Regulation of Liver Tyrosine Aminotransferase by Endogenous Factors in the Mouse

CALEB E. FINCH, HARRIS S. HUBERMAN, and ALFRED E. MIRSKY

From The Rockefeller University, New York 10021

ABSTRACT The levels of mouse liver tyrosine aminotransferase (TAT) are shown to increase rapidly and transiently during and after various challenges (e.g., exposure to cold or shaking) to the homeostatic machinery of the fasted mouse. The increase of TAT is dependent on gene activity. Recent feeding and adrenalectomy are shown to inhibit the induction of TAT during the challenge of cold.

INTRODUCTION

The response of a mammal to a challenge from its environment may result in major shifts in metabolic activities. Analyzed below is the regulation of a liver enzyme, tyrosine aminotransferase (1-tyrosine:2-oxoglutarate aminotransferase, E.C.2.6.1.5), whose levels are observed to be sensitively varied through a gene-dependent process in concurrence with demands made on the body. Tyrosine aminotransferase (TAT) catalyzes the first stage of the catabolism of tyrosine in the liver (Knox and LeMay-Knox, 1951; Schepartz, 1951) and is considered to have an important role in gluconeogenesis in vivo (Feigelson and Feigelson, 1966).

A characteristic feature of TAT is the rapidity with which its levels are increased in response to injected hormones in the rat. For instance, transient five- to sixfold increases of TAT occur within 5 hr after injections of hydrocortisone (Lin and Knox, 1957), and insulin or glucagon (Holten and Kenney, 1967). An increase of TAT also follows the perfusion of hydrocortisone (Goldstein et al., 1962), and insulin or glucagon (Hager and Kenney, 1968) through the isolated rat liver, thereby proving a direct action of these hormones on the liver. These hormonally stimulated increases of TAT are inhibited by actinomycin D (Greengard and Acs, 1962; Hager and Kenney, 1968) and therefore may be considered dependent on gene activity. The stud-

ies of Kenney, based on a radioimmunological assay, have elegantly demonstrated that the increase of TAT activity stimulated by hydrocortisone, insulin, and glucagon is the result of a selective increase in the rate of synthesis of the polypeptide chains of TAT (Kenney, 1962; Hager and Kenney, 1968). The dependence of hydrocortisone-, insulin-, and glucagon-stimulated TAT increases on gene activity and on specific protein synthesis characterizes the increase of TAT activity under these conditions as an *enzyme induction*. It should be noted that rapid increases in activity of other liver enzymes, e.g. glucose-6-phosphatase (Shimazu and Amakawa, 1968) and tryptophan pyrrolase (Greengard and Feigelson, 1961; Greengard and Acs, 1962) can be mediated by entirely different processes not dependent on gene activity or increased protein synthesis.

Various stressful procedures, such as intraperitoneal injection of large amounts of insoluble materials (diatomaceous earth, tyrosine, or tryptophan (Kenney and Flora [1961]) and the procedure of laparotomy (Tsukada et al., 1968) are known to stimulate a rapid increase of TAT in rats. We have demonstrated in experiments described below, that certain stressful environmental challenges (exposure to cold and shaking) may also stimulate rapid increases of TAT in mice by a process dependent on gene activity.

METHODS AND MATERIALS

1. *Source and Husbandry of the Mice* C57Bl/6J male mice were obtained from Jackson Laboratories, Bar Harbor, Me., at the age of 10 wk or 8 months. The mice were held at least 3–4 wk before use. Although age is known to be an important variable in the regulation of TAT activity (Schapiro et al., 1966; Finch et al., 1969), no age differences were found between mice 4–10 months old (20–35 g).

Mice were caged at random in groups of five. Purina Lab Chow was available except as indicated. The room was maintained at 74–78°F; lights were turned on at 7 a.m. and off at 7 p.m.

2. *Assay of TAT* TAT activity was assayed by the method of Lin and Knox (1957); tautomerase (beef kidney, grade I) was obtained from Sigma Chemical Co., St. Louis, Mo., and all batches were found to be free of detectable TAT activity. The final product of the enzyme reactions, the enol-borate complex of *p*-hydroxyphenylpyruvic acid, was identified by its characteristic E_{max} at 310 mμ. The assay was conducted in covered 1 cm^2 cuvettes at 34°C.

As a rule, 0.2 g of the right median lobe of the liver, which had been rapidly excised and frozen immediately on dry ice, was homogenized at 2°C in 8.0 ml of 0.14 M KCl in a motor-driven TenBroeck homogenizer (six double strokes). After centrifugation (5,000 *g* for 10 min) at 2°C, the supernatant extract, which contained 95% of the total activity of the homogenate, was decanted and analyzed for TAT. All assays were performed in duplicate and gave highly reproducible values (±5%). The variations in TAT activity therefore appear to represent true differences from animal to animal.

TAT activity is generally expressed per unit DNA as suggested by Grossman and

Mavrides (1967). In two experiments, TAT activity was measured per unit liver cell mass; to enable comparison of all experiments, these activities have been converted to "equivalent" TAT/DNA units, indicated on the ordinates of the graphs in Figs. 1 and 3 as "equiv." TAT/DNA. The DNA content of the pellet of the centrifuged liver homogenate (consisting principally of intact nuclei) was measured by the Burton modification of the diphenylamine reaction (Burton, 1956). The characteristic spectrum of the reaction of pure DNA with the diphenylamine reagent (E_{max} 600 mμ; OD 600 mμ/640 mμ = 2.1) was obtained in 0.5 M PCA extracts of the above centrifugal pellet (70°C/30 min) after a single washing with cold 0.25 M PCA.

