Multiphasic analysis of growth

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Multiphasic Analysis of Growth

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STELLINGEN

I Wiskundige groeifuncties moeten worden gezien als hulpmiddel ter vergroting van het biologisch inzicht in groei en niet als resultaat van groeiwetten. (Zeger, S.L. & Harlow, S.D., 1987. Growth 51, 1-21)

II De meerfasen-groeifunctie heeft eigenschappen die duidelijke overeenstemming vertonen met het op biologische gronden te verwachten groeipatroon en kan daardoor een gereedschap zijn voor de bioloog en zoötechnicus om meer inzicht te krijgen in de biologie van de groei. (Dit proefschrift)

III Verschillende produktiekenmerken als melk- en ei-produktie vertonen een meerfasig verloop. Toepassing van de meerfasenfunctie behoeft daarom niet beperkt te blijven tot de beschrijving van groei.

IV Gebruik van het volwassen gewicht als "genetic size factor" in de "genetic scaling rules" van Taylor (1985) brengt de variatie tussen diersoorten in een groot aantal biologische kenmerken terug tot het niveau van de variatie binnen soorten. Binnen soorten is het echter noodzakelijk een nauwkeuriger schatting te krijgen van de "genetic size factor" om dezelfde reductie in variatie tussen rassen te krijgen. (Taylor, St. C.S 1985. J. Anim. Sci. 61 (Suppl. 2) 118-143.)

V Bij invoering van biotechnologische technieken in het veeteeltkundig onderzoek zullen statistische technieken nodig zijn die verschillen van de gebruikelijke. Het is voor de onderzoekers noodzakelijk hiermee in een vroeg stadium vertrouwd te raken.

VI Robuuste schattingstechnieken dienen in de statistische verwerking van veeteeltkundige proeven meer aandacht te krijgen.

VII Bij het beoordelen van verschillen tussen statistische schattingen wordt te vaak de nadruk gelegd op de significantie van de verschillen in plaats van op de grootte van de verschillen.

VIII De te benoemen hoogleraar 'geïntegreerde dierlijke produktie', zal in de huidige constructie meer tijd nodig hebben voor het integreren van vakgebieden dan van modellen.

IX De kledingindustrie anticipeert onvoldoende op de snelheid waarmee de gemiddelde lichaamslengte van de Nederlandse man en vrouw toeneemt.

X Om het getalbegrip van kinderen te bevorderen zal het rekenonderwijs meer elementen van schatten en benaderen moeten bevatten.

XI Uit het feit dat de hedendaagse veehouder de stroom informatie die hem wordt verstrekt door de fokkerij-instanties, weet te verwerken, blijkt dat hij niet van gisteren is.

XII Het oudhollanse gezegde, 'van geld en mest heeft een boer nooit teveel', is niet voor niets in onbruik geraakt.

Proefschrift Wiebe J. Koops,
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VOORWOORD

Het onderzoek beschreven in dit proefschrift is tot stand gekomen bij de vakgroep Veefokkerij. Ik ben de vakgroep zeer erkentelijk voor de kans en de ruimte die mij is gegeven om dit mogelijk te maken.

De titel van het proefschrift is geheel in overeenstemming met de wijze waarop de plannen zijn gegroeid tot het resultaat wat thans voor u ligt. In de eerste fase, rond 1983, is de aanleiding tot het onderzoek onderkend en is het onderzoek met groeicurven gestart. Als tweede fase kan het 'sabbatical year' (86-87) van prof. dr. M. Grossman van de Universiteit van Illinois (USA) worden gezien. In deze periode is een intensieve samenwerking tot stand gekomen wat betreft het onderzoek in groei- en lactatiecurven. Toen zijn ook de plannen geboren om te komen tot een afronding van dit onderzoek in de vorm van een proefschrift. De derde fase, kort maar intensief, is de periode geweest waarin het meeste schrijfwerk heeft plaatsgevonden.

Voor de stimulerende rol die mijn promotoren prof. dr. ir. R.D. Politiek en prof. dr. M. Grossman hebben gespeeld, wil ik hun hartelijk dank zeggen.

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Met prof. Grossman heb ik gedurende de periode dat hij als gastmedewerker aan de vakgroep Veefokkerij was verbonden, intensief samengewerkt. Uit het feit dat hij bij de meeste hoofdstukken als medeauteur wordt genoemd moge blijken dat deze samenwerking nog lang niet is uitgeput. Hij heeft er onder andere voor gezorgd dat de Engelse tekst leesbaar geworden. Ik kan me voorstellen dat het niet altijd even leuk was steeds weer tekstfiles via Bitnet te moeten ontvangen en te corrigeren. Mike, zeer veel dank voor al het vele werk wat je hebt gedaan.

Arnoud ik hoop dat je niet het 'heen en weer' hebt gekregen van alle files die je van PC naar Vax hebt gestuurd en andersom. Bedankt daarvoor.

Tevens een woord van dank aan het LEB-fonds voor de financiële ondersteuning bij de afronding van het proefschrift.

Tot slot, maar niet in het minst, wil ik Bertha, André en Annejo bedanken voor de steun en de belangstelling, en het geduld wat ze met mij hebben gehad. Zij weten nu ook hoe het is iemand in hun midden te hebben met vierkante oogjes en een nietszeggende blik.

CONTENTS

INTRODUCTION

Extensive research on growth of living organisms has been conducted during the last century. The phenomenon of growth was and is a fascinating subject for many researchers. Nevertheless there are many questions to answer about how and why an individual grows. Growth in biological terms is related to increase in size and shape. The processes of hyperplasia, hypertrophy and cell differentiation are the basis for biological growth (Brody, 1945).

Many studies have been published in which growth was analyzed to quantify change in body weight, height or other body measures. In the use of mathematical functions to describe growth, most of the time growth is considered to be a homogeneous process that results in a smooth S-shaped curve. From birth to death, body measures follow a continuous curve, although only a few points are observed. Under closer examination, however, random deviations of observations from the estimated (smooth) line emerge; these deviations are caused partly by short-duration irregularities. In many cases, systematic deviations over longer periods appear; these deviations can be a result of autocorrelation or can be explained in biological terms. Random deviations assume that the mathematical growth function is correct and that such deviations can be explained statistically. For systematic deviations, however, it is assumed that the growth function is incorrect, on biological grounds, and that for such deviations to disappear another function must be found that can describe the observations correctly. The statistical interpretation is used only if the growth function is believed to be correct, for instance if functions are based on biological laws. Many of the well known growth functions are based on 'growth laws' (*e.g.*, Robertson, 1923 and Von Bertalanffy, 1960). Zeger and Harlow (1987) showed a tendency in the use of growth functions, for better understanding biology, from functions based on biological laws to functions used as analytical tools.

The dilemma of explaining systematic deviations, statistically or biologically, was the reason for the investigation in this thesis. If there is any evidence for a biological interpretation of deviations, automatically the statistical explanation can be rejected. Therefore, the growth literature will be reviewed to find biological evidence for such an explanation. Based on these findings, objectives will be formulated for further investigation.

Quantitative analysis of growth

The first growth data set known in the history of growth studies is the famous series of body height measurements on the son of Count Philibert de Montbeillard taken between 1759 and 1777 (Scammon, 1927). This growth data set was used by many researchers to test new growth models (*e.g.*, Sandland and McGilchrist, 1979; Sager, 1982; Koops, 1986). The literature in mathematical modelling of growth has been reviewed recently by Zeger and Harlow (1987), who mentioned that the earliest quantitative growth study was published in 1835 by L. A. Quetelet. As with many other early growth studies, the subject of that study was human growth in body weight and height.

In animals, influential contributions to growth studies have come from the research of Robertson (1923), Brody (1945), Von Bertalanffy (1960) , Pruitt *et al.* (1979) and Parks (1982). Robertson (1923) is cited frequently as having first used the logistic growth function in animals.

Another aspect mentioned by Robertson, one that is almost never cited, however, is the discovery of cycles in growth curves of mice, rats, cattle and poultry. In the gain or velocity curves of body weight, two or three cycles could be distinguished. This is in contrast with the current 'laws of growth', and therefore it can be a possible explanation of systematic deviations mentioned as a motivation for this study. The multiphasic property of growth, which is based on the existence of growth 'phases', 'cycles' or 'spurts', can be very important, and therefore, special attention will be paid to the literature on this subject.

Growth phases

The term 'growth cycle' is first quoted by Sachs in 1874 and cited by Courtis (1937), who said:

> *" We can say, therefore, that the characteristic of a cycle is that in its course the rate of growth varies continually, and passes through a maximum. The variations of this rate as a function of time are represented by a bell shaped curve."*

Conditions that gave rise to growth cycles (also called waves, impulses and phases) were differences in maturation of different components of the organism and changes in environmental conditions (Courtis, 1937).

During the years 1900 to 1945, there was intensive discussion in the literature about the existence of growth cycles. Robertson (1908) stated the existence of three cycles during mammalian growth. Brody (1921) found two cycles in postnatal growth curves of cows and concluded that at least one cycle was before birth. Robertson (1923) detected at least two cycles in growth curves of a number of species. Robertson, as well as Brody, used the logistic growth function to describe growth in each cycle. A few years later Robb (1929) concluded:

> *"Robertson's explanation of observed deviations (by assuming a succession of 'growth cycles') has met with but partial success. For example, the early optimism as to the possibility of resolving the growth curve of man into three growth cycles superimposed one upon another has been found unsatisfactory."*

The application of three cycles to the human growth curve was later widely accepted (Tanner, 1962; Cheeck, 1968; Marshall, 1977; Gasser *et al.*, 1984).

Zucker *et al.* (1941) discussed different approaches to cyclic growth in the rat, according to growth curve formulae of Robertson (1923), Pearl (1924), Crozier (1926), Schmalhausen (1930) and Von Bertalanffy (1938). It was suggested that causes for cyclic growth could be:

1. changes in environment and nutrition accompanying birth and weaning,
2. special changes and hormonal stimuli of puberty,
3. temporary predominance of some glands of internal secretion,
4. hormonal activity, relative activity of the various glands is not constant throughout life,
5. distinctions related to increase in number of cells, increases in size and weight of cells, and
6. the successive development of various tissues in a morphological and chemical sense.

In 1945, when Brody published his famous book, 'Bioenergetics and growth', he mentioned the existence of growth cycles in animals. Brody also used the term 'phases', but in a different way. He called the part before the inflection point of the S-curve the 'accelerating phase' and the part after inflection point, the 'self-retarding phase.' In contrast with Brody, in the present study, the term 'cycle' and 'phase' will be used as a synonym, indicating one entire S-curve.

As mentioned before, in human growth three cycles or phases were distinguished. In terms of gain in height, the first peak (maximum gain) is within the first year; the second (small) peak, the so-called 'mid-growth spurt,' is at about age 7 years; and the third peak, the so-called 'pubertal' or 'adolescence growth spurt,' is at about age 12 for girls and at about age 14 for boys. Tanner (1962) compared the human growth curve with growth curves of several species for the existence of the 'adolescence spurt'. He concluded that this spurt seems to be an evolutionary step taken by primates.

In the meantime, focus of growth studies shifted from growth of the entire body to growth of body components. Growth of parts of the body were seen to be related to growth of the whole body or to other parts of the body. These studies, frequently indicated as 'allometric growth studies,' showed differences in rate of maturation for different parts of the body (*e.g.*, Palsson, 1955; Berg and Butterfield, 1976; Walstra, 1980). The sequence of maturation of the main parts of the body is: first, nervous system; second, bone; third, muscle; and last, fat (Palsson, 1955).

Gall and Kyle (1968) discovered two phases of growth in mice and called the phenomenon the 'diphasic nature' of the growth curve of mice. The same pattern was found by Eisen (1976), who suggested analyzing the growth curve in two parts. Although these results could be a motive to improve current mathematical growth functions that will allow more than one growth cycle or phase, only a few such examples were found in literature.

Multiphasic growth functions

Assuming multiphasic growth, a function with n phases could be written as:

$$Y_t = y_{1t} + y_{2t} + \ldots + y_{it} + \ldots + y_{nt} \qquad (1)$$

where Y_t is total growth (*e.g.*, weight, height or length) at age t and y_{it} is amount of growth at age t in phase i. The Y is an observed or measured value and y's are partly observed or estimated.

Brody (1921) and Robertson (1923) used a logarithmic form of the logistic growth function to describe growth in body weight for each phase:

$$\ln [y_{it}/(A_i - y_{it})] = K_i \ (t - D_i) \tag{2}$$

where y_{it} is actual body weight at age t (counted from conception) minus amount grown in previous phases; for each phase i: A_i is asymptotic weight, K_i is the growth parameter and D_i is the inflection point. Parameters A, K and D are estimated separately for each phase, with separation of phases by inspection.

Courtis (1937) assumed growth in each cycle according to the Gompertz function and used a double logarithmic transformation to make the relation linear between weight in a particular phase and age:

$$\ln \ln y_{it} = m_i t + b_i \tag{3}$$

where y_{it} and t have the same meaning as in (2), m_i and b_i are parameters estimated separately for each phase. Separation of phases was by inspection.

Other examples were found only in human growth literature. To describe human height curves, Bock *et al.* (1973) and Bock and Thissen (1976) suggested a multi-component growth model containing a summation of two or three logistic functions. They called it a double-logistic and triple-logistic model. The triple-logistic was of the form:

$$\begin{aligned} Y_t = a_1 \{ & q/[1+e^{-b_1(t-c_1)}] + p/[1+e^{-b_2(t-c_2)}] \} \\ & + (f-a_1)/[1+e^{-b_3(t-c_3)}] \end{aligned} \tag{4}$$

where Y_t is height at age t and a_1, q, p, f, b_1, c_1, b_2, c_2, b_3 and c_3 are parameters. For meaning of these parameters, see Bock and Thissen (1976). Parameters were fitted simultaneously.

