 1974) which was obtained

Fig. 4. Interrelationships between respiratory CO_2 output and plasma FFA oxidation (A) and its uptake rate (B) in warm- (□, ■) and cold-acclimated rats (○, ●) under the basal state (open symbols) and during norepinephrine infusion (closed symbols).

with an indirect indicator of thermogenesis. Thus, the magnitude of oxidation, instead of uptake, of plasma FFA is of direct importance.

Average value of oxidation rates before norepinephrine infusion was significantly larger in cold- than in warm-acclimated rats (Table 2). The result is consistent with the finding that liver slices from cold-acclimated animals oxidized more labeled palmitate to labeled CO_2 than controls (BAUMBER and DENYES, 1965; FELT and MASORO, 1959). Increases in the oxidation rate of plasma FFA under the hormone stimulation were also significantly greater in the former than in the latter (Table 2). Similarly, average value of uptake of plasma FFA during norepinephrine infusion was $4.45 \pm 0.24 \mu\text{Eq}/\text{min}/100 \text{ g body weight}$ ($n=20$) in cold-acclimated rats which was significantly greater than that of controls, 3.54 ± 0.23 ($n=20$). However, increases in the uptake under catecholamine load were not significantly different between both groups (Fig. 3). These results indicate that norepinephrine caused more enhancement in the oxidation than in the uptake of plasma FFA. In fact, percentages of oxidation to uptake of plasma FFA under norepinephrine infusion became much higher in cold- than in warm-acclimated rats (Table 3). Nevertheless, the enhanced activation of FFA oxidation in cold-acclimated rats did not accompany corresponding increases in percentage of CO_2 derived from plasma FFA. This fact suggests an occurrence of simultaneous stimulation of oxidation of substrates other than plasma FFA under these conditions. Similar findings were obtained in rats even after evisceration (Table 4). All of these results indicate that norepinephrine infusion in the cold-acclimated rats stimulates more oxidation of plasma FFA than its uptake, even though both parameters were increased simultaneously. The stimulation of FFA oxidation probably occurs in muscles, since the whole pattern of changes in the parameters was not affected by evisceration.

However, before arriving at the above conclusion, we have to exclude the following possibilities. The first is whether or not the stimulatory effect of norepinephrine on the FFA oxidation may be due to its effect on blood flow. KUROSHIMA *et al.* (1967) showed that the cardiac output in cold-acclimated rats increased 2-fold over controls. They also showed that intravenous infusion of norepinephrine ($2 \mu\text{g}/\text{min}$ for 10 min) into control rats caused an 1.9-fold increase in the cardiac output, but no further increment in cold-acclimated rats. In the present study, this dose of norepinephrine did not cause a greater change of parameters in the control rat as compared with cold-acclimated rats. Therefore, it is very unlikely that the norepinephrine-induced change in hemodynamics is the cause of the enhanced oxidation observed. The second is the finding in the present study that percentages of FFA oxidation to total oxidative metabolism were much lower than in other reports (ISSEKUTZ and PAUL, 1966; PAUL and HOLMES, 1973). As shown in Fig. 5, continuous infusion of ^{14}C -palmitate-albumin complex failed to yield a plateau in specific activity of respiratory CO_2 , while a plateau was observed in 10 min when ^{14}C -labeled bicarbonate was infused (Fig. 2). MASIRONI and DEPO-

CAS (1961) observed the same phenomena in conscious rats. Extensive recycling of labeled compounds in compartments which are not on the direct pathway of oxidation of FFA, such as incorporation into adipose tissue and plasma and tissue glycerides and also conversion to ketone bodies and their subsequent utilization (MAEKUBO *et al.*, 1977), could account for this observation. In the present study, CO_2 expired was trapped for five minutes from 15 to 20 min after the start of ^{14}C -palmitate infusion, but other authors utilized the much later period. Therefore, difference in the timing of sampling would explain the difference in the percentage.

Fig. 5. Respiratory $^{14}\text{CO}_2$ production in terms of total CO_2 , CO_2SA and $^{14}\text{CO}_2$ output in the exhaled air (as percentage of ^{14}C infused) during continuous intravenous infusion of ^{14}C -palmitate-albumin complex to warm- ($\square$, $\blacksquare$) and cold-acclimated rats ($\circ$, $\bullet$). Open symbols represent values in the basal state under urethane anesthesia and closed symbols values during norepinephrine infusion at a rate of $2 \mu\text{g/min}$ into rats under pentobarbital anesthesia.

The third is the effect of anesthetics used. These anesthetics are known to diminish cellular respiration by primarily inhibiting electron transfer in the area of NADH dehydrogenase and their ability of diminishing respiration *in vitro* relates to narcotic potency *in vivo* (COHEN, 1973). In fact, CO₂ expiration rate in this study was almost one-half of respiration in conscious rats reported by others (TSUKADA *et al.*, 1965; BARTUKOVA *et al.*, 1970). Nevertheless, norepinephrine was shown in this study to stimulate more oxidation of plasma FFA than its uptake, even though further experiments with conscious animals would be warranted. Finally, according to MASIRONI and DEPOCAS (1961), although the oxidation rate of plasma FFA in cold-acclimated rats in conscious state was higher in environment at 6°C than at 30°C, the rate at 6°C was not different from that in warm-acclimated rats. Since cold exposure stimulates norepinephrine release, which results in an increase in the oxidation rate of plasma FFA, their results appear difficult to interpret. However, it is possible that animals exposed acutely to cold may release more norepinephrine as well as other lipolytic hormones such as ACTH, than cold-acclimated rats (LEDUC, 1961). According to HANNON and LARSON (1962), the effects of norepinephrine infusion and cold exposure on the calorogenesis were not identical. Therefore, we cannot estimate the direct effect of norepinephrine alone from their results.

On the rate-limiting step of the FFA oxidation, carnitine acyltransferase has been implicated in several studies from other laboratories (FRITZ and MARQUIS, 1965; ORAM *et al.*, 1973). This enzyme is associated with the membrane of mitochondria which are intracellular site of FFA oxidation and respiration. When carnitine is present the enzyme allows FFA to penetrate the mitochondria so that the substrate may gain access to β -oxidation by enzyme system. Furthermore, it has been reported that in cold-acclimated rats the carnitine content increased eight times and its turnover time was one-half of the control (DELISLE and RADOMSKI, 1968; THERRIAULT and MEHLMAN, 1965). Therefore, it remains to examine changes in the enzyme activity of acylcarnitine transferase in cold-acclimated animals and the influence of norepinephrine on it.

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