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EFFECTS OF EPINEPHRINE ADMINISTRATION ON THE PRODUCTION AND PERIPHERAL METABOLISM OF THYROID HORMONES IN RATS

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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TO

MY PARENTS

ABSTRACT

The effects of a single intramuscular injection of epinephrine on the plasma levels of thyroid hormones and their degradation and secretion were studied in male Sprague-Dawley rats held at room temperature.

When compared to vehicle-injected controls, epinephrine significantly lowered plasma protein-bound ^{125}I, L-thyroxine (T_4) and 3,5,3'triiodo-L-thyronine (T_3) levels. The effects of epinephrine persisted for approximately 7 hours.

In a series of experiments in which the kinetics and metabolism of ^{125}I-T_4, ^{125}I-T_3 and $^{125}I^-$ were followed in perchlorate-blocked and non-blocked rats, it was shown that epinephrine increased the fractional disappearance and clearance rates of plasma ^{125}I-T_4 and ^{125}I-T_3, at least in part, by increasing their deiodination. Epinephrine increased T_4 degradation rate but decreased T_3 degradation rate despite the increase in plasma T_3 clearance.

A significant depression of T_4 secretion rate and elevation of thyroidal T_3/T_4 ratios indicated that epinephrine inhibited thyroidal hormone release.

In conclusion, epinephrine lowered plasma concentrations of thyroid hormones by increasing their clearance and inhibiting their secretion.

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LIST OF ABBREVIATIONS AND SYMBOLS

cAMP : 3',5'-cyclic adenosine monophosphate
Ci : curie(s)
cpm : counts per minute
CR : clearance rate (ml/hr/100 g)
DR : degradation rate (ng/hr/100 g)
DS : distribution space (ml/100 g)
GH : growth hormone
HP : human plasma
hr : hour(s)
I : iodine (stable)
I^- : iodide (stable)
*I : iodine (^{125}I-labelled)
*I^- : iodide (^{125}I-labelled)
$\underline{i}$: extrapolated level of radioactivity at the time of tracer injection
$\underline{k}$: fractional disappearance rate (fraction/hr)
min : minute(s)
mRNA : messanger ribonucleic acid
nRNA : nuclear ribonucleic acid
PB : protein-bound
PTU : propylthiouracil
$\underline{r}$: regression coefficient
RIA : radioimmunoassay
rRNA : ribosomal ribonucleic acid
rT_3 : 3,3'5'-triiodo-L-thyronine
sp. act. : specific activity (μCi/μg)
SR : secretion rate (ng/hr/100 g)
T_2 : 3,3'-diiodo-L-thyronine
T_3 : 3,5,3'-triiodo-L-thyronine (stable)
*T_3 : 3,5,3'-triiodo-L-thyronine (^{125}I-labelled)
T_4 : L-thyroxine (stable)
*T_4 : L-thyroxine (^{125}I-labelled)
TBG : thyroxine-binding globulin

LIST OF ABBREVIATIONS AND SYMBOLS (continued)

TCA : trichloroacetic acid
TRH : thyrotropin-releasing hormone
TSH : thyrotropin
[ ] : hormone concentration

INTRODUCTION

Reports on the effects of epinephrine on the mammalian thyroid system are numerous and conflicting. Thyroid activities have been shown to increase (Melander, 1970), decrease (Reiss, 1953) or remain unaltered (Whitman et al., 1976) after epinephrine administration. Differences in species, the physiological state of animals, dose and the route of injection, the time of observation and the parameter measured may be reasons for the discrepencies. At present, all the above possibilities remain open and the results are at variance even within a single species (eg. the albino rat).

Most previous investigations (Eskelson et al., 1954; and others) have shown that plasma thyroid hormone levels are depressed by epinephrine. But recently, Udupa et al. (1976) and Whitman et al. (1976) reported that circulating thyroid hormone concentrations were either unaltered or increased by epinephrine. As plasma thyroid hormone levels are net results of hormone addition to plasma (thyroidal secretion) and removal from the plasma (hormone degradation), plasma hormone concentrations are not very informative as general indices of thyroid activities.

Increases in thyroid hormone degradation and secretion after epinephrine administration in rats have been indicated by an increased urinary excretion of $^{131}I^-$ after ^{131}I-labelled hormone injection (Williams et al., 1949; and others) and

a decreased thyroidal protein-bound (PB) ^{131}I content after $^{131}I^-$ pretreatment (Botkin and Jensen, 1952). However, the rates of hormone secretion, degradation, plasma fractional disappearance and clearance in rats under the influence of epinephrine have not been reported. Changes in thyroid hormone secretion and degradation due to epinephrine should be studied.

In an attempt to determine the direction of changes in thyroid activities in rats under the influence of epinephrine, four series of experiments were performed.

A. The time course of the response to epinephrine was studied by monitoring plasma PB^{125}I (PB*I) levels after $^{125}I^-$ ($*I^-$) pretreatment.

B. Changes in plasma L-thyroxine (T_4) and 3,5,3'-triiodo-L-thyronine (T_3) concentrations after epinephrine injections were measured.

C. T_4 and T_3 degradation, plasma fractional disappearance and clearance rates and deiodination under the influence of epinephrine were estimated by following the disappearance of injected radio-labelled T_4 and T_3 and $*I^-$. Knowledge on the effects of epinephrine on $*I^-$ metabolism itself was required before the significance of the changes in plasma $*I^-$ derived from the injected radio-labelled T_4 or T_3 could be interpreted.

D. Alterations in thyroidal hormone secretion were investigated by measuring T_4 secretion rates and thyroidal T_3/T_4 ratios. The latter provided information on T_3

secretion.

Both perchlorate-treated (blocked) and non-blocked rats were used. Perchlorate treatment was used to block radioiodide recycling when the formation of endogenously-labelled thyroid hormones might interfere with the measurement of radio-labelled thyroid hormones.

LITERATURE REVIEW

A. Rat thyroid system.

The rat thyroid system resembles that of other mammals. Thyrotropin-releasing hormone (TRH) stimulates secretion of thyrotropin which, in turn, stimulates the thyroid. The thyroid hormones (T_4 and T_3) act on various target organs and exert negative feed-back control at various levels of the thyroid-pituitary-hypothalamic axis. Short-loop feed-backs by TSH and TRH on their organs of secretion may also occur.

The hypothalamic content of TRH, a tripeptide, ranges from 7.1 to 8.4 ng/rat (Bassiri and Utiger, 1974). TRH maintains basal TSH secretion and production, probably by controlling intracellular cAMP levels (Wilber, 1971; Pawlikowski et al., 1977; Szabo et al., 1978; Harris et al., 1978). The activity of TRH peptidases, located in tissues (eg. hypothalamus) and serum, has been positively correlated with thyroid status and thyroid hormone levels (Griffiths et al., 1975; Bauer, 1976; White et al., 1976). TRH secretion is controlled by neurotransmitters (Reichlin et al.,1976; Krulich et al., 1977). Dopamine inhibits and norepinephrine stimulates TRH release (Tuomisto et al., 1973, 1975; Mueller et al., 1976). Other biogenic amines such as serotonin, histamine and melatonin also affect TRH release (Chen and Meites, 1975; Onaya and Hashizume, 1976; Relkin, 1978).

TSH synthesis and release are stimulated by TRH and inhibited by T_3 and T_4 (Schally and Redding, 1967; Vale et al., 1968; Wilber and Utiger, 1969). Although T_3-inhibition may be incomplete in some instances, it is usually as pronounced, or more pronounced, than that for T_4 (Surks and Oppenheimer, 1976; Lewis et al., 1977a, b; Berthier and Lemarchand-Béraud, 1977). T_4 may inhibit TSH secretion without involving cAMP (Pawlikowski et al., 1977). Somatostatin also inhibits TSH release (Arimura and Schally, 1976; Gordin et al., 1976). Estimated TSH secretion rate and plasma TSH half-life in rats are 18.2mU/hr and 10.2-13.6 min respectively (Bakke et al., 1974; D'Angelo et al., 1976).

TSH stimulates thyroidal blood flow, I^- uptake, protein synthesis (including thyroglobulin), organification of I^-, iodothyronine synthesis, intermediary metabolism, and the secretion of thyroid hormones (T_4 and T_3)(Ekholm and Strandberg, 1968; Kapitola et al., 1969; Dumont, 1971). Protein synthesis may be stimulated at the translational and transcriptional levels (Wäger et al., 1973; DeNaya, 1978). By stimulating thyroglobulin exocytosis, iodination and coupling (catalysed by the membrane-bound peroxidase) are enhanced at apical surfaces of follicle cells (Greer and Haibach, 1974; Ekholm and Wollman, 1975; Björkman et al., 1978). The secretory response includes resorption of colloid, hydrolysis, and release of liberated iodothyronines (Malan et al., 1974).

TSH mediates most of these processes by binding to specific plasma membrane receptors of both high and low

affinities, stimulating cAMP formation and subsequently, the activity of protein kinases (Zakariji et al., 1973; Field, 1975; Goldfine et al., 1976). The activation of the iodide pump is not by cAMP (Granner and Halmi, 1972). Intrathyroidal amines mediate some of the TSH responses (Melander and Sundler, 1972b, Ericson et al., 1972; Melander et al., 1975).

Autoregulation occurs as excess iodide inhibits the iodide pump, and cAMP fromation and accumulation (Sherwin and Tong, 1974, 1975; Van Sande et al., 1975). Short-loop negative feed-back by thyroid hormones may be via inhibition of thyroid adenyl cyclase or TSH-receptor binding (Yu et al., 1976; Kielczynski and Nauman, 1977; Friedman et al., 1977). Recently, TSH-controlled secretion of 3,3',5'-triiodo-L-thyronine (rT_3) was demonstrated in man (Hooper et al., 1978).

T_4, T_3, rT_3 and 3,3'-diiodo-L-thyronine (T_2) have been detected in rat plasma (Roche et al., 1956), where they are mostly reversibly bound to plasma proteins. In rats, there is no TBG equivalent but T_4 and T_3 bind to albumin and a pre-albumin-like protein (Refetoff et al., 1970: Sutherland and Brandon, 1976). Total plasma or serum T_4 and T_3 concentrations in various strains of rats are listed in Table I.

Plasma free T_4 and T_3 are in constant exchange with various tissues of the body and are subjected to degradation, and subsequent urinary and fecal excretion, via deiodination, conjugation, oxidative decarboxylation and deamination (Tata

Table I.

Plasma or serum T_4 and T_3 concentrations in rats of different strains, sexes and body weights.

Reference	strain[+]	sex	weight(g)	T_4(µg/dl)	T_3(ng/dl)
Refetoff et al. (1970)	H	M	---	3.9	---
Sartin et al. (1977)	H	M	330	1.46	161
ibid.	H	M	517	0.96	105
Abrams & Larsen (1973)	S	M	150-200	4.2	44
Schwartz et al. (1971)	S	---	---	3.4	115
Azizi et al. (1974)	S	M	250-350	3.7	26
Silva & Larsen (1978)	S	M	---	---	38
Döhler et al. (1977)	S	M	---	4.9	48
Fukuda et al. (1975b)	S	F	200-250	5.7	73
Zaninovich et al. (1977b)	W	---	200	4.8	---
Kojima et al. (1975)	W	M	285	5.0	82
Gregerman & Crowder (1963)	W	M	453	4.7	---
ibid.	W	F	335	5.4	---
ibid.	OM	M	266	5.9	---
ibid.	OM	F	197	5.3	---
Tal et al. (1972)	SA	M	120	2.6	95
Kieffer et al. (1976)	CH	M	---	4.0	---
ibid.	CH	F	---	4.2	---
Azizi (1975)	---	M	400	3.0	---
ibid.	---	M	300	4.7	---

[+] H, Holtzman; S, Sprague-Dawley; W, Wistar; OM, Osborne-Mendel; SA, Sabra; CH, Charles River; ---, not specified.

et al., 1957; Ramsden et al., 1976; Fishman et al., 1977). Extravascular binding sites in tissues (eg. liver) withdraw T_4 from the plasma (Irvine, 1974; Järnerot et al., 1976; Langer et al., 1977). The organic products of degradation, including conjugated and unconjugated hormones, and the inorganic iodide from deiodination are excreted mainly in the urine and feces respectively (DiStefano and Fisher, 1976). Metabolic clearance rates of T_4, T_3 and rT_3 in rats are shown in Table II. Conjugation and deiodination are the major degradation routes.

Conjugation occurs predominantly in the liver and kidney. T_4 is glucuronide conjugated in the liver by microsomal glucuronyl transferase but T_3 is mainly sulphoconjugated in the kidney due to the different tissue location of the glucuronide and sulphoconjugating systems (Isselbacher, 1956; Roche et al., 1959a; Flock et al., 1960). These conjugates appear in blood and bile. Biliary conjugates may be hydrolysed by bacterial glucuronidase or sulfatase in the gut but the free hormones are not extensively resorbed at physiological concentrations (Galton and Nisula, 1972).

T_4 and T_3 share similar deiodination sites (Zaninovich, 1976; Zaninovich et al., 1977a). Monodeiodination of T_4 in rats was first confirmed by Larson et al. (1955). Monodeiodination of T_4 to T_3 or rT_3 may be different from a nonspecific deiodinating system described by Hillier (1972) which can produce T_3 as an intermediate product (Höffken et al., 1978). The enzymatic conversion of T_4 to

Table II.

T_4, T_3 and rT_3 clearance rates in rats of different strains and sexes.

Reference	strain[+]	sex	clearance rate (ml/hr/100 g)		
			T_4	T_3	rT_3
Cullen et al. (1973)	CH	M	0.98	----[a]	----
Gregerman & Crowder (1963)	OM	M	0.77	----	----
ibid.	OM	F	0.81	----	----
ibid.	W	M	0.88	----	----
ibid.	W	F	0.97	----	----
Zaninovich et al. (1976)	W	----	----	5.5	----
Zaninovich et al. (1977a)	W	----	1.40	18.5	----
Zaninovich et al. (1977b)	W	----	0.96	----	----
Balsam et al. (1978)	S	M	----	17.7	250.0
Schwartz et al. (1971)	S	----	0.71	14.2	----
Oppenheimer et al. (1970)	S	M	0.83	15.3	----
ibid.	S	M	----	20.9	----
Balsam & Sexton (1975)	S	M	0.81	11.3	----
Zimmerman et al. (1978)	S	M	0.77	28.0	----

[+] CH, Charles River; OM, Osborne-Mendel; W, Wistar; S, Sprague-Dawley.

[a] Not Specified.

T_3 or rT_3 occurs primarily in the liver and kidney, and is oxygen independent. It is stimulated by iron or thiol groups and inhibited by propylthiouracil (PTU) (Visser et al., 1975; Chopra, 1976, 1977, 1978; Cavalieri et al., 1977a; Chiraseveenuprapund et al., 1978).

Conversion of T_4 to T_3 has been found to be associated with purified plasma membranes, microsomes and mitochondria, but not with nuclei or cytosol. It has a pH optimum of 6.0-7.0, can be enhanced by T_4 and T_3, and inhibited by rT_3. The presence of a stimulatory cytosol cofactor has been postulated (Hüfner and Grussendorf, 1977a; Balsam and Ingbar, 1977; Chiraseveenuprapund et al., 1978; Höffken et al., 1978). Further deiodination of T_3 to T_2 is slow under physiological or slightly acidic conditions but is enhanced by an alkaline pH (Höffken et al., 1977).