Tanner (1977) proposed to fit the human height growth curve from birth to adulthood in two parts:

$$Y_t = a + bt + c \ln t \qquad \text{for } t < 10 \tag{5}$$

$$Y_t = P + Q/(1 + e^{s-rt}) \qquad \text{for } t > 10 \tag{6}$$

where Y_t is height at age t (years) and a, b, c, P, Q, s and r are parameters.

Peil (1979) and Peil and Helwin (1981) fitted many different growth functions to human height data. Results of their studies were almost the same as those of Robertson (1923) and Bock and Thissen (1976). Peil and Helwin (1981) came up with a function with three phases, each phase based on logistic growth and written in hyperbolic tangent form:

$$Y_t = a_0 + \sum_{i=1}^{3} \{a_i \tanh[b_i(t - c_i)]\} \tag{7}$$

where Y_t is height at age t and a_0 is a parameter; for each phase, parameters a_i, b_i and c_i should be estimated. Peil and Helwin (1981) found three phases for data on human height from 7 to 20 years of age. In analyses including embryo-fetal period and ages before age 6, however, they detected nine phases. Extrapolation of these functions to animal growth was suggested. Phases should reflect what they called the 'biological program of the growth process.'

Discussion and objectives

In light of the motivation for reviewing growth literature, it can be concluded that single-phase growth functions are strongly simplified descriptions of growth from birth to death. Empirical results in older literature of animal growth and recent descriptions of human growth curves support this conclusion. Studies in animal body composition (morphometric and allometric studies) showed biological evidence for rise of growth phases. It is remarkable that during the last four decades almost no attention has been given to the existence of growth phases in quantitative animal growth literature. Reasons for this could be:

1. There is an almost sacred believe that growth laws are the basis for single-phase growth functions, as presented by authorities such as Robertson, Brody and Von Bertalanffy.
2. For the purpose of application, many researchers are satisfied with only a few parameters to summarize the growth curve for body weight of an individual.
3. Mathematicians are not interested in developing growth functions if they receive no stimulation from biologists.

4. Phases are almost undetectable if measurements are not taken frequently, relative to lifetime.
5. Complexity of the problem.

The only motivation to apply the multiphasic approach to animal growth has come from Peil and Helwin (1981). The multiphasic approach of growth seems to be a solution to give a biological interpretation of systematic deviations in growth curves fitted by single-phase growth functions. Based on this information, the original motivation, can be translated into the following main objective:

To investigate application of a suitable mathematical growth function to describe multiphasic growth.

A multiphasic function is chosen to be applied in this study and is based on findings in the literature and a comparison of different multiphasic functions in a pilot study. The function is a modification of functions presented by Robertson (1923) and Peil and Helwin (1981); it is a summation of n logistic growth functions. The logistic function is chosen as the basic function because, in many growth data sets, gain showed up as symmetrical bell-shaped phases; in that case, the Gaussian or logistic function can be applied. The logistic function is chosen for favorable mathematical properties. The function is of the form:

$$Y_t = \sum_{i=1}^{n} a_i \, \{1 + \tanh \, [\, b_i \, (t-c_i) \,] \, \} \tag{8}$$

where Y_t is a measure of growth at age t, tanh is the hyperbolic tangent, n is the number of phases, and within each phase i: a_i is half asymptotic value, b_i is a growth parameter and c_i is age at maximum gain (peak).

Because application of a growth function has many aspects (purpose, computation, circumstances, interpretation of results, *etc.*), it is impossible to be complete. Therefore concentration will be on some selected aspects.

In Chapter 1, the function will be applied to growth data sets of different origins so as to get an impression how successful application can be. Application of the function to individual body weights of mice will be the subject of study in Chapter 2. In Chapter 3, properties of the function will be discussed and the function will be applied to growth data obtained under different circumstances. Well known single-phase growth functions will be compared with the multiple-phase growth function in Chapter 4. For interpretation purposes, the function will be applied to body weights and tail lengths of transgenic and non-transgenic mice in Chapter 5. In the last Chapter, consequences of use of the multiphasic function for morphometric or allometric research will be demonstrated and discussed.

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Chapter 1

MULTIPHASIC GROWTH CURVE ANALYSIS

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ABSTRACT

Application of a multiphasic growth curve is demonstrated with 4 data sets, adopted from literature. The growth curve used is a summation of n logistic growth functions. Human height growth curves of this type are known as 'double logistic' (n=2) and 'triple logistic' (n=3) growth curves (Bock and Thissen, 1976). In the literature there is also some evidence for the existence of growth phases in weight growth curves of animals. The fit of the multiphasic growth curve, applied to pika, mice and rabbit weights, was superior to the monophasic model in terms of residual variances and absence of autocorrelation of residuals.

INTRODUCTION

For the biologist who wishes to describe size-age relationships for growing animals, there is an extensive number of growth models available. Names such as Brody, von Bertalanffy, Gompertz, Richards or logistic are associated with asymptotic growth models (Richards, 1969). New nonlinear models with slightly different properties appear regularly (Pruitt *et al.*, 1979; Sandland and McGilchrist, 1979; Schnute, 1981).

Parameters of these nonlinear models should be 'interpretable biologically', although it is hard to believe that a model with 3 or 4 parameters could describe so complicated a process as growth from birth to death.

In the human growth curve of body weight and height, the inadequacy of simple a sigmoid model is clear. In the case of human height, maximum growth is within the first year and roughly half of the mature height is reached at the age of 2 years. There is a small peak at about age 7 years, the so called 'mid growth spurt' (Gasser *et al.*, 1984). Around age 12 for girls and 14 for boys there is again an acceleration in growth, the so called 'pubertal' or 'adolescence growth spurt' (Marshall, 1977).

Robertson (1923) distinguished three growth cycles for man and various species of animals. This theory is completely accepted only for the human growth curve (Cheek, 1968).

For animal growth curves, however, single cycle growth curves are used mostly. Recently Peil and Helwin (1981) proposed a growth model capable of fitting many growth cycles simultaneously. Like Robertson (1923) they used a growth function in which each cycle was based on a separate logistic growth curve.

Bock *et al.* (1973) combined two logistic functions into a 'double logistic model', to model individual human height growth from birth to adulthood. Bock and Thissen (1976) improved the model by modifying it to a 'triple logistic'.

In animal growth, many workers have shown the existence of growth phases (Robertson, 1923; Zucker *et al.*, 1941; Murphy and Dunn, 1948; Crary and Sawin, 1960; Gall and Kyle, 1968; Eisen, 1976 and Scholtz and Roux, 1981). Tanner (1962) compared growth curves of several species of animals with the human growth curve for the existence of the adolescence spurt. This spurt seems to be an evolutionary step taken by the primates, he concluded. In many curves, however, he showed more than one growth phase could be distinguished.

The aim of this study is to demonstrate the application of a multiphasic growth curve to suitable growth data of different origins.

MATERIALS

To demonstrate the existence of growth phases or cycles in growth curves, it is necessary to have data available with frequent measurements in time. As examples, therefore, four data sets (Table 1) were selected from the literature:

I. <u>Human height data</u>.
Height measurements of the son of the Count de Montbeillard, taken between 1759 and 1777 (an often used example from Sandland and McGilchrist, 1979).

II. <u>Pika weight data</u>.
Mean weights of the North America pika (*Ochotona princips*) (estimated from Figure 1 of Whitworth and Southwick, 1981).

III. <u>Mice weight data</u>.
Mean weights of 480 mice originated from a Polish selection experiment (Michalska *et al.*, 1984).

IV. <u>Rabbit weight data</u>.
Mean weights of Polish male rabbits up to an age of 180 days (Robb,1929).

Table 1. Data sets used[1].

			cont'd		cont'd		cont'd	
data set[2]	t	y	t	y	t	y	t	y
I. Human height	0	51.4	60	111.7	115.4	140.1	186.3	177.5
	6	65.0	67	115.5	120	141.9	195.3	181.4
	12	73.1	72	117.8	138	141.6	198.2	183.3
	18	81.2	78.6	122.9	144	149.9	204.1	184.6
	24	90.0	84	124.3	152	154.1	205.3	185.4
	30	92.8	87	127.0	156	155.3	209.2	186.5
	36	98.8	90	128.9	162	158.6	211.1	186.8
	42	100.4	96	130.8	168	162.9		
	48	105.2	102	134.3	174.3	169.2		
	55	109.5	108	137.0	180.1	175.0		
II. Pika weight	0	12	31	75	60	115	88	150
	7	21	39	93	67	127		
	15	34	47	103	74	134		
	23	57	53	110	81	145		
III. Mice weight	3	2.06	18	8.81	33	19.26	48	23.84
	6	3.77	21	9.63	36	20.85	51	24.39
	9	5.44	24	11.34	39	21.72	54	25.15
	12	6.91	27	13.76	42	22.29	57	25.26
	15	8.10	30	16.76	45	23.01	60	25.69
IV. Rabbit weight	0	35	50	479	100	892	150	1168
	10	108	60	561	110	958	160	1195
	20	181	70	641	120	1018	170	1208
	30	300	80	720	130	1072		
	40	395	90	810	140	1118		

[1] for sources of data sets see text.

[2] for data set I, y is in cm and t in months; all others, y in grams and t in days.

METHODS

The mathematical growth function used is based on logistic growth for each growth phase. The basis for this assumption is the symmetrical form of the average daily gain curve in each phase. In such a case it is possible to use the logistic or the Gaussian distribution. Because of favorable mathematical properties, the logistic was chosen. The number of phases is not always the same; different species and frequency of measuring can influence this number. The frequency can also obscure the determination of the number of phases. Probably this is the reason why the single phase growth curve is normally used. The logistic growth function can be written in different forms; the most familiar forms are:

$$y_t = A \ / \ \{1 + e^{-k(t-c)}\} \qquad (1)$$

and:

$$y_t = a \ [1 + \tanh \ \{b(t - c)\}] \qquad (2)$$

where:

y_t is the prediction of the measure at age t; A is the upper asymptotic value of y (a = .5 A); k and b are growth-parameters (b = .5 k); c is age at the inflection point.

Robertson (1923) used a summation of equation 1. He determined the number of phases by observation and calculated the constants for (1) in each phase separately. The model of Peil and Helwin (1981) is a summation of equation 2 with an extra constant a_0, the initial level, which represents the asymptotic level of foregoing phases not in the equation. Bock and Thissen (1976) added 3 logistic functions of form (1). The multiphasic growth curve used here is a summation of n logistic functions of form (2):

$$y_t = \sum_{i=1}^{n} [a_i \ \{1 + \tanh \ (b_i(t - c_i)) \ \}] \qquad (3)$$

with a first derivative at age t of:

$$y_t' = \sum_{i=1}^{n} [a_i b_i \ \{1 - \tanh \ (b_i(t - c_i)) \ \}] \qquad (4)$$

Constants to be estimated are:

n, the number of phases; a_i, half the asymptotic value of y in phase i;
b_i, growth parameter in phase i; c_i, age at the inflection point of phase i.

The number of constants to be estimated is 3n. For estimating the constants of equation 3, a modified nonlinear regression program was used: BMDX85 (Dixon, 1973). Residual variances, R-squares and Durbin-Watson statistics were calculated to judge goodness of fit. The Durbin-Watson statistic is a measure of autocorrelation. This statistic has an expectation of 2, a value less than 2 indicates a positive autocorrelation and a value greater than 2 a negative autocorrelation (Theil, 1971).

RESULTS

Estimates of the parameters of function (3) for each data set are in Table 2. To determine the final number of phases, (3) was fitted for n = 1, 2, 3 and 4 in a stepwise procedure (Table 3). The model with the smallest residual variance was assumed to have the right number of phases. The pika weight data set showed 2 phases, all others 3. In all final runs, the constants stabilized within 10 iterations.

Table 2. Constant estimates for the multiphasic growth curve.[1]

data set	a_1	b_1	c_1	a_2	b_2	c_2	a_3	b_3	c_3
Human height	51.9	.035	0.0	19.2	.030	79.7	23.5	.034	169.7
Pika weight	56.6	.046	23.4	21.1	.065	72.5	-	-	-
Mice weight	4.4	.137	7.2	6.5	.154	28.5	2.0	.119	48.1
Rabbit weight	198.0	.048	24.8	352.0	.025	82.7	62.0	.051	140.2

[1] Constants a_i for Human height in cm and c_i in months, for other data sets a_i in g and c_i in days. Constants b_i in 1/age units.

Figure 1 (A to D) show the fitted growth curve for each data set. The first derivatives (4) of the curves in Figure 1 are in Figure 2 (A to D). The deviations of the observations from the estimated curve are presented in Figure 3 (A to D). In each case, the final model showed no systematic deviations. This is in agreement with the Durbin-Watson statistics in Table 3, none of which are less than 2.

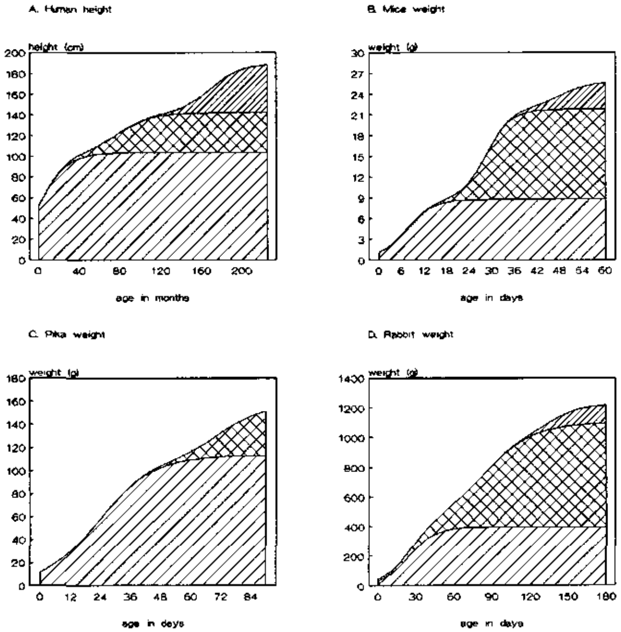

Figure 1. Multiphasic growth curves for four different data sets.

