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SHORT PAPER

Genetic variation in plasma thyroxine levels and minimal metabolic rates of the mouse, *Mus musculus*

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SUMMARY

A study of inbred strains of the mouse using competitive protein-binding techniques revealed significant strain variation in the total plasma thyroxine levels and in the plasma-free thyroxine index. Measurements of minimal metabolic rates also showed strain variation, but the positive correlation which might be expected between the plasma-free thyroxine index and the minimal metabolic rate did not obtain. Possible explanations are discussed.

There are numerous reports of genetic variation in the rate of production and plasma levels of thyroid hormones in various vertebrate species, as determined by indirect measures of hormone production rate (for example, rates of radioiodine uptake and release) or of hormone levels (for example, protein-bound iodine in plasma) (Silverstein, Sokoloff, Mickelsen & Jay, 1960; Chai & Dickie, 1966; Farrington & Mellen, 1967; Chai & Melloh, 1972; Synenki, Eisen, Matrone & Robison, 1972; Davis, Laird & Fox, 1974). We present here the first report of the range of plasma thyroxine values in different strains of the mouse obtained with a competitive protein-binding technique of high specificity. In the rat, Feuer (1969) has found strain differences in circulating thyroxine levels, but using a rather less specific method; and although Kojima and colleagues (1976) have made specific measurements of plasma thyroxine and tri-iodothyronine, they found a strain difference only of marginal statistical significance in thyroxine values between SHR and Wistar animals. In addition, as thyroid hormones are known to affect the minimal metabolic rate (Denckla, 1970, 1973), we also report measurements of this parameter.

All mice were bred and maintained under controlled conditions of lighting, temperature, diet and housing density. In the first series of assays, all 10-week-old male animals of the strains CBA/FaCam, C57Bl/Fa, C3H, Balb/c and Peru which became available during the study period were anaesthetized with ether and weighed. Blood was obtained by cardiac puncture, and plasma was separated by centrifugation and stored at −20 °C until assay. Thyroxine was then extracted by treatment of the plasma with 75% (v/v) ethanol; the supernatant was kept

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and evaporated to dryness. Thyroxine was then determined by a competitive protein-binding method, as described by Seth (1973*a*, *b*).

In a further series of experiments, using plasma samples from animals of the strains C3H, C57Bl/Fa and CBA/FaCam, the residual binding capacity of the plasma thyroxine-binding globulin was also measured using a commercial test kit (the STA T_3 (TBG) uptake test, Oxford Laboratories). The principle behind the assay is that plasma is incubated with an excess of labelled tri-iodothyronine; the proportion of this which is bound by the plasma indicates the residual binding capacity of the plasma proteins, mainly thyroxine binding globulin, as tri-iodothyronine binds to the same sites as thyroxine but with a lower affinity (Clark, 1967; Standeven, Cullen & Irvine, 1967; Clark & Brown, 1970*a*). Combining the measurements of residual binding capacity and total plasma thyroxine concentration yields a free thyroxine index (Clark & Horn, 1965; Clark & Brown, 1970*b*) which correlates well with the concentration of free thyroxine which would be available to the tissues in the intact animal (Wellby & O'Halloran, 1966). In a third series of experiments, the minimal metabolic rate of 15 males from each of the CBA/FaCam, C57Bl/Fa, C3H and Peru strains was measured (Denckla, 1970, 1973). Each mouse was fasted for 8 h, weighed and anaesthetized by an intraperitoneal injection of Nembutal (1·2–1·3 μl/g body weight; Abbott Laboratories). It was then placed in a sealed glass chamber; rectal temperature was monitored by a thermocouple and the heating of the chamber manipulated to maintain the animal under thermoneutral conditions. Carbon dioxide was absorbed by soda lime (Carbosorb, BDH, self-indicating), facilitated by circulation of the gas in the chamber with a small electric fan. The gaseous pressure inside the chamber was monitored by a manometer. 5 ml of oxygen (British Oxygen Company) were admitted to the chamber, and the time taken for the pressure to return to its original value was noted. Three determinations were made for each animal.

