representative for circulating radioactivity and a 'liver region' representative for the storing organ. Figure 2 shows the time course of radioactivity detected in these 2 defined regions: A. Sequestration of neuraminidase-treated erythrocytes is completed after 6 min. Increase in the liver region and decrease in the heart region are closely correlated. B. After preinjection of glycolipids sequestration of neuraminidase-treated erythrocytes is delayed by a factor of about 3. Here again, the decrease in the heart region corresponds with the increase in the liver region. The results of experiments with different erythrocytes and different competing agents are listed in the table. The observed homing of erythrocytes, either neuraminidase-treated or xenogeneic is irreversible: after 24 and 48 h the radioactivity is still localized in the liver region (the term 'last homing' would therefore be more appropriate). It is probable that the erythrocytes reach the liver as intact cells: a) in vitro incubation of the used erythrocytes with rat serum did not lead to fragmentation. b) Another argument comes from homing experiments with lysed ^{111}Indium labelled erythrocytes. When this lysate was injected into rats, the radioactivity stayed first in circulation, slowly accumulating in the kidneys rather than in the liver. (In this lysate Indium radioactivity is associated to more than 95% with hemoglobin)[9]. This may indicate that surface components are recognized by liver cells. Preinjected glycolipids and glycoproteins may block presumptive recognition sites. This is in line with Ashwell's experiments; however, there seems to be a major difference: Desialiated glycoproteins are obviously trapped and incorporated by liver parenchymal cells[5] whereas erythrocytes homing to liver appear to be taken up by sinusoidal cells[10]. Thus it may be concluded that RES cells of the liver recognize altered or xenogeneic cells by surface components. This could be relevant not only for the elimination of erythrocytes. Tumor cells could show similar signs of 'foreigness' and indeed, tumor cells have been found to home to liver irreversibly[4,11]. The functional integrity of liver sinusoidal cells may thus play an important part in the control of tumor cell populations.

Liver sequestration kinetics

Erythrocytes and competing agents	Time for maximal sequestration	
Rat erythrocytes neuraminidase-treated	5 ± 1.5	(7)
Gangliosides (5 mg/animal)	14 ± 4	(4)
(10 mg/animal)	25 ± 5	(2)
Methylglucose	> 15	(2)
Galactose	> 15	(2)
Bovine erythrocytes	18 ± 4	(6)
Glycopeptides (10 mg/animal	> 30	(4)
Bovine albumine (10 mg/animal)	15 ± 5	(3)

The agents mentioned were preinjected 3 min before injection of the labelled erythrocytes. Details see text. Number of animals given in parentheses.

9 I. Kampfer, K. Nissler and J. Lobe, XIII. Symposion der Sektion Nuklearmedizin der Gesellschaft für Medizinische Radiologie, 17.–21. März 1976 Reinhardsbrunn, in press.
10 D. M. Bissell, L. Hammaker and R. Schmid, J. Cell Biol. *54*, 107 (1972).
11 S. Draz, J. E. Byfield, P. Byfield and E. W. Fonkalsrud, Arch. Surg. *102*, 574 (1971).

Changes in the T_4/T_3 molar ratio following thyrotrophin releasing hormone injection in cattle

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Summary. The injection of thyrotropin releasing hormone into cattle resulted in a rapid decrease in the T_4/T_3 molar ratio. 2 breeds of cattle, Shorthorn and Africander Cross were studied. The decrease in the T_4/T_3 molar ratio was significantly greater in the Shorthorn breed. It is concluded that acute stimulation of the thyroid gland with TRH results in enhanced release of both T_3 and T_4 and that T_3 is discharged more rapidly than T_4.

There are marked differences among species in the ratio of thyroxine (T_4) to triiodothyronine (T_3) in the thyroid gland. Thus the T_4/T_3 ratio in man has been reported to be 20:1[1] and in rat 6:1[2]. Since there appears to be only very limited conversion of T_4 to T_3 in the thyroid[3,4] it is reasonable to expect that, in general, T_3 and T_4 will be secreted in the same ratio as they occur in the gland. However, little is known about the variation of the thyroid T_4/T_3 ratio within a particular species or of conditions which may alter the secretion rate of T_4 to T_3. In this study we report acute changes in the serum T_4/T_3 molar ratio in 2 breeds of cattle following the injection of TRH.