Table 3. Information about the fit in a stepwise procedure.

data set	number of phases (n)	error df	residual variance	R-square	Durbin-Watson statistic
Human height	1	34	33.84	.9769	.31
	2	31	6.11	.9962	1.03
	3	28	1.67	.9991	2.19
	4	25	1.70	.9991	2.59
Pika weight	1	10	33.82	.9872	.66
	2	7	2.15	.9994	3.66
	3	4	3.76	.9994	3.66
	4	1	15.04	.9994	3.66
Mice weight	1	17	.552	.9926	.65
	2	14	.275	.9970	.71
	3	11	.009	.9999	3.22
	4	8	.012	.9999	3.22
Rabbit weight	1	15	1268	.9928	.35
	2	12	97	.9996	1.39
	3	9	40	.9999	2.78
	4	6	47	.9999	3.19

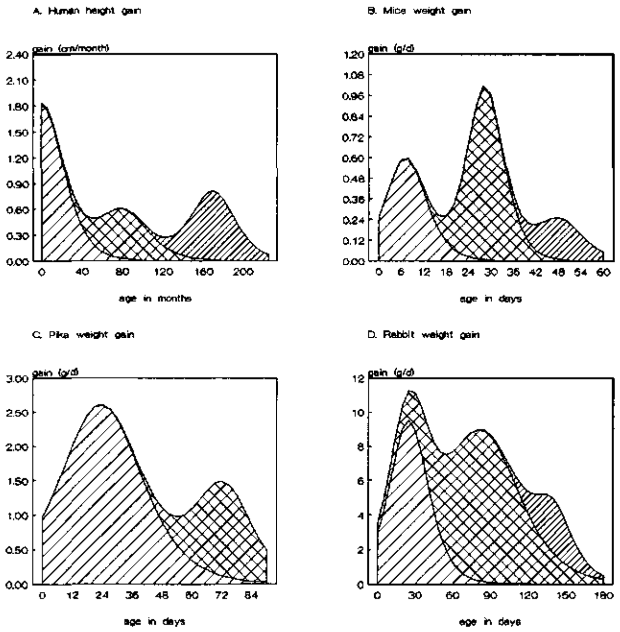

Figure 2. First derivatives (gains) of multiphasic growth curves of four different data sets.

DISCUSSION

Describing growth with the multiphasic growth curve appears successful. The fit is nearly perfect, without systematic deviations. The role of each parameter is easy to understand, although the biological meaning may not yet be clear (Peil and Helwin, 1981).

The Human height data set is used frequently to demonstrate the application of new growth curves (Sandland and McGilchrist, 1979 and Sager, 1982). The constants of the human height data set (Table 1) are in good agreement with the findings of Gasser *et al.* (1984). The peak of the "mid growth spurt" is estimated at 6.6 years and the peak of the "adolescence spurt" at 14.1 years. The three spurts, mentioned in literature, are isolated properly. The application of a multiphasic growth curve to this kind of data is widely accepted, although there is some criticism about interpretation of parameters (El Loshy, 1978).

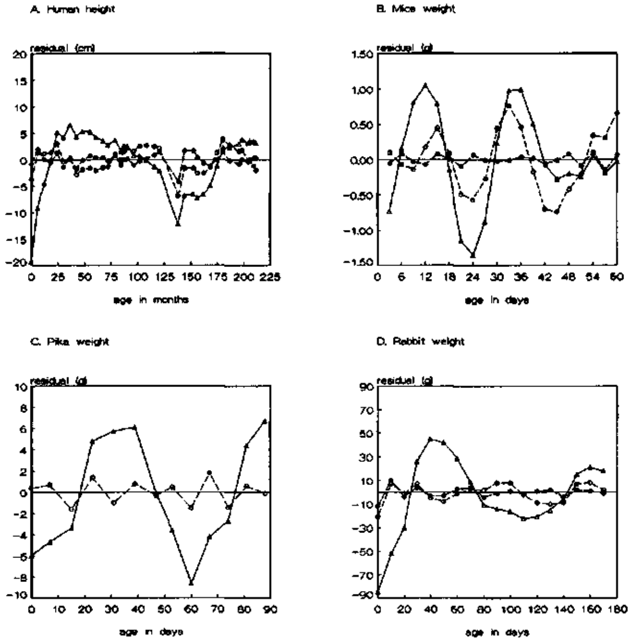

Figure 3. Residuals for a mono- (open triangles), di- (open circles) and triphasic (closed circles) growth model for four different data sets.

Growth in height which is related to bone growth, shows fewer fluctuations in individual growth curves than growth in weight does, which is a summation of growth of different components of the body, some of which (gut fill, digestive organs and adipose tissue) are subject to larger fluctuations especially on an individual basis.

The weight data sets used here are mean growth curves in which most of the fluctuations are greatly reduced by averaging. The advantage of mean curves is that different phases become clearer in the absence of short-term fluctuations. It is well known that growth of the whole body follows an S-shaped curve, but also that some parts of the body follow S-shaped curves different from the whole body curve. Some components of the body matured earlier than others (Palsson, 1955);*e.g.*, weight of brain and eyeballs mature especially early. In contrast muscle and fat are later in maturing. If in the weight data three phases appear, then it is possible that, with this frequency of measuring, three groups of components of the body are being distinguished. Mice data can serve as an example. In a more detailed analysis of these data set we found no difference between sexes in the first phase but strong differences in the second and third phases. The third phase was very pronounced in the male but hardly detectable in the female. Maternal influences are important in the first phase. The most essential parts of the body (organs, nervous system) develop in this phase. In the second phase muscle and bone growth will be an important part. The third phase is almost certainly fat growth.

To be certain about the possibility of distinguishing growth of different parts of the body with the multiphasic growth curve, further investigation is needed with frequently measured weights of body components.

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Chapter 2

MULTIPHASIC GROWTH CURVE ANALYSIS IN MICE

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ABSTRACT

Growth curves of mean body weights were compared to those of individual weights when fitted to data of male and female mice using monophasic (logistic) and triphasic growth functions. Goodness-of-fit was determined by residual variances and Durbin-Watson statistics. These criteria suggest that the triphasic function, with smaller and less correlated residuals, describes the data better than the monophasic function.

For the triphasic function, residual variances were higher when fitting individual weights than mean weights. Males had higher residual variances than females. Autocorrelation was negligible when fitting individual weights for males and for females. Parameters of the triphasic function were higher when fitting curves of individual weights than curves of mean weights; differences between curves within sex were small. Parameters were similar for males and females, especially in the first phase of growth. Half asymptotic weights for the second and the third phases were higher for males than for females.

From these results, it should be clear that using a multiphasic function to describe growth curves in mice provides greater insight for understanding the biology of growth.

INTRODUCTION

Nonlinear growth functions generally are used to describe increase in body weight in mice over time. Eisen *et al.* (1969) compared three growth functions for use on mice data. Of the three, the logistic function gave smallest residual variance when applied to individual growth data.

Many others working on growth in mice (*e.g.*, Timon and Eisen, 1969; and Bakker, 1974) have reached the same conclusion. In each case, however, there were systematic deviations of computed from observed values. For example, weight at birth and weight at weaning were always overestimated.

The reason for these systematic deviations is what Gall and Kyle (1968) and Lang and Legates (1969) observed as the diphasic nature of the growth (weight-age) curve in mice. This means that the average-daily-gain curve rarely has only one peak; there are usually two and perhaps more peaks. The associated weight-age curve, therefore, usually has more than one point of inflection. This pattern of growth can only be recognized with frequent measurements.

Taking this diphasic phenomenon into account, Koops (1986) hypothesized a multiphasic growth function based on summation of logistic functions. Growth curves of mean body weights of mice and some other species were fitted with functions having one through four phases. The triphasic function fitted best, with smallest residual variance, and had less systematic deviations with age than the monophasic function. Results are graphed here to summarize the weight-age curve (Figure 1A), analysis of residuals (Figure 1B) and average-daily-gain curve (Figure 1C). The assumption of logistic growth is probably correct, because for each phase, average daily gain appears symmetrical around its maximum (that is, the point of inflection on the weight-age curve).

Multiphasic growth functions, however, have not been applied to curves of individual body-weight measurements. Such individual growth curves often show temporary irregularities because of problems with health or feed intake. The aim of this study, therefore, was to compare growth curves of mean body weights with those of individual weights when fitted to data of male and female mice using monophasic (logistic) and triphasic functions.

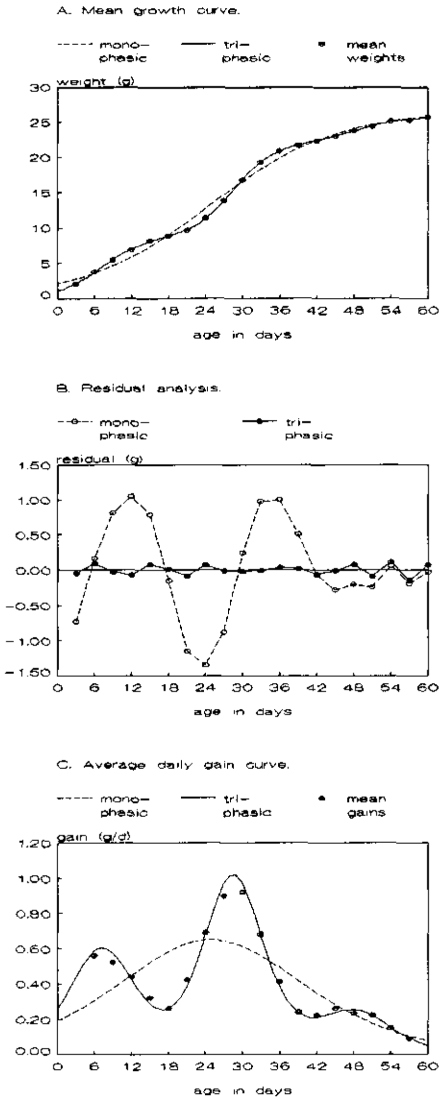

Figure 1. Mean growth curve (A), residuals (B) and average daily gain curve (C) for 480 mice.

MATERIAL

Data were from a long-term divergent selection experiment in mice. Lines were selected for high and low 21-day (weaning) body weight; a concurrent control line was randomly selected. At generation 25, a separate experiment involved a 3x3 diallel cross including each of the three lines. Male and female progeny from each of these nine crosses were weighed individually at 3-day intervals, from 3 to 60 days of age. In total, 242 males and 238 females were measured, with a mean number and standard deviation per cross of 26.9 ± 6.2 males and 26.4 ± 6.9 females. In this analysis, no attention was paid to differences between crosses.

METHODS

The function used to describe the growth curves was that of Koops (1986) for multiphasic growth:

$$y_t = \sum_{i=1}^{n} [a_i \{1 + \tanh(b_i(t - c_i))\}] + e_t \quad (1)$$

where y_t is body weight at age t ($t = 3,6,\ldots,60$ days); n is number of growth phases; for each phase i, parameter a_i is half asymptotic weight (grams), b_i is growth rate relative to a_i (days^{-1}) and c_i is age at point of inflection (days); and e_t is random error at age t.

Equation (1) is a summation of n logistic functions (Koops, 1986) and may also be written in the more familiar form of:

$$y_t = \sum_{i=1}^{n} [\ 2a_i \ / \ \{1 + e^{-2b_i(t - c_i)}\}] + e_t \quad (2)$$

Meanings of the variables and parameters in (2) are the same as those in (1). Number of parameters depends on number of phases; for example, a monophasic function has 3 parameters and a triphasic has 9.

Equation (1) was fitted to individual weight data for the monophasic ($n = 1$) and triphasic ($n = 3$) functions by nonlinear regression (computer program

BMDX85 using a modified Gauss-Newton iteration technique; Dixon, 1973). When fitting a triphasic function to individual weights, it is difficult to interpret estimates of parameters if an individual fails to show one of the phases. Such difficulties can be dealt with by eliminating from the data those animals that show by inspection irregular patterns of growth or by setting limits to parameters. Rather than discard potentially useful information, it was decided to set limits to some parameters (Table 1) based on standard deviations of parameters estimated from the curve of mean weights (Koops, 1986).

Table 1. Limits for each parameter of the triphasic function and number of estimates reaching those limits for males and females.

Parameter[1]	Estimate[2]	limit		No. estimates reaching limits	
		Lower	Upper	Males	Females
a_1	4.36	0	free	0	0
b_1	.137	.06	.22	6	6
c_1	7.2	3.0	11.4	5	2
a_2	6.53	0	free	0	0
b_2	.154	.06	.27	10	9
c_2	28.5	22.0	35.0	3	1
a_3	2.03	0	free	1	6
b_3	.12	.12	.12	-	-
c_3	48.1	48	48	-	-

[1] a's (grams), b's (days^{-1}), c's (days).

[2] Koops, 1986.

In these data, only the third phase of growth was not present in all mice. This led to a zero value for a_3 and to indeterminate values for b_3 and c_3. To overcome this difficulty, limits for b_3 and c_3 were fixed at their mean values (Table 1).

Monophasic and triphasic functions for growth curves of mean weights and of individual weights for males and for females were compared on goodness-of-fit using residual variance and the Durbin-Watson statistic (d). The d is a measure of serial correlation (Theil, 1971) and is related to the first order autocorrelation coefficient (r) of residuals as 2(1 - r), approximately.

A value for d around 2 indicates no autocorrelation (see *e.g.*, Theil, 1971 for tables). A lower value of d indicates more positive autocorrelation and a higher value indicates more negative autocorrelation.

RESULTS AND DISCUSSION

Estimates of parameters for males reached limits more often than estimates for females, with some individuals reaching limits for more than one parameter (Table 1). Limits set for b_2 were reached most often for males and for females. Estimates of a_3 reached the lower limit for females more often than for males.

Means and standard deviations of parameters, residual standard deviations and Durbin-Watson statistics for fitting curves of mean weights and of individual weights for males and for females are in Table 2 for the monophasic function and in Table 3 for the triphasic function.