The values of the total plasma thyroxine concentration for the five strains of mice are shown in Table 1. Analysis of variance indicates that there are significant differences between the strains ($P < 0{\cdot}001$). In the second series of measurements using 10 C3H, 9 CBA/FaCam and 8 C57Bl/Fa males with total thyroxine values ranging from 3·5 to 6·6 μg/ml, a correlation coefficient of 0·91 ($P < 0{\cdot}001$) was found between total thyroxine and the free thyroxine index. Analysis of variance did not reveal any significant strain difference in the relation between these two parameters.

There are significant strain differences in the minimal metabolic rates of the four strains of mice (Table 2, $P < 0{\cdot}001$ by analysis of variance).

These results confirm the existence of significant genetic variation in total plasma levels of thyroxine. The determination of residual plasma binding capacity in three of the strains indicates that the differences in total plasma levels between them will be reflected in the amount of free thyroxine available to the tissues, although investigation of further strains would quite possibly reveal variation affecting the plasma hormone binding capacity in mice (as has been reported in rabbits (Davis *et al.* 1974)). Further studies, which should also include tri-iodothyroxine, directed towards an elucidation of the physiological and biochemical

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factors responsible for this variation would be worthwhile, and would lead to an improved understanding of the synthesis and metabolism of the thyroid hormones. In particular, Gross & Schwartz (1951) found that radioiodine excretion, which is probably correlated with thyroxine breakdown, was much more rapid in C57Bl than in C3H mice. Since the plasma thyroxine levels for these strains which we

Table 1. *Plasma thyroxine concentrations in five strains of mice*

(Values ± standard error.)

Strain	Number of animals	Total plasma thyroxine (μg/ml)	Body weight (g)
Balb/c	13	7·4 ± 0·5	23·7 ± 0·5
CBA/FaCam	20	6·1 ± 0·4	22·8 ± 0·7
C57Bl/Fa	19	5·2 ± 0·4	24·8 ± 0·7
C3H	15	4·6 ± 0·3	23·0 ± 0·5
Peru	12	4·0 ± 0·3	17·4 ± 0·7

Table 2. *Minimal metabolic rates in four strains of mice*

(Values ± standard error.)

Strain	Number of animals	Minimal metabolic rate (ml O_2/min/100 g body wt.)	Body weight (g)
CBA/FaCam	15	1·89 ± 0·06	24·6 ± 0·8
C57Bl/Fa	15	2·11 ± 0·04	23·4 ± 0·7
C3H	15	2·25 ± 0·04	23·2 ± 0·4
Peru	15	2·65 ± 0·10	18·1 ± 0·6

report are not very different, this implies that C57Bl mice may also synthesize thyroxine more rapidly than C3H mice. Thus the similar plasma levels may result from differing biochemical mechanisms (Shire, 1976), providing useful material for future investigation.

Perhaps the most interesting result is the apparent lack of a positive correlation between plasma thyroxine levels and minimal metabolic rate. In pigeons, thyroid size (probably reflecting plasma thyroid hormone levels) and metabolic rate are in general correlated, but particular stocks are clearly exceptions, suggesting that the pattern of results described here for mouse strains is not unreasonable (Riddle, 1947). Since it is well established that an increased hormone level leads to an increased minimal metabolic rate within an individual animal (Denckla, 1970, 1973), it seems probable that there is genetic variation between the strains studied affecting other components of the endocrine system too. These could include plasma binding capacity (in the case of Peru mice), the plasma concentration of tri-iodothyronine relative to thyroxine and the response of target organs (especially liver) to thyroid hormones as affected by both endogenous and exogenous factors (Denckla, 1974), in addition to unrelated factors which might affect minimal metabolic rate. Analysis of these possibilities will require further, more co-ordinated studies using biochemically specific techniques to examine segregating hybrid generations (Shire, 1976).

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REFERENCES

Chai, C. K. & Dickie, M. M. (1966). Endocrine variations. In *Biology of the Laboratory Mouse*, 2nd ed. (ed. E. L. Green), pp. 387–403. New York: McGraw-Hill.