Methods. 2 groups of 6 cattle, one comprised of Shorthorns (SH), a temperate climate adapted breed and the other of Africander Crosses (AX), a tropically adapted breed, were injected i.v. with TRH (Calbiochem, Lot 30075). 3 injection schedules were used: a) a single injection of either 0.4, 1, 2.5 or 5 μg/kg live weight (l.wt), b) 2 injections of 3 μg/kg l.wt 24 h apart or c) repeated injections of increasing amounts of TRH (1, 2, 3, 4 and 5 μg/kg l.wt) hourly over 4 h. This latter injection schedule gave a total dose of 15 μg/kg l.wt per animal. Blood samples were taken at intervals from a jugular vein and the resulting serum samples analyzed using radioimmunoassays for thyroid stimulating hormone (TSH), T_3 and T_4. Experiments were carried out at least 1 week apart to allow a recovery period before the next injection of TRH.

1 T. J. Chopra, D. A. Fisher, D. H. Solomon and G. N. Beall, J. clin. Endocr. Metab. *36*, 311 (1973).
2 R. Pitt-Rivers and J. E. Rall, Endocrinology *68*, 309 (1961).
3 H.Haibach, Endocrinology *88*, 918 (1971).
4 H. Haibach, Metabolism *20*, 819 (1971).

Results. The mean preinjection T_4/T_3 molar ratio ± SE calculated from 4 separate estimates throughout the experimental period was 52.1 ± 3.1–57.5 ± 5.5 for the SH group and 46.7 ± 2.9 for the AX group ($p = 0.1–0.05$). There was no dose-response relationship following single injections of TRH for either TSH, T_3 or T_4 and results from all doses have been combined. The maximal TSH response occurred within 15 min of TRH injection, maximal T_3 at 4.5 h and maximal T_4 at 6–7 h (figure 1). There was a significant difference ($p = 0.001$) between breeds at the maximal T_3 response.

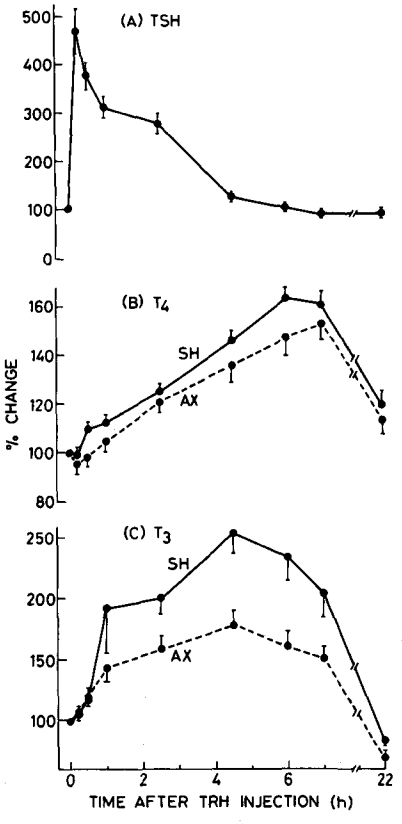

Fig. 1. Percentage changes in serum TSH, T_3 and T_4 in AX and SH breeds of cattle, following a single injection of TRH. Mean ± SE. For TSH results, both breeds combined.

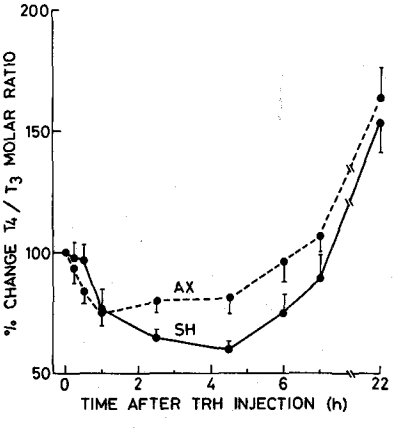

Fig. 2. Percentage change in serum T_4/T_3 molar ratio in AX and SH breeds of cattle following a single injection of TRH. Mean ± SE.

The percentage changes in the T_4/T_3 molar ratios from the initial value is shown in figure 2. The ratio began to decline almost at once and by 1 h the ratio for both breeds was 75% of the initial value. In the AX group the ratio then began to rise while in the SH group it continued to fall, reaching at 4.5 h a minimum of 60% of the initial value. There was a significant difference between the responses of the 2 breeds at 2.5, 4.5 and 6 h ($p = 0.02$, 0.01 and 0.05 respectively). By 22 h both breeds had T_4/T_3 molar ratios well above the initial value, 153% in the SH group and 164% in the AX group. Following 2 injections of TRH 24 h apart a decrease in the T_4/T_3 molar ratio was observed on both days. This decrease was greater in the SH group, particularly on the second day (table).