Table 2. Means and standard deviations (sd) for parameters of the monophasic function, residual standard deviations (s_e) and Durbin-Watson statistics (d) for males and females.

Curve		Parameter				
		a	b	c	s_e	d
Males						
Mean wts.	mean	14.60	.050	26.01	.822	.6
(N = 1)[1]	sd[2]	.31	.003	.70	-	-
Ind. wts.	mean	14.92	.050	26.54	.905	1.0
(N = 242)	sd[3]	1.63	.005	2.74	.339	.3
Females						
Mean wts.	mean	11.90	.048	23.42	.669	.7
(N = 1)	sd[2]	.24	.003	.69	-	-
Ind. wts.	mean	12.16	.048	23.90	.624	1.0
(N = 238)	sd[3]	1.57	.005	2.56	.294	.3

[1] N is number of curves fitted.

[2] Asymptotic standard deviation.

[3] Sampling standard deviation.

Residual variance (s_e^2) for the monophasic function was higher than for the triphasic function. Autocorrelation was positive for the monophasic function and negative or negligible for the triphasic function. These criteria suggest clearly that the triphasic function, with smaller and less correlated residuals, described the data better than the monophasic function.

For the monophasic function (Table 2), parameters were generally higher when fitting curves of individual weights than when fitting a curve of mean weights; the reason for this is the nonlinear nature of the function. Parameters were higher when fitting weights of males than weights of females, especially for a, half asymptotic weight, and for c, age at point of inflection. For growth rate (b), however, differences were small. Parameters were estimated with about equal precision for males and females.

Residual variance for males was higher when fitting individual weights than when fitting mean weights, as might be expected. For females, however, residual variance was higher when fitting mean weights; the reason for this is not clear.

Table 3. Means and standard deviations (sd) for parameters of the triphasic function, residual standard deviations (s_e) and Durbin-Watson statistics (d) for males and females.

		Parameter										
Curve		a1	b1	c1	a2	b2	c2	a3	b3	c3	s_e	d
---------- Males ----------												
Mean wts.	mean	4.3	.14	7.1	7.3	.16	28.4	2.5	.12	47.2	.097	3.4
(N = 1)[1]	sd[2]	.1	.01	.2	.3	.01	.1	.2	.02	.6	-	-
Ind. wts.	mean	4.3	.14	7.2	7.6	.16	28.8	2.4	.12	48.0	.165	2.0
(N = 242)	sd[3]	.9	.03	1.3	1.5	.04	1.6	.8	-	-	.135	.6
---------- Females ----------												
Mean wts.	mean	4.4	.14	7.2	5.7	.15	28.5	1.6	.11	49.5	.095	3.0
(N = 1)	sd[2]	.1	.01	.2	.2	.01	.2	.3	.03	.9	-	-
Ind. wts.	mean	4.4	.14	7.3	5.8	.16	28.7	1.5	.12	48.0	.143	2.0
(N = 238)	sd[3]	.9	.03	1.3	1.2	.04	1.4	.7	-	-	.164	.5

[1] N is number of curves fitted.

[2] Asymptotic standard deviation.

[3] Sampling standard deviation.

Males had higher residual variance than females, consistent with higher asymptotic weights for males than for females. There were significant positive autocorrelations ($d < 2$) when fitting mean weights. The higher value of d when fitting individual weights indicates less, but still positive, autocorrelation. Values of d were about the same for males and females. For the triphasic function (Table 3), parameters were generally higher when fitting individual weights than when fitting mean weights, especially in the second phase of growth; differences, however, were small. Parameters were generally similar for males and females, especially in the first phase. Parameters a_2 and a_3 (half asymptotic weight in phase 2 and in 3), however, were higher for males than for females. The lower value of a_3 for females explains the greater number of estimates for females that reached the lower limit (Table 1). Age at point of inflection in the third phase (c_3) was 2.3 days earlier for males.

Differences between males and females within curve of mean weights or of individual weights were nearly constant. Sexes did not differ in the first phase of growth. However, differences between sexes appeared soon after the second phase started, which was just before weaning at 21 days of age. Gall and Kyle (1968) showed that the start of the second phase was not caused by the weaning process *per se*. This 'critical' age, at the end of the first phase and the beginning of the second phase, probably is associated with changes in metabolism and onset of sexual maturity. In the third phase of growth, half asymptotic weight was about one gram higher for males than for females or about 17% of half total asymptotic weight for males and about 14% for females. Growth in the third phase probably is associated with fat deposition; males reached maximum growth in this phase about 2.3 days earlier than females.

Residual variances were higher when fitting individual weights than when fitting mean weights, as expected. Males had higher residual variance than females, consistent with higher asymptotic weight for males. Autocorrelation was negative ($d > 2$) when fitting mean weights and slightly more negative for males than for females, but was negligible ($d = 2$) when fitting individual weights for males and for females.

Correlations between parameters of the triphasic function for males and for females are in Table 4. Within phase 1, correlations between parameters were relatively high, with only small differences between males and females. Within phase 2, however, correlations between parameters were relatively low, except for a_2 with b_2; differences between males and females ranged from .10 to .15.

Correlations between parameters in phase 1 and those in phase 2 were relatively low for b_2 and for b_1 with a_2. Differences between males and females were small, except for a_1 with c_2. Correlations involving a3 were relatively low, except with b2 which also showed highest difference between males and females.

Table 4. Correlations between parameters of the triphasic function for males (above diagonal, N = 242) and for females (below diagonal, N = 238).

	Parameter						
Parameter	a_1	b_1	c_1	a_2	b_2	c_2	a_3
a_1	-	-.58	.67	-.22	.63	-.36	.22
b_1	-.60	-	-.74	.53	-.64	.05	-.13
c_1	.74	-.72	-	-.36	.60	.08	.16
a_2	-.12	.50	-.30	-	-.54	-.03	-.23
b_2	.63	-.56	.59	-.45	-	-.30	.29
c_2	-.03	-.12	.18	-.16	-.15	-	-.14
a_3	.26	-.19	.21	-.18	.40	-.11	-

It is possible to compare parameters of the monophasic function with those of the triphasic function. Parameter a for the monophasic function is the sum of a's for the triphasic function because equation (1) is linear in parameter a. The sum of a's for males is 14.1 and 14.3 for curves of mean weights and of individual weights, respectively, and for females the sum of a's is 11.7 for each curve. These values for the triphasic function are lower than corresponding values for the monophasic function.

Parameter b for the monophasic function is approximately the reciprocal of the sum of reciprocals of b's (harmonic sum of b's) for the triphasic function because b is measured as reciprocal of days. The harmonic sum of b's is .046 for three of the four curves and is .044 for the curve of mean weights for females. These values are slightly lower than corresponding values for the monophasic function.

Parameter c for the monophasic function is approximately the weighted average of c's for the triphasic function, weighted by corresponding values of parameter a. This procedure gives more statistical weight to the phase with higher asymptotic weight. In these data, parameter c_2 received the most weight because the second phase (Figure 1C) is the most pronounced phase and there are only small differences in the first and the third phase.

For males the average c is 25.2 and 25.5 for curves of mean weights and of individual weights, respectively, and for females is 23.4 and 23.1. These values for the triphasic function are lower than corresponding values for the monophasic function, especially for males.

To examine statistically how each parameter of the monophasic function is related to parameters of the triphasic function, a multiple regression analysis was performed (computer program BMDP1R; Dixon, 1983). Squared multiple correlation coefficients (R^2) ranged from .86 to .99. Regression coefficients, their standard errors and standardized coefficients are in Table 5 for males and for females. From the standardized regression coefficients (B'), one can see that the monophasic parameter a (half asymptotic weight) is associated with triphasic parameters a_1, a_2 and a_3, with a_2 being the most important and with only small differences between sexes. R^2 values were .99 for males and .98 for females, which supports the formulation of parameter a as a linear combination of the a's.

The monophasic parameter b is associated relatively little with triphasic parameters b_1 and b_2 because the relation of parameter b with the b's is nonlinear. The b is most associated with parameters of the second phase, with c_2 being most important. These results are supported by relatively low R^2 values of .86 for males and .88 for females.

The monophasic parameter c is associated with triphasic parameters a_1, a_2, c_2 and a_3, with the a's being more important for females and c_2 being most important for males and for females. R^2 values were .97 for males and .91 for females. These results support the formulation of c as a weighted average of the c's.

Table 5. Coefficients (B), their standard errors (se) and standardized coefficients (B′) for regression of each parameter of the monophasic function on parameters of the triphasic function for males and females.

	Monophasic parameter								
	a			b			c		
Triphasic parameter	B	se	B′	B	se	B′	B	se	B′
Males (N=242)									
a_1	1.10	.02	.59	-.002	.000	-.34	-1.39	.06	-.44
b_1	.46	.75	.01	-.017	.008	-.08	-4.68	1.99	-.05
c_1	-.14	.02	-.12	.002	.000	.48	-.01	.04	-.01
a_2	.95	.01	.87	.002	.000	.62	.13	.03	.07
b_2	-1.76	.50	-.04	.046	.006	.33	-2.98	1.34	-.04
c_2	.25	.01	.25	-.002	.000	-.68	1.20	.02	.70
a_3	1.08	.02	.55	-.002	.000	-.38	1.64	.04	.50
Females (N=238)									
a_1	1.01	.03	.59	-.001	.000	-.20	-1.75	.09	-.63
b_1	.33	.85	.01	-.016	.008	-.08	-2.69	3.16	-.03
c_1	-.14	.02	-.12	.002	.000	.62	-.02	.07	-.01
a_2	1.01	.02	.76	.002	.000	.47	.53	.06	.24
b_2	.06	.54	.00	.033	.005	.25	4.34	2.02	.07
c_2	.20	.01	.19	-.002	.000	-.66	1.21	.04	.67
a_3	1.08	.02	.50	-.003	.000	-.45	2.25	.08	.64

Eisen *et al.* (1969) suggested that because of the "diphasic nature" of growth in mice, data should be analyzed in two parts, from birth to 21 days of age and from 21 to 56 days of age. Our results support the importance attributed by Eisen *et al.* (1969) to the second phase of growth relative to the first phase. The difficulty in analyzing data in parts, however, is in deciding where to separate the parts. The multiphasic function obviates this difficulty because it treats the data as a continuous growth curve.

Eisen *et al.* (1969) also expected more precise results if only data from the second phase were used. They concluded, however, that results were similar when analysis was applied to the second part as when applied to the entire growth curve. Our results support their expectation of more precise results when using a multiphasic function compared to a monophasic function. For example, any differences between sexes that might exist in the first and third phases of growth would be overlooked when using a monophasic function.

By ignoring the multiphasic nature of the growth curve, one can misinterpret results, which could lead to wrong conclusions. The most illustrative example comes from curves of mean weights in Tables 2 and 3. For the monophasic function (Table 2), age at point of inflection (c) for males was 2.6 days later than for females. For the triphasic function (Table 3), however, differences in c between sexes were negligible in the first and the second phase, but in the third phase age at point of inflection for males was 2.3 days earlier than for females. Therefore, one can conclude from the monophasic function (Table 2) that males reach maximum gain later than females, and from the triphasic function (Table 3) that males reach maximum gain at the same age as females for the first and the second phase but earlier than females for the third phase. This difference in conclusions can be explained by the higher value of half asymptotic weight (a_3) for males than for females; this information would not be available using the monophasic function.

With respect to the aim of this study, we conclude that it is feasible to fit growth curves of individual body weights using a multiphasic function. With the triphasic function, the curve of individual weights showed less precision, as expected, but also showed an absence of autocorrelation when compared to the curve of mean weights. Values of parameters were slightly higher when fitting individual weights, but differences between curves within sex were small. Values of parameters were similar for the sexes, especially for the first phase of growth. Half asymptotic weight in the second and the third phases were higher for males than for females. From these results, it should be clear that using a multiphasic function to describe growth curves in mice provides greater insight for understanding the biology of growth.

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Chapter 3

PROPERTIES AND APPLICATIONS OF THE MULTIPHASIC GROWTH FUNCTION

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ABSTRACT

Properties of the multiphasic growth function are shown and discussed. The function is applied to problems of growth in different circumstances.

Seasonal influence on growth in length of Northsea herring is an example of an external factor causing phases of growth. The most important internal factor causing phases of growth is the difference in growth rate of body components.

For applications in growth studies related to body composition, the general multiphasic function was modified and applied to growth data of pigs. In a first example, growth of total dry matter was analyzed with a diphasic function, and growth of fat and fat-free components were analyzed with monophasic functions. Results for total dry matter showed clearly that parameter estimates of the diphasic function for the two distinguishable phases were closely related to estimates of the monophasic function for each of the two components. In a second example it was shown that phases in the multiphasic function contain groups of body components, where grouping is based on similarity of ages where gain is maximum.

By restricting parameters of the general multiphasic function, and treating other parameters as constants, manageable growth functions were constructed with parameters that are easy to interpret.

INTRODUCTION

A multiphasic growth function, suggested by Koops (1986) and used in mice (Koops *et al.*, 1987) and in chickens (Grossman and Koops, 1988), has been shown to provide an excellent fit to growth data where measurements are precise and frequent over the course of a lifetime.

The multiphasic function is a summation of logistic functions:

$$y_t = \sum_{i=1}^{n} [a_i(1 + \tanh(b_i(t - c_i)))] \qquad (1)$$

with a first derivative:

$$y'_t = \sum_{i=1}^{n} [a_i b_i(1 - \tanh (b_i(t - c_i)))] \qquad (2)$$

where y_t is a growth measurement (weight or length) at age t and y'_t is gain; n is number of growth phases; tanh is the hyperbolic tangent; for each phase i, parameter a_i is half asymptotic value; b_i is growth rate relative to a_i (age^{-1}), and c_i is age at maximum gain (a_ib_i).

