radioimmunoassay[10] and the results expressed as a percentage of the pretreatment level because of variations in basal concentrations. Statistical differences were determined by Student's t-test.

Results and discussion. It can be seen from table 1 that injection and serial bleeding of conscious birds had very little effect on the levels of plasma growth hormone. Handling and bleeding has been shown to increase[13] or decrease[9] the plasma growth hormone levels in mammals although not in the rabbit[14]. In contrast, in this study anaesthesia resulted in a marked depression of the plasma growth hormone levels (table 1). The concentration of plasma growth hormone was significantly lower than the pretreatment levels in all anaesthetized birds 30, 60 and 90 min after treatment. Throughout the period of study the levels of plasma growth hormone in these birds were also significantly different from the conscious controls. There were no significant differences in the levels of plasma growth hormone in birds treated with different anaesthetics. The effects of Althesin, Rompun and Chloral hydrate on plasma growth hormone levels have not been reported in other animals. However, whereas sodium pentobarbitone is known to be a provocative stimulus of growth hormone secretion in the rat[8], Sagatal and Equithesin (sodium pentobarbitone based) had the opposite effect in these experiments. The effect of ether anaesthesia can be seen in table 2. The concentration of plasma growth hormone was significantly lower than the pretreatment levels in anaesthetized birds 5, 10, 20, 40 and 80 min after treatment. Moreover, serial bleeding of the control birds had no effect on the plasma growth hormone levels and were significantly higher than their anaesthetized counterparts. Similar results of ether anaesthesia have been seen in the rat and mouse[8, 9] although in primates ether treatment elevates the concentration of plasma growth hormone[6].

The results of this investigation clearly demonstrate that several commonly used anaesthetics have a profound and consistent effect on the secretion of plasma growth hormone. Furthermore, physiological studies in the domestic fowl may be affected by the use of anaesthetics.

13 V. Meyer and E. Knobil, Endocrinology *80*, 163 (1967).
14 J. F. Garcia and I. I. Geschwind, Ed. A. Pecile and E. E. Muller. in: Growth Hormones, p. 267. Excerpta Medica Foundation, Amsterdam 1968.

Mathematical model of pituitary thyrotropic function

F. J. Seif

Medizinische Poliklinik, University of Tübingen, Liebermeisterstrasse 14, D-7400 Tübingen 1 (Federal Republic of Germany), 26 January 1977

Summary. A nonlinear differential equation is used to develop a mathematical model describing the time course of thyrotropin (TSH) concentration in human plasma after thyroliberin (TRH) stimulation. The application of the model to real data shows that pituitary responsiveness to TRH is highest in euthyroidism, reduced in primary hypothyroidism, and lowest in hyperthyroidism.

The secretion by the anterior pituitary gland of thyrotropin (TSH) is stimulated by thyroliberin (TRH) originating from the hypothalamus, and is inhibited by triiodothyronine and thyroxine, which feed back from the thyroid gland. The thyrotropic secretory capacity of the pituitary is tested by the increase of plasma TSH after injection of TRH. The dynamics of TRH and TSH together with triiodothyronine and thyroxine in plasma can serve as data to formulate a mathematical model of thyrotropic secretion in man.

Origin of biological data. 20 healthy male and female volunteers (group A), 12 hyperthyroid patients (group B) and 7 patients with primary hypothyroidism (group C) were diagnosed by clinical signs and by a TRH test: About 8.00 a.m. samples of venous blood were withdrawn for concentration measurements of basal TSH in plasma (h_0), of total plasma triiodothyronine (T_3), of total plasma thyroxine (T_4) by radioimmune assay, and for estimation of relative serum binding capacity (R) for radioactive triiodothyronine by equilibrium distribution in presence of resin. Thereafter, at time t = 0, 400 µg of TRH (r_0) were injected i.v. as a bolus in order to stimulate TSH secretion. In general the reactive peak level of plasma TSH is reached between 20 and 35 min after TRH injection. Hence t = 20 min was usually chosen as a second time point to measure TSH in plasma h(t).

Mathematical description. The feedback inhibition of TSH in the thyrotropic cells of the pituitary is brought about by a moiety of triiodothyronine and of thyroxine, the intracellular concentration of which (x_3, x_4) is nearly equal to that of the free plasma fraction of T_3 and T_4 not bound to plasma proteins. The respective concentrations of the free fractions we call F_3 and F_4.