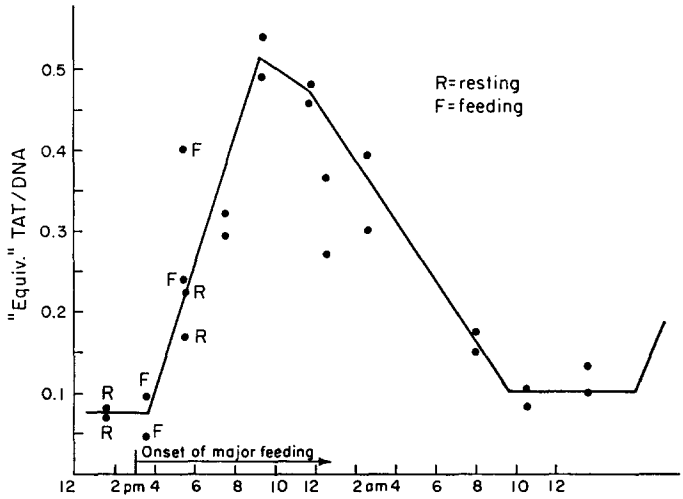

FIGURE 1. Diurnal variation of TAT during ad lib. feeding. TAT levels were determined in mice randomly sampled during a 24 hr period. Food and water were available ad lib. The lights were scheduled to go on at 7 a.m. and off at 7 p.m. Note the differences in TAT levels at 5 p.m., depending on whether mice were resting (*R*) or actively feeding (*F*). Each point represents TAT activity in the liver from one mouse. TAT activity is expressed per equivalent unit of DNA (see Materials and Methods).

3. *Diurnal Variations of TAT* A prerequisite for any study of TAT regulation is a knowledge of the schedule of the cyclic change of TAT activity during a 24 hr period (Potter et al., 1966; Wurtman and Axelrod, 1967). The cyclic changes of TAT in our colony during ad lib. feeding are shown in Fig. 1. In order to minimize individual differences in the daily schedule of ad lib. feeding (see Fig. 1), a brief 20 hr fast (from noon of the previous day) preceded all experiments unless otherwise indicated. A 20 hr fast will eliminate stores of liver glycogen which would vary according to the recentness of feeding (Ekman and Holmgren, 1949); large liver glycogen stores would be expected to alter the response to exposure to cold. The recentness of feeding is shown to be an important factor in the regulation of TAT (section A4 below). The period of least change in TAT, when its activity is at the lowest, extends at least from 9 a.m. to 1 p.m. in fasted mice. During this period, the increase of TAT was measured to be less than +0.005 TAT/DNA per hr (10%/hr)

of the basal levels (Finch, unpublished observations). Mice are observed to be asleep or quiescent at this time. All our experiments were performed during these hours unless otherwise indicated. Thus, increases in TAT activity can be properly attributed to the circumstances of the experiment and do not represent the ascending limb of the 24 hr cycle of TAT changes within the duration of the experiment.

4. *Adrenalectomy and Corticosterone Assay* Retroperitoneal, bilateral adrenalectomy was performed on 10 wk-old mice anesthetized with Nembutal. Electrolyte balance after adrenalectomy was maintained by supplying 0.14 M NaCl solutions for drinking. The completeness of the adrenalectomy was tested by exposing mice to cold (2°C) 5–10 days after the operation and examining the quantity of corticosterone in circulation. Corticosterone was assayed by the method of Peterson (1957) in the serum of blood collected from the jugular veins immediately after the mice had been killed by cervical dislocation. Approximately 80% of the adrenalectomies were judged complete by the absence of serum corticosterone 45 min after the onset of exposure to cold.

RESULTS

A. *The Regulation of TAT Levels during Exposure to Cold*

1. INCREASE DURING EXPOSURE TO COLD

Mice were placed singly in individual, 2 quart, prechilled glass jars without food, water, or nesting material. An increased activity of TAT was found to be rapidly stimulated by exposure to cold between 1 and 10°C. As exposure to temperatures below 5°C causes the collapse and death of many fasted mice within a few hours (see Fig. 5), most experiments were conducted at 9–10°C. An example of such an experiment is shown in Fig. 2. As a rule, an increase of TAT was detectable within 30 min and proceeded as a linear function of time from the beginning of the exposure to cold. The rate of increase of TAT activity (0.04 TAT/DNA per hr, in this experiment) was always in marked excess of the rate of TAT increase in control animals (0.005 TAT/DNA per hr) during the same period of time. Thus, the increase of TAT activity following exposure to cold can be properly attributed to this stress.

2. INHIBITION BY ACTINOMYCIN D

Actinomycin D,[1] injected intraperitoneally (2 mg/100 g body weight in solution of 0.14 M NaCl) 15 min before exposure to 9°C, was found to almost completely inhibit the increase of TAT in fasted mice (Fig. 2). (Preliminary studies verified that this dose of actinomycin D is more than sufficient to reduce the incorporation of ^{3}H-orotic acid into high molecular weight, phenol-purified liver RNA by more than 95% within 15 min, in agreement with other published studies [Trakatellis et al., 1964].) The increase of TAT

[1] Actinomycin D was generously provided by Mr. W. B. Gall of Merck, Sharp & Dohme, Rahway, N. J.

during exposure to cold in fasted mice thus requires DNA-dependent RNA synthesis. Therefore, we consider increases of TAT activity to be dependent on gene activity and to represent an enzyme induction, as discussed in the Introduction to this paper.