Function (1) appears to be flexible in describing growth, assuming the existence of different phases. Phases in growth patterns of individuals or groups of individuals can be the result of external or internal influences. Examples of external influences include season, feeding, and housing. An example of internal influences is heterogeneity of growth rate at many biological levels (Piantadosi, 1987). In considering mean growth curves of individuals, the differences in individual curves will cause heterogeneity in the mean curve. In fact, this same type of heterogeneity exists between growth curves of body parts within individual growth curves. It is known that different parts or components of the body have different growth rates (Palsson, 1955). If these differences are considerable, they possibly can lead to phases in the curve of total body growth. The objective of this study is to demonstrate some applications of the multiphasic function in different circumstances. To fit various growth curves, the multiphasic function (1) can be modified, or parameters can be bounded or replaced by constant values. A decrease in number of parameters is often desirable because in many cases it leads to less correlated parameters that are easier to interpret.

To choose the right modification, insight is needed in the function and in the meaning of parameters. Therefore a brief description of properties of the function and meaning of parameters is included. The first application is to growth of fish, influenced by environmental factors. Secondly, two applications are demonstrated to growth of body components in pigs. Functions used in these applications are special cases of function (1). Parameters of each function were estimated by the nonlinear regression procedure NLIN using DUD (SAS Institute Inc, 1985).

THE MULTIPHASIC GROWTH FUNCTION

A typical growth pattern, as result from the multiphasic growth function, is in Figure 1 for a diphasic (n=2) function, which is the accumulation of the corresponding gain pattern in Figure 2, consisting of two symmetrical bell-shaped phases.

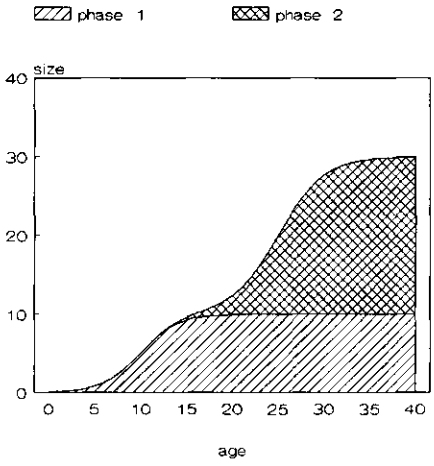

Figure 1. A typical growth pattern for a diphasic function.

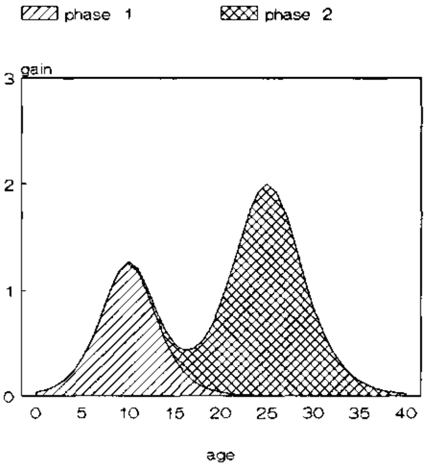

Figure 2. A typical gain pattern for a diphasic function.

The assumption of symmetrical gain phases, is based on appearance of the gain curve in practical growth data (Robertson, 1923; Peil and Helwin, 1981). For description purposes, the logistic function is chosen, because of its favorable mathematical properties (Koops, 1986).

Each phase of gain is defined by three characteristics: 1) location, 2) height of maximum gain (peak), and 3) duration. Location of the peak is determined by parameter c_i (age), height of peak by a_ib_i (size/age), and duration by a function of b_i (1/age), as in Figure 3. Location and height of peak are easily determined: 50 to 10, respectively, from Figure 3. To determine duration, two lines are drawn in the figure, from ages $c_i \pm 2/b_i$ to the peak. The area of the triangle formed by these lines, one-half base ($4/b_i$) times height (a_ib_i), is equal to the area under the curve, namely, $\frac{1}{2}(4/b_i)(a_ib_i) = 2a_i$, the asymptotic value. About 96.4% of total area under the curve is

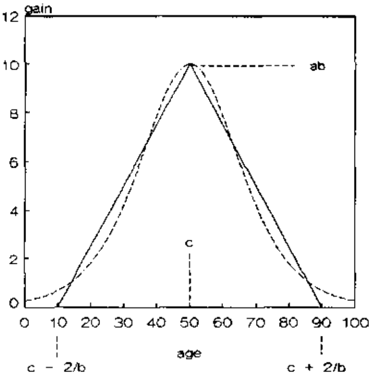

Figure 3. One phase of gain from the multiphasic function.

within ages $c_i \pm 2/b_i$. It is useful, therefore, to define duration of phase as $4/b_i$. Thus, parameter b_i can be estimated, in Figure 3, by $4/b_i = 90 - 10$, so that $b = .05$. This approach is helpful to determine correct starting values for fitting the multiphasic function.

The summation of n such phases can result in many different shapes, from almost straight lines to stairstep patterns, as will be demonstrated in following sections.

CONSTANCY OF PARAMETERS OVER PHASES

If parameters a_i and b_i in function (1) are held constant to a and b, for each of n phases, and the successive difference (d) of c's ($d = c_i - c_{i-1}$) is taken constant, a regular stairstep pattern is the result. If d is taken equal to 1.53/b, curves appear straight in the middle over longer periods, depending on number of phases. Then function (1) becomes:

$$y_t = \sum_{i=1}^{n} [a\{1 + \tanh(b(t - (c + (i-1)1.53/b))))\}] \qquad (3)$$

which is illustrated in Figure 4 for n = 1 to 4. Parameters used for these lines are $a = 12/(2n)$, $b = .10$, and $c = 30$.

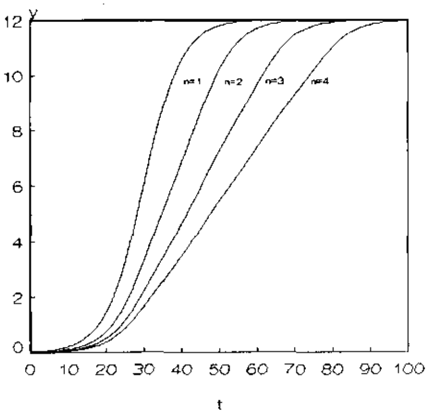

Figure 4. Multiphasic functions which are straight in the middle.

Such a pattern of growth can arise, for example, when there is a series of equal growth phases at fixed intervals in time. If intervals are smaller than 1.53/b, periods where lines are straight will be smaller; if intervals are larger than 1.53/b, a stairstep pattern is seen. For example, growth in length of Northsea herring (presented in Figure 5, with data points estimated from Figure 13.13 in Daan and Zijlstra, 1984) is influenced by water temperature, so that fish begin growing in spring, continue through summer, slow down in fall, and cease in winter. In this case, we have nearly-fixed annual intervals, and for each year we can expect similar duration of phases, so that only the parameter describing total growth (2a) in each year is different.

Collection of data, presented in Figure 5, was started in January at age 2; therefore, an "initial" level (a_0), which is the level reached in the two previous years, was added to the function.
Then function (1) becomes:

$$y_t = a_0 + \sum_{i=1}^{n} [a_i(1 + \tanh(b(t - (c + (i-1)12))))] \qquad (4)$$

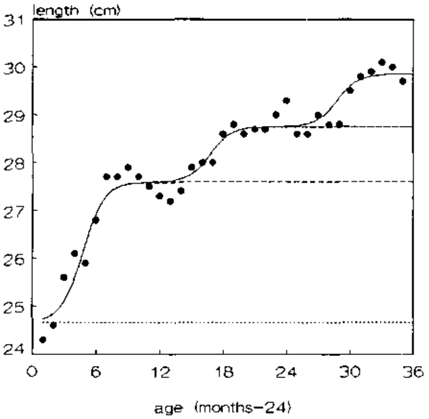

Figure 5. Length growth of Northsea herring, fitted by a multiphasic function.

Parameter estimates $a_0 = 24.7$, $a_1 = 1.46$, $a_2 = .58$, $a_3 = .54$, $b = .50$, and $c = 4.78$ resulted in the estimated line in Figure 5. This means that maximum gain (c) appeared about May (4.78 months) and duration (4/b) was about 8 months. Total growth in length each year ($2a_i$) decreased with age: during age 3, fish averaged 3 cm growth; and during ages 4 and 5, about 1 cm growth.

In this example, where growth phases were produced by external influences, the multiphasic function gave satisfactory prediction and parameters were easy to interpret.

DURATION DEPENDENT ON AGE

One example of an internal influence producing phases in growth of body weight comes from the theory of the 'Hammond school' (Palsson, 1955), which states that different components of the body have maximum growth at different ages: first the nervous system, second the frame (bone), third muscles, and finally fat depots.

To analyze body composition using the multiphasic function, it is assumed that growth of each body component starts before birth and that different body components have maximum gain at different ages (Palsson, 1955). Further, it is assumed that components having maximum gain at older ages have phases of longer duration. Therefore, duration of phase i is taken to be a function of age at maximum gain, by expressing $1/b_i$ in terms of c_i in function (1).

If function (1) is reparameterized so that $k_i = b_ic_i$, it can rewritten as:

$$y_t = \sum_{i=1}^{n} [a_i(1 + \tanh(k_i(t/c_i-1)))] \qquad (5)$$

The parameter k_i is without units because b_i is expressed as 1/age and c_i as age. To help interpret k_i, we return to Figure 3. With duration of phase i defined as $4/b_i$, ages of interest are $c_i \pm 2/b_i$. If $k_i = b_ic_i$, then these ages can expressed as: $c_i(1 \pm 2/k_i)$, and duration is now defined as $4/k_i$ times c_i. Because of the assumption that growth of each body component starts before birth, we can expect k_i to be similar for each component; therefore, k_i in function (5) can be changed to k.

In the following examples it will be shown that phases determined by the multiphasic function (5) are related to growth of different body components.

The first example contains data from Table 2.9 in Pfeiffer *et al.* (1984) on pig growth (body weight and composition) from birth to 222 days of age. For purposes of demonstration, only two components were examined, total dry matter growth and fat growth. A third component, fat-free dry matter, was computed by subtraction of fat from total dry matter. Gestation length (113 days) was added to each age, thus measuring age as 'conception age'.

For simplicity, data on total dry matter growth in the pig was described by a diphasic function and data on the components fat and fat-free dry matter each by a monophasic function. If the method performs well, parameter estimates for the monophasic functions for each of the two components are expected to be related to estimates for the two phases of the diphasic function for total dry matter. Results of parameter estimation and 95% confidence limits for estimates are in Table 1. Predicted values for total dry matter showing the two distinguishable phases are in Figure 6 and predicted values for fat and for fat-free dry matter are in Figure 7.

Results in Table 1 show small confidence intervals for estimates of each of the two components, relative to total dry matter. Each parameter estimate of the monophasic function is included in the confidence interval for the corresponding estimate of the diphasic function, except for k for fat-free dry matter. Thus, the two phases of total dry matter are strongly related to the growth pattern of each of the two components (Figures 6 and 7).

Table 1. Parameter estimates, with asymptotic 95% confidence limits, of diphasic function for dry matter and of monophasic for components fat and fat-free dry matter, in growth data for pigs (Pfeiffer *et al.*, 1984).

Total dry matter[1]				Components of dry matter[2]			
Parameter	Estimate	95% Conf.limits Lower	Upper	Parameter	Estimate	95% Conf.limits Lower	Upper
Phase 1				Fat-free			
a_1	12.96	6.54	19.39	a	8.81	8.58	9.03
k	3.97	3.84	4.11	k	3.46	3.31	3.61
c_1	242.73	227.36	258.10	c	237.92	235.06	240.77
Phase 2				Fat			
a_2	29.93	27.27	32.59	a	28.44	26.71	30.17
k	3.97	3.84	4.11	k	4.03	3.89	4.18
c_2	325.10	292.07	358.13	c	299.14	294.01	304.27

[1] $y_t = a_1[1 + \tanh\{k(t/c_1 - 1)\}] + a_2[1 + \tanh\{k(t/c_2 - 1)\}]$

[2] $y_t = a[1 + \tanh\{k(t/c - 1)\}]$

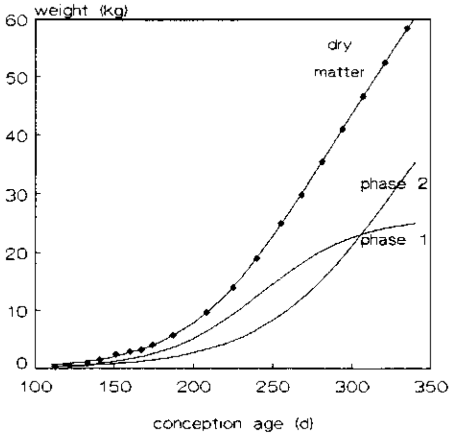

Figure 6. Growth in dry matter weight in pigs, fitted by a diphasic function.

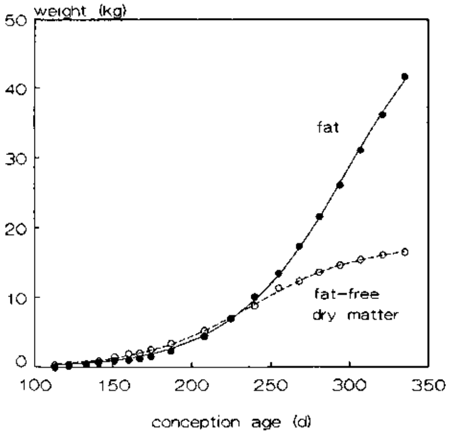

Figure 7. Growth of fat and fat-free dry matter in pigs, each fitted by a monophasic function.

Data for a second example on body composition in pigs were from Tables 4.6 and 4.11 in Walstra (1980). At eight different dissection stages, mean weights for carcass side and components (offal, skin, fat, muscle and bone) were reported for Dutch Landrace sows from the *ad libitum* feeding level group. The number of sows slaughtered at each stage ranged from five to eight. Dissections were at birth and at 12, 19, 25, 30, 37, 50 and 135 weeks of age. By adding gestation length (16 weeks), these ages were transformed to 'conception ages'. These data were chosen because they illustrate how the multiphasic function behaves on data with few observations over a wide range of ages. Because the number of weights for each component was eight, a monophasic function was fitted to weights for each of the components and a diphasic function to carcass side weights. Parameter estimates and residual standard deviations for fits of the monophasic function to body components are in Table 2.