When repeated injections of TRH were given on the same day, the T_4/T_3 molar ratio was 60%, 4 h after the first TRH injection, and although still low, had increased to 68% by 8 h, despite the continued injections.

Discussion. The T_4/T_3 molar ratio significantly and rapidly decreased following a single injection of TRH into cattle. The magnitude of this response varied between individuals and also between the 2 breeds. The more marked decrease in the SH breed suggests there is a real difference in thyroid function between the 2 breeds. This could well be a consequence of adaptive changes to the different climates for which they have been bred.

There is little evidence about conditions which may change the serum T_4/T_3 ratio in normal animals. In the rat iodine deficiency lowers the T_4/T_3 ratio in the thyroid gland[5] and changes in the ratio have also been reported in the neonatal rat[6]. The daily injection of TSH increases the ratio of T_3 to T_4 in stored thyroglobulin[7]. Whether there were corresponding changes in the relative secretion rates of T_3 and T_4 were not reported.

The changes in serum T_4/T_3 molar ratio which were observed following TRH injection could have been brought about in several ways. The rate of secretion of T_3 from the thyroid gland could have changed relative to that of T_4. The relative rate of clearance of T_3 and T_4 from the blood could also have changed or there could have been increased peripheral conversion of T_4 to T_3.

5 R. Pitt-Rivers and R. R. Cavaliere, in: The Thyroid Gland, p. 87. Ed. R. Pitt-Rivers and W. R. Trotter. Butterworths, London 1964.

6 W. H. Florsheim, M. A. Faircloth, N. L. Corcorran and P. Rudko, Acta endocr. *52*, 375 (1966).

7 M. A. Greer and C. Rockie, Endocrinology *85*, 244 (1969).

Effect of 2 injections of TRH (3 μg/kg lwt) 24 h apart on the T_4/T_3 molar ratio of 2 breeds of cattle (% change from pre experimental value)

Time	Day 1 0 h	Day 1 4 h	Day 1 8 h	Day 2 0 h	Day 2 4 h	Day 2 8 h
Shorthorn	100	52.8 ±6.5	78.3 ±11.2	112.0 ±21.3	73.3 ±14.3	96.7 ±19.6
Africander	100	63.3 ±4.7	115.7 ±13.6	170.7 ±22.4	124.3 ±13.5	144.5 ±14.4
Probability		NS	0.05	NS	0.05–0.02	0.01–0.05

NS, not significant.

There is no evidence that either TRH or TSH is capable of directly altering the rate of peripheral conversion of T_4 to T_3. The daily injection of T_4 into man, increased plasma T_3 concentration and the clearance rate of both T_3 and T_4 resulted in a nett increase in the T_4/T_3 ratio[8]. On the other hand a single i.v. injection of T_4 did not alter the fractional turnover rate of T_3 for at least 1 h after injection, although it had decreased by 24 h. Thus increased secretion of T_4 alone, while altering the plasma T_3 concentration and clearance rate does not lead to an immediate decrease in the T_4/T_3 ratio.

It appears therefore that the decrease in the T_4/T_3 molar ratio that follows the acute stimulation of the thyroid gland is due to increased secretion of T_3 relative to T_4. This relative increase in T_3 to T_4 secretion is probably underestimated. This is because although binding of T_3 to thyroid binding globulin does occur[9], it is probably not tightly bound[10] and therefore will be removed more rapidly from the circulation than T_4, most of which is bound to plasma proteins. The short term release of stored thyroglobulin ought not cause rapid changes in the T_4/T_3 ratio. However, by whatever mechanism this increased T_3 secretion is brought about, the nett result is clearly an increase in the active form of the circulating thyroid hormones.

We conclude that in non-pathological conditions 2 mechanisms operate to meet tissue thyroid hormone requirements. Normally, T_3 requirements are met largely by peripheral deiodination of T_4 to T_3, the active form of the hormone. However, under conditions of increased demand for thyroid hormones the release of both hormones is enhanced, with T_3 being discharged more rapidly than T_4.