Chai, C. K. & Melloh, A. (1972). Selective breeding for variations in thyroidal iodine release rate in mice. *Journal of Endocrinology* **55**, 233–243.

Clark, F. (1967). The estimation of thyroid hormone binding by plasma proteins and of unbound levels of thyroxine in plasma. *Journal of Clinical Pathology* **20**, 344–352.

Clark, F. & Brown, H. J. (1970*a*). Evaluation of Thyopac-3 test in the *in vitro* assessment of thyroid function. *British Medical Journal* **1**, 713–715.

Clark, F. & Brown, H. J. (1970*b*). Free thyroxine index. *British Medical Journal* **1**, 543 and 672.

Clark, F. & Horn, D. B. (1965). Assessment of thyroid function by the combined use of serum protein-bound iodine and resin uptake of ^{131}I-tri-iodothyronine. *Journal of Clinical Endocrinology and Metabolism* **25**, 39–45.

Davis, P. J., Laird, C. W. & Fox, R. R. (1974). Binding of thyroid hormones in rabbit serum: definition of a prealbumin and effects of strain variation on binding. *Journal of Endocrinology* **63**, 389–398.

Denckla, W. D. (1970). Minimal oxygen consumption in the female rat: some new definitions and measurements. *Journal of Applied Physiology* **119**, 28–33.

Denckla, W. D. (1973). Minimal O_2 consumption as an index of thyroid status: standardisation of method. *Endocrinology* **93**, 61–73.

Denckla, W. D. (1974). Role of the potuitary and thyroid glands in the decline of minimal O_2 consumption with age. *Journal of Clinical Investigation* **53**, 572–581.

Farrington, A. J. & Mellen, W. G. (1967). Thyroid activity and endocrine gland weight in fast- and slow-growing chickens. *Growth* **31**, 43–59.

Feuer, G. (1969). Differences in emotional behaviour and in function of the endocrine system in genetically different strains of albino rat. In *Physiology and Pathology of Adaptation Mechanisms* (ed. E. Bajusz), pp. 214–233. Oxford: Pergamon Press.

Gross, J. & Schwartz, S. (1951). The metabolism of thyroxine in C57 mice and C3H mice with and without mammary tumours. *Cancer Research* **11**, 614–618.

Kojima, A., Kubota, T., Sato, A., Yamada, T., Harada, A., Utsumi, M., Sakoda, M., Baba, S., Yamori, Y. & Okamoro, K. (1976). Abnormal thyroid function in spontaneously hypertensive rats. *Endocrinology* **98**, 1109–1115.

Riddle, O. (1947). Endocrines and constitution in doves and pigeons. *Carnegie Institution of Washington Publication*, no. 572.

Seth, J. (1973*a*). Work simplification of a competitive protein binding analysis for serum thyroxine, and a study of some laboratory factors affecting assay results. *Clinical Chimica Acta* **46**, 431–441.

Seth, J. (1973*b*). Work simplification of a competitive protein binding analysis for serum thyroxine – a study of some analytical variables affecting accuracy and precision. *Clinica Chimica Acta* **46**, 443–453.

Shire, J. G. M. (1976). The forms, uses and significance of genetic variation in endocrine systems. *Biological Reviews* **51**, 105–141.

Silverstein, E., Sokoloff, L., Mickelsen, O. & Jay, G. E. (1960). Thyroid function in various strains of mice: T/S ratio PBI and thyroid weight. *American Journal of Physiology* **199**, 203–208.

Synenki, R. M., Eisen, E. J., Matrone, G. & Robison, O. W. (1972). Thyroid activity in lines of mice selected for large and small body weight. *Canadian Journal of Genetics and Cytology* **14**, 483–494.

Standeven, R. M., Cullen, D. R. & Irvine, W. J. (1967). Estimation of degree of saturation of thyroxine binding globulin by uptake of triiodothyronine using albumin-coated charcoal. In *Thyrotoxicosis. Proceedings of an International Symposium* (ed. W. J. Irvine), pp. 164–174.

Wellby, M. & O'Halloran, M. W. (1966). Measurement of plasma free thyroxine level as a test of thyroid function. *British Medical Journal* **2**, 668–670.

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