Table 2. Parameter estimates and residual standard deviations (s_e) for fits of monophasic functions to different body components in growth data for sows (Walstra, 1980).

	body component				
parameter[1]	offal	skin	fat	muscle	bone
a	3.24	2.40	32.16	19.38	3.43
k	1.52	1.67	2.08	1.76	1.65
c	49.15	55.48	61.62	49.67	48.60
s_e	.49	.32	3.15	2.72	.51

[1] $y_t = a[1 + \tanh\{k(t/c - 1)\}]$

Looking to ages where gain is maximum, it can be concluded that maximum gain for offal, muscle and bone was about 49 weeks, for skin at about 55 weeks, and for fat at about 62 weeks. Based on age of maximum gain in these components, two groups are obvious, an early group for offal, muscle and bone, and a late group for skin and fat. If there is any relation of body components to phases in a diphasic function for total body weight, it is expected that these two groups will be isolated. Therefore monophasic functions were fitted to weights of offal+muscle+bone and to skin+fat.

Table 3. Parameter estimates, with asymptotic 95% confidence limits, of diphasic function for carcass side weights and of each monophasic function for components muscle+bone+offal and fat+skin in growth data for sows (Walstra, 1980).

Carcass side weight[1]				Components of weight[2]			
Parameter	Estimate	95% Conf.limits Lower	95% Conf.limits Upper	Parameter	Estimate	95% Conf.limits Lower	95% Conf.limits Upper
Phase 1				Muscle+bone+offal			
a_1	27.22	15.91	38.52	a	25.94	21.25	30.62
k	3.15	1.79	4.50	k	1.72	.99	2.44
c_1	38.05	31.94	44.17	c	49.31	41.28	57.43
Phase 2				Fat+skin			
a_2	34.31	22.88	45.74	a	34.57	30.10	39.05
k	3.15	1.79	4.50	k	2.05	1.41	2.68
c_2	78.95	51.06	103.84	c	61.26	54.30	68.22

[1] $y_t = a_1[1 + \tanh\{k(t/c_1 - 1)\}] + a_2[1 + \tanh\{k(t/c_2 - 1)\}]$

[2] $y_t = a[1 + \tanh\{k(t/c - 1)\}]$

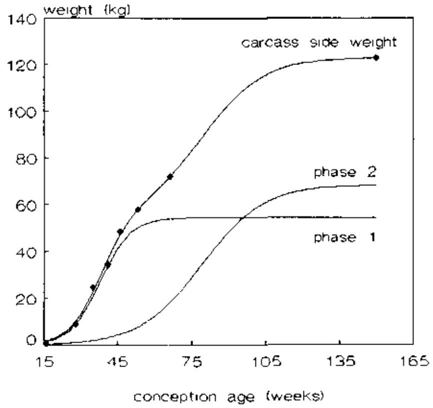

Figure 8. Growth of carcass side weight in pigs, fitted by a diphasic function.

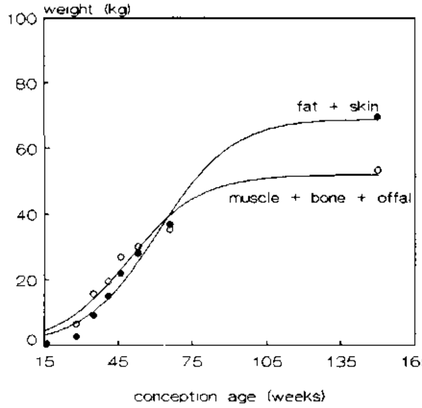

Figure 9. Growth of two groups of body components in pigs, fitted by monophasic functions.

Residual standard deviation for a diphasic fit of carcass side weights, with parameter k assumed to be equal for both phases, was significantly lower than for a monophasic fit, 2.25 to 7.03 respectively. Parameter estimates and 95% confidence limits for a monophasic fit to weights of each of the two groups of body components are in Table 3, together with parameter estimates and 95% confidence limits for a diphasic fit to carcass side weight.

Results in Table 3 show similar estimates for parameter a of phases and groups, but there is less agreement for parameters k and c. That means that the amount grown for the two groups of components at mature stage was predicted well by phases, but there was less agreement at earlier stages. One of the reasons for this is the absence of observations in the period where the second phase is located, as can be seen in Figures 8 and 9. In these figures, observations and predicted lines are presented for a diphasic fit to carcass side weights, including the two predicted phases, and for a monophasic fit to weights of each of the two groups of components. In Figure 9 it can seen also that a monophasic fit was not very good for weights of each group, especially at younger ages. However, given the number of observations and the unbalanced range of observations, results obviously showed that the fraction fat+skin in the body at maturity was predicted well by the second phase of the diphasic function for carcass side weight.

CONCLUSIONS

Properties of the multiphasic function are discussed and demonstrated. Examples of growth in Northsea herring and of growth of body components in pigs indicate that application of the multiphasic growth function to various situations is attractive, if appropriate assumptions are made. The general multiphasic function is easy to adapt to different assumptions, according to circumstances of growth. In the fish growth example, effect of season could be incorporated easily into the general multiphasic function. This function gave satisfactory predictions with parameters easy to interpret.

Analysis of growth in weight of body components of pigs, showed that growth of these components were related to the phases produced by a slightly modified diphasic growth function applied to total weights of the components. Grouping of growth of body components into phases seemed to be based on ages where gain is maximum.

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Chapter 4

MULTIPLE- VERSUS SINGLE-PHASE GROWTH FUNCTIONS

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ABSTRACT

A multiple-phase growth function is compared with four single-phase growth functions to study the relation between these two approaches for describing growth curves. A simulation study was conducted on four functions, selected from a general five-parameter function to differ in shape; included were Johnson-Schumacher, Michaelis-Menten, Gompertz and Logistic functions. The multiphasic function was fitted to the simulated data from the four functions.

Body weights of a cow and a boar were analyzed with the four single-phase functions and with the multiphasic function, with one, two and three phases.

Results of simulation showed a satisfactory description of a triphasic function to data sets of the four functions in terms of residual standard deviation and number of runs. It can be concluded that single-phase functions with early inflection points show relatively high fractions of asymptotic value in the first phase, using triphasic functions.

Using a single-phase function, cow and boar data could be described best with the Johnson-Schumacher function. Using a diphasic function for each data set, systematic deviations disappeared and residual standard deviation was similar to the fit with the Johnson-Schumacher function. Results of the analyses of the cow and boar data were in agreement with the findings in the simulated data.

This comparison showed that a multiple-phase function is a reasonable alternative to a single-phase function. An important advantage is not having to select the 'best' single-phase function. The multiphasic function can be used in almost every case. Problems of having to estimate a larger number of parameters for a multiple-phase function can be overcome because parameters are less correlated than for a single-phase function.

INTRODUCTION

A multiphasic growth function (Koops, 1986) theorizes growth to result from different growth phases, also called growth spurts or cycles. This theory is supported by the acceptance of a triphasic pattern in the human growth curve (Tanner, 1962 and Bock *et al.*, 1973). Use of a multiphasic function on mean growth data has been demonstrated for various species (Koops, 1986), for chickens (Grossman and Koops, 1988) and on individual data for mice (Koops *et al.*, 1987). Koops (1989) demonstrated application of the multiphasic function in growth of fish and body composition of pigs. In general, the function shows excellent fit to data where measurements are precise and taken frequently over the course of a lifetime. The multiphasic function used was a summation of logistic functions:

$$y_t = \sum_{i=1}^{n} [a_i(1 + \tanh(b_i(t - c_i)))] \tag{i}$$

with the first derivative:

$$y'_t = \sum_{i=1}^{n} [a_i b_i(1 - \tanh^2(b_i(t - c_i)))] \tag{ii}$$

where y_t is body weight measured at age t and y'_t is body-weight gain; n is number of growth phases; tanh is the hyperbolic tangent; and for each growth phase i, parameter a_i is half asymptotic weight; b_i is growth rate relative to a_i (age^{-1}) and c_i is age at the inflection point.

Well-known growth functions, including the Gompertz, logistic and the von Bertalanffy function, (Richards, 1959) are three-parameter functions that assume single-phase growth. Frequently, these functions show systematic deviations of predicted from observed values. Nevertheless, they give a general impression of overall growth. If more detailed insight into the growth pattern is needed, however, four-parameter functions are suggested, such as the modified von Bertalanffy or the Richards function (Richards, 1959). The additional parameter provides a flexible point of inflection. In most situations, however, these functions are difficult to fit because of high pairwise correlations between parameters. The Richards function can be considered to comprise a 'family' of growth functions, including many of the well-known three-parameter growth functions. Often the Richards function is used to decide which member of the family fits 'best'; then a three-parameter function is used for further application. To decide which function fits best, the following criteria often are used: small residual standard deviation, absence of systematic deviations (trends), and realistic parameter estimates.

Results from testing criteria sometimes are in conflict; *e.g.*, functions with a good fit can give poor estimates, especially for mature weight.

There is a tendency in the literature to assign specific three-parameter growth functions to different species of animals. For example, the Gompertz function is accepted to be best to describe growth in birds (Laird *et al.* (1965); the logistic, for growth in mice and rats (Bakker, 1974 and Eisen, 1976); and the von Bertalanffy for growth in fish (Fabens, 1965 and Ricker, 1975). These choices generally are based on location of point of inflection. The inflection point for the logistic function is one-half asymptotic weight, whereas the inflection point for the Gompertz is asymptotic weight divided by *e*, the base of the natural logarithms. No biologically sound arguments are given in the literature, however, to explain differences in location of inflection point, except it is believed that the inflection point coincides with the onset of puberty (Brody, 1945). Zeger and Harlow (1987) noted, quite rightly, that available functions should be considered to be tools for biological analysis and not to be results from laws of growth.

The multiphasic growth function is based on the underlying existence of one or more logistic growth functions. In practical terms, this means that total gain is a summation of one or more symmetrical, bell-shaped curves. As mentioned before, one of the characteristic differences among single-phase growth functions is in the point of inflection; that is, in the symmetry of the curves. Under the assumption that gain in body weight appears symmetrical and bell-shaped (Robertson, 1923), the question arises: is it possible to express an asymmetrical single-phase growth function by two or more symmetrical functions?

The objectives of this study are to investigate whether differences in location of point of inflection can be explained by the existence of more than one growth phase and to examine how a multiple-phase growth function can be used in practical growth studies to help overcome difficulties in having to select the "best" single-phase growth function.

SINGLE-PHASE GROWTH FUNCTIONS

A large number of three- or four-parameter growth functions are presented in the literature (*e.g.*, Grosenbaugh, 1965; Pruitt *et al.*, 1979; Schnute, 1981; and Sager and Sammler, 1982). Three-parameter functions are used often because of simplicity of computation and interpretation. The current ease and speed of computing complicated nonlinear functions, however, has made it more difficult to decide which function to use.

We have found a general five-parameter function that contains most of the single-phase, three- or four-parameter functions. The function is not suitable for use directly, but should be considered as a tool to find the best three-parameter function by varying parameters p and m. The function is of the form:

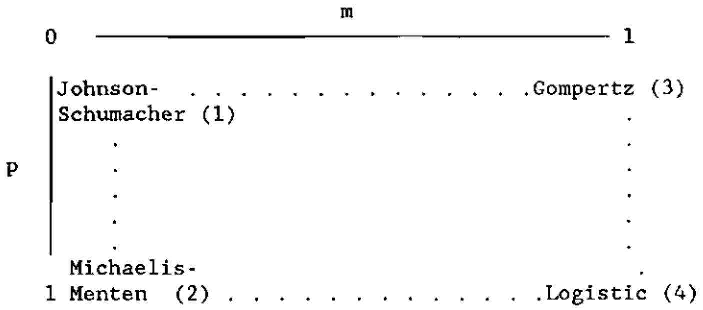
$$y_t = A/[1 + p\, e^{-h((t/d)^m-1)/m}]^{1/p} \qquad \text{(iii)}$$

where y_t is the observation at age t and A, h, d, p, and m are parameters to be estimated. Parameter A is the asymptotic value, h is the growth rate parameter, d is center (for $m = 1$, d is point of inflection), p is the transformation parameter for observation scale, and m is the transformation parameter for age scale. Function (iii) can be written in hyperbolic tangent form, with the same parameters:

$$y_t = \tfrac{1}{2} A[1 + \tanh\{\tfrac{1}{2} h((t/d)^m-1)/m + \ln(p)\}]^{1/p} \qquad \text{(iv)}$$

Different combinations of p and m yield most of the well-known functions. For instance, $m \to 0$ includes the Johnson-Schumacher (Grosenbaugh, 1965) and the Michaelis-Menten functions (Jolicoeur, 1985) and, $m = 1$ is the Richards function. For our purpose, we shall consider only functions with positive values of y_t for t positive, which is true for $p>0$ and $m>0$. Most interesting functions are within the square: $0 < p \leq 1$ and $0 < m \leq 1$.

To investigate the relation of the multiple-phasic function to single-phase functions, we have selected four functions corresponding to the four extreme combinations of p and m because they differ fundamentally in shape. Schematically:



The four selected functions are in Table 1, with the number in front of the name of each function corresponding to the number in the scheme above.

Table 1. Well-known, three-parameter growth functions derived from a basic, five-parameter function.

Function		Equation	Values for m and p
Number	Name		
0.	Basic function[1]:	$y_t = A/[1 + p\, e^{-h((t/d)^{m}-1)/m}]^{1/p}$	free
1.	Johnson-Schumacher:	$y_t = A_1\, e^{-(d_1/t)^{h_1}}$	$(m \to 0,\ p \to 0)$
2.	Michaelis-Menten:	$y_t = A_2 \,/\, [1 + (d_2/t)^{h_2}]$	$(m \to 0,\ p = 1)$
3.	Gompertz:	$y_t = A_3\, e^{-e^{-h_3(t/d_3-1)}}$	$(m = 1,\ p \to 0)$
4.	Logistic:	$y_t = A_4 \,/\, [1 + e^{-h_4(t/d_4-1)}]$	$(m = 1,\ p = 1)$

[1] y_t = observation at age t,
A = asymptotic value,
h = growth rate parameter,
d = center (for $m = 1$, d is point of inflection),
m = transformation parameter for age scale and
p = transformation parameter for observation scale.