3. TRANSIENT NATURE OF THE CHANGES FOLLOWING EXPOSURE TO COLD

In this experiment, fasted mice were exposed at 2°C for 45 or 90 min and

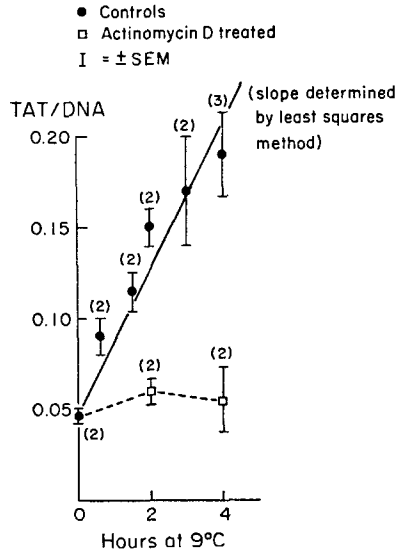

FIGURE 2. Inhibition of cold induction of TAT by actinomycin D. Mice, fasted for 20 hr, were exposed to 9°C temperature as described in the text. Four mice were given intraperitoneal injections of actinomycin D (2 mg/100 g body weight) 15 min before the beginning of exposure to cold. The mice injected with actinomycin D did not appear less able to bear the cold stress than the untreated controls. The number of mice is given in parentheses. TAT activity is expressed per unit DNA (see Materials and Methods).

then removed from the cold jars and returned to a cage at room temperature (24°C). As seen in Fig. 3, the increase of TAT continues beyond the termination of the exposure to cold. The final extent of increase was greatest in the mice exposed to cold for the longest time. In each case, the increase of TAT activity was transient and TAT activity returned toward basal levels in a few hours. The activity of TAT is thus sensitively adjusted by endogenous factors, which may be considered to reflect specific shifts in body metabolism during the reaction to and the recovery from exposure to cold.

A similar pattern of transient change was also observed after exposure to cold at 9–10°C (data not shown).

4. INFLUENCE OF THE POSTPRANDIAL INTERVAL ON THE INDUCTION OF TAT

In a preliminary experiment on the induction of TAT during exposure to cold, it was found that mice fasted overnight tended to have a greater net increase of TAT than unfasted mice. The influence of feeding on the induction of TAT during exposure to cold was investigated by testing the response of mice at various times after feeding. It is necessary to reduce individual variations in the schedule of feeding (see Fig. 1) for the successful performance of this experiment. Hence, mice were trained to eat on a schedule by making

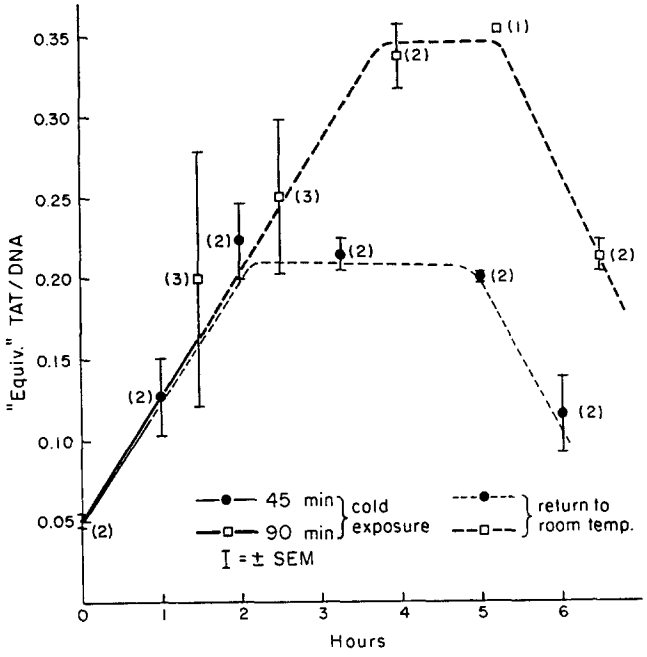

FIGURE 3. Levels of TAT as a function of the length of exposure to cold (1–2°C). Mice fasted for 20 hr, were exposed to cold. At 45 min and 90 min mice were removed from the cold jars and returned to a cage at room temperature. The number of mice is given in parentheses. TAT activity is expressed as described in the legend of Fig. 1.

food available only between 1 and 5 p.m.; this schedule was rigidly adhered to for 7 days before the experiment. Water was available at all times. The room was lighted from 7 a.m. to 7 p.m. The mice were singly caged to minimize social interaction (which might influence feeding behavior) and quickly appeared to become synchronized in their patterns of feeding and spontaneous locomotor activity. In these experiments, the postprandial interval is designated as the length of time from the *beginning* of the most recent period of feeding to the beginning of the experiment. The 24 hr cycle of TAT activ-

ity during scheduled feeding is represented semidiagrammatically in the small graph on the right in Fig. 4.

The results of such an experiment are shown in Fig. 4. There is no induction of TAT after 120 min of exposure to cold in recently fed mice (12 hr postprandially). However, the usual induction of TAT results from exposure to cold in fasted mice (20 hr postprandially).