Formation of rT_3 from T_4 occurs in the cytosol, mitochondria and microsomes. Under physiological pH and in the presence of microsomes, rT_3 is rapidly deiodinated to T_2. Under alkaline conditions (pH 9.5), production of rT_3 is maximal and its deiodination to T_2 is decreased (Cavalieri et al., 1977a,b; Grussendorf and Hüfner, 1977; Grussendorf et al., 1977; Hüfner and Grussendorf, 1977b). Whether T_3 or rT_3 are produced from T_4 by separate enzyme systems is still debated (Cavalieri et al., 1977a , b; Höffken et al., 1977).

Enzymatic monodeiodination of T_3 or rT_3 to T_2 occurs predominantly in the liver and kidney. It is stimulated by sulphydryl groups and inhibited by PTU (Roche et al.,

1959b; Chopra, 1978; and Chopra et al., 1978).

Deiodination may also be an activation process. In rats, 17-27% of the T_4 degraded may be monodeiodinated to T_3, producing 20.5-80% of the T_3 utilized (Schwartz et al., 1971;Abrams and Larsen,1973; Höffken et al., 1978; Zimmerman et al., 1978). Since T_3 is the more potent form of thyroid hormone, this peripheral conversion is important.

Tata and Widnell (1966) proposed that thyroid hormones exert their effects by regulating transcription. Semi-specific or specific high affinity and low capacity T_3 nuclear receptor sites (proteins) which bind to DNA have been demonstrated in the rat pituitary, kidney, liver, cerebral hemispheres, lungs, spleen, testis and cultured GH_1 cells (Oppenheimer et al., 1972; Surks et al., 1973; Samuels and Tsai, 1973, 1974; DeGroot and Strausser, 1974; Oppenheimer et al., 1974; Latham et al., 1976; MacLeod and Baxter, 1976; Morishige and Guernsey, 1978; Eberhardt et al., 1978). In liver and GH_1 cells, T_3 stimulates the activity of RNA polymerases I & II, and the synthesis of nuclear protein, poly(A)-containing nRNA, mRNA and rRNA (Jothy et al., 1975; Samuels and Shapiro, 1976; DeGroot et al., 1977; Bernal et al., 1978; Dillman et al., 1978). The T_3-induced stimulation of hepatic mitochondrial α-glycerophosphate dehydrogenase and malic dehydrogenase, stimulation of growth hormone (GH) and inhibition of prolactin production in GH_1 cells, and suppression of TSH release in hypothyroid state have been correlated with

nuclear T_3 binding (Samuels et al., 1973, 1976, 1977: Tsai and Samuels, 1974; Oppenheimer et al., 1975, 1977; Silva and Larsen, 1977). Although these data suggest that T_3 is the active form of thyroid hormone acting via protein synthesis to produce thyroid hormone effects, T_3 can also act at a post-translational level (Bernal and Refetoff, 1977; Kempson et al., 1978). Low affinity, high capacity cytosol T_3-binding proteins also exist. These may regulate the amount of intracellular free T_3 available for hormone action following T_3 uptake (Murthy et al., 1978). T_3 uptake into the cell may be an active process (Eckel et al., 1978).

Whether T_3 is the only active form of thyroid hormone is still debated. The manifestation of thyroid hormone actions in tissues of T_4-injected, PTU-treated rats, and the proposed existence of a blood-tissue barrier for the transport of T_3 in the pituitary of mice suggest that T_4 posesses intrinsic activities (Takaishi et al., 1975: Larsen and Frumess, 1977;du Breuil and Galton, 1978). However, PTU-treatment might not prevent localized (eg. intrapituitary) conversion of T_4 to T_3 (Silva and Larsen, 1978). rT_3, traditionally thought of as an antithyroidal agent, is as potent as T_3 in stimulating hepatic L-T_3 aminotransferase in rats (Fishman et al., 1977). T_2 and rT_3 stimulate GH production and glucose consumption, while inhibiting nuclear T_3 binding in GH_1 cells (Papavasiliou et al., 1977).

B. Effects of epinephrine on the mammalian thyroid system.

1. Thyroidal tissue.

Thyroidal accumulation of $^{131}I^-$ has been the most frequently used measure of thyroid activity. Radioiodide accumulation depends on the rate of iodide influx and efflux, iodide organification, and organic iodine efflux.

In rabbits, the rate of thyroidal $^{131}I^-$ accumulation was decreased by a single injection or infusion of epinephrine (Reiss, 1953; Brown-Grant and Gibson, 1956). Contrary to the report by Reiss et al. (1949), Hays (1965) observed that the 24-hr thyroidal $^{131}I^-$ accumulation in man was decreased when $^{131}I^-$ was injected up to 1 hr after epinephrine. The thyroidal ^{131}I radioactivity was only increased when $^{131}I^-$ was injected 5 hr after epinephrine injection. In rats, Williams et al. (1949) found that the accumulation of ^{131}I radioactivity in the thyroid was decreased at 1 and 2 hr, but was increased at 24 hr after epinephrine injection. Soffer et al. (1949) observed that the 24-hr thyroidal $^{131}I^-$ accumulation was decreased by epinephrine.

Some of the above contradictions could be explained if epinephrine, acting via α-adrenergic receptors, inhibited active iodide transport while stimulating the organification of iodide and iodothyronine synthesis as shown in isolated calf thyroid cells (Maayan and Ingbar, 1968, 1970; Maayan, 1977). Although the involvement of α-adrenergic receptors and the epinephrine-induced

depression of active iodide transport were confirmed in *in vivo* studies with rats, Joasoo and Murray (1974a, b, 1975) observed that epinephrine decreased the organification of iodide and iodothyronine synthesis without altering thyroidal $^{131}I\text{-}T_4/^{131}I\text{-}T_3$ ratios.

Both TSH and epinephrine act via cAMP, stimulating thyroidal iodide efflux, glucose metabolism, and RNA and protein synthesis in isolated porcine and calf thyroid cells (Maayan and Ingbar, 1970; Maayan *et al.*, 1973; Dumas and Guibout, 1978). But unlike TSH, epinephrine decreased the cAMP-independent ^{32}P incorporation in calf thyroid cells (Zor *et al.*, 1969; Maayan and Ingbar, 1970).

2. Thyroid hormone release.

Early workers used plasma $PB^{131}I$ level and/or the loss of thyroidal radioactivity after $^{131}I^-$ pretreatment as indices of thyroid hormone secretion. In rats, epinephrine decreased the thyroidal $^{131}I^-$ and $PB^{131}I$ contents (Botkin and Jensen, 1952). These observations suggested an increase in hormone secretion, but without concurrent measurements of radioiodide recycling, a definite conclusion could not be made. Botkin and Jensen (1952) and Eskelson *et al.* (1954) reported that epinephrine depressed plasma $PB^{131}I$ in rats, suggesting a depression in hormone secretion. Assuming that plasma $PB^{131}I$ measurements represented the levels of circulating thyroid hormones, this might only reflect an increased utilization of thyroid hormones.

In mice not influenced by endogenous TSH (T_4-blocked or hypophysectomized), but not in normal mice, epinephrine, like TSH, induced an increase in thyroidal release of ^{131}I-radioactivity, total blood $^{131}I^-$ and $PB^{131}I$ radioactivities and formation of colloid droplets (Melander, 1969, 1970; Ericson et al., 1970; Melander and Sundler, 1972a). In T_4-blocked mice on a low iodide diet, the TSH- and epinephrine-stimulated release of thyroid hormones were additive if epinephrine and TSH were administered simultaneously but were mutually antagonistic when the two hormones were injected at an interval of 2 hr (Melander and Sundler,1972a).Epinephrine inhibits the TSH-induced increase in intracellular cAMP level by interferring with cAMP accumulation (Maayan et al., 1977a , b; Sherwin, 1978). Thus the net effect of epinephrine on thyroid hormone secretion might depend on the balance between direct stimulation and epinephrine/TSH interaction.

Some of the above variations may also be the result of differences in experimental treatments. T_4 blockage, hypophysectomy and the treatment with a low iodide diet may alter thyroid hormone and catecholamine economy and hence the interpretation of the data (Eartly and Leblond, 1954; Gafni et al., 1975; Ismahan et al., 1977).

3. Thyroidal blood flow.

Brown-Grant and Gibson (1956), Ackerman and Arons (1958) and Mowbray and Peart (1960) observed a reduction in thyroidal blood flow with epinephrine administration.

This suggested that the effects of epinephrine on the thyroid gland might be secondary to the reduced blood flow (Harrison, 1964).

This possibility was studied in dogs with a cannula implanted in the thyroid vein (Ahn et al., 1969). Epinephrine infusion reduced thyroidal blood flow but increased the efficiency of the thyroid to extract iodide. Although the amount of organic iodine secreted was unaffected, the amount of iodide released from the thyroid was increased. The latter usually accompanies an increase in thyroid hormone secretion. These effects were duplicated by withdrawing 200-500 ml of femoral arterial blood.

The above results showed that epinephrine could act on the thyroid gland by altering its blood supply but did not preclude other possible mechanisms of actions. It should be noted that the removal of 200-500 ml of blood constituted a change in approximately 30% of the total blood volume and the results should be interpreted with caution.

4. Circulating thyroid hormone and their metabolism.

In rats, most of the measurements of plasma PB^{131}I radioactivity (Botkin and Jensen, 1952; Eskelson et al., 1954; and others) suggest a depression of circulating thyroid hormone levels after epinephrine injection. In contrast, Udupa et al. (1976) observed an increase in plasma PB^{127}I level after epinephrine injection. Whitman et al. (1976) reported that epinephrine increased plasma

T_3 concentrations in a dose related fashion without altering T_4 levels. Variation in epinephrine injection sites was not likely to be the cause of these differences. Eskelson et al. (1954) and Botkin and Jensen (1952), as well as Udupa et al. (1976) and Whitman et al. (1976), all injected epinephrine intraperitoneally.

After removing thyroids prelabelled with $^{131}I^-$, epinephrine decreased plasma $PB^{131}I$ levels in rats (Williams et al., 1949), suggesting an increased degradation of thyroid hormones.

Epinephrine did not alter the urinary excretion of injected radioiodide but increased the urinary $^{131}I^-$ content after injections of ^{131}I-labelled T_4, T_3 and D-thyroxine in intact, thyroidectomized, and T_4 maintained (2 µg/day) perchlorate-blocked rats on low iodide diet (Kallman and Starr, 1959; Escorbar del Rey and Morreale de Escorbar, 1963; Galton, 1965; Hillier, 1968). In thyroidectomized, ^{131}I-T_4 injected mice, epinephrine increased the ^{131}I-T_3 content in the pituitary and other brain tissues (Grinberg, 1964). These in vivo studies indicated that epinephrine enhanced the deiodination of thyroid hormones.

Reports on the effects of epinephrine on in vitro deiodination of thyroid hormones are conflicting. Although the in vitro deiodination of ^{131}I-T_4 by liver homogenates of mice treated with epinephrine in vivo was increased (Galton, 1965), the deiodination of ^{131}I-T_4 and ^{131}I-T_3 by rat muscle homogenates was decreased in a dose-related manner with in vitro application of epinephrine

(Kobayashi et al., 1966).

5. TSH release and circulating TSH levels.

Observations on the effects of epinephrine on TSH release and circulating TSH levels are conflicting. Von Euler and Holmgren (1956) reported that local injection of epinephrine into the anterior pituitary of rabbit decreased TSH secretion as measured by a reduction of thyroidal ^{131}I radioactivity. In rats and thyroidectomized dogs, epinephrine administration increased serum and decreased pituitary bioassayable TSH concentration respectively (D'Angelo, 1956; Soffer et al., 1947a , b). In non-lactating Guernsey cows, plasma TSH levels were increased during epinephrine infusion (Goret et al., 1974). These conflicts cannot be resolved without investigating the influence of epinephrine on TSH metabolism.

6. Summary.

Epinephrine generally stimulates the thyroid system in studies where the influence of TSH has been removed. Conflicts are more common between studies where TSH is available.

MATERIALS AND METHODS

A. Experimental animals and their maintenance.

Adult male Sprague-Dawley rats weighing an average of 448 g (range 324 to 726 g) were obtained from an inbred colony held in the Department of Zoology, University of Manitoba and from Bio-breeders (Ottawa). Body weight differences within each experiment did not exceed 100 g. Rats of one sex were chosen since plasma TSH, T_4 and T_3 concentrations may differ between male and female rats (Fukuda *et al.*, 1975a). To eliminate possible variations in thyroid activities during the estrous cycle (Brown-Grant, 1966), only adult male rats were used.

Rats were housed in individual cages of 17.7 cm x 25.4 cm x 17.8 cm at room temperature with free acess to drinking water (tap water under normal conditions; 1% $KClO_4$ solution (w/v) during perchlorate treatment). Unless otherwise stated, perchlorate treatment commenced at 24 hr before tracer or epinephrine or vehicle injection. Rats were kept under a controlled photoperiod (light, 08:00 to 22:00). They were maintained on an *ad libitum* diet of Lab-Blox F6 food pellets (Allied Mills Inc., Chicago, Ill.; approximately 27 μg I/g). Commencing 1-2 days before the start of each experiment, they were fed 3% of their body weight per day. Rats were fed during experiments as starvation reduces hepatic T_3 binding capacity and T_4 to T_3 monodeiodination (Melander *et al.*,

1977; Burman et al., 1977).

B. Injections and blood sampling.

All rats were anaesthetized prior to injection or blood sampling with anaesthetic-grade ether (Mallinckrodt Inc., St. Louis, Mo.). L-epinephrine (Sigma Chemical Co., St, Louis, Mo.) was dissolved in acidified 0.9% saline containing 0.1% sodium metabisulfite to give an epinephrine concentration of 1 μg/μl. Dilutions were made from this stock solution where necessary. Doses of epinephrine, ranging from 0.007 to 0.9 μg/g body weight, were injected into thigh muscles from 1-ml tuberculin syringes (needle size 30G, ½"). The frequently used doses, 0.7 and 0.9 μg/g, were similar to those commomly reported in the literature (0.5 to 1.0 μg/g). Intramuscular injection was used to permit slow release of the epinephrine into the blood and thereby offset the rapid degradation that would result following its direct introduction into the vascular system (Hillier, 1968; Callingham, 1975). Injection volume varied between 0.2 and 0.5 ml. Control rats were injected with equivalent volumes of epinephrine vehicle.

In rats, the hypothalamic TRH content, suprarenal epinephrine content, serum or plasma TSH concentration, and iodide metabolism follows a diurnal rhythm (Leppäluto et al., 1974, 1978; Singh et al., 1967; Scheving et al., 1968; Pallardo et al., 1976). To facilitate comparison and pooling of results, epinephrine or vehicle was injected between mid-morning and early afternoon.

Plasma samples were expelled from the tubes with a gentle stream of air and collected in 2-ml plastic beakers.

Terminal blood samples were obtained via cardiac puncture with preheparinized 3-ml disposable syringes (needle size 25G, 7/8"). Blood samples were ejected into 1-ml polystyrene centrifuge tubes and held on ice until centrifuged at 15,000 x g for 3 min (International Micro-capillary Centrifuge, model MB). Plasma samples were stored, covered with Parafilm, at -20°C until processed. Rats were killed after each experiment with a blow to the neck region.