The two most essential differences in functions are the point of inflection and the rate of approach to the asymptote. Functions 1 and 2 have flexible inflection points in contrast to functions 3 and 4, which have fixed points. Function 1 approaches the asymptote slowly, function 4 quickly, and the other functions approach at intermediate rates.

To study the behavior of these functions, 100 observations (y_t) were simulated for each of the four functions over an age period from 1 to 100, rounded to the fourth decimal. Assuming measurement errors of 1%, observations (y_t) were multiplied by (1+.01e), where e is a pseudo-random deviate drawn from a N(0,1) distribution. Parameters for function (iv) were chosen to meet conditions that $y_t = .01A$ at $t = 10$ and $y_t = .99A$ at $t = 90$, to ensure that the range of simulated values is the same for all functions. Values for parameters and results for fitting the four functions to the simulated data are in Table 2.

Table 2. Parameters used for simulation and their estimates after simulation of data for four single-phasic growth functions[1].

Function	Parameters			Point of inflection		Parameter estimates			Residual standard deviation
(j)	A_j	d_j	h_j	Age	y/A_j	A_j	d_j	h_j	
1	1.0	17.3	2.79	15.5	.257	1.0	17.3	2.77	.0092
2	1.0	30.0	4.18	26.7	.380	1.0	30.0	4.16	.0074
3	1.0	29.9	2.29	29.9	.368	1.0	29.9	2.28	.0078
4	1.0	50.0	5.74	50.0	.500	1.0	49.9	5.79	.0067

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[1] Description of functions in Table 1.

If other functions are used to approximate the simulated data the obtained residual standard deviations can be compared with the s_e based on random deviates in Table 2, to judge goodness of fit.

Figure 1 shows graphs of the four functions resulting from deterministic modeling (without random deviates) using parameters in Table 2.

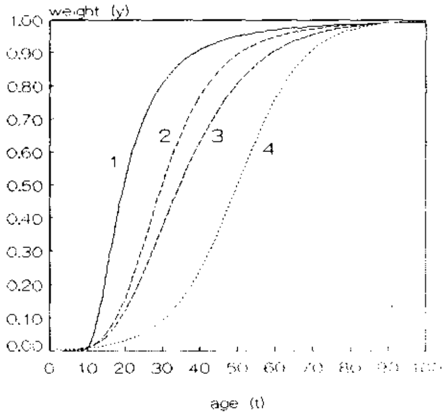

Figure 1. Four selected single-phase growth functions. (Numbers correspond with equations in Table 1.)

RELATION OF MULTIPLE-PHASE TO SINGLE-PHASE GROWTH FUNCTIONS

Simulation

Simulated observations from the four selected functions (Table 1 and Figure 1) were fitted by the multiphasic function (iv), using a nonlinear regression procedure (PROC NLIN, method DUD; SAS Institute Inc., 1985). For the multiphasic function (i) to be comparable to functions (iii) and (iv), parameter b in (i) is replaced by k = bc, leading to:

$$y_t = \sum_{i=1}^{n} [a_i(1 + \tanh(k_i(t/c_i - 1)))] \qquad (v)$$

Parameter b is in age^{-1} units, c is in units of age, so k is without units. A discussion on treating k = bc is given by Koops (1989). Parameter k is related to the value of y_t at age 0; larger values for k, indicate smaller values for y_0.

Table 3 shows results of fitting the multiphasic function (v) to the simulated data, for n = 1, 2, and 3. To judge goodness of fit, three criteria were used: residual standard deviation (s_e), number of runs to test for presence of trends in residual deviations, and estimates of asymptotic values. The runs test is a quick test for trend, where a run is defined as a series of equally signed deviations (Clark and Schkade, 1969).

Because the multiphasic function with n = 1, is equal to the logistic function, function 4 data were described completely by a monophasic function. Therefore, further concentration will be only on data from function 1, 2 and 3. As expected, the monophasic function did not fit well simulated data from these functions, because they differ basically in shape from the logistic. This is illustrated in Table 3, where monophasic fits to data of function 1, 2 and 3 have high residual standard deviations and small numbers of runs, which indicates large series of residuals with same signs, and have low estimates for the asymptotic value ($2a_1$). Results of the monophasic fit to Johnson-Schumacher (function 1) data (largest s_e, smallest number of runs, lowest a) indicated that the shape of function 1 differs most from the logistic shape. This is because the Johnson-Schumacher differs from the logistic function in m and p, whereas the other functions differ in m or p.

Table 3. Residual standard deviation (s_e) and estimated parameters for the multiple-phase growth function [1], by number of phases (n), fitted to data simulated for four single-phase growth functions[2].

Number of phases (n)	Function no.[2]	s_e	Number of runs[3]	Phase (i)	Parameters a_i	k_i	c_i
1	1	.0360	12 *	1	.483	2.17	20.6
	2	.0226	14 *	1	.487	2.25	30.4
	3	.0231	18 *	1	.488	2.06	35.2
	4	.0067	41	1	.500	2.89	49.9
2	1[4]	.0168	34 *	1	.369	3.08	17.8
				2	.122	3.08	35.3
	2[4]	.0098	44	1	.350	2.81	26.7
				2	.144	2.81	44.3
	3[4]	.0104	34 *	1	.276	2.65	28.4
				2	.218	2.65	46.9
3	1[4]	.0109	43	1	.289	3.77	16.4
				2	.161	3.77	26.9
				3	.045	3.77	51.0
	2[4]	.0079	54	1	.169	3.19	22.6
				2	.252	3.19	32.5
				3	.075	3.19	52.2
	3[4]	.0084	38 *	1	.141	3.02	23.7
				2	.251	3.02	36.6
				3	.105	3.02	56.1

[1] $y_t = \sum_{i=1}^{n} [a_i\{1 + \tanh(k(t/c_i - 1))\}]$

[2] See Table 1 for description of functions and Table 2 for values of parameters used.

[3] * Significant trend ($P<.05$).

[4] Assumed k_i to be equal for all phases.

For the diphasic function (n=2), high asymptotic standard errors were obtained for k_1 and k_2. Confidence limits for these estimates overlapped completely; therefore, $k_1 = k_2$ was assumed. Results showed slight differences in estimates, but similar s_e and number of runs. Asymptotic standard errors for most estimates showed a substantial decrease when using equal k's;

therefore, analyses with $k = k_1 = k_2$ were conducted. Using the diphasic function, residual standard deviations for these fits were about two times smaller (Table 3) than when using the monophasic model. Compared with residual standard deviations in Table 2, however, they were higher still. For fits to each of functions 2 and 3 data, s_e was at a reasonable level (.0098 and .0104), but for the fit to function 1 data, s_e was almost twice as high (.0168) as s_e from Table 2 (.0092). Number of runs indicated for function 1 and 3 data significant trends in residual deviations, but deviations for functions 2 data seemed to be random. Estimates of asymptotic values $2(a_1+a_2)$ were close to 1.

For the triphasic function, similarly, there were high standard errors for parameters, especially for k_1, k_2, and k_3. Assuming equal k's caused a remarkable decrease in standard errors, so again the situation with $k = k_1 = k_2 = k_3$ will be considered. Residual standard deviations, compared to Table 2, were almost minimum and number of runs were not alarming (Table 3). Estimates for asymptotic values $2(a_1+a_2+a_3)$ were similar and close to 1.

It can be concluded that simulated data from single-phase growth functions could be described well by a multiphasic function. The monophasic function fitted data of function 4 completely, but failed to fit the other data. Using a diphasic function, goodness of fit criteria were not entirely met. Residual standard deviations were too large and number of runs too small, especially for function 1 data. Results of fitting triphasic functions showed satisfactory descriptions of simulated data of the three remaining single-phase functions. Parameter estimates (Table 3) show more than half the asymptotic value in the first phase for Johnson-Schumacher data, whereas the Michaelis-Menten and Gompertz data had about half the asymptotic value in the second phase. There is an indication of higher fractions in the first phase for data of functions with early inflection points. The smallest value for k was estimated for the Johnson-Schumacher data, which agrees with the abrupt rise at $t = 10$ in Figure 1. The estimated value for k decreased with later points of inflection of the original single-phase functions. Ratios of age at inflection point for the phases (c_i in Table 3) in the triphasic function to age at inflection point in the original functions (Table 2) were respectively for Johnson-Schumacher data: 1.06, 1.74, 3.29; for Michaelis-Menten data: .85, 1.22, 1.96; and for Gompertz data: .79, 1.22 and 1.88. Characteristics of parameter estimates for a triphasic function fitted to simulated data of single-phase growth functions can be summarized as: earlier inflection points in the single-phase function result in large fractions of asymptotic values in the first phase, larger estimates for k, and later inflection points for the three phases, especially for the third phase.

Actual data

To show the application of the multiple-phase and single-phase approach in actual data, two data sets were used, one containing body-weight data from a cow and the other from a boar. Individual weights were chosen instead of averaged group weights, to demonstrate the approach on data with increased variability.

The cow originates from a Holstein Friesian x Dutch Friesian crossbreeding experiment (Politiek *et al.*, 1982), in which body weight was measured on days 1, 7, and 21 after birth, and monthly from day 21 to day 546. Two additional body weights were measured one day after each calving, on days 805 and 1127. The Dutch Landrace boar was part of an experiment to study growth and carcass composition (Walstra, 1980). Body weight of the boar was measured monthly from day 67 after birth to day 621.

To compare growth curves for the period from conception to maturity, age should be counted from conception (Jolicoeur, 1985). Therefore, length of gestation was added to age and the new age was called 'conception age'. Gestation length was assumed to be 279 days for cows and 114 days for pigs.

The four selected functions, as well as the multiphasic functions with values for $n = 1$, 2 and 3, were fitted to body-weight data (Table 4). Results for the four single-phase growth functions show that function 1 had smallest residual standard deviation and largest number of runs. Compared to the other three functions, therefore, the Johnson-Schumacher was considered to be 'best.' For this function, age at the inflection point can be calculated as $d\ [h/(h+1)]^{1/h}$, and weight at the inflection point as $Ae^{-(h+1)/h}$. For the cow, this results in .17A at 420 days from conception, and for the boar, in .21A at 256 days from conception. Results for fitting the monophasic ($n=1$) function (Table 4) were similar to those for fitting function 4: high residual standard deviation, few number of runs, and low mature weight.

Table 4. Estimated parameters and measures for goodness of fit (residual standard deviation, s_e, and number of runs) using four single-phase growth functions[1] and a multiphasic (n=1, 2 and 3)[2] function to fit body weight data for cow and boar.

Data set	Number obs.	Function no.[2]	s_e	Number of runs[3]	Phase (i)	Parameters A	d	h
				Single phase				
cow	22	1	7.7	6 *		816	651	1.30
		2	13.1	4 *		638	684	2.67
		3	14.9	4 *		579	531	1.80
		4	23.3	4 *		545	624	3.42
boar	22	1	5.2	9		359	329	1.78
		2	7.5	8		307	365	3.25
		3	7.9	6 *		290	301	2.13
		4	11.7	4 *		276	349	3.77
				Multiple phase				
						a_i	c_i	k_i
cow	22	n=1	23.3	4 *	1	273	624	1.71
		n=2[4]	6.3	14	1	127	427	2.71
					2	163	896	2.71
		n=3[4]	5.3	12	1	114	409	2.92
					2	112	767	2.92
					3	84	1224	2.92
boar	22	n=1	11.7	4 *	1	138	349	1.89
		n=2[4]	5.6	12	1	86	281	2.79
					2	67	552	2.79
		n=3[4]	5.2	12		unrealistic estimates		

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[1] See Table 1 for description of functions and Table 2 for values of parameters used.

[2] $y_t = \sum_{i=1}^{n} [a_i(1 + \tanh(k_i(t/c_i - 1)))]$

[3] * Significant trend (P<.05).

[4] Assumed k_i to be equal for all phases.

Using the diphasic (n=2) function (Table 4), results were similar to those in the simulation study: high asymptotic standard errors for estimates, especially for a_i and k_i. Assuming equal k's improved results (Table 5).

Table 5. Parameter estimates for diphasic function, with asymptotic standard errors and asymptotic correlations between parameters for cow (above diagonal) and for boar (below diagonal) data sets.
A: with parameters k_1 and k_2 free, B: with $k = k_1 = k_2$.

A. Diphasic function with k_1 and k_2 free

parameter			a_1	k_1	c_1	a_2	k_2	c_2
	estimate		104	3.11	419	189	2.23	852
		asympt. se	31	.73	7	36	.57	59
			asympt. corr.					
a_1	64	34	*	-.98	.30	-.99	.98	.97
k_1	3.41	1.45	-.98	*	-.30	.97	-.96	-.94
c_1	272	8	-.28	.29	*	-.23	.14	.41
a_2	88	38	-.99	.98	.37	*	-.99	-.93
k_2	2.12	1.00	.97	-.96	-.47	-.99	*	.92
c_2	493	78	.97	-.94	-.16	-.93	.91	*

B. Diphasic function with $k = k_1 = k_2$

parameter			a_1	k	c_1	a_2	-	c_2
	estimate		127	2.71	427	163	-	896
		asympt. se	4	.10	7	5	-	22
			asympt. corr.					
a_1	86	5	*	-.06	.80	-.52	-	.85
k	2.79	.18	-.32	*	-.43	-.30	-	-.24
c_1	281	7	.88	-.50	*	-.32	-	.69
a_2	67	4	-.22	-.29	-.17	*	-	-.07
-	-	-	-	-	-	-	-	-
c_2	552	32	.87	-.37	.74	.26	-	*

In table 5A, with k_1 and k_2 free to vary, correlations with c_1 were low; others were above .90. In table 5B, with $k = k_1 = k_2$, no correlation was larger than .90, indicating that equal k's decreased the pair-wise dependence between parameters estimates. This is important, because high pair-wise correlations between parameters is often a problem in the application of nonlinear growth functions. For instance, asymptotic correlations for the Johnson-Schumacher function with the cow data were: r(A,d)=.99, r(A,h)=-.95, and r(d,h)=-.95; and with the boar data were: r(A,d)=.96, r(A,h)=-.95, and r(d,h)=-.88. Favourable consequences of lower correlations include lower standard errors of parameter estimates and, consequently, smaller confidence limits for estimates; faster convergence in the iteration process; and easier interpretation of parameters. There is little doubt that equal k's improved asymptotic standard errors (Tables 5A and 5B).