The bound fraction of T_3 is set equal to the bound fraction p of radioactive triiodothyronine of patient serum. A normal standard serum yielding a bound fraction of $p_0 = 0.7$ served as control for measurements of R. With $R = p/p_0$, $R < 1/p_0 = 1.42$, T_3 in ng/dl, and c as proportionality factor we define

$$x_3 = T_3 - pT_3 = T_3(1 - p_0R) \approx cF_3. \quad (1)$$

As T_4 binds reversibly to plasma proteins, mainly to thyroxine-binding globulin (TBG), the law of mass action is applicable as soon as equilibrium is obtained. With [TBG] and [TBG-T_4] standing for concentration of TBG and TBG-thyroxine complex respectively, and K_1 = const., we have

$$F_4 = \frac{K_1[\text{TBG-}T_4]}{[\text{TBG}]}.$$

Normally 99.97% of total plasma T_4 is bound to TBG. Therefore [TBG-T_4] is essentially equal to T_4. Furthermore free [TBG] is approximately proportional to $p = p_0R$. With [TBG] $\approx K_2p_0R$ we can write:

$$F_4 \approx \frac{K_1T_4}{K_2p_0R}.$$

With $K_3 = K_2p_0/K_1$ we define (T_4 in µg/dl):

$$x_4 = \frac{T_4}{R} \approx K_3F_4. \quad (2)$$

The injected bolus of TRH r_0 is diluted by the individual plasma volume v taken from nomographs as function of body weight, age and sex[1]. Dye dilution curves show that maximal concentrations are reached within less than 30 sec[2]. This time interval is adopted for the distribution of TRH. Since it is very short in comparison with the half life of TRH $\tau_1 = 5$ min[3], it is assumed that the maximal concentration of TRH r_0/v is reached instantaneously. Thus the change of the time course of the TRH concentration r(t) can be approximated by $dr/dt = -\alpha r$, with the coefficient of TRH elimination $\alpha = \ln 2/\tau_1 = 0.139$ min^{-1}. The solution of this differential equation describes the time course of TRH and is given by

$$r(t) = \frac{r_0}{v} \exp\{-\alpha t\} \qquad (3)$$

with $r(t) = r_0/v$ at time $t = 0$; r_0 in μg and v in ml.
The stimulation of TSH secretion, i.e. the increase of secretion rate by TRH is thought to be directly proportional to r(t). The inhibition, i.e. the decrease of secretion rate by triiodothyronine and thyroxine is assumed to be inversely proportional to the product of x_3 and x_4. The inhibition is by logarithmic scale[4] and $\ln(x_3x_4)$ can be considered constant for the time of 120 min under consideration. The secretion rate of TSH dH/dt is also proportional to the amount of TSH secreted H, as it reflects the number of thyrotropic cells in the pituitary and allows for intrinsic basal TRH stimulation. The plasma half life of TSH τ_2 is about 50 min[5]. From this we calculate the rate constant of elimination $\beta = \ln 2/\tau_2 = 0.014$ min^{-1}. Altogether the time-dependent change of TSH can be described approximately by the following differential equation with k as rate constant of secretion:

$$\frac{dH}{dt} = k\frac{r(t)}{\ln(x_3x_4)} H - \beta H = \left(k\frac{r_0\exp\{-\alpha t\}}{v\ln(x_3x_4)} - \beta\right) H; \qquad (4)$$

x_3, x_4 and r(t) as in equations 1, 2 and 3 respectively. Equation 4 is rearranged and integrated:

$$\int_{H_0}^{H(t)} \frac{dH}{H} = \frac{kr_0}{v\ln(x_3x_4)} \int_0^t \exp\{-\alpha t\}dt - \beta\int_0^t dt;$$

$$\ln H(t) - \ln H_0 = \frac{kr_0}{\alpha v\ln(x_3x_4)}\left(1 - \exp\{-\alpha t\}\right) - \beta t. \qquad (5)$$

As H distributes in v, we can write $H(t) = vh(t)$ and $H_0 = vh_0$, substitute into equation 5, and solve explicitely for k:

$$k = \frac{(\ln h(t) - \ln h_0 + \beta t)\alpha v\ln(x_3x_4)}{r_0(1 - \exp\{-\alpha t\})}. \qquad (6)$$

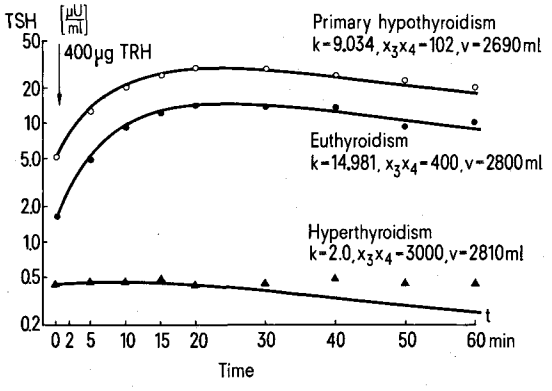
Hence the time course of TSH plasma concentration with h(t) and h_0 in μU/ml, is given by:

$$h(t) = h_0\exp\left\{\frac{kr_0}{\alpha v\ln(x_3x_4)}(1 - \exp\{-\alpha t\}) - \beta t\right\}. \qquad (7)$$