The above sampling procedure did not alter the haematocrit ratio significantly (Appendix I).

C. Plasma PB*I determinations.

A volume of 0.5 ml of 10% trichloro-acetic acid (TCA, w/v) was added to 50-100 µl of plasma in a 1-ml polystyrene centrifuge tube. After mixing, the tube was centrifuged at 15,000 x g for 10 min (International Micro-capillary Centrifuge, model MB). The supernatant was removed with a Pasteur pipet. The pellet was washed twice by resuspending in 10% TCA and centrifuging at 15,000 x g for 10 min. The washed pellet, dissolved in 1 ml of 0.1 N NaOH, was transferred to a counting tube and made up to a total volume of 4 ml with 0.1 N NaOH. This constituted the PB*I fraction. Radioactivity was expressed as % injected dose/ml/100 g body weight.

D. T_4 and T_3 determinations.

T_4 and T_3 in plasma and thyroidal extracts were measured by radioimmunoassays (RIA) modified from Brown & Eales (1977). Standard stock solutions of T_4 and T_3 were made by dissolving the anhydrous sodium salt of T_4 (Eltroxin, sodium L-thyroxine pentahydrate) or T_3 (Sigma) in 0.1 N NaOH. Working standards containing 0-0.8 ng hormone in 100 µl were prepared by further dilution with 0.1 N NaOH. Barbital buffer (pH 8.6; 75 mmol/l) were prepared (Brown, 1977).

Lyophylized rabbit antiserum to T_3 human serum albumin (T_3-antibody) or to T_4 human serum albumin (T_4-antibody) were obtained from K & T Biological Services, Edmonton, Alberta. The supplier specified that the antibodies were highly specific and had low cross reactivity with other iodothyronines. T_4- and T_3-antibody, reconstituted and diluted 1:5,000 and 1:22,000 with barbital buffer, were used in T_4 and T_3 determinations respectively.

*T_3 and *T_4 tracers were diluted with 0.1 N NaOH to give 5,000-7,000 cpm/100 µl in a gamma well detector of about 50% efficiency.

Miniature columns (5-ml syringe barrels), each containing 0.5 g G-25 (fine) Sephadex (Pharmacia) equilibrated in 0.1 N NaOH (pH 13), were used in the assays. Before addition of T_4 or T_3 standards (100 µl) or plasma samples (10 µl, T_4-RIA or 100-200 µl, T_3-RIA), the columns were drained and their bottoms capped. *T_4 or *T_3 (100 µl) was added to each column.

Columns were swirled and allowed to drain to waste. At pH 13, plasma T_4 and T_3 dissociated from their binding proteins and became adsorbed onto the Sephadex (Brown, 1977). Four ml (T_4-RIA) or 3 ml (T_3-RIA) barbital buffer was added to each column to remove contaminating radioiodide and plasma proteins.

Each column was positioned over an empty counting tube. One ml of diluted T_4- or T_3-antibody was added to each column in sequence and allowed to equilibrate for 90 min before barbital buffer (3 ml, T_4-RIA or 2 ml, T_3-RIA) was added to each column in the same sequence. All radioactivity bound to antibody was removed with the first 2 ml of buffer (Brown & Eales, 1977). Standard curves were obtained by plotting antibody-bound radioactivity (cpm) against hormone concentration. Sample hormone concentrations were determined by interpolation. Detection limits (mean ± SE) for all RIA assays conducted were 8.45 ± 0.57 pg for T_4 and 10.75 ± 0.97 pg for T_3; precision indices (mean ± SE) were 0.041 ± 0.003 for T_4 and 0.052 ± 0.005 for T_3.

Columns were regenerated by successive elutions with 10 ml distilled deionized water, 8 ml of out-dated human plasma (diluted 1:10 with barbital buffer), 10 ml deionized water and 8 ml of 0.1 N NaOH.

E. T_4 and T_3 kinetics.

1. Separation of plasma radioactive materials.

Plasma radio-labelled materials were separated by a modified method of Eales (1977a). Miniature columns were prepared as in the T_4- or T_3-RIA. Radioactive plasma samples (up to 0.2 ml) and 0.1 ml aliquots of 0.1 N NaOH were added onto drained columns with capped bottoms. After mixing by gentle swirling, the mixtures were drained into the columns. Eluates were collected in counting tubes. At pH 13, T_4 and T_3 (both stable and radio-labelled) bind to the Sephadex (Brown, 1977) and plasma proteins and iodide can be eluted with 2.8 ml barbital buffer, pH 8.6. All tubes were made up to a standard counting volume of 4 ml with deionized water. This fraction contained $*I^-$ (Eales, 1977a).

A new counting tube was placed beneath each column. A volume of 0.5 ml of T_3-antibody (diluted 1:300 with barbital buffer) was added to each column. After a 60-min incubation, the antibody-bound T_3 (including $*T_3$) was eluted with 3.5 ml barbital buffer.

$*T_4$, if present in the sample, remained on the column (Eales, 1977a). This was removed with two 4-ml washes of human plasma (diluted 1:10 with barbital buffer). Both washes , when collected and counted, formed the $*T_4$ fraction. $*T_4$ fractions obtained in this manner compared well with those obtained by eluting with 2 ml barbital buffer after a 1-hr incubation with 2 ml T_4-antibody, diluted 1:10

with barbital buffer (Appendix II). Columns were regenerated by washing with 10 ml deionized water and 8 ml of 0.1 N NaOH.

Radioactivity in the plasma $*I^-$, $*T_3$ and $*T_4$ fractions was expressed as % dose/ml/100 g body weight. Recovery efficiencies were checked each time by replacing radioactive plasma samples and 0.1 ml of 0.1 N NaOH with pooled stable plasma and 0.1 ml $*T_4$ or $*T_3$ tracer (diluted with 0.1 N NaOH to give 5,000-7,000 cpm/100 µl in a gamma well dectector of about 50% efficiency). Extraction efficiencies (mean ± SE) for all separations conducted were 93.26 ± 1.03% for $*T_3$ and 96.33 ± 0.81% for $*T_4$.

2. Calculation of T_4 and T_3 kinetics.

Parameters of plasma T_4 and T_3 kinetics (degradation rate (DR), clearance rate (CR), fractional disappearance rate (k), and distribution space (DS)) were derived by following the loss of injected $*T_4$ and $*T_3$ from the plasma. Levels of $*T_4$ and $*T_3$ decreased exponentially with time. Regressions were calculated for each semilogarithmic plot.

T_4 or T_3 DR (ng/hr/100 g body weight) was calculated for epinephrine-injected (experimental) or control groups over the period (7 hr, T_4; 8 hr, T_3) when epinephrine was effective in influencing thyroid function.

$$DR = CR\ (ml/hr/100\ g) \times \text{the average stable plasma hormone concentration at 4 hr after epinephrine or vehicle injection (ng/ml).}$$

CR = $\underline{k}$ (fraction/hr) x DS (ml/100 g).

$\underline{k}$ = slope of the control or experimental $*T_4$ or $*T_3$ regression covering 0-7 hr (T_4) or 0-8 hr (T_3) after vehicle or epinephrine injection.

DS = $100/\underline{i}$,

where $\underline{i}$ represented the level of radioactivity at the time of tracer injection, as extrapolated from the regression line from control rats covering the entire time interval studied. In the case of T_4DS, $\underline{i}$ was corrected for the excretion of $*T_4$ during the uptake phase immediately following $*T_4$ injection, by multiplying $\underline{i}$ with a factor of 0.72 estimated from a preliminary experiment (Appendix III). A similar adjustment was not made for T_3 because of its rapid uptake (Appendix IV). Due to the nature of the experimental design, transient changes in distribution spaces could not be detected. Hence, distribution spaces were assumed to remain unaltered throughout a given experiment.

F. $\underline{T_4 \text{ secretion rate } (T_4SR)}$.

T_4SR (ng/hr/100 g body weight) was estimated for individual control or experimental blocked rats over the 7-hr period during which epinephrine was effective in influencing thyroid function.

$$T_4SR = \frac{T_4 \text{ plasma pool}_{7hr} - T_4 \text{ plasma pool}_{0hr} + T_4 \text{ degraded in 7 hr}}{7},$$

where:-

1. T_4 plasma $pool_{0hr}$ (ng/100 g) = $T_4DS \times [T_4]plasma_{0hr}$.

T_4DS (ml/100 g) was estimated from the corrected intercept of the regression describing disappearance of exogenous $*T_4$ from plasma of control rats. $[T_4]plasma_{0hr}$ was taken as the average stable plasma T_4 concentration (ng/ml) before epinephrine or vehicle injection.

2. T_4 plasma $pool_{7hr}$ (ng/100 g) = $T_4DS \times [T_4]plasma_{7hr}$.

$$[T_4]plasma_{7hr} = [T_4]plasma_{0hr} \times \frac{\text{endogenous } *T_4 \text{ at 7 hr}}{\text{endogenous } *T_4 \text{ at 0 hr}}.$$

Endogenous $*T_4$ at 0 and 7 hr were obtained by counting the $*T_4$ plasma fraction of rats injected 24 hr earlier with $Na^{125}I$. In calculating $[T_4]plasma_{7hr}$ (ng/ml), it was assumed that the sp. act. of endogenous $*T_4$ remained unaltered during the 7-hr period.

3. T_4 degraded in 7 hr (ng) = $[T_4]$plasma x T_4DS x $\underline{k}$ x 7.

$[T_4]$plasma was taken as the average stable T_4 concentration (ng/ml) at 4 hr after epinephrine or vehicle injection. $\underline{k}$ was equivalent to the slope of the semi-logarithmic regression describing exogenous $*T_4$ loss from the plasma of control or experimental blocked rats.

G. Thyroidal T_3/T_4 ratio.

The modified method of Chopra et al. (1973) was used.

1. Homogenization.

Thyroids, together with adjacent sections of trachea, were removed from freshly-killed rats and rinsed with cold

Tris-buffered saline (0.04 M Tris, 0.11 M NaCl, 0.05 M methylmercaptoimidazole, pH 8.4) to remove surface blood contamination. Each thyroid/cartilage complex was cut up into fine pieces and placed into a 16- x 150-mm glass test tube. Five ml of cold Tris-buffered saline was added and the thyroid homogenized at 4°C for 2 minutes at maximum speed with a Polytron homogenizer (Brinkmann Instruments Ltd., Toronto, Ont.). Thyroids not processed immediately were stored at 4°C. The thyroid homogenate was poured into a 10-ml graduated cylinder. The homogenizer was washed by running it at maximum speed for 30 seconds in 4 ml cold Tris-buffered saline. The washing was added to the homogenate. The total volume was adjusted to 10 ml. Thyroid homogenates were stored at 4°C until all thyroids were processed.

2. Hydrolysis

Duplicate volumes of 1 ml of homogenate were added to 16- x 150-mm glass test tubes. A volume of 0.25 ml pronase (purified non-specific protease from Streptomyces griseus, 45,000 proteolytic units/g, Sigma; at a concentration of 40 mg/ml of Tris-buffered saline) and 0.1 ml toluene were added to each tube. To estimate recovery, the remaining homogenates were pooled and 1-ml aliquots were added to 16- x 150-mm glass test tubes in quadruplicate. Volumes of 0.25 ml pronase, 0.1 ml toluene and 0.1 ml $*T_4$ or $*T_3$ tracer (with known contaminating $*I^-$ level and diluted in 0.05 N NaOH to give 5,000-7,000 cpm

in a well-type gamma radiation detector of about 50% efficiency) were added to each recovery tube. The tubes were vortexed, plugged with cotton wool, and incubated at 37°C in a Metabolyte Water Bath Shaker (New Brunswick Scientific Co. Inc., New Brunswick, N.J.) for 16 hr under N_2. Under these anaerobic conditions, the recovery of iodothyronines and reproducibility of results were maximized (Inoue and Taurog, 1967).

3. Extraction.

After pronase digestion, each tube was vortexed and two 0.5-ml aliquots of hydrolysate were transferred into 10- x 75-mm glass test tubes. One ml of butanol:ethanol (1:1, v:v) was added to each tube. After vortexing, the tubes were centrifuged at 1,100 x g for 10 min in a Sorvall GLC-1 centrifuge. Except for recovery tubes, supernatants were decanted into 13-ml Autoclear centrifuge tubes (Fisher Scientific) and evaporated to dryness at 45°C under streams of air. Dried extracts were reconstituted with 2 ml of 0.1 N NaOH. To estimate recovery, the supernatants were decanted into counting tubes and made up to total volumes of 4 ml with distilled water before counting. T_4 or T_3 contents in 0.2-ml aliquots of a 100x or 10x dilution of the reconstituted extract were estimated by radioimmunoassays. After adjusting for T_4 and T_3 recovery efficiencies, thyroidal T_3/T_4 ratios were calculated.

H. Radiation counting.

All samples were counted twice consecutively for 10 min or 10,000 counts in a Nuclear Chicago Automatic Gamma Radiation Detector System containing a 2-inch (DS202) NaI crystal. Discrimator settings were optimized for ^{125}I.

I. Statistical analysis.

Results from duplicate experiments were pooled. Data sets without initial sample values due to mishaps in processing, and those from animals that died during the experiments were excluded. To eliminate variations between rats, values were often expressed as a percentage of the initial value for a given individual. All differences were considered to be insignificant when $P>0.05$.

Differences between final and initial values were compared with a paired-difference test.

When means from two groups were compared, heterogeneity of variances was tested with a two-tailed F-test. A two-tailed unpaired-t-test was used when the variances were homogeneous. A Behrens-Fisher's test was used if the variances were not homogeneous.

When more than two means were compared, a Bartlett's X^2 test was used to test for homogeneity of variances. Where necessary, a $\log_e$ transformation was used to reduce the heterogeneity of variances prior to comparison by a one-way analysis of variance (ANOVA). If the ANOVA showed

that significant differences existed between groups, a Student-Newman-Keul test was used to locate those means that differed.

Analysis of covariance was used to compare variances, slopes and intercepts of regression lines.

These statistical techniques are outlined in Steel and Torrie (1960), Mendenhall (1971) and Snedecor and Cochran (1971).

RESULTS

A. Plasma PB*I.

Six rats were each injected with carrier-free $Na^{125}I$ (10 μCi). At 24 hr after tracer injection, blood was sampled and epinephrine (0.9 μg/g) or vehicle was injected into 3 experimental or 3 control rats. Blood samples were taken at 1, 2, 4, 7 and 24 hr later. Plasma PB*I was measured.

The experimental PB*I values were significantly ($P<0.05$) lower than those of the controls as of 2 hr after epinephrine injection (Fig. 1A). Alterations of experimental plasma PB*I levels (expressed as % control values) were stablized at 7 hr after epinephrine injection (Fig. 1B).