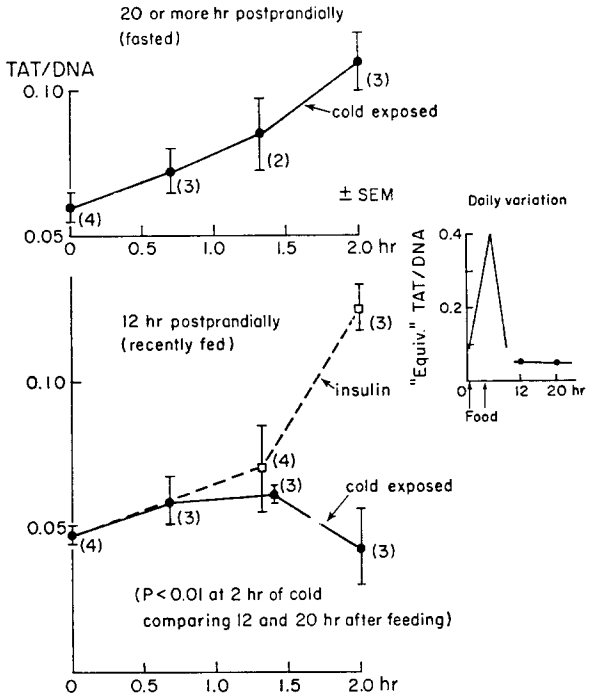

FIGURE 4. The influence of the postprandial time on the induction of TAT by exposure to cold. Mice were trained to eat their daily rations between 1 p.m. and 5 p.m. Mice were exposed to cold at 12 hr or 20 hr post-prandially (recently fed vs. fasted). At 12 hr postprandially some mice (interrupted line in lower graph) were injected with insulin (0.75 IU Iletin/100 g body weight) but were not exposed to cold. The daily variation of TAT is indicated semidiagrammatically in the small graph on the right; the two points on the line of TAT activity indicate when groups of mice were removed from the colony and exposed to cold. TAT activity is expressed as described in the legend of Fig. 2.

5. INDUCIBILITY OF TAT BY INSULIN IN THE RECENTLY FED MOUSE

The results of the previous section lead one to question the ability of liver cells in recently fed mice to respond to the endogenous stimulae which operate during exposure to cold in fasted mice. The ability of liver cells in a recently fed mouse to respond to at least one hormonal stimulus was verified by the injection of insulin, a hormone known to induce TAT in the isolated, perfused liver (Hager and Kenney, 1968). As shown in Fig. 4, the intraperi-

toneal injection of 0.75 IU insulin (Iletin)/100 g body weight 12 hr postprandially results in a large induction of TAT. It is concluded that the biosynthetic machinery of the liver which is concerned with TAT induction can function in the insulin-induced synthesis of TAT 12 hr postprandially. The endogenous inducers of TAT which are elicited by exposure to cold in fasted mice are either not elicited in recently fed mice which are replete with metabolic reserves, or their action is inhibited. The results of this experiment demonstrate another aspect of the sensitivity of the regulation of TAT levels to the physiological state of the mouse.

The fact that the liver cells are capable of renewed synthesis of TAT in response to insulin immediately after the major TAT increase which follows feeding corroborates the similar results of repeated injections of hydrocortisone which cause a "superinduction" of TAT (Grossman and Mavrides, 1967). The ability of the liver to make TAT is evidently not exhausted by rapidly succeeding stimulae.

6. EFFECT OF ACTINOMYCIN D ON SURVIVAL IN THE COLD

The induction of TAT might be presumed to contribute significantly to the increasing tempo of metabolic processes upon which a mouse is dependent to maintain its body temperature during severe and stressful exposure to cold. This possibility was examined by a study of the effect of actinomycin D, an inhibitor of TAT induction, on survival during cold stress (measured as the length of time before collapse occurred). Determination of the precise moment of death in the cold is difficult because of variations in the period between collapse and death. As seen in Fig. 5, actinomycin D (2 mg/100 g body weight, as described above) does not affect survival at 2°C. This surprising result implies that the induction of TAT and presumably other gene-dependent processes in the liver and other organs do not determine the ability to survive at 2°C. Actinomycin D at this dosage is lethal to 50% of fasted mice after a much longer period (about 18 hr) (Finch, unpublished data).

B. *The Effect of Stressful Shaking on TAT Levels and Body Temperature*

1. INCREASES OF TAT FOLLOWING SHAKING

A cage containing six mice previously fasted for 20 hr was fixed on a linear shaking apparatus (4 inch stroke length); the motion of the shaking was perpendicular to the long side of the metal cage (12 x 5 x 6 inches high). The top was removed and the mice were subjected to 180 excursions/min for 30 min. Within a few minutes, the mice arranged themselves within the cage so as to avoid collision with each other or with the walls of the cage. A ½ inch layer of Lab Litter (Carworth, Inc., New City, Rockland County, New York) covered the floor of the cage. At the end of the shaking, the cage remained

in the same room (not the room containing the mouse colony). As demonstrated in Table I, treatment by shaking resulted in a rapid increase in TAT activity to levels comparable to those resulting from exposure to cold within the same period of time (compare with Fig. 2).