Applications and conclusions. Since the right hand side of equation 6 comprises only measurable values of constants and variables, this equation was used to calculate the individual rate constants k_i of control persons (group A) and patients (group B and C) from experimental data as outlined above. The resulting single k_i-values and their means are listed in the table. The overall null hypothesis, that the k_is of the 3 groups stem from the same population, is rejected by the nonparametric rank test of Kruskal and Wallis using as criterion $\chi^2 > C(0.0005; 2)$[6,7]. By Scheffé's procedure[8] all linear contrasts among the means of the 3 groups are significant with $p < 0.01$ by post hoc criterion except for group C versus A and B. Therefore it can be concluded that the large rate constants k_i in euthyroidism are different from the small ones in hyperthyroidism, whereas the k_is in hypothyroidism have an intermediary position.

Rate constants k_i of pituitary TSH secretion stimulated by TRH injection in euthyroid controls (A), patients with hyperthyroidism (B) and primary hypothyroidism (C)

Group Number n of individuals	Individual rate constants k_i i = 1,2,...,n			Means of k_is and SD $\bar{k} \pm s$
A	22.378	20.521	18.695	
	17.998	17.420	17.138	
	16.559	16.262	16.156	
	15.979	15.625	15.068	
	14.981	14.846	14.635	
	14.547	14.299	14.158	
n = 20	13.578	13.396		16.2 ± 2.3
B	2.667	2.506	2.497	
	2.426	2.303	2.264	
	2.018	1.755	1.739	
n = 12	1.712	1.707	1.647	2.1 ± 0.4
C	12.952	11.298	9.034	
	8.499	8.355	7.279	
n = 7	7.156			9.2 ± 2.1



Time course of TSH concentration h(t) after stimulation with 400 μg TRH at time $t = 0$. The calculated points are represented by lines, whereas the corresponding measured data are single symbols. Examples: ●, euthyroid control; ▲, hyperthyroidism; ○, primary hypothyroidism. k, rate constant of TSH secretion; x_3x_4, product of fractions of free triiodothyronine and thyroxine; v, plasma volume.

1 Documenta Geigy, Wissenschaftliche Tabellen, 7th ed., p. 552. J. R. Geigy AG, Basel 1968.
2 R. Hegglin, W. Rutishauser, G. Kaufmann, E. Lüthy and H. Scheu, Kreislaufdiagnostik mit der Farbstoffverdünnungsmethode. Georg-Thieme-Verlag, Stuttgart 1962.
3 R. M. Bassiri and R. D. Utiger, J. clin. Invest. *52*, 1616 (1973).
4 F. J. Seif, W. Klingler, K. Zech and W. Voelter, Experientia *31*, 992 (1975).
5 J. M. Hershman and J. A. Pittman, New Engl. J. Med. *285*, 997 (1971).
6 J. Pfanzagl, Allgemeine Methodenlehre der Statistik, vol. II, p. 146. Walter de Gruyter & Co., Berlin 1962.
7 E. P. Billeter, Grundlagen der erforschenden Statistik, p. 113. Springer-Verlag, Vienna 1972.
8 R. J. Harris, A Primer of Multivariate Statistics, p. 96. Academic Press, New York 1975.

The rate constants k_i of TSH secretion can be considered as measure of pituitary thyrotropic responsiveness to TRH normalized for triiodothyronine and thyroxine. The responsiveness is highest in euthyroidism, reduced in primary hypothyroidism, and lowest in hyperthyroidism. These differences indicate that the responsiveness to TRH is not only a function of feedback inhibition by triiodothyronine and thyroxine, but also a function of hitherto unknown factors. Neurogenic amines and polypeptides of hypothalamic origin are to be considered as possible candidates.

Equation 7 can be applied to predict the time course of TSH concentration in plasma of individuals after TRH stimulation, especially values at time t other than $t = 0$ and $t = 20$ min. Examples, one out of each group, are shown in the figure. The congruency of the measured data with the calculated points is fairly good, thus supporting the validity of the assumptions taken into account.