The residual standard deviation (Table 4) for the diphasic function was lower for cow data and slightly higher for the boar data, compared with the s_e of the Johnson-Schumacher function. Number of runs indicated no systematic deviations. Estimates of asymptotic weight were 580 kg for cow and 306 kg for boar. Observations together with predictions for the Johnson-Schumacher and diphasic functions are in Figure 2 for cow and in Figure 3 for boar. Residuals after fitting the Johnson-Schumacher and diphasic functions are in Figure 4 for cow and in Figure 5 for boar.

The triphasic function gave reasonable parameter estimates for cow data despite that the third phase was determined only by the two last observations. For boar data, however, there were unrealistic parameter estimates. It is concluded, therefore, that it was not possible to detect more than two phases using these data sets. This is in agreement with the simulation study. In the simulated Johnson-Schumacher data, about 90% of the asymptotic value was reached in the first two phases of a triphasic function. The last observation of the cow data is about 560 kg, which is 69% of the estimated asymptote (816 kg) of the Johnson-Schumacher fit; for boar data the last observation (295 kg) is 82% of the estimated asymptote (359 kg). According to the results of the simulation study, this means that the third phase is not reached yet, within this observation period, and the diphasic functions to cow and boar data has to be considered as the first two phases of a triphasic model.

The fraction of the asymptotic value in the first phase can be calculated now as: $2a_1/816 = .31$ for the cow, and $2a_1/359 = .48$ for the boar. Ratios of age of inflection points of the two phases to that estimated age using the Johnson-Schumacher function were: 1.02 and 2.13 for the cow data, and 1.10 and 2.16 for the boar data. These findings are in agreement with the results of the simulation study.

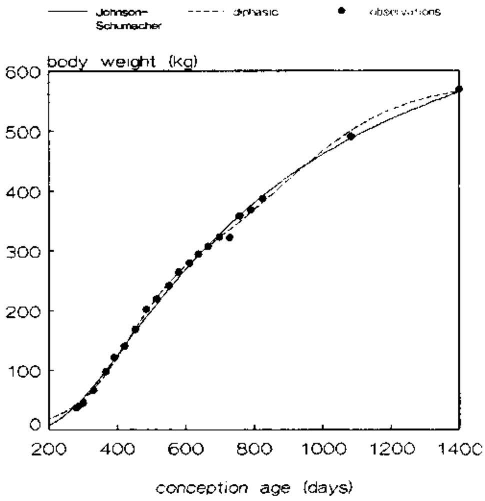

Figure 2. Body weights of a cow, fitted by Johnson-Schumacher and a diphasic function.

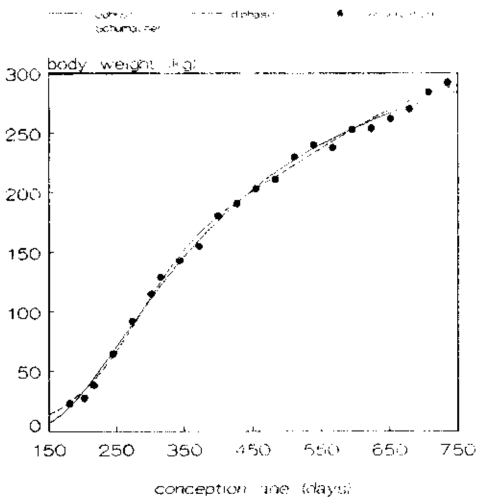

Figure 3. Body weights of a boar, fitted by Johnson-Schumacher and a diphasic function.

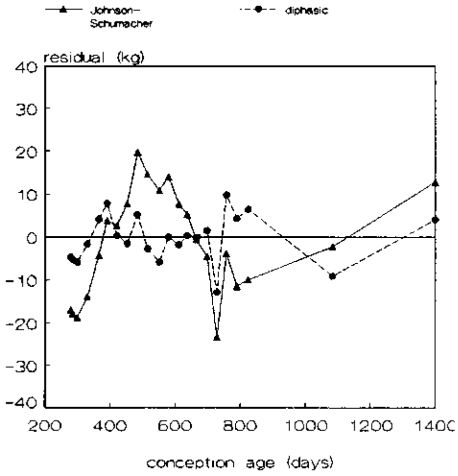

Figure 4. Residuals in body weights for the cow, after fitting the Johnson-Schumacher and diphasic function.

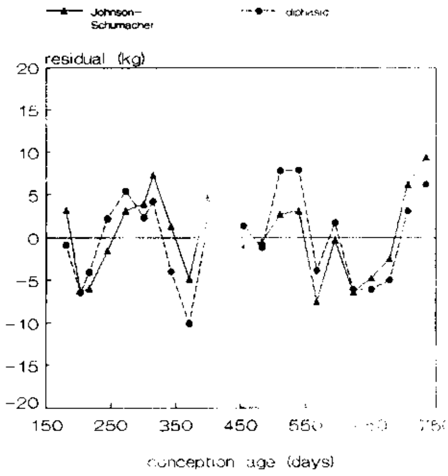

Figure 5. Residuals of body weights of the boar, after fitting the Johnson-Schumacher and diphasic function.

DISCUSSION AND CONCLUSIONS

The multiple-phase growth function suggested by Koops (1986) was compared to selected single-phase growth functions, with special attention to asymmetry, to investigate the relation between these two approaches. First, a simulation study was conducted with four single-phase, three-parameter functions from a general five-parameter function, including (1) Johnson-Schumacher, (2) Michaelis-Menten, (3) Gompertz and (4) the logistic function. Functions 1 and 2 have flexible points of inflection, whereas functions 3 and 4 have fixed points of inflection. With the general function as the basis, functions differ in the transformed y and/or t scale: no transformation leads to logistic, ln-transformed y-scale to Gompertz ($p \to 0$), ln-transformed t-scale ($m \to 0$) to Michaelis-Menten, and ln-transformed y-scale and t-scale to the Johnson-Schumacher (p and $m \to 0$). These S-shaped functions differ essentially in shape. A multiphasic function was fitted to the simulated data using the four functions. Second, actual data using body weights from a cow and a boar were analyzed using the four single-phase functions and the diphasic function.

Results of simulation indicated that the triphasic function satisfactorily described data sets of the four functions, in terms of residual standard deviation and number of runs. Using triphasic functions, it can be concluded that single-phase growth functions with early inflection points show relatively high fractions of asymptotic weight achieved in the first phase, and high ratios of age at the point of inflection of each phase to that age of the original single-phase function.

Equating k's for different phases gave similar results for goodness-of-fit criteria, but improved accuracy of estimates considerably. This is shown in the analysis of the cow and boar data. The reason for improved accuracy is less pairwise correlation between parameter estimates.

Within the group of single-phase functions, the cow and boar growth data could be described best by the Johnson-Schumacher function. For the cow, however, residuals showed significant trends and mature weight appeared to be overestimated. For the boar, this function gave a good fit. Using a diphasic function caused systematic deviations to disappear. Residual standard deviation was similar to the Johnson-Schumacher function for each data set. Characteristics of parameter estimates agreed with results of the simulation study.

This comparison showed the multiple-phase growth function to be a reasonable alternative to a single-phase function. An important advantage for the multiple-phase function is not having to select the "best" single-phase function. The multiphasic function is usable in almost every case. The need to estimate a larger number of parameters is not a problem, if parameters are less correlated.

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Chapter 5

MULTIPHASIC ANALYSIS OF GROWTH CURVES OF PROGENY OF A TRANSGENIC (Somatotropin) MALE MOUSE

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ABSTRACT

Multiphasic growth functions were applied to growth in body weight and tail length of 20 transgenic mice and 20 non-transgenic littermates from 3 to 26 weeks of age. Mice were progeny of one male, carrying the human growth hormone, and 'random bred' NMRI females. At week 12, ten litters were chosen from which four females or males could be selected, two with highest and two with lowest body weight. Body weight and tail length of these 40 mice were measured about weekly to week 26.

Transgenic mice reached a body weight at week 26 that averaged 1.6 (females) times and 1.9 (males) times that of non-transgenic littermates. Using a diphasic growth function for body weight, transgenic females and males grew 30% more in first phase than non-transgenic littermates. Transgenic females grew 52% more and males 144% more in second phase.

Applying a diphasic function to tail length, transgenic mice had significantly shorter tail lengths than non-transgenic mice in the first phase: females had .64 cm shorter tails and males .92 cm shorter. Tails of transgenic mice grew 1.4 cm (females) more and 1.58 cm (males) more in second phase than non-transgenic littermates. This indicated a different relation between growth in weight and in length for transgenic and non-transgenic mice. Multiphasic growth analyses fitted data for body weight and tail length satisfactorily and provided greater insight to differences in growth patterns of transgenic mice.

INTRODUCTION

A large number of mathematical growth functions are available to describe growth curves in mice (Eisen, 1976). Recently growth of body weight has been described with a multiphasic growth function in mice (Koops, 1986; Koops *et al.*, 1987) and in chickens (Grossman and Koops, 1988). Application of this function to growth of fish and to growth in body composition in pigs has been demonstrated and discussed (Koops, 1989). A comparison was made for use of single-phase growth functions with the multiphasic function (Koops and Grossman, 1989). The multiphasic function assumes that increase in body weight, and in other body characteristics, from birth to maturity is the result not of a single S-shaped growth curve but of a sum of more than one

S-curve or 'growth phase'. Koops *et al.* (1987) showed a three-phase pattern for the body-weight growth curve of mice to 8 weeks of age. However, detection of number of phases depends on the length of the measurement period and the frequency of measurements.

In cases that require flexibility of the growth function and for which large variation between weights or measurements of individuals exists, it seems appealing to apply a multiphasic growth function. Such large variation is created nowadays by introducing foreign growth hormone genes into animals. Introduction of foreign genes into embryos of mice has resulted in improved performance for some traits. Palmiter *et al.* (1982), Hammer *et al.* (1985) and Wagner (1985) reported that mice grew 100% larger when the human or bovine growth hormone gene was introduced. Higher growth hormone level, achieved by injection or genetically, yielded higher gain in body weight for mice and rats (Wagner, 1985). Positive effects of growth hormone injections in rats were already discovered 50 years ago in growth experiments of Evans and Simpson, as mentioned by Wagner (1985). Machlin (1972) reported positive effects of porcine growth hormone in swine, with increased muscle growth and decreased fat deposition.

Wagner (1985) reported high variability in expression of the growth hormone gene in transgenic animals. Nieuwhof and Kanis (1988) were able to classify offspring of three types of transgenic males and normal female mice into transgenic or non-transgenic groups, based on body weight at 12 weeks. At younger ages, however, they reported more overlap of distributions for weight. After correction for sex and litter, they distinguished two distributions for body weights at 12 weeks. To understand better the expression of growth caused by transgeneity, growth curves for transgenic animals should be compared with those for non-transgenic animals.

The objectives of this study were to apply the multiphasic growth function to body measurements of transgenic and non-transgenic mice and to obtain more insight into the effect of a transgene on growth in mice from birth to maturity by means of the multiphasic approach. Body weight and tail length measurements of one type of transgenic (somatotropin) mice from the experiment of Nieuwhof and Kanis (1988) were available for applying the growth function. This study offers the opportunity to examine growth in two dimensions of the body; body weight as indicator of growth of body mass and tail length representing length growth of the skeleton.

MATERIAL

Data for this study were from an experiment in which one male mouse, originating from a line (hybrid cross C57BL6/C3H) into which the human growth hormone gene was introduced, was mated to random-bred NMRI females.

The young were weaned at 3 weeks of age, after which males and females were housed separately in groups of 3 or 4 per cage. There was free access to water and feed (RMH-B standard diet, Hope Farms B.V., Woerden, The Netherlands). A brief description of the experiment is given by Nieuwhof and Kanis (1988). Weekly body weight was measured at the same age, by litter before weaning and by individual after weaning. Weekly tail length also was measured individually after weaning.

For this study we needed groups of transgenic and non-transgenic mice. Only a fraction of the offspring of each mating, however, carried the gene for human growth hormone. To have the greatest chance of obtaining transgenic and non-transgenic groups, 20 mice with high and 20 with low body weights at week 12 were selected from 10 litters. Litters were selected with at least four females or at least four males. Within a litter, the two males (females) with highest body weights and the two males (females) with lowest body weights were chosen, such that the ratio for mean weights of high to low littermates was at least 1.48, which is the ratio for mean weights of transgenic (somatotropin) to non-transgenic mice classified at week 12 by Nieuwhof and Kanis (1988).

Table 1. Weights (g) at week 12 of the 40 selected mice by sex and transgenic group (+) or non-transgenic group (-).

	Females			Males	
Dam	(+)	(-)	Dam	(+)	(-)
5	43 42	26 27	6	45 40	26 30
35	40 39	28 25	19	62 55	32 28
37	49 48	27 27	20	48 54	30 32
38	43 39	27 27	33	43 45	27 29
39[1]	42 41	27 27	39[1]	51 42	32 30
mean	42.6	26.8		48.5	29.6
st.dev.	3.44	0.79		6.92	2.12
no. obs.	10	10		10	10

[1] Same dam. In the analysis no allowance is made for that.

These 40 mice were measured to 26 weeks of age. It was