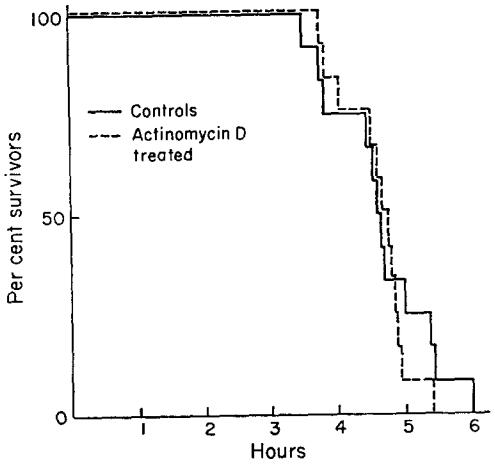

FIGURE 5. The effect of actinomycin D on survival at 2°C. Mice fasted for 20 hr, were given intraperitoneal injections of actinomycin D (2 mg/100 g body weight) 15 min before the beginning of exposure to cold (12 treated mice; 12 controls). No effects of actinomycin D on the ability to withstand cold were observed.

TABLE I

INCREASE OF TAT STIMULATED BY SHAKING AND THE EFFECT OF ACTINOMYCIN D

Group	TAT/DNA*	Range	No. of mice
Controls	$6.0\pm0.4\times10^{-2}$	5.0–6.5	4
Shaken	$21.0\pm1.5\times10^{-2}$	18.5–22.5	4
Shaken after actinomycin	$8.5\pm0.2\times10^{-2}$	7.0–10.0	4

The mice were shaken for 30 min and killed 90 min later.
* Mean ± SEM.

2. INHIBITION BY ACTINOMYCIN D

Actinomycin D (2 mg/100 g body weight, as described above in section A2) almost completely inhibits the increase of TAT as shown in Table I. The increase of TAT stimulated by the shaking of fasted mice requires DNA-dependent RNA synthesis and we consider it to represent an enzyme induction.

3. TRANSIENT NATURE OF CHANGES FOLLOWING SHAKING

Examination of longer time intervals after the cessation of shaking revealed that the increase of TAT is transient: the basal level is nearly regained by 180 min after the start of the experiment as is shown in Fig. 6. Another study of the effect of shaking on liver enzymes of adult rats did not reveal a change

in the level of TAT, although a marked increase in TAT was observed in very young rats (Schapiro et al., 1966). This variance with our results in mice seems clearly due to the fact that TAT levels in the study of Schapiro et al. (1966) were measured at a single time point 240 min after the end of the shaking. TAT levels in the experiment shown in Fig. 6, as well as in several other experiments, have nearly returned to basal levels by 150 min after the end of the shaking.

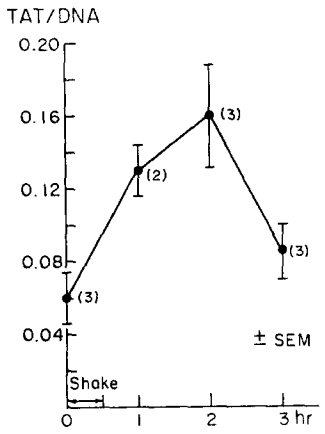

FIGURE 6. The induction of TAT by shaking. Mice, fasted for 20 hr, were shaken in their cages (six mice per cage; cage tops removed) on a reciprocating shaker (180 excursions per min, 4 inch stroke length) for 30 min. The mice remained in their cages at room temperature (24°C) until sacrificed 90 min later. The number of mice is given in parentheses.

TABLE II

BODY TEMPERATURE AND SHAKING

Mouse	Before shaking	After 30 min of shaking	30 min after end of shaking
	(1)	(2)	(3)
1	36.0°C	34.0°C	36.0°C
2	36.5	34.0	37.0
3	37.0	35.0	37.0
4	37.5	35.0	37.0
Mean ±SEM	36.75±0.3	34.5±0.3	36.75±0.3
P of (1) vs. (2)	<0.001		

4. EFFECT OF SHAKING ON BODY TEMPERATURE

The maintenance of balance during shaking obviously required a major muscular exertion and severely challenged the metabolic resources of the mouse. Measurements of internal temperature were made by inserting a lubricated thermocouple 4 cm through the anus into the colon as described by Barnett (1956). As shown in Table II, fasted mice had a significant and transient decline of body temperature following shaking (180 excursions/min). Thus, exposure to both cold and shaking has similar effects on chal-

lenging the level of body temperature and causing transient induced increases of TAT. It is therefore possible that the endogenous physiological stimulae which ultimately act on the liver to control the synthesis of TAT are the same in both experimental circumstances.

C. *Adrenal Secretions and the Induction of TAT*

The necessity of adrenal secretions for the induction of TAT during exposure to cold was demonstrated in fasted adrenalectomized and sham-operated

TABLE III

EFFECT OF ADRENALECTOMY ON THE INDUCTION OF TAT BY EXPOSURE TO COLD

Group	*16 days postadrenalectomy (experiment I)*		*5 days postadrenalectomy (experiment II)*	
	*TAT/DNA**	*Range*	*TAT/DNA**	*Range*
Sham, 120 min/2°C	$11.4\pm1.0\times10^{-2}$	9.0–17.0 (7)	$8.5\pm0.5\times10^{-2}$	8.0–10.0 (5)
Sham, basal levels	$3.0\pm0.3\times10^{-2}$	1.8–3.7 (7)	$4.6\pm0.6\times10^{-2}$	4.0–6.1 (5)
	Increase of 8.4×10^{-2}		Increase of 3.9×10^{-2}	
Adrenalectomized, 120 min/2°C	$5.3\pm0.5\times10^{-2}$	4.2–6.7 (5)	$3.5\pm0.3\times10^{-2}$	3.2–4.1 (4)
Adrenalectomized, basal levels	$2.9\pm0.2\times10^{-2}$	2.6–4.0 (5)	$2.5\pm0.3\times10^{-2}$	2.1–3.3 (5)
	Increase of 2.4×10^{-2}		Increase of 1.0×10^{-2}	