B. Plasma T_4 and T_3.

1. Non-blocked rats - single dose.

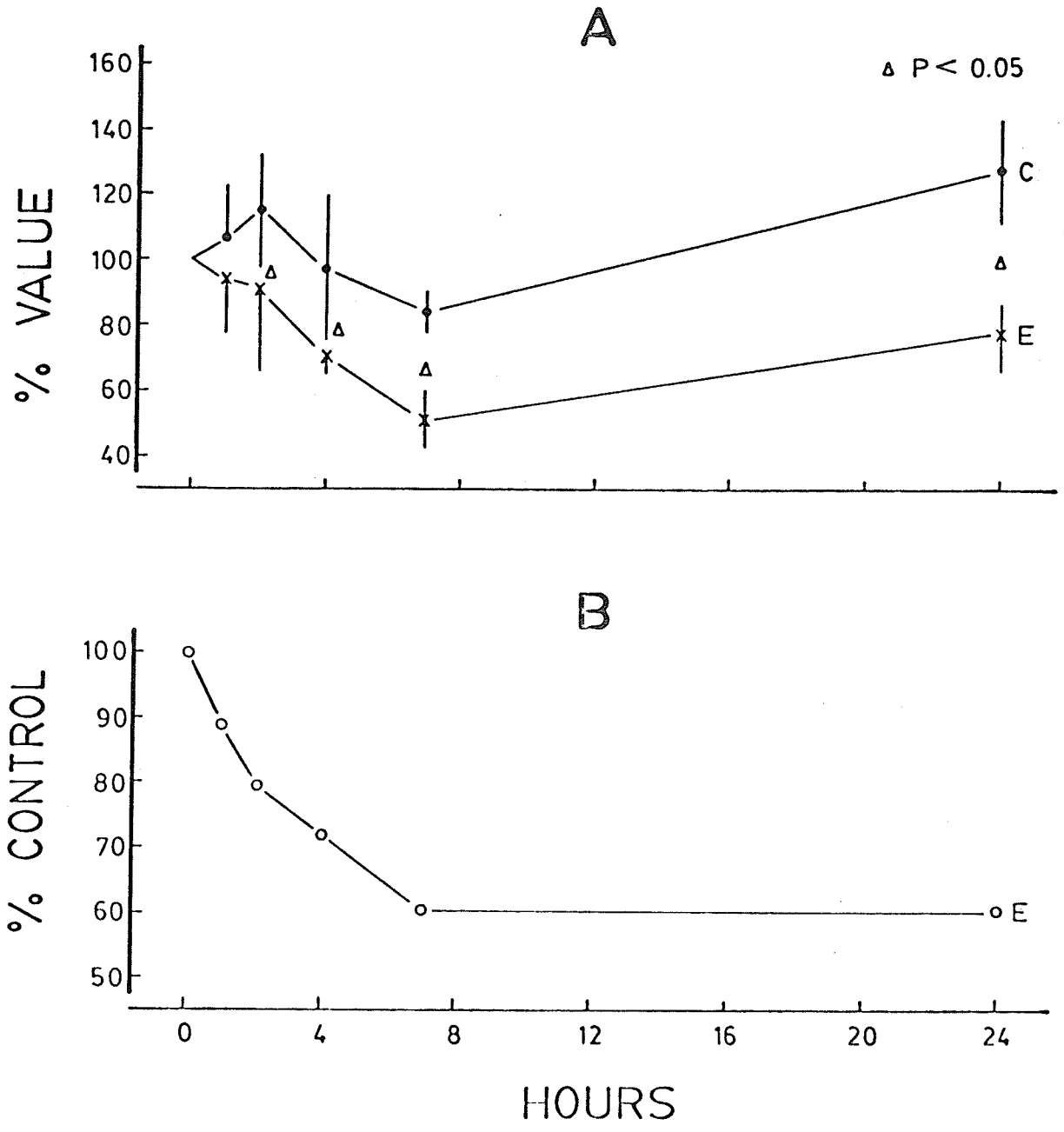
Immediately following initial blood sampling, epinephrine (0.9 μg/g) or vehicle was injected into 5 experimental or 5 control rats. Blood was sampled from each rat 2, 4 and 7 hr later. Plasma T_4 and T_3 concentrations were measured. The experiment was repeated twice and the results from the three experiments pooled.

When compared to the controls, epinephrine significantly

Figure 1. Control (C) or experimental (E) plasma PB*I levels in non-blocked rats pretreated with carrier-free $Na^{125}I$ at various times after vehicle or epinephrine injection.

A. Plasma PB*I levels are expressed as % value at the time of epinephrine or vehicle injection. Means and 95% confidence intervals are plotted. (n = 3; Δ, significantly different from control values ($P<0.05$) as shown by Behrens-Fisher's test or unpaired-t-test.)

B. Experimental plasma PB*I levels are expressed as % control values.


A
Δ P < 0.05
% VALUE
160
140
120
100
80
60
40
C
E
Δ
B
% CONTROL
100
90
80
70
60
50
E
0
4
8
12
16
20
24
HOURS

($P<0.01$) lowered plasma T_4 at 4 and 7 hr and plasma T_3 at 7 hr post injection (Table III).

2. Blocked rats - single dose.

Epinephrine (0.7 μg/g) or vehicle was injected into 10 experimental or 10 control rats pretreated with perchlorate, following initial blood sampling. Five rats from each group were bled at 2 hr and the remaining five at 4 hr post injection.

Experimental T_4 levels were significantly lower ($P<0.05$) than those of the controls at 2 and 4 hr. Control and experimental T_3 levels were not significantly different (Table IV).

3. Dose-response.

Four groups of 5 blocked rats were bled and then injected with vehicle (control) or various doses of epinephrine (0.007, 0.07 and 0.7 μg/g). They were bled 4 hr later. Plasma T_4 concentrations were determined.

The T_4 values decreased with increasing doses of epinephrine (Table V). However, the 0.7 μg/g group was the only group that differed significantly ($P<0.05$) from the controls.

C. Thyroid hormone (T_4 and T_3) degradation rates and deiodination.

1. T_4 (non-blocked rats).

Ten rats were each injected with 2 μCi $*T_4$ after removing an initial blood sample. Blood was sampled 24 hr

Table III.

Control (C) or experimental (E) plasma T_4 and T_3 concentrations in non-blocked rats at various times after vehicle or epinephrine injection (n=14, T_4; n=13, T_3).

hormone		hours after vehicle or epinephrine injection							
		0		2		4		7	
		C	E	C	E	C	E	C	E
T_4 ($\mu g/dl$)	$\bar{x}$	4.56	3.86	4.60	3.48	4.51	2.77[a,b]	4.02	2.01[a,b]
	SD	1.75	1.63	1.08	1.83	1.60	1.62	1.34	1.02
T_3 (ng/dl)	$\bar{x}$	64.6	51.0	----	----	48.0	37.6[a]	72.2	19.8[a,b]
	SD	25.2	19.4	----	----	21.4	12.4	22.2	5.3

[a] Significantly different from 0 hr value ($P<0.01$) as shown by paired-difference test.

[b] Significantly different from control values ($P<0.01$) when expressed as % initial values (determined by Behrens-Fisher's test or unpaired-t-test).

Table IV.

Control (C) or experimental (E) plasma T_4 and T_3 concentrations in blocked rats at various times after vehicle or epinephrine injection (n=5 in all cases).

hormone	experiment		hours after vehicle or epinephrine injection					
			0		2		4	
			C	E	C	E	C	E
T_4 (μg/dl)	1	$\bar{x}$	3.97	4.01	4.01	3.20[a,b]	----	----
		SD	0.77	0.47	0.29	0.85	----	----
	2	$\bar{x}$	3.79	3.76	----	----	3.92	2.72[a,b]
		SD	0.61	0.44	----	----	0.55	0.70
T_3 (ng/dl)	1	$\bar{x}$	22.2	39.5	27.5	29.7[a]	----	----
		SD	13.1	9.5	8.0	6.4	----	----
	2	$\bar{x}$	24.0	30.6	----	----	42.0[a]	21.6
		SD	11.7	10.5	----	----	7.1	9.2

[a] Significantly different from 0 hr value ($P<0.05$) as shown by a paired-difference test.

[b] Significantly different from control values when expressed as % initial values ($P<0.05$, as shown by unpaired-t-test).

Table V.

Changes in plasma T_4 concentrations in blocked rats due to various doses of epinephrine injected 4 hr earlier. For each individual rat , the T_4 concentration at 4 hr was expressed as a percentage of the T_4 concentration just prior to injection. Prior to statistical analysis, a $\log_e$ transformation was required to reduce the heterogeneity of variances. Means that are similar (as shown by a Student-Newman-Keul test) are identified by similar superscripts.

epinephrine doses (μg/g)	n	$\bar{x}$ (%)	SD
0.0	5	117.7^{a}	48.4
0.007	4	$90.1^{a,b}$	13.7
0.07	4	$88.7^{a,b}$	10.3
0.7	5	66.3^{b}	20.0

later and 5 rats were then injected with epinephrine (0.9 μg/g) and 5 rats with vehicle. Blood samples were taken at 2, 4, 7, 12 and 24 hr after epinephrine or vehicle injection. The radioactivity in plasma iodide, T_3 and T_4 fractions was separated. The experiment was repeated and the data from the two experiments pooled.

The disappearance of $*T_4$ from plasma in control rats was exponential (Fig. 2A) with a slope (k) of -0.046/hr, a 'y-intercept' of 12.1% dose/ml/100 g and a correlation coefficient (r) of -0.910 ($P<0.01$). The disappearance of plasma $*T_4$ in the experimental rats was biphasic with a 'fast' phase persisting for the first 7 hr. The slope of this 'fast' phase (-0.116/hr) was significantly different ($P<0.01$) from that of the controls (-0.032/hr) over the same time interval. The slopes of the $*T_4$ regression lines for both groups from 7 to 24 hr after epinephrine or vehicle injection were not significantly different.

When expressed as % value at the time of epinephrine or vehicle injection (Fig. 2B), experimental $*T_4$ levels were significantly lower ($P<0.05$) than those of the controls at 4, 7, 12 and 24 hr after epinephrine or vehicle injection. The biphasic nature of the change in the experimental plasma $*T_4$ radioactivity in response to epinephrine injection was also observed.

The experimental T_4DR and other kinetic parameters, calculated using the above information, were higher than those for the controls (Table VI).

Figure 2. Changes in control (C) and experimental (E) plasma $*T_4$, $*I^-$ and $*T_3$ radioactivity levels in non-blocked rats injected with $*T_4$. The time of vehicle or epinephrine injection is indicated by the arrow (↓).

A. Radioactivity in the various fractions is expressed as % dose/ml/100 g. Geometric means and 95% confidence intervals are plotted. The slope of the experimental $*T_4$ regression line from 0-7 hr after epinephrine is significantly different ($P<0.01$) from that of the control over the same interval (Appendix V).

B. Radioactivity in the various fractions is expressed as % value at the time of epinephrine or vehicle injection. Means and 95% confidence intervals are shown. (Δ, significantly different from control values ($P<0.05$) as shown by Behrens-Fisher's test or unpaired-t-test.)

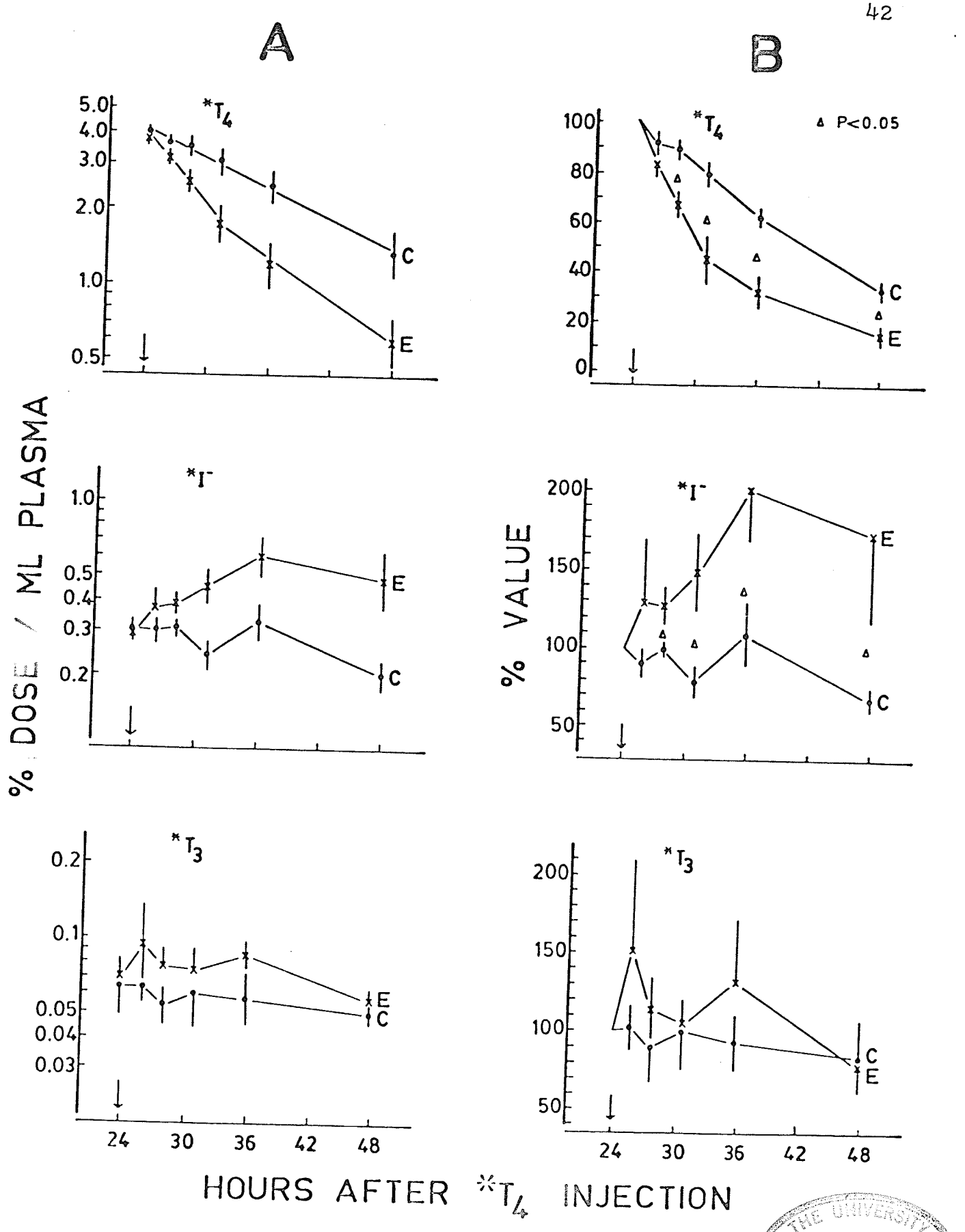


Table VI.

Plasma T_4 concentrations and kinetic parameters for non-blocked and blocked control (C) or experimental (E) rats during the first 7 hr after vehicle or epinephrine injection. The plasma T_4 concentrations were average plasma T_4 values at 4 hr after vehicle or epinephrine injection, determined from previous experiments. The kinetic parameters were based on the disappearance of injected $*T_4$ from the plasma.

parameters	non-blocked		blocked	
	C	E	C	E
T_4DS (ml/100 g)	11.4	11.4	15.3	15.3
T_4 concentration (μg/dl)	4.51	2.77	3.68	2.75
T_4-$\underline{k}$ (fraction/hr)	-0.032	-0.116	-0.042	-0.078
T_4CR (ml/hr/100 g)	0.37	1.33	0.65	1.20
T_4DR (ng/hr/100 g)	16.4	36.7	25.3	32.8

When expressed as % value at the time of epinephrine or vehicle injection, the control and experimental $*I^-$ values were significantly different as of 4 hr after epinephrine or vehicle injection (Fig. 2B). The elevation of experimental $*I^-$ levels coincided with the 'fast' phase of the experimental $*T_4$ disappearance (Fig. 2A and B). Control and experimental $*T_3$ values were not significantly different at any time (Fig. 2B).