8 L. E. Braverman, A. Vagenakis, P. Downs, A.E. Foster, K. Sterling and S. H. Ingbar, J. clin. Invest. *52*, 1010 (1973).
9 K. A. Woeber, E. Hecker and S. H. Ingbar, J. clin. Invest. *49*, 650 (1969).
10 A. A. Zaninovich, H. Farach, C. Ezrin and R. Volpé, J. clin. Invest. *45*, 1290 (1966).

Effect of growth factors on hepatic drug metabolism in diabetic-hypophysectomized rats

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Summary. In vivo administration to diabetic-hypophysectomized rats of either the growth factor produced by the plerocercoid larvae of the tapeworm, Spirometra mansonoides, or mammalian growth hormone caused inhibition of hepatic drug metabolism measured in vitro.

Hormonal control of hepatic metabolism is, at best, only vaguely understood. Recent investigations have shown that the growth factor produced by the plercoceroid larvae of the tapeworm, Spirometra mansonoides, causes inhibition of hepatic drug metabolism in hypophysectomized rats[1]. The inhibition of drug metabolism by the plerocercoid growth factor (PGF) is accompanied by enhanced growth[1] and is similar to the response observed when mammalian growth hormone is administered to hypophysectomized rats[2]. Since alloxan diabetes, a condition of abnormally low insulin levels, is known to affect drug metabolism in rats[3,4] and since GH is reported to have some insulin-like as well as anti-insulin-like activity[5], it is important to know whether the effect of growth factors on hepatic drug metabolism in hypophysectomized rats is dependent on or independent of normal insulin levels. Therefore, the present study was designed to determine the effect of PGF and bovine growth hormone (BGH) on hepatic drug metabolism in diabetic-hypophysectomized rats.

Methods. 3 weeks after hypophysectomy (Hormone Assay, Chicago, Ill.) male Sprague-Dawley rats (approximately 100 g) were injected i.p. with alloxan monohydrate (Eastman) (250 mg/kg b.wt) to induce diabetes. Serum sugar concentrations were determined[6] with blood collected by orbital sinus puncture. Only those rats with fed serum sugar concentrations greater than 300 mg/100 ml 4 days after alloxan injection were considered to be diabetic. In one experiment rats were treated with PGF by the s.c. injection of 10 plerocercoids/rat[7] 4 days after alloxan treatment. In another experiment rats were treated with BGH (NIH-GH-B-18, a gift of the Endocrine Study Section, NIH, Bethesda, Maryland) by daily s.c. injection of 500 µg of the hormone dissolved in 0.85% NaCl beginning 9 days after alloxan injection. 7 days subsequent to the initiation of either treatment, the rats were sacrificed after a 12-h fast and the livers used for in vitro drug metabolism studies as previously described[1].

Table 1. Effect of in vivo treatment of diabetic-hypophysectomized rats with plerocercoid growth factor (PGF) on hepatic drug metabolism of aminopyrine and aniline in vitro[a]

Drug substrate	Control	PGF-treated[b]	Inhibition (%)
	(µmoles/min g liver)	(µmoles/min g liver)	
Aminopyrine	51.72 ± 2.86 (9)	38.05 ± 3.37 (6)[c]	26
Aniline	12.81 ± 0.59 (9)	7.02 ± 0.58 (6)[d]	45

[a] The results are expressed as formaldehyde-formed or p-aminophenol-formed with aminopyrine or aniline as substrate, respectively. The numbers given are mean ± SEM (number of animals). [b] Treatment conditions are described in methods. [c] < 0.02 versus control. [d] $p < 0.01$ versus control.

1 D. E. Cook and C. K. Phares, Biochem. Pharmac. *24*, 1919 (1975).
2 E. Wei and J. T. Wilson, J. Pharmac. exp. Ther. *177*, 227 (1971).
3 R. L. Dixon, L. G. Hart and J. R. Fouts, J. Pharmac. exp. Ther. *133*, 7 (1961).
4 R. L. Dixon, L. G. Hart, L. A. Rogers and J. R. Fouts, J. Pharmac. exp. Ther. *142*, 312 (1963).
5 T. J. Merimee and D. Rabin, Metabolism *22*, 1235 (1973).
6 A. Hyrärinen and E. A. Nikkila, Clinica chem. Acta 7, 140 (1963).
7 J. F. Mueller, J. Parasit. *55*, 167 (1968).