The values of TAT in resting mice and mice exposed to cold, and sham-operated mice were not different from similarly handled, nonoperated mice; both intact and sham-operated mice are grouped together in experiment I. In experiment II, the TAT levels of two adrenalectomized mice exposed to cold (not given) were equal to the average TAT levels in sham-operated mice exposed to cold; this clear deviation from the other adrenalectomized mice indicates incomplete adrenalectomy and the values of these mice were *not* included in the calculations.

The increases of TAT in adrenalectomized mice exposed to cold were statistically significant ($P < 0.05$). In both experiments, the average increment of TAT in adrenalectomized mice during exposure to cold was about 25% of that in sham-operated or normal mice.

* Mean ± SEM. The number of mice is indicated in parentheses.

mice. After 2 hr of exposure to cold (2°C), all mice showed a statistically significant ($P < 0.05$) increase in TAT levels in comparison with their controls, as is shown in Table III. However, the net increase of TAT in the adrenalectomized mice was about 25% of that in sham-operated mice. Thus, adrenal secretions are necessary for the full, normal induction of TAT during exposure to cold, although a much reduced increase of TAT can occur in their absence.

DISCUSSION

These studies have been concerned with the regulation of the mouse liver enzyme, tyrosine aminotransferase (TAT), in various environmental circum-

stances and in different physiological states. We have observed that rapid, transient increases of TAT are initiated by exposure to cold or shaking.

The induction of TAT in fasted mice by exposure to cold or by shaking was found to be inhibited by actinomycin D; we therefore consider that the increase of TAT resulting from exposure to cold or shaking is dependent on gene activity and may properly be designated as an enzyme induction.

The genomically dependent increases of TAT activity initiated by exposure to cold or shaking in fasted mice are transient; within a few hours, TAT activity has nearly returned to the base line. Other evidence indicates that the usual decline of TAT from peak levels after induction by casein hydrolysate (Pitot et al., 1965) or hydrocortisone (Garren et al., 1964) is inhibited by actinomycin D. This implies that gene activity may be necessary for induction of both TAT and its eventual return to the base line. It is not known how changes of TAT during its 24 hr cycle depend on gene activity and on the rate of TAT synthesis.

Although measurements were made only on TAT, the circumstances which result in changes of its levels probably also affect the activity of other gene loci in addition to the genes concerned with TAT synthesis. The facts that (*a*) hormones (e.g. hydrocortisone) can alter the pattern of gene activity in the liver (Drews and Brawerman, 1967) and (*b*) that the induction of TAT by various hormones is dependent on gene activity, do not necessarily imply that the controlling step in TAT induction is the transcription of those genes which code for the polypeptide chains of TAT, for it is evident that the activities of numerous genes are involved in the process of enzyme induction. In general, the existence of rapid and reversible changes in the pattern of gene activity of higher cells is not well-appreciated. Extremely rapid, hormonally induced changes in gene activity in mammalian tissues have been directly demonstrated in the experiments of Kidson and Kirby (1964) and Means and Hamilton (1966).

The endogenous factors which regulate TAT levels are incompletely characterized at present. A brief and selective survey of factors which influence the regulation of TAT in rats and mice is given in Table IV. Although published data are sometimes at variance with the conclusions in Table IV (see note at foot of Table IV), it may be concluded that the 24 hr cycle of TAT activity is not dependent on secretions from the adrenals, pancreas, or pituitary gland, but is, in fact, principally regulated by the amount of dietary protein. This is in marked contrast to the induction of TAT by exposure to cold or by intraperitoneal injection of large amounts of tyrosine, in which adrenal secretions appear to be *necessary* for the full, normal response. In the present study we observed that adrenalectomy reduces the induction of TAT during exposure to cold to 25% of that in intact mice. The increase of TAT during these latter procedures may be mediated by corticosteroids and/or

epinephrine. Therefore, we conclude that *several* endogenous factors (recently ingested nutrients, adrenal secretions) are important in the regulation of TAT.

The impact of exposure to cold, monitored through changes in TAT levels, varies markedly according to the state of the body and does not necessarily constitute a stress by the criterion of a change in TAT levels. For instance, no induction of TAT occurs during exposure of recently fed mice to cold 12 hr postprandially, whereas a vigorous induction occurs during exposure to cold in fasted mice 20 hr postprandially. It is therefore apparent that an experience may have an entirely different physiological consequence according to the state of the body.