2. T_4 (blocked rats).

The experimental protocol was identical to that used in the previous experiment except that rats were treated with perchlorate and an epinephrine dose of 0.7 μg/g was used.

The disappearance of $*T_4$ from the plasma in control rats was exponential (Fig. 3A) with a slope of -0.051/hr, a 'y-intercept' of 9.03% dose/ml/100 g and a $\underline{r}$ value of -0.918 ($P<0.01$). The disappearance of plasma $*T_4$ in the experimental rats was biphasic. The 'fast' phase lasted for 7 hr after epinephrine injection and was followed by a 'slow' phase. The slopes of these two phases (-0.078/hr, 'fast'; -0.069/hr , 'slow') could not be compared to those of the controls covering similar time intervals (-0.042/hr, 'fast'; -0.057/hr, 'slow') due to heterogeneity of variances (Fig. 3A).

When expressed as % value at the time of epinephrine or vehicle injection (Fig. 3B), the experimental $*T_4$ levels were significantly lower ($P<0.02$) than those of

Figure 3. Changes in control (C) and experimental (E) plasma $*T_4$ radioactivity in blocked rats injected with $*T_4$. ↓ indicates the time of epinephrine or vehicle injection.

A. Geometric means and 95% confidence intervals of $*T_4$ values (% dose/ml/100 g) are plotted. Experimental and control $*T_4$ regression lines cannot be compared due to heterogeneity of variances (Appendix VI).

B. Means and 95% confidence intervals of $*T_4$ values (% value at the time of epinephrine or vehicle injection) are shown. (Δ, significantly different from control values ($P<0.02$) as shown by unpaired-t-test or Behrens-Fisher's test.)

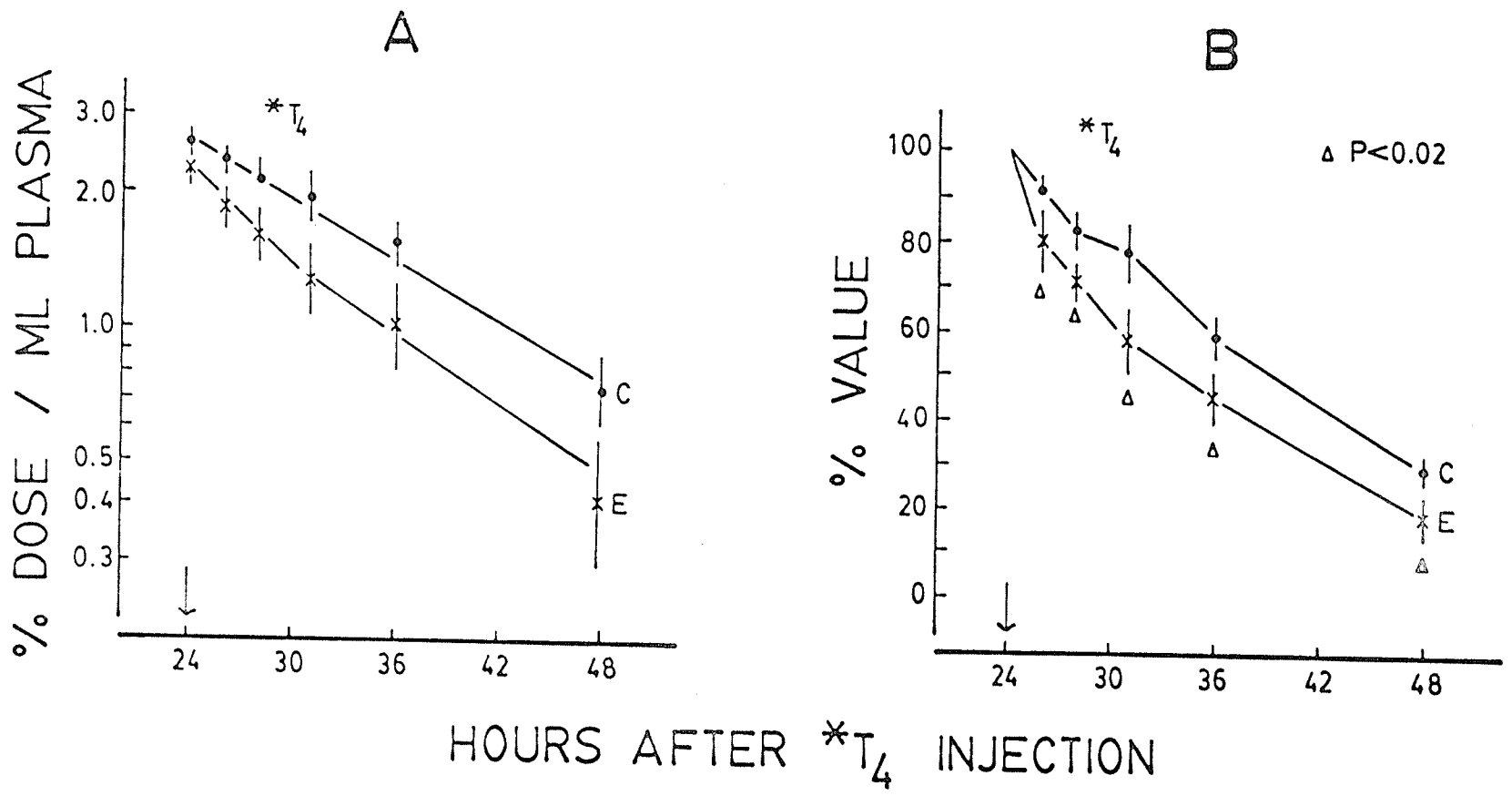
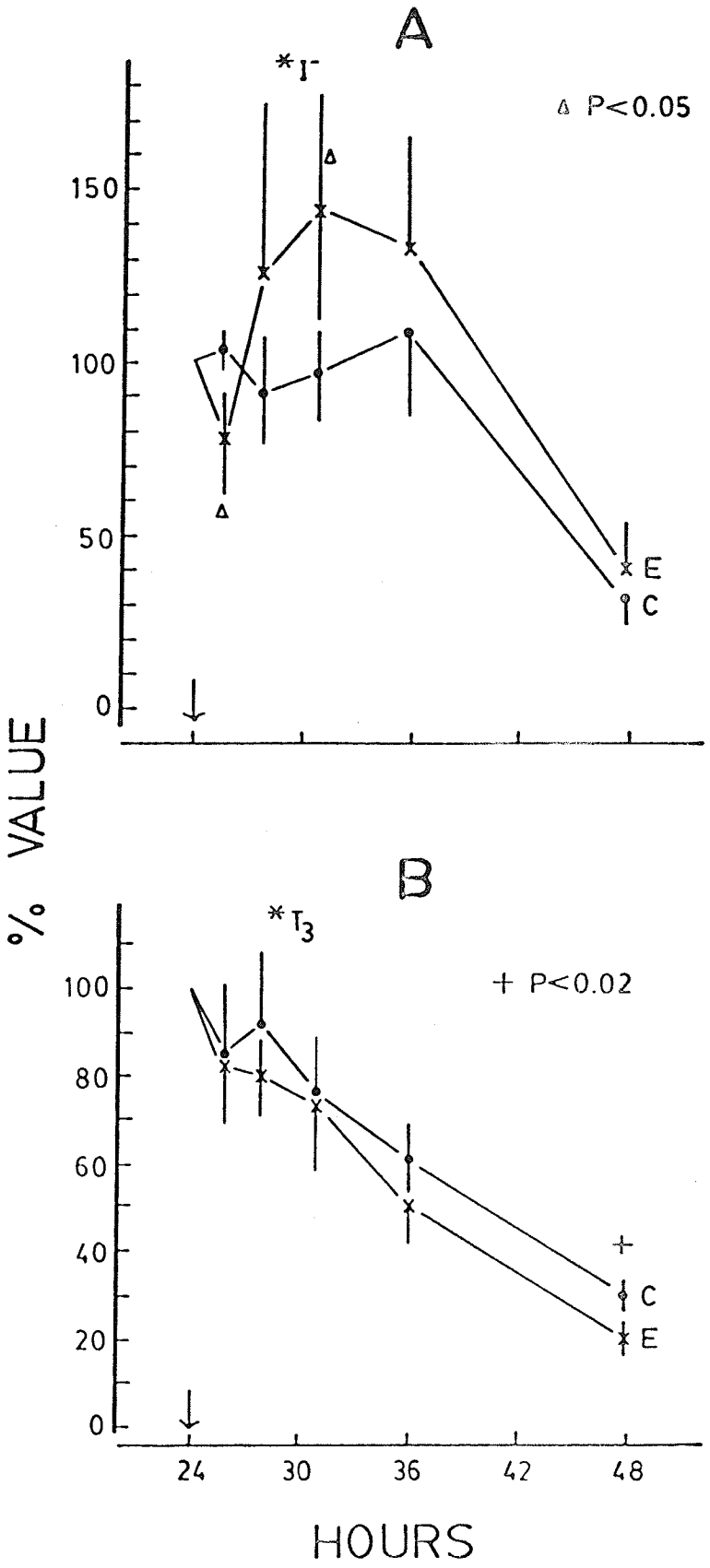

A
B
% DOSE / ML PLASMA
% VALUE
*T4
Δ P<0.02
C
E
3.0
2.0
1.0
0.5
0.4
0.3
100
80
60
40
20
0
24
30
36
42
48
HOURS AFTER *T4 INJECTION

Figure 4. Control (C) and experimental (E) plasma $*I^-$ and $*T_3$ radioactivity levels (% value at the time of vehicle or epinephrine injection) in blocked rats at various times after $*T_4$ injection. The arrow (↓) indicates the time of epinephrine or vehicle injection.

A. Means and 95% confidence intervals of $*I^-$ values are plotted. (Δ, significantly different from control values ($P<0.05$) as shown by Behrens-Fisher's test.)

B. Means and 95% confidence intervals of $*T_3$ values are shown. (+, significantly different from control values ($P<0.02$) as determined by an unpaired-t-test.)


A
*I⁻
Δ P<0.05
150
100
50
0
E
C
B
*T3
+ P<0.02
100
80
60
40
20
0
C
E
24
30
36
42
48
% VALUE
HOURS

the controls at all times.

The experimental T_4 kinetic parameters (including T_4DR), calculated using the above information, were higher than those for the controls (Table VI).

Although the experimental $*I^-$ level (expressed as % value at the time of epinephrine injection) was significantly lower ($P<0.05$) at 2 hr after epinephrine injection, it was higher than control values at other times (significant, $P<0.05$, at 7 hr after epinephrine injection; Fig. 4A). Experimental $*T_3$ levels were not significantly different from those of the controls except at 24 hr after epinephrine injection (Fig. 4B).

3. T_3 (blocked rats).

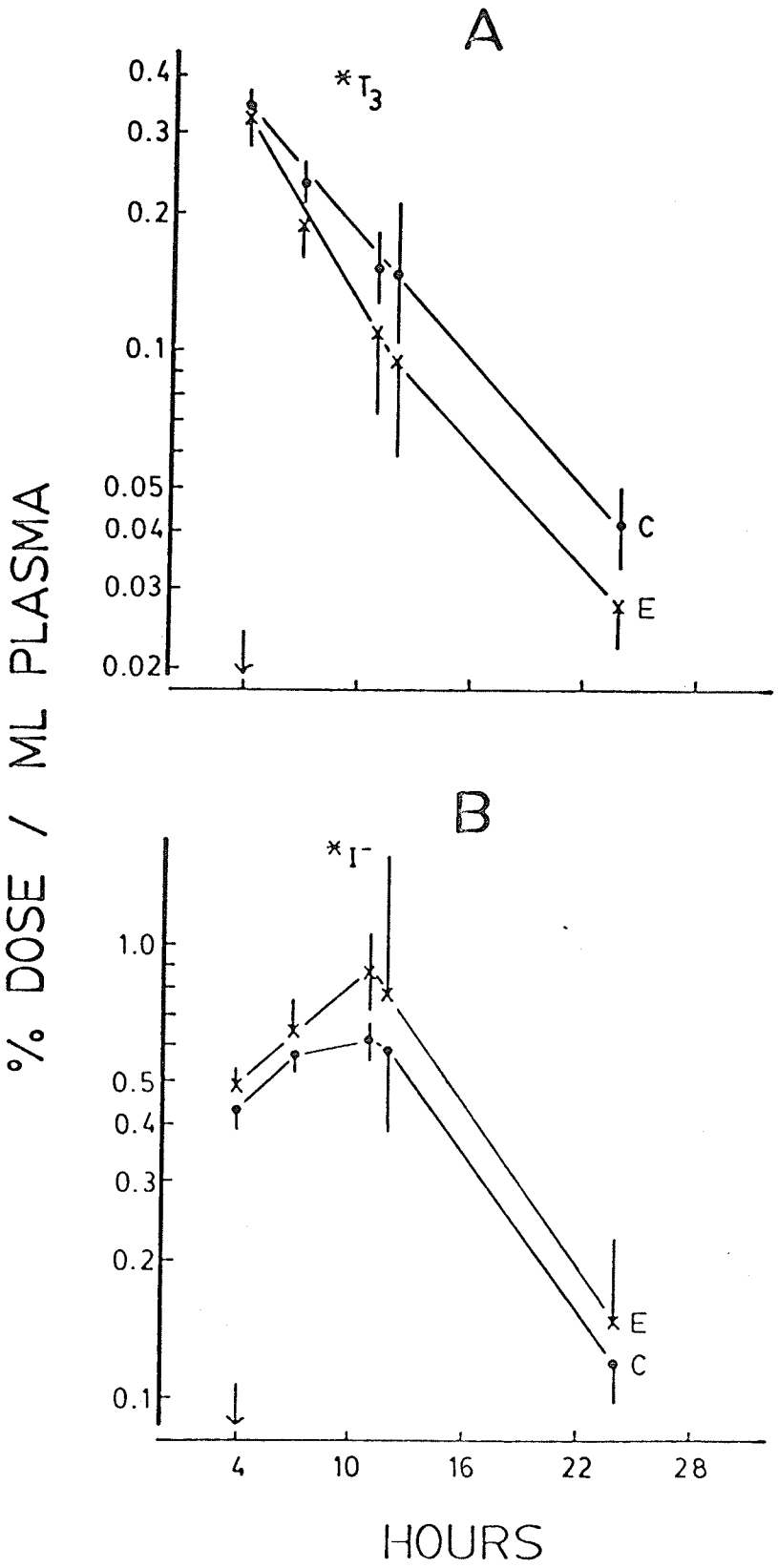
Ten perchlorate-treated rats were each injected with 5 μCi $*T_3$. Blood was sampled at 4 hr after tracer injection and 5 rats were injected with epinephrine (0.7 μg/g) and 5 rats with vehicle. Blood samples were taken at 3, 8 and 20 hr after vehicle or epinephrine injection. The radioactivity in plasma iodide and T_3 fractions was separated. The experiment was repeated with a minor adjustment. Blood was sampled at 7 hr instead of 8 hr after epinephrine or vehicle injection. Results from both experiments were pooled.

