Table 3. Effects of intraperitoneal administration of α-terpineol and isobornyl acetate on the induction of the liver mixed-function oxidase system in male rats*

Parameters	Control	α-Terpineol		Isobornyl acetate	
		A	B	C	D
Cytochrome b_5 content†	0·31 ± 0·03	0·16 ± 0·04	0·15 ± 0·04	0·58 ± 0·03‡	0·58 ± 0·04‡
Cytochrome P-450 content†	0·40 ± 0·04	0·42 ± 0·05	0·29 ± 0·03	0·67 ± 0·04‡	0·97 ± 0·05§
Aminopyrine *N*-demethylase	4·1 ± 0·4	3·0 ± 0·5	2·6 ± 0·6	6·6 ± 0·3§	10·7 ± 0·5§
Ethylmorphine *N*-demethylase‖	4·5 ± 0·3	3·8 ± 0·3	3·2 ± 0·3	7·1 ± 0·5§	11·5 ± 0·6§
NADPH cytochrome *c* reductase¶	43 ± 2	42 ± 2	46 ± 3	61 ± 3**	86 ± 3§

* Each group of four male rats was injected with various concentrations of α-terpineol or isobornyl acetate for 3 days. A and B 40 and 80 mg α-terpineol/100 g of body weight respectively; C and D, 50 and 100 mg isobornyl acetate/100 g of body weight. Values represent mean ± S. E.

† nmoles per mg of microsomal protein.

‡ $P < 0{\cdot}01$.

§ $P < 0{\cdot}001$.

‖ nmoles formaldehyde formed per mg of microsomal protein per min.

¶ nmoles cytochrome *c* reduced per mg of microsomal protein per min.

** $P < 0{\cdot}05$.

pyrine, *p*-nitroanisol and aniline *in vitro*. Although, α-terpineol does not induce the mixed-function oxidase system, it interacts with the latter, as evidenced by the intense type I spectral change and the competitive inhibition of aminopyrine *N*-demethylation.

Based on these results and others, once again, one cannot over-emphasize the importance of the controlled chemical-free environment in which experimental animals are housed. Without such controls, data obtained from studies concerning kinetics of drug metabolism, induction of the enzyme system by various xenobiotics, the mechanism of induction, and the purification and reconstitution of the drug-metabolizing enzyme system would be meaningless.

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Perifused fat cells—Kinetic analysis of epinephrine-stimulated lipolysis

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Many reports have appeared demonstrating that epinephrine increases the rate of hydrolysis of triglyceride in adipose tissue, presumably by activation of triglyceride lipase [1, 2]. Recently, a perifused fat cell system has been described which allows for continuous monitoring of the changes in lipolytic activity in isolated fat cells and provides a technique by which the kinetics of these changes can be observed [3]. Based on results from this system, a kinetic model has been constructed which describes the increase in lipolytic rates after the addition of epinephrine (10^{-5} M final concentration) and the decrease in lipolytic rates after cessation of the hormone.

It was assumed that sufficient triglyceride exists within the cell to completely saturate the lipase enzymes. Based on this assumption, the rate of glycerol production was taken to be proportional to the amount of activated lipase present in the fat cells. One unit of lipase enzyme was defined as that amount which produced 1 nmole glycerol/

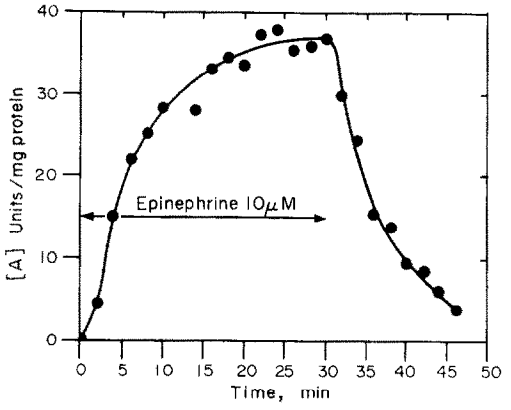

Fig. 1. Amount of "active lipase" in fat cells after the initiation and cessation of epinephrine-stimulated lipolysis in the perifused fat cell. Two ml of packed fat cells was perifused as described previously. Cells were perifused for a 10-min equilibration period (not shown) after which epinephrine was infused into the cell chamber at such a concentration and rate to produce a final concentration of 10 μM in the cell chamber. Basal rate of glycerol release (7 μmoles/mg of protein/min) has been subtracted from all values.