TABLE IV
FACTORS INFLUENCING TAT REGULATION

A Amplitude of 24 hr cycle of TAT levels in rats	B Increase which follows i.p. injection of tyrosine in rats	C Induction by exposure to cold in mice (data from this paper)
Adrenalectomy: slight effect (Civen et al., 1967; Shambaugh, 1967; Wurtman and Axelrod, 1967; Black and Axelrod, 1968*)	Nearly but not completely abolishes (Grossman and Mavrides, 1967; Kenney and Flora, 1961; Lin and Knox, 1957; Kenney and Albritton, 1965*; Rosen and Milholland, 1963*)	Nearly but not completely abolishes
Hypophysectomy: slight effect (Wurtman and Axelrod, 1967; Shambaugh et al., 1967*)	Abolishes (Kenney and Albritton, 1965)	?
Pancreatectomy: no effect (Fuller et al., 1969)	?	?
Diet: amplitude "proportional" to protein content (Watanabe et al., 1968)	?	No induction after recent feeding

A complete review of the literature on TAT regulation is not intended. References indicated by an asterisk are at variance with the conclusion given in the above table. Variations in the results of the same experimental procedure, performed under apparently similar circumstances on different dates by the same workers, have been observed, e.g., in the induction of TAT by hydrocortisone (Kenney and Flora, 1961) and repression of TAT by growth hormone (Kenney, 1967); in any single experiment, the animals appeared to respond uniformly. Variations in the regulation of TAT in individual animals in the same experiment have also been observed in the experiments of Grossman and Mavrides (1967). The sensitivity of TAT regulation to many physiological variables, expecially food intake, may be the basis for this variation. Sufficiently stringent control of the physiological state of animals for use in studies of TAT regulation is difficult to achieve.

The functional significance of changes in the levels of TAT, an enzyme which is concerned with the catabolism of tyrosine (Knox and LeMay-Knox, 1951; Schepartz, 1951), is not clear at present. The induction of hepatic TAT by hydrocortisone is considered by some to play an important role in

mediating increased gluconeogenesis, also stimulated by hydrocortisone in vivo (Feigelson and Feigelson, 1966). However, increased enzyme levels do not necessarily imply an increased traffic of substrates through the metabolic pathway with which an enzyme is concerned, e.g. Kim and Miller (1969) have observed that the large increases in TAT activity which follow hydrocortisone induction do not result in a corresponding increase in the oxidation of l-tyrosine-^{14}C to $^{14}CO_2$ in the perfused, isolated rat liver. The observations described above of rapid and transient changes in TAT activity following exposure to cold or shaking demonstrate that TAT activity is increased in concurrence with demands made on the homeostatic machinery of the mouse and returns toward basal levels during the recovery from these treatments. A physiological relevance of the *factors* regulating TAT is thereby implied. It is possible that the induction of large quantities of TAT, which may not be necessary for increased oxidation of tyrosine (Kim and Miller, 1969), was evolutionarily selected for by rare circumstances requiring an extra "factor of safety" (Meltzer, 1906) in the capacity for tyrosine catabolism.

Received for publication 9 June 1969.

BIBLIOGRAPHY

BARNETT, S. A. 1956. Endothermy and ectothermy in mice at −3°C. *J. Exp. Biol.* **33**:124.

BLACK, I. B., and J. AXELROD. 1968. Regulation of the daily rhythm in tyrosine transaminase activity by environmental factors. *Proc. Nat. Acad. Sci. U.S.A.* **61**:1287.

BURTON, K. 1956. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62**:315.

CIVEN, M., R. ULRICH, B. M. TRIMMER, and C. B. BROWN. 1967. Circadian rhythms of liver enzymes and their relationship to enzyme induction. *Science (Washington).* **157**:1563.

DREWS, J., and G. BRAWERMAN. 1967. Alterations in the nature of ribonucleic acid synthesized in rat liver during regeneration and after cortisol administration. *J. Biol. Chem.* **242**:801.

EKMAN, C-A., and H. HOLMGREN. 1949. The effect of alimentary factors on liver glycogen rhythm and the distribution of glycogen in the liver lobule. *Anat. Rec.* **104**:189.

FEIGELSON, M., and P. FEIGELSON. 1966. Relationships between hepatic enzyme induction, glutamate formation, and purine nucleotide biosynthesis in glucocorticoid action. *J. Biol. Chem.* **241**:5819.

FINCH, C. E., J. R. FOSTER, and A. E. MIRSKY. 1969. Ageing and the regulation of cell activities during exposure to cold. *J. Gen. Physiol.* **54**:690.

FULLER, R. W., G. T. JONES, H. D. SNODDY, and I. H. SLATER. 1969. Daily rhythm of liver tyrosine transaminase and of plasma tyrosine and glucose after removal of the pancreas of rats. *Life Sci.* **8**:685.

GARREN, L. D., R. R. HOWELL, G. M. TOMKINS, and R. M. CROCCO. 1964. A paradoxical effect of actinomycin D: The mechanism of regulation of enzyme synthesis by hydrocortisone. *Proc. Nat. Acad. Sci. U.S.A.* **52**:1121.

GOLDSTEIN, L., E. J. STELLA, and W. E. KNOX. 1962. The effect of hydrocortisone on tyrosine-α-ketoglutarate transaminase and tryptophan pyrrolase activities in the isolated, perfused rat liver. *J. Biol. Chem.* **237**:1723.

GREENGARD, O., and G. ACS. 1962. The effect of actinomycin on the substrate and hormonal induction of liver enzymes. *Biochim. Biophys. Acta.* **61**:652.

GREENGARD, O., and P. FEIGELSON. 1961. The activation and induction of rat liver tryptophan pyrrolase in vivo by its substrate. *J. Biol. Chem.* **236**:158.

GROSSMAN, A., and C. MAVRIDES. 1967. Studies on the regulation of tyrosine aminotransferase in rats. *J. Biol. Chem.* **242**:1398.