The disappearance of $*T_3$ from the plasma in control rats was exponential (Fig. 5A) with a slope of -0.104/hr, a 'y-intercept' of 0.5% dose/ml/100 g and a r value of -0.970 ($P<0.01$). The disappearance of plasma $*T_3$

Figure 5. Changes in control (C) and experimental (E) plasma $*T_3$ and $*I^-$ radioactivity levels (% dose/ml/100 g) in blocked rats injected with $*T_3$. The arrow (↓) indicates the time of epinephrine or vehicle injection.

A. Geometric means and 95% confidence intervals of $*T_3$ values are plotted. The slope of the experimental $*T_3$ regression line from 0-8 hr after epinephrine injection is significantly different ($P<0.05$) from that of the control (Appendix VII). (When expressed as % value at the time of epinephrine or vehicle injection, experimental and control $*T_3$ levels are not significantly different (Appendix VIII).

B. Geometric means and 95% confidence intervals of $*I^-$ values are shown. (When expressed as % value at the time of epinephrine or vehicle injection, experimental and control $*I^-$ levels are not significantly different (Appendix VIII).


A
*T3
0.4
0.3
0.2
0.1
0.05
0.04
0.03
0.02
C
E
B
*I-
1.0
0.5
0.4
0.3
0.2
0.1
E
C
4
10
16
22
28
% DOSE / ML PLASMA
HOURS

in the experimental rats was biphasic. Because of the difference in the time of blood sampling, the 'fast' phase was considered to extend over the first 8 hr after epinephrine injection. The slope of this 'fast' phase (-0.151/hr) was significantly different ($P<0.05$) from that of the control (-0.106/hr) over the same interval.

Control and experimental T_3 degradation rates during the 8 hr when epinephrine was effective in altering $*T_3$ metabolism were calculated from these results. Despite a higher T_3-$\underline{k}$ and T_3CR, the experimental T_3DR was lower than that of the control (Table VII).

Although plasma *I levels were generally higher in the experimental than the control rats (Fig. 5B), no significant difference was found when control and experimental plasma *I values (expressed as % value at the time of vehicle or epinephrine injection) were compared.

4. Iodide kinetics.

Ten perchlorate-treated rats were each injected with carrier-free $Na^{125}I$ (5 μCi). Blood was sampled at 2 hr after tracer injection and epinephrine (0.7 μg/g) or vehicle was injected into 5 experimental or 5 control rats. Blood samples were taken at 2, 4 and 7 hr after epinephrine or vehicle injection.

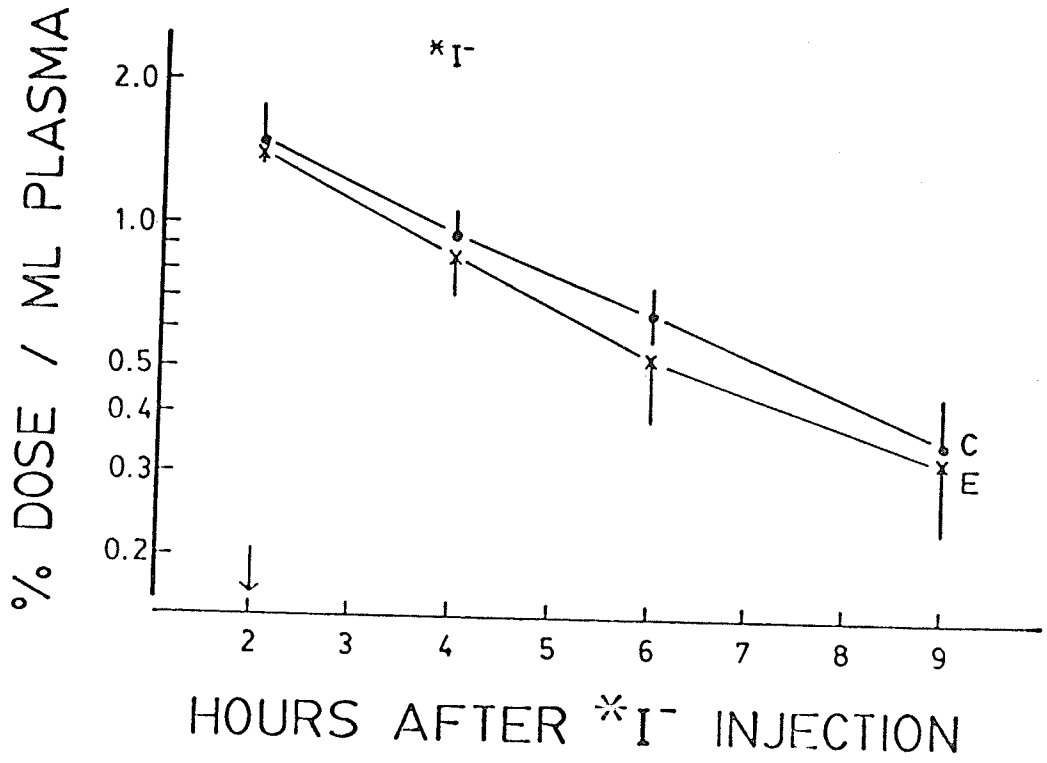
The disappearance of plasma $*I^-$ was exponential in both experimental and control rats (Fig. 6). Although the control and experimental $*I^-$ regression lines covering

Table VII.

Plasma T_3 concentrations and kinetic parameters in blocked control (C) and blocked experimental (E) rats during the first 8 hr after vehicle or epinephrine injection. Plasma T_3 concentrations were the average plasma T_3 values at 4 hr after vehicle or epinephrine injection, determined from previous experiments. Kinetic parameters were based on the metabolism of injected $*T_3$.

parameter	C	E
T_3DS (ml/100 g)	200	200
T_3 concentration (ng/dl)	42.0	21.6
T_3-$\underline{k}$ (fraction/hr)	-0.106	-0.151
T_3CR (ml/hr/100 g)	21.2	30.2
T_3DR (ng/hr/100 g)	8.92	6.54

Figure 6. Changes in control (C) and experimental (E) plasma $*I^-$ radioactivity levels (% dose/ml/100 g) in blocked rats injected with $*I^-$. The arrow (↓) indicates the time of epinephrine or vehicle injection. Geometric means and 95% confidence intervals are plotted. The experimental $*I^-$ regression line from 0-4 hr after epinephrine injection was not significantly different from that of the control over the same interval (Appendix IX).


% DOSE / ML PLASMA
*I⁻
2.0
1.0
0.5
0.4
0.3
0.2
C
E
2
3
4
5
6
7
8
9
HOURS AFTER *I⁻ INJECTION

the entire 7-hr period could not be compared due to heterogeneity of variances, the slopes and elevations of both control and experimental $*I^-$ regressions over a shorter interval (0-4 hr after vehicle or epinephrine administration) were not significantly different.

When the experiment was repeated with non-blocked rats, a similar trend emerged.

D. Thyroid hormone secretion.

1. T_4 secretion.

Two groups of 5 rats were injected with 10 μCi $Na^{125}I$ at 12 hr before the commencement of perchlorate treatment. Blood was sampled at 24 hr after tracer injection and epinephrine (0.7 μg/g) or vehicle was injected into experimental or control rats. Blood samples were taken at 2, 4 and 7 hr after epinephrine or vehicle injection. Plasma $*T_4$ radioactivity was measured. This experiment was repeated and the results from both experiments were pooled.

The experimental $*T_4$ levels (expressed as % value at the time of epinephrine injection) were significantly lower ($P<0.05$) than those of the controls at 4 and 7 hr after epinephrine or vehicle administration (Table VIII).

Using the plasma $*T_4$ levels stated above, in conjunction with data obtained from previous experiments on $*T_4$ kinetics and plasma T_4 concentrations, the T_4SR was calculated for individual control and experimental

Table VIII.

Control (C) and experimental (E) levels of endogenously-produced $*T_4$ (expressed as % value at the time of vehicle or epinephrine injection) in blocked rats preinjected with carrier-free $Na^{125}I$ (n=10, C; n=6, E).

		hours after vehicle or epinephrine injection					
		2		4		7	
		C	E	C	E	C	E
endogenous $*T_4$	$\bar{x}$	103	95	96	76$^+$	102	49$^+$
(% initial value)	SD	20	15	16	15	26	8

$^+$ Significantly different from control values ($P<0.05$, as determined by Behren-Fisher's test or unpaired-t-test).

rats (Table IX). The estimated experimental T_4 secretion rates were significantly lower ($P<0.05$) than those of the controls.

2. Thyroidal T_3/T_4 ratios.

Epinephrine (0.7 μg/g) or vehicle was injected into 15 experimental or 15 control rats pretreated with perchlorate. Thyroids were obtained from 5 rats in each group at 2 hr and from the remaining rats at 4 hr post injection. Thyroidal T_3/T_4 ratios were calculated.

Experimental thyroidal T_3/T_4 ratios were not significantly different at 2 hr but were significantly lower ($P<0.05$) than those of the controls at 4 hr after epinephrine or vehicle injection (Table X).

Table IX.

Individual control (C) and experimental (E) T_4SR values as estimated from changes in the plasma endogenously-labelled $*T_4$ levels from 0-7 hr after vehicle or epinephrine injection in blocked rats. $[T_4]plasma_{0hr}$, as estimated from all available values in blocked rats, was 3.97 μg/dl.

	T_4SR (ng/hr/100 g)	
	C	E
	77.0	-3.0
	27.9	-12.8
	3.6	-21.5
	31.6	-9.8
	22.2	-14.9
	16.0	-7.1
	21.5	
	23.0	
	50.1	
	2.9	
$\bar{x}$	27.6	-11.5[+]
SD	22.1	6.4

[+] Significantly different ($P<0.05$) from control values as determined by Behrens-Fisher's test.

Table X.

Control (C) and experimental (E) thyroidal T_3/T_4 molar ratios in blocked rats at 2 and 4 hr after vehicle or epinephrine injection.

hours after vehicle or epinephrine injection	thyroidal T_3/T_4 ratio					
	C			E		
	n	$\bar{x}$	SD	n	$\bar{x}$	SD
2	5	0.076	0.018	5	0.080	0.014
4	7	0.076	0.012	9	0.129^{+}	0.030

$^{+}$ Significantly different from control values ($P<0.05$, as shown by an unpaired-t-test).

DISCUSSION

A. Effects of epinephrine on the thyroid system.

As shown by the depression of experimental plasma PB*I levels from those of the controls, the effect of epinephrine was transient, lasting for approximately 7 hr (Fig. 1B). This brief action of the epinephrine pulse agrees with the findings of Botkin and Jensen (1952), and is substantiated by the transitory alteration of plasma $*T_4$ and $*T_3$ fractional disappearance rates in experimental animals (Figs. 2, 3 and 4).

A significant depression of plasma PB radioiodine levels was observed also by Williams et al. (1949), Eskelson et al. (1954) and Kallman and Starr (1959), suggesting a lowering of circulating thyroid hormone concentrations by epinephrine. The depression of plasma T_4 and T_3 by epinephrine in this study (Tables III and IV) supports this trend but contradicts the reports by Udupa et al. (1976) and Whitman et al. (1976). Dosage variation was likely not the cause of this difference. Although the depression of plasma T_4 was significant only at the highest dose (0.7 μg/g), the low doses (0.007 and 0.07 μg/g) also depressed plasma T_4 concentrations in blocked rats (Table V). This range of epinephrine doses covered that used by Whitman et al. (1976) (0.01 to 0.5 μg/g) and Udupa et al. (1976) (0.1 μg/g). As Whitman and co-workers

also used Sprague-Dawley rats, differences due to strains can be disregarded.

Depression of plasma thyroid hormone concentrations may result from changes in hormone degradation and/or production. To investigate these possibilities, the effects of epinephrine on plasma $*T_4$, $*T_3$ and $*I^-$ disappearance and T_4SR were followed.

Epinephrine transiently increased the fractional disappearance rates and clearance rates of plasma T_4 and T_3 as judged from changes in the plasma levels of radio-labelled hormones and/or the slopes of their disappearance regressions induced by epinephrine injection (Figs. 2, 3 and 5). In the case of T_4, the DR was also increased by epinephrine despite the lowering of plasma T_4 (Table VI). In the case of T_3, epinephrine depressed both the plasma level and DR (Table VII). Increased experimental T_3 and T_4 fractional disappearance rates could result from increased activities in one or more of the degradation pathways.

Plasma $*I^-$ fractions following $*T_4$ and $*T_3$ injections were generally increased by epinephrine (Figs. 2, 3, 4 and 5). Some of these increases were significant and followed the pattern of changes in $*T_4$ and $*T_3$ disappearance. The less dramatic increase of plasma $*I^-$ radioactivity in blocked rats might have arisen from an increased irreversible loss of plasma radioiodide after blockage of iodide recycling. The temporary increases in plasma radioiodide levels in

experimental rats could be due to alterations in iodide input (eg. deiodination) and/or removal.

The major routes for plasma iodide removal are thyroid uptake and urinary excretion. In non-blocked rats, the total clearance of iodide (including iodide recycling) can be measured, whereas, in blocked rats, the disappearance of iodide approximates the urinary iodide clearance. As indicated by the fractional loss of plasma $*I^-$ (Fig. 6), epinephrine altered neither urinary nor total body clearance of iodide. This supports the report that epinephrine did not affect urinary iodide excretion (Kallman and Starr, 1959).

If the removal of plasma iodide is unaffected by epinephrine, then changes in plasma iodide levels induced by epinephrine will be due to an altered input of iodide. The main sources of $*I^-$ after $*T_4$ or $*T_3$ injections are assumed to be peripheral deiodination of thyroid hormones and thyroidal release after $*I^-$ recycling. Because the total iodide clearance was not affected by epinephrine, an increase in thyroidal $*I^-$ release in non-blocked rats, as observed by Dumas and Guibout (1978), was unlikely. This, together with the knowledge that the iodide derived from intrarenal T_4 and T_3 deiodination is preferentially excreted via the urine (Shimoda and Greer, 1972; Shimoda et al., 1977), indicates that the increase in plasma $*I^-$ after $*T_4$ or $*T_3$ injection in the epinephrine treated rats was the result of an increased deiodination of thyroid

hormones. This does not preclude possible changes in other degradation routes such as, conjugation, deamination and decarboxylation.

The influence of epinephrine on T_4 and T_3 deiodination agrees with reports from other workers (Kallman and Starr, 1959; Escorbar del Rey and Morreale de Escorbar, 1963; Galton, 1965; Hillier, 1968). Liver and kidney are common sites for deiodination (Chopra, 1977). In vitro ^{131}I-T_4 deiodination was increased by epinephrine in mouse liver (Galton, 1965) but decreased in rat muscle homogenates (Kobayashi et al., 1966). Species and tissue differences might be the cause of this conflict. The present study did not provide enough information to further explain this contradiction but suggested the involvement of other deiodination sites besides the kidney. Despite the preferential excretion of $*I^-$ from intrarenal deiodination of $*T_3$ and $*T_4$ (Shimoda and Greer, 1972; Shimoda et al., 1977), plasma $*I^-$ levels were significantly elevated at various times after $*T_4$ injection (Figs. 2 and 4). Other tissues must have contributed to the plasma $*I^-$ pool.