The effects of continuous chloroform and halothane anesthesia on plasma prolactin levels in ovariectomized estrogen-treated rats[1]

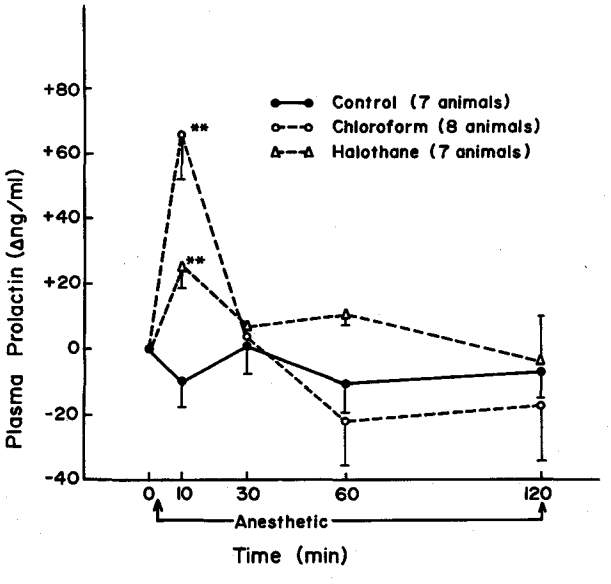
Marappa G. Subramanian and R. R. Gala[2]

Department of Physiology, Wayne State University School of Medicine, 540 E. Canfield, Detroit (Michigan 48201, USA),
17 January 1977

Summary. In ovariectomized, estrogen-treated rats bearing indwelling aortic catheters, continuous inhalation of chloroform or halothane resulted in increases in plasma prolactin levels 10 min after the exposure to the anesthetics. The plasma prolactin levels over the subsequent 2 h, however, were not significantly different from that of the control animals.

Previous studies from our laboratory examined the influence of injectable and inhalation anesthetics on prolactin release[3–5]. Ether increased plasma prolactin levels in both ovariectomized and ovariectomized, estrogen-treated rats[3–5] whereas methoxyflurane (MF) increased plasma prolactin only in estrogen-treated rats[5]. The present study was performed to determine the effects of 2 additional inhalation anesthetics, chloroform and halothane, on plasma prolactin levels in ovariectomized, estrogen-treated rats.

Materials and methods. Mature female Sprague-Dawley rats weighing 225–250 g were ovariectomized and housed in a room with controlled temperature (23 ± 2 °C) and lighting (14 h light, 10 h darkness, lights on at 06.00 h). 2–3 weeks later, a catheter was inserted into the left carotid as described in detail elsewhere[3]. At the time of catherization 0.5 mg of polyestradiol phosphate (1 mg Estradurin®, Ayerst Laboratories) was administered s.c. to each rat. The withdrawal of blood samples began 1 week after catheterization. The experimental procedure was as follows: on the day of sampling between 08.00 and 09.00 h an extension was attached to the indwelling catheter and the animals were left undisturbed for at least 60 min. After this equilibration period, a control sample (0.6 ml) was withdrawn (time 0). After each blood sample was obtained, the volume was replaced with an equal volume of saline warmed to 37 °C. In the control group subsequent blood samples were obtained at 10, 30, 60 and 120 min. In the experimental groups, after the 0 min sample, the animals were anesthetized in a large jar saturated with either chloroform or halothane (Fluothane®, Ayerst Laboratories) vapor and maintained under continuous anesthesia using a nose cone for the duration of the experiment (120 min). Additional blood samples were obtained 10, 30, 60 and 120 min after the initiation of anesthesia. The blood samples were immediately diluted with an equal volume of chilled phosphate buffer saline (pH 7.6) and centrifuged at 3 °C. The plasma obtained was stored frozen at −20 °C. Plasma prolactin levels were assayed by the double antibody radioimmunoassay[6] at 2 dilutions each in duplicate. Rat prolactin NIAMDD-RP-1, with a potency of 11 IU/mg, was the standard. Plasma prolactin values are expressed as ng/ml



The influence of continuous chloroform and halothane anesthesia on plasma prolactin levels in ovariectomized, polyestradiol phosphate-injected rats. Plasma prolactin levels are expressed as ng/ml change from the initial levels. Values represent the mean ± SEM at each point. Statistical comparisons were made between the control and experimental groups at the same time periods. ** $p < 0.01$.

1 Supported by NSF Research Grant BMS 74-17332.
2 The authors wish to express their appreciation to Mrs Cynthia Van De Walle for her outstanding assistance in the performance of the prolactin RIA and the art work.
3 D. M. Lawson and R. R. Gala, J. Endocr. *62*, 75 (1974).
4 D. M. Lawson and R. R. Gala, J. Endocr. *66*, 151 (1975).
5 M. G. Subramanian, D. M. Lawson and R. R. Gala, Life Sci. *18*, 305 (1976).
6 E. Y. H. Kuo and R. R. Gala, Biochim. biophys. Acta *264*, 462 (1972).