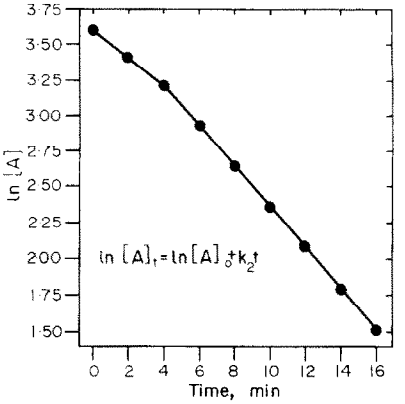

Fig. 2. Plot of the natural log of the amount of "active lipase" in fat cells at various times after cessation of epinephrine infusion. At time zero, the infusion of epinephrine was stopped. The natural log of the amount of "active lipase" was plotted vs time.

min under the conditions of these experiments. For calculations of kinetic data, amounts of active lipase were expressed as units/mg of protein.

In the perifused fat cells preparation, epinephrine caused a rapid increase in the glycerol release from the fat cells (Fig. 1). The rate of lipolysis was elevated in the first 2 min and continued to increase for about 20 min, after which a plateau was reached. The maximum lipolytic rate was 38 nmoles glycerol/min/mg of protein. When the infused epinephrine was stopped, lipolysis rapidly returned to basal rate. For all calculations of kinetic data, it was assumed that no active lipase existed in the absence of hormone stimulation. For this reason, the basal lipolytic rate (7 nmoles/min/mg of protein) was subtracted from all subsequent values.

It has been shown that, after the removal of the hormone stimulant, lipolytic rates return rapidly to basal values [3]. It is logical to assume that this is accomplished by an inactivation of the active lipase and a reduction in its tissue concentration.

If the inactive form of the lipase is represented by lipase B and the active form by lipase A, then the reaction for the activation and inactivation can be described as:

$$\text{Lipase B} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Lipase A} \qquad (1)$$

where k_1 is the reaction velocity for the activation and k_2 is the rate constant for the inactivation reaction.

It was assumed that, under all conditions, excess amounts of lipase B were present, thus the activation reaction would follow zero-order kinetics. It was also assumed that under basal conditions that the amount of lipase A was zero and after stimulation increased to an amount insufficient to saturate the inactivation process. Thus, the inactivation reaction would follow first-order kinetics. The activation reaction (V_1) may be described as:

$$V_1 = d[\text{A}]/dt = k_1 \qquad (2)$$

and the inactivation reaction (V_2) as:

$$V_2 = -d[\text{A}]/dt = k_2[\text{A}] \qquad (3)$$

Because both the activation and inactivation reaction occur simultaneously, the observed velocity (V_{ob}) would be the sum of the two and can be represented:

$$V_{ob} = (d[\text{A}]/dt)_{ob} = V_1 + V_2 \qquad (4)$$

Substituting equation 2 and 3:

$$V_{ob} = (d[\text{A}]/dt)_{ob} = k_1 + k_2[\text{A}] \qquad (5)$$

Integrating equation 5:

$$[\text{A}] = \frac{k_1 e^{k_2 t} - k_1}{k_2} \qquad (6)$$

where t = time after addition of epinephrine and [A] is the concentration of lipase A at that point in time. As can be seen from this equation, the concentration of lipase A could be increased by either increasing k_1 or decreasing k_2. Epinephrine, via increased cyclic AMP and activation of protein kinase, increases k_1. Whether or not epinephrine also has an effect on k_2 is considered below.

Equation 6 predicts that the [A] would approach a maximum value in an asymptotic manner. Experimentally, it is found that maximum concentration of lipase A ($[\text{A}]_{max}$) was obtained in 20–30 min after epinephrine addition. After this time $(d[\text{A}]/dt)_{ob} = 0 = k_1 + k_2[\text{A}]_{max}$, thus

$$[\text{A}]_{max} = -\left(\frac{k_1}{k_2}\right) \qquad (7)$$

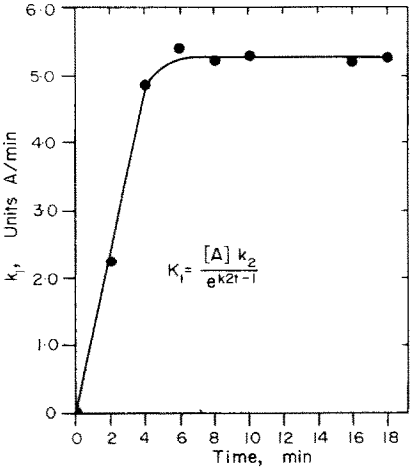

Fig. 3. Changes in apparent k_1 after the infusion of epinephrine. Using the experimental data for [A] from Fig. 1, and a value of k_2 of $-0{\cdot}14$/min, apparent k_1 values were calculated for various times after the start of epinephrine infusion.

Equation 7 shows that the maximum concentration of lipase A is defined by the ratio of the activation reaction velocity and the inactivation rate constant.