The increased T_4 deiodination may be a means of maintaining plasma T_3 levels in experimental animals. Although the experimental $*T_3$ levels after $*T_4$ injections in both blocked and non-blocked rats did not differ significantly from the control values during the 7-hr period when epinephrine influenced $*T_4$ metabolism (Figs. 2 and 4), an increased monodeiodination of T_4 to T_3

after epinephrine injection could not be discounted. As T_3 had a higher turnover rate than T_4, the level of radioactivity associated with the plasma $*T_3$ fraction after $*T_4$ (2 μCi) injection was low. Alterations in plasma $*T_3$ contents might remain undetected. With T_3 being mainly an intracellular hormone (Oppenheimer et al., 1970), increases in intracellular T_3 might not be reflected in plasma measurements.

Grinberg (1964) observed that epinephrine increased pituitary and brain ^{131}I-T_3 contents after ^{131}I-T_4 injection. An increased monodeiodination of T_4 to T_3 might be outstripped by the epinephrine-induced increase in T_3 fractional disappearance rate, resulting in a net decrease in plasma T_3 levels, and subsequently, a reduced average experimental T_3DR. An available method for calculating T_4 to T_3 conversion (Zimmerman et al., 1978) cannot be applied to the present data because of the low levels of radioactivity in fractions containing $*T_3$ derived from $*T_4$. Without concurrent measurements of T_4 to T_3 conversion, this issue cannot be resolved.

Changes in the experimental T_4 fractional disappearance rates were more dramatic in blocked than in non-blocked rats (Figs. 2 and 3). This might be due to the difference in the amount of epinephrine injected. However, a decrease in T_4 secretion might also be involved due to the presence of iodide recycling.

T_4SR estimations indicated that epinephrine significantly

depressed thyroidal T_4 release (Table IX). The negative value in the experimental group might result from an error in one of the assumptions used in the calculation. It might also signify a complete cessation of secretory processes accompanied by an increase in T_4 degradation.

Without measurements of T_4 to T_3 conversion, T_3 secretion rates could not be calculated. The significant increase in experimental thyroidal T_3/T_4 ratios at 4 hr after epinephrine injection (Table X) refuted the possibility of continual T_3 secretion and supported the hypothesis of complete cessation of secretory processes after epinephrine administration. An intrathyroidal T_4 deiodinating system exists and produces part, if not all of the T_3 released from the thyroid (Greer and Haibach, 1974). With perchlorate blockage, the iodide supply to the thyroids of control and experimental rats were terminated. As iodothyronine synthesis could be inhibited by epinephrine (Joasoo and Murray, 1974a, 1975), the increased thyroidal T_3/T_4 ratio not only suggested that epinephrine increased the intracellular monodeiodination of T_4 to T_3 but also the cessation of T_3 secretion.

The observed epinephrine-induced inhibition of T_4 secretion was at variance with other findings in non-blocked rats (Botkin and Jensen, 1952) and hypophysectomized or T_4-blocked mice (Melander, 1969, 1970; Ericson et al., 1970; Melander and Sundler, 1972a). Differences between species and treatments of experimental animals may partly

explain this conflict. As epinephrine could interfere with TSH stimulation of thyroid activities (Maayan et al., 1977a , b; Sherwin, 1978), the interference with TSH action might be more important than the direct stimulation of thyroid hormone release observed by Melander and co-workers.

Although a reduction in thyroidal blood flow was not accompanied by a similar change in thyroid hormone secretion (Ahn et al., 1969), an epinephrine-induced localized complete occlusion of the thyroidal blood supply could explain the depression of T_4SR and plasma T_4 and T_3 concentrations. Without simultaneous measurements of thyroidal blood flow, this possibility cannot be excluded.

Further investigations should also be performed to locate the sites of increased deiodination after epinephrine administration and the mechanism by which deiodination is induced. Possible effects on other degradation routes should also be explored. Measurements of T_4 to T_3 conversion will not only clarify the nature of the increased T_4 deiodination but also permit the estimation of T_3SR. Knowledge of the effects of epinephrine on TSH metabolism and action, including possible interactions with TSH-receptor binding, may resolve or explain some of the reported conflicts.

If considered separately, the epinephrine-induced depression of T_4SR, stimulation of plasma T_4 and T_3 clearance and depression of plasma T_3 and T_4 concentrations

would suggest that epinephrine had an antithyroidal effect. Knowing the role that thyroid hormones and epinephrine played in altering cardiac function, lipolysis and metabolic rates in response to cold, emotional stress and other environmental stimuli (Swanson, 1956; Spaulding and Noth , 1975 ; Kaciuba-Uścilko et al., 1976; Brzezińska and Kaciuba-Uścilko, 1977), the present findings suggested a mechanism whereby the interactions between thyroid hormones and epinephrine might be manifested and controlled.

Stress increases the metabolism of thyroid hormones and epinephrine (Habermann et al., 1977). T_3 increases the number of β-receptors and potentiates the epinephrine-induced increase in cAMP (Tsai and Chen, 1977: Williams et al., 1977; Issekutz, 1978). Epinephrine increases heart rate; atrial contractibility and conduction rate; ventricular contractibility, automaticity and conduction rate; conduction velocity of A-V node; mobilization of glucose; free fatty acid level; and dilation of arteries via β-receptors (Moran, 1975). Hypothyroidism increases monoamine oxidase and catechol-O-methyltransferase activities (Landsberg and Axerod, 1968). Epinephrine, by directly or indirectly stimulating T_4 deiodination, could increase the production of T_3. A localized increase in T_3 level would potentiate the action of epinephrine. The depression of thyroid hormone secretion and stimulation of T_4 and T_3 clearance would create a 'hypothyroid' situation which could accelerate epinephrine metabolism.

In conclusion, epinephrine depressed plasma thyroid hormone concentrations by transiently increasing plasma hormone clearance and inhibiting thyroid hormone release. Increased plasma hormone clearance was accomplished, at least partly, by increasing deiodination.

B. Other factors that might influence plasma T_4 and T_3 metabolism.

1. Ether anaesthetic and serial blood sampling.

As operative procedures were short, ether anaesthetic was used as suggested by Ben et al. (1969). Although ether might inhibit TRH release (Ohtake and Bray, 1975). the combined process of animal transfer and ether anaesthesia did not alter serum TSH and T_4 levels (Fenske and Wuttke, 1977; Wong et al., 1977). Unlike serial orbital sinus puncture under ether anaesthesia (Simpkins et al., 1978), the current procedure (serial venipuncture under ether) did not affect the plasma T_4 concentrations in the vehicle-injected controls (Tables III and IV). Changes in the control PB*I values (Fig. 1A) were observed but could be explained by the diurnal variation in thyroid iodine metabolism (Pallardo et al., 1976; Jolin and Tarin 1974). Plasma T_3 levels in the non-blocked controls were also not significantly altered by the anaesthesia/venipuncture procedure (Table III). Alterations of control T_3 levels in blocked rats (Table IV) might be the result of the perchlorate treatment.

2. Body weight.

As in other studies (Azizi, 1975; Sartin et al., 1977), a significant negative correlation ($r = -0.567$, $P<0.01$) existed between plasma T_4 concentration and body weight when data from non-blocked rats used in this study and preliminary experiments were pooled (Fig. 7A). The possible influence of perchlorate treatment on measurements of plasma thyroid hormone concentrations was eliminated by excluding values from blocked rats. The absence of a significant negative relationship between T_3 concentration and body weight (Fig. 7B) conflicts with the report by Sartin et al. (1977). It might be the result of the small T_3 sample size used in this investigation.

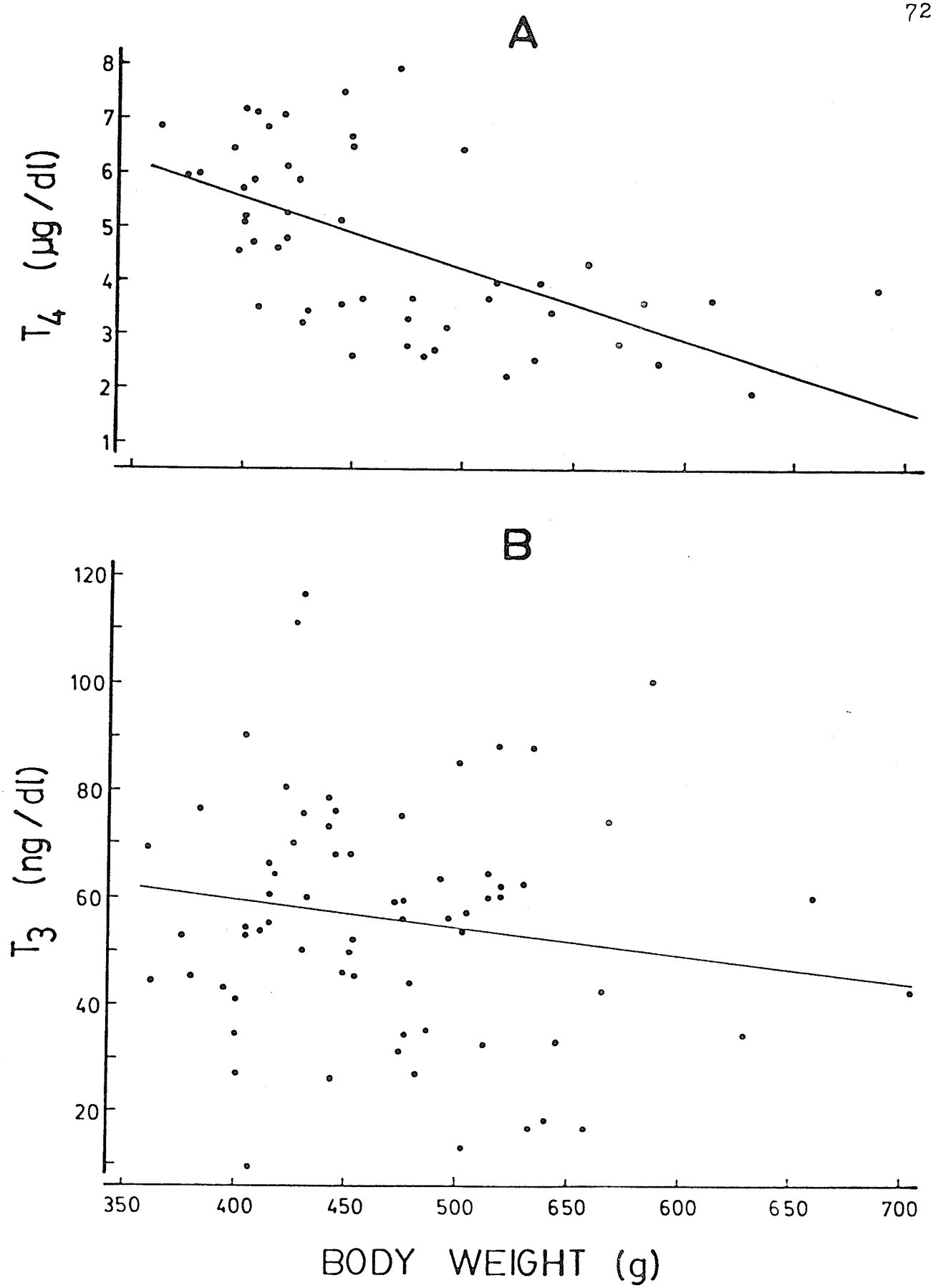
Generally, age and body weight are positively correlated. An increased T_4 degradation, decreased T_4 and T_3 secretion, depressed thyroidal response to TSH, and an increased number of hyperplastic thyroid follicles and vacuolated thyrotrophs are associated with ageing rats (Gregerman and Crowder, 1963; Kumaresan and Turner, 1967 Azizi, 1975; Garner and Bernick, 1975; Sartin et al., 1977).

In each experiment, the influence of body weight was minimized by using control and experimental rats of similar sizes and by expressing plasma T_3 and T_4 concentrations as % original values before control and experimental hormone levels were compared.

Figure 7. Relationship between plasma thyroid hormone concentration and body weight for non-blocked rats.

A. The relationship between plasma T_4 concentration and body weight is shown. Regression equation: Hormone Concentration = 10.6 - 0.013(weight). Regression Coefficient = -0.567 ($P<0.01$).

B. The relationship between plasma T_3 concentration and body weight is shown. Regression Coefficient = -0.106 (not significant).


A
T_4 (μg/dl)
8
7
6
5
4
3
2
1
B
T_3 (ng/dl)
120
100
80
60
40
20
350
400
450
500
650
600
650
700
BODY WEIGHT (g)

3. Perchlorate treatment.

Results from this study and preliminary experiments showed that perchlorate treatment not only lowered plasma T_3 and T_4 concentrations ($P<0.05$) in rats (Table XI), but also the response of plasma T_3 levels to vehicle injections (Tables III and IV). Body weight difference was not the cause of the depressed plasma T_4 level in blocked rats (Table XI). As no significant relationship existed between body weight and plasma T_3 concentration in the non-blocked rats used in this study (Fig, 7B), variation in body weight was not likely the reason behind the difference in T_3 concentrations between blocked and non-blocked rats.

Perchlorate treatment did not alter the effects of epinephrine on plasma T_4 concentrations and T_4 degradation (Tables III, IV and VI). However, the diminished response of plasma T_3 concentrations in blocked rats to epinephrine injection (Table IV) might be the result of the perchlorate-induced depression in 'basal' T_3 level (Table XI).

As perchlorate not only could lower plasma T_3 and T_4 concentrations but also displace ^{131}I-T_4 from rat plasma prealbumin (Yamada and Jones, 1968), it should only be used when blockage of recycling is essential.

Table XI.

Comparison of plasma T_4 or T_3 concentrations (conc) between blocked and non-blocked rats of various sizes.

		blocked			non-blocked		
		n	$\bar{x}$	SD	n	$\bar{x}$	SD
T_4	conc (μg/dl)	38	3.97[+]	0.99	50	4.62	1.64
	weight (g)	38	493	122	50	466	72
T_3	conc (ng/dl)	30	33.2[+]	12.5	35	59.3	21.8
	weight (g)	30	381[+]	33	35	420	27

[+] Significantly different from non-blocked rats ($P<0.05$, as determined by Behrens-Fisher's test or unpaired-t-test).

SUMMARY

A. As determined by changes in plasma PB*I levels, the effect of a single intramuscular injection of epinephrine on the thyroid system lasted for approximately 7 hr.

B. Epinephrine lowered plasma T_4 and T_3 concentrations significantly.

C. Depression of plasma T_4 levels was dose related.

D. Depression of plasma thyroid hormone concentrations was achieved by inhibiting hormone release from the thyroid and accelerating the plasma clearance of T_4 and T_3.

E. Increased plasma clearance was accounted for, at least partly, by increased T_4 and T_3 deiodination.

F. Further studies are required to determine the mechanism by which increased deiodination is achieved.

G. The effects of epinephrine on some other facets of the thyroid system still need to be determined.

H. As in other studies, a significant negative correlation existed between plasma T_4 concentration and body weight.

I. Besides other known effects on the thyroid system, $KClO_4$ lowered plasma T_4 and T_3 values significantly.

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APPENDIX

Appendix I.