HAGER, C. B., and F. T. KENNEY. 1968. Regulation of tyrosine-α-ketoglutarate transaminase in rat liver. VII. Hormonal effects on synthesis in the isolated, perfused liver. *J. Biol. Chem.* **243**:3296.

HOLTEN, D., and F. T. KENNEY. 1967. Regulation of tyrosine-α-ketoglutarate transaminase in rat liver. VI. Induction by pancreatic hormones. *J. Biol. Chem.* **242**:4372.

KENNEY, F. T. 1962. Induction of tyrosine-α-ketoglutarate transaminase in rat liver. IV. Evidence for an increase in the rate of enzyme synthesis. *J. Biol. Chem.* **237**:3495.

KENNEY, F. T. 1967. Regulation of tyrosine-α-ketoglutarate transaminase in rat liver. V. Repression in growth hormone-treated rats. *J. Biol. Chem.* **242**:4367.

KENNEY, F. T., and W. L. ALBRITTON. 1965. Repression of enzyme synthesis at the translational level and its hormonal control. *Proc. Nat. Acad. Sci. U.S.A.* **54**:1693.

KENNEY, F. T., and R. M. FLORA. 1961. Induction of tyrosine-α-ketoglutarate transaminase in rat liver. I. Hormonal nature. *J. Biol. Chem.* **236**:2699.

KIDSON, C., and K. S. KIRBY. 1964. Selective alterations of mammalian messenger-RNA synthesis: evidence for differential action of hormones on gene transcription. *Nature (London).* **203**:599.

KIM, J. H., and L. L. MILLER. 1969. The functional significance of changes in activity of the enzymes, tryptophan pyrrolase and tyrosine transaminase, after induction in intact rats and in the isolated, perfused rat liver. *J. Biol. Chem.* **244**:1410.

KNOX, W. E., and M. LEMAY-KNOX. 1951. The oxidation in liver of l-tyrosine to acetoacetate through *p*-hydroxyphenylpyruvate and homogentisic acid. *Biochem. J.* **49**:686.

LIN, E. C. C., and W. E. KNOX. 1957. Adaptation of the rat liver tyrosine-α-ketoglutarate transaminase. *Biochim. Biophys. Acta.* **26**:85.

MEANS, A. R., and T. H. HAMILTON. 1966. Early estrogen action: concomitant stimulations within two minutes of nuclear RNA synthesis and uptake of RNA precursors by the uterus. *Proc. Nat. Acad. Sci. U.S.A.* **56**:1594.

MELTZER, S. J. 1906. The factors of safety in animal structure and animal economy. *Harvey Lect.* (1906-07):139.

PETERSON, R. E. 1957. The identification of corticosterone in human plasma and its assay by isotope dilution. *J. Biol. Chem.* **225**:25.

PITOT, H. C., C. PERAINO, C. LAMAR, JR., and A. L. KENNAN. 1965. Template stability of some enzymes in rat liver and hepatoma. *Proc. Nat. Acad. Sci. U.S.A.* **54**:845.

POTTER, V. R., R. A. GEBERT, H. C. PITOT, C. PERAINO, C. LAMAR, JR., S. LESHER, and H. P. MORRIS. 1966. Systematic oscillations in metabolic activity in rat liver and in hepatomas. I. Morris hepatoma No. 7793. *Cancer Res.* **26**:1547.

ROSEN, F., and R. J. MILHOLLAND. 1963. Glucocorticoids and transaminase activity. VII. Studies on the nature and specificity of substrate induction of tyrosine-α-ketoglutarate transaminase and tryptophan pyrrolase. *J. Biol. Chem.* **238**:3730.

SCHAPIRO, S., A. YUWILER, and E. GELLER. 1966. Maturation of a stress-activated mechanism inhibiting the induction of tyrosine transaminase. *Science (Washington).* **152**:1642.

SCHEPARTZ, B. 1951. Transamination as a step in tyrosine metabolism. *J. Biol. Chem.* **193**:293.

SHAMBAUGH, G. E., D. A. WARNER, and W. R. BEISEL. 1967. Hormonal factors altering the rhythmicity of tyrosine-α-ketoglutarate transaminase in rat liver. *Endocrinology.* **81**:811.

SHIMAZU, T., and A. AMAKAWA. 1968. Regulation of glycogen metabolism in liver by the autonomic nervous system. II. Neural control of glycogenolytic enzymes. *Biochim. Biophys. Acta.* **165**:335.

TRAKATELLIS, A. C., A. E. AXELROD, and M. MONTJAR. 1964. Actinomycin D and messenger-RNA turnover. *Nature (London).* **203**:1134.

TSUKADA, K., H. OURA, S. NAKASHIMA, and N. HAYASAKI. 1968. Adaptive response of tryptophan pyrrolase and tyrosine transaminase after partial hepatectomy and laparotomy. *Biochim. Biophys. Acta.* **165**:218.

WATANABE, M., V. R. POTTER, and H. C. PITOT. 1968. Systematic oscillations in tyrosine transaminase and other metabolic functions in liver of normal and adrenalectomized rats on controlled feeding schedules. *J. Nutr.* **95**:207.

WURTMAN, R. J., and J. AXELROD. 1967. Daily rhythmic changes in tyrosine transaminase activity of the rat liver. *Proc. Nat. Acad. Sci. U.S.A.* **57**:1594.