If it is assumed that in the absence of epinephrine stimulation k_1 equals zero, and if V_2 is a first-order reaction, then the fall in [A] after epinephrine removal should follow the equation for a first-order reaction: $\ln[A]_t = \ln[A]_0 + k_2 t$ and a plot of $\ln[A]_t$ vs time should result in a straight line with a slope equal to k_2. Such a plot is shown in Fig. 2. A linear relationship does exist between times 4 and 16 min. The deviation from linearity during the first 4 min probably represents incomplete washout of epinephrine. Previous work has demonstrated a 2- to 4-min washout period for epinephrine [3]. These data are compatible with the assumptions that k_1 is zero in the absence of stimulation and V_2 is a first-order reaction. Calculation of k_2 using the data between 4 and 16 min gives a value of $-0{\cdot}14$/min.

Rearranging equation 6 to: $k_1 = [A]k_2/(e^{k_2 t} - 1)$ and using a k_2 of $-0{\cdot}14$/min, the apparent k_1 can be calculated from all experimental values of [A] in Fig. 1. This is plotted in Fig. 3. The apparent k_1 increases during the first 6 min after which it remains constant. These results can be explained in either of two ways: (1) time was required for epinephrine to elevate k_1 to its maximum value, that is, the step leading to activation of lipase A required time to produce maximum activation, or (2) epinephrine decreased k_2 as well as instantaneously increasing k_1. This means that epinephrine produced a decrease in the rate of inactivation of active lipase as well as an increase in the rate of activation.

Kinetic models can be constructed for these two possibilities. If it is assumed that epinephrine had no effect on k_2 and, therefore, k_2 in the presence of epinephrine was the same as that in its absence, then using equation 7 and the $[A]_{max}$ from Fig. 1 (38 units/mg of protein), a value of 5·32 units/mg/min can be calculated for k_1. This value would be valid at any time after 6 min. Using equation 6, $k_2 = -0{\cdot}14$/min, and this value for k_1, the theoretical curve shown in Fig. 4, was constructed.

In the kinetic model in which there is a change in both k_1 and k_2, it was assumed that at time zero [A] = 0, then V_2 would also equal zero. Substituting in equation 5, it can be shown that for initial conditions, $V_{ob} = k_1$. Using the experimental value for [A] at 2 min (5·5 units/mg of protein), an initial value of k_1 of 2·25 units/mg of protein/min was calculated. If this initial k_1 was constant during the presence of epinephrine, it can be calculated from equation 7 that k_2 must have been changed by epinephrine to $-0{\cdot}06$/min. Using these values and equation 6, another theoretical curve was constructed (Fig. 4).

Theoretical curves constructed for the two different models differ substantially. When the experimental values for [A] were plotted vs time (Fig. 4), there was a good correlation between these values and the theoretical values for the model which assumes that epinephrine has no effect on k_2. This mathematical model does not take into account that time was required for the elevation of k_1 to a maximum value. This explains the less exact correlation between experimental and theoretical values at the 2-min time. No correlation exists between the experimental values and the theoretical values for the model which assumes a change in k_2.

Thus, the experimental data from the perifused fat cell system are consistent with a model system in which epinephrine produces a time-dependent increase in the rate of activation of a lipase without any effect on the rate of inactivation. Based on current concepts of hormone-stimulated lipolysis [4–6], the effect on activation would be through the cyclic AMP-dependent protein kinase secondary to stimulation of adenylate cyclase and an elevation in cyclic AMP levels. The 6 min required to reach maximum value for k_1 (Fig. 3) is similar to the time necessary to achieve peak cyclic AMP levels after the addition of epinephrine [7]. The remaining time lag to peak glycerol output conceivably represents the time necessary for the activated protein kinase to convert the lipase B to lipase A.

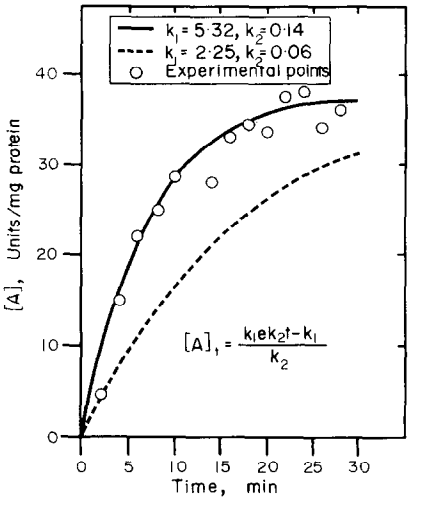

Fig. 4. Theoretical curves for amount of "active lipase" at various times after epinephrine infusion. The solid line represents conditions of $k_1 = 5{\cdot}32$ and $k_2 = -0{\cdot}14$. The broken line represents conditions of $k_1 = 2{\cdot}25$ and $k_2 = -0{\cdot}06$. The open circles are the experimental values from Fig. 1.

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