Individual haematocrit ratios in the 1st and 5th blood sample taken from serially sampled rats. The 5th sample was taken 12 hr after the first. Changes in the haematocrit ratios were not significant as shown by a paired-difference test.

	haematocrit ratio (%)		
	1st sample	5th sample	changes (1st - 5th)
	47.5	47.0	-0.5
	52.0	52.5	0.5
	50.0	50.0	0.0
	47.5	50.5	3.0
	47.5	45.0	-1.5
	53.0	52.5	-0.5
	49.0	49.0	0.0
	51.0	52.0	1.0
$\bar{x}$	49.7	49.8	0.3
SD	2.2	2.7	1.3

Appendix II.

Comparison of plasma $*T_4$ fractions in $*T_4$-injected rats obtained by diluted human plasma (HP) or T_4-antibody. Results were averages of duplicate runs.

total plasma radioactivity (TPR) in cpm	$*T_4$ fraction radioactivity			
	HP		T_4-antibody	
	cpm	% TPR	cpm	% TPR
137	93	68	98	72
1568	1083	69	1068	68
6079	5755	95	5780	95

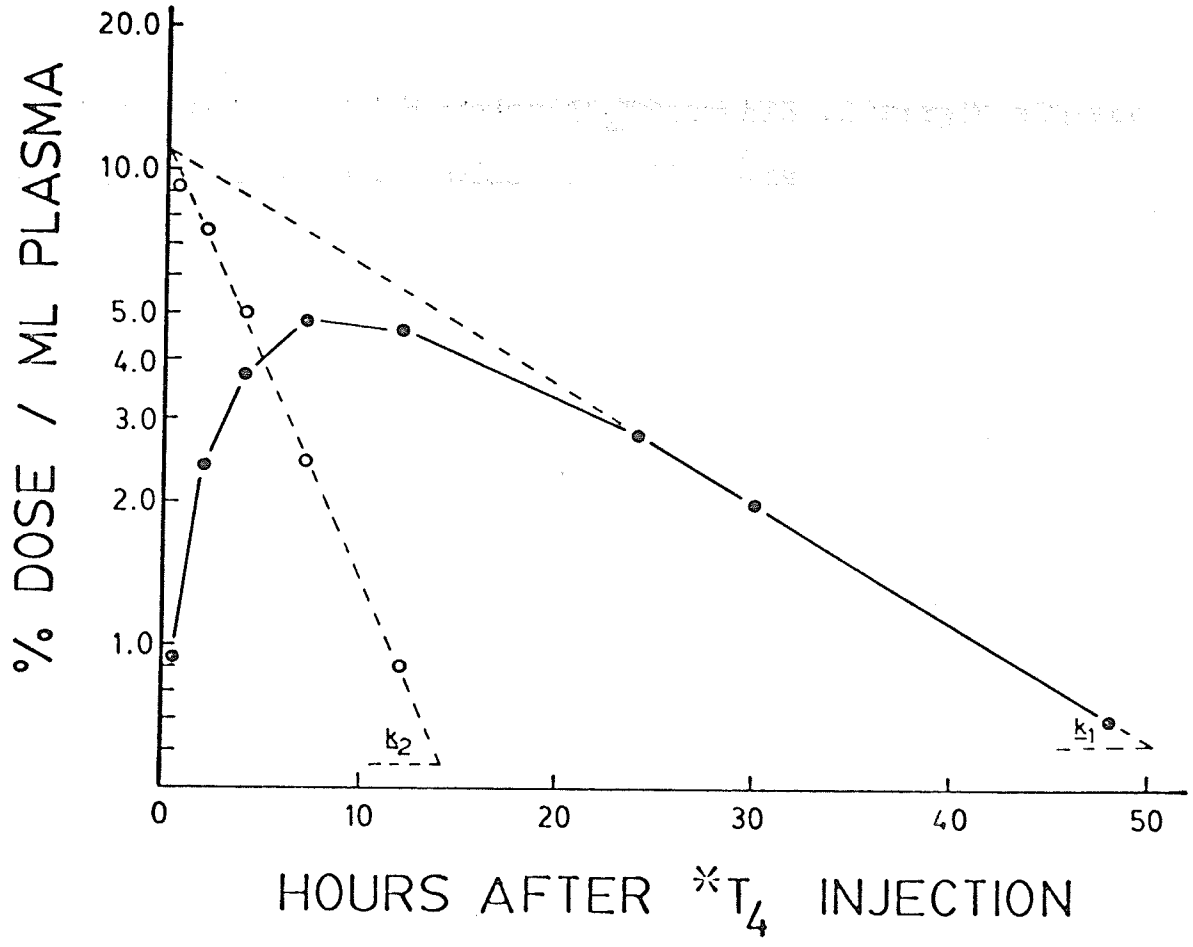
Appendix III.

Time course of $*T_4$ disappearance and correction for $*T_4$ loss during uptake: a preliminary study.

$*T_4$ tracer (2 μCi) in 40 μl of 50% propylene glycol was injected intraperitoneally into a rat. Plasma $*T_4$ radioactivity was evaluated at ½, 2, 4, 7, 12, 24, 30 and 48 hr post-injection. These values were plotted semi-logarthmically against time. A first regression line was fitted to the final portion of the $*T_4$ disappearance curve (24-48 hr) and its slope ($\underline{k}_1$) determined (Appendix Fig. 1). The differences between the observed $*T_4$ levels in the non-linear portion and the extrapolated values at these times (o) were plotted. The slope ($\underline{k}_2$) of the line of best fit between these points and the observed y-intercept was calculated. The corrected intercept was calculated as in Eales (1977b):-

$$\text{corrected intercept} = \text{observed intercept} \times \frac{\underline{k}_2 - \underline{k}_1}{\underline{k}_2}.$$

Appendix Figure 1. Plasma $^*T_4$ levels at various times after $^*T_4$ injection. ($\underline{k}_1 = -0.057$; $\underline{k}_2 = -0.206$.)

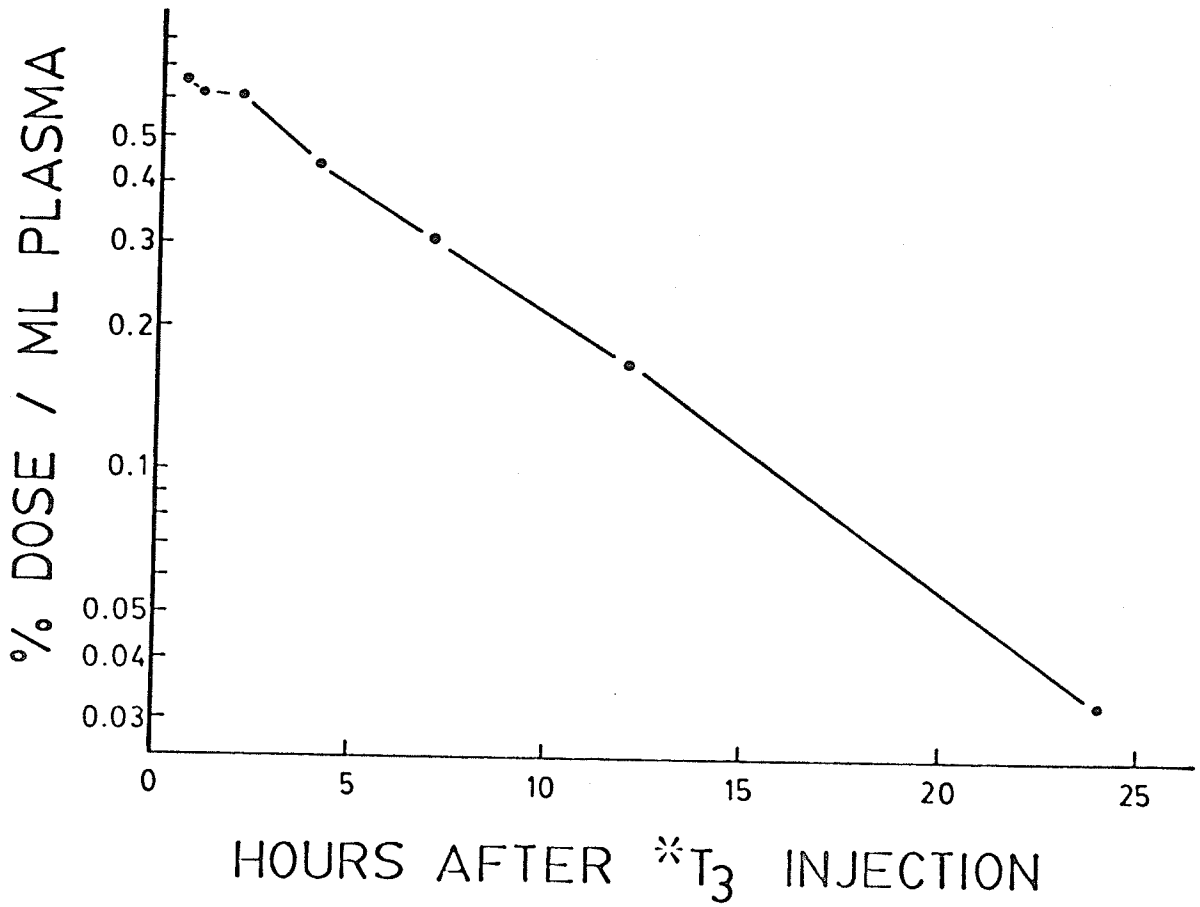

20.0
10.0
5.0
4.0
3.0
2.0
1.0
% DOSE / ML PLASMA
k2
k1
0
10
20
30
40
50
HOURS AFTER *T4 INJECTION

Appendix IV.

Time course of $*T_3$ disappearance: a preliminary study.

$*T_3$ tracer (5 μCi) in 40 μl of 50% propylene glycol was injected intraperitoneally into a blocked rat. Plasma $*T_3$ radioactivity was evaluated at ½, 2, 4, 7, 12 and 24 hr post-injection. These values were plotted semilogarithmically against time. An uptake phase was not observed (Appendix Fig. 2).

Appendix Figure 2. Disappearance of injected *T_3 from the plasma of a blocked rat : a preliminary time course study.


% DOSE / ML PLASMA
0.5
0.4
0.3
0.2
0.1
0.05
0.04
0.03
0
5
10
15
20
25
HOURS AFTER *T3 INJECTION

Appendix V.

Analysis of covariance for control (C) and experimental (E) $*T_4$ regression from 24-31 and 31-48 hr after $*T_4$ injection in non-blocked rats. Vehicle or epinephrine was injected 24 hr after $*T_4$ administration.

group	regression		correlation	$(SD)_x$	$(SD)_y$	df
	a	b	coefficient			
A. C 24-31 hr	2.115	-0.032	-0.522$^+$	2.619	0.159	38
E 24-31 hr	4.150	-0.116	-0.875$^+$	2.580	0.241	33
B. C 31-48 hr	2.704	-0.051	-0.884$^+$	7.256	0.416	28
E 31-48 hr	2.377	-0.061	-0.856$^+$	7.261	0.514	24

Tests:-

A. 24-31 hr

1. Homogeneity of within group variances:
   F=1.622 (df=33,38) Not Significant (NS)

2. Comparison of slopes:
   F=34.988 (df=1,71) P<0.01

B. 31-48 hr

1. Homogeneity of within group variances:
   F=1.875 (df=24,28) NS

2. Comparison of slopes:
   F=1.287 (df=1,52) NS

$^+$ Significant, P<0.01.

Appendix VI.

Analysis of covariance for control (C) and experimental (E) $*T_4$ regression from 24-31 and 31-48 hr after $*T_4$ injection in blocked rats. Vehicle or epinephrine was injected 24 hr after $*T_4$ administration.

group	regression		correlation	$(SD)_x$	$(SD)_y$	df
	a	b	coefficient			
A. C 24-31 hr	1.930	-0.042	-0.593$^+$	2.619	0.184	38
E 24-31 hr	2.658	-0.078	-0.685$^+$	2.619	0.297	38
B. C 31-48 hr	2.437	-0.057	-0.891$^+$	7.256	0.463	28
E 31-48 hr	2.394	-0.069	-0.787$^+$	7.256	0.635	28

Tests:-

A. 24-31 hr

1. Homogeneity of within group variances:

F=2.143 (df=38,38) P<0.05 ∴no further comparison is possible.

B. 31-48 hr

1. Homogeneity of within group variances:

F=3.479 (df=28,28) P<0.01 ∴no further comparison is possible.

$^+$ Significant, P<0.01.

Appendix VII.

Analysis of covariance for control (C) and experimental (E) $*T_3$ regression from 4-12 hr after $*T_3$ injection in blocked rats. Vehicle or epinephrine was injected 4 hr after $*T_3$ administration.

group	regression		correlation	$(SD)_x$	$(SD)_y$	df
	a	b	coefficient			
C 4-12 hr	-0.677	-0.106	-0.877^+	3.149	0.381	28
E 4-12 hr	-0.542	-0.151	-0.892^+	3.107	0.527	22

Tests:-

1. Homogeneity of within group variances:
   F=1.708 (df=22,28) NS

2. Comparison of slopes:
   F=5.628 (df=1,50) P<0.05

$^+$ Significant, P<0.01.

Appendix VIII.

Control (C) and experimental (E) plasma $*T_3$ and $*I^-$ levels (expressed as % value at the time of vehicle or epinephrine injection) in blocked rats injected with $*T_3$. Vehicle or epinephrine was injected 4 hr after $*T_3$ administration.

		hours after vehicle or epinephrine injection							
		3		7		8		20	
		C	E	C	E	C	E	C	E
$*T_3$ (%)	$\bar{x}$	70.2	38.6	44.1	32.1	46.2	36.0	11.9	8.7
	SD	12.1	11.5	7.9	11.8	8.9	3.6	4.0	2.3
	n	10	8	5	5	5	3	10	8
$*I^-$ (%)	$\bar{x}$	133	130	153	183	139	160	29	33
	SD	21	19	39	55	61	37	10	28
	n	10	8	5	5	5	3	10	8

Appendix IX.

Analysis of covariance for control (C) and experimental (E) $*I^-$ regression from 2-9 and 2-6 hr after $*I^-$ injection in blocked rats. Vehicle or epinephrine was injected at 2 hr after $*I^-$ administration.

group	regression		correlation	$(SD)_x$	$(SD)_y$	df
	a	b	coefficient			
A. C 2-9 hr	0.820	-0.207	-0.977^+	2.653	0.563	18
E 2-9 hr	0.757	-0.217	-0.936^+	2.653	0.472	18
B. C 2-6 hr	0.840	-0.213	-0.962^+	1.690	0.374	13
E 2-6 hr	0.873	-0.236	-0.928^+	1.690	0.472	13
combined	0.873	-0.236	-0.928^+	1.661	0.423	28

Tests:-

A. 2-9 hr

1. Homogeneity of within group variances:
   F=3.026 (df=18,18) P<0.05 ∴no further comparison is possible.

B. 2-6 hr

1. Homogeneity of within group variances:
   F=2.911 (df=13,13) NS
2. Comparison of slopes:
   F= 1.954 (df=1,26) NS
3. Comparison of elevations:
   F=4.930 (df=1,27) NS

$^+$ Significant, P<0.01.

Appendix X.

Diagrammatic representation of the metabolism of T_4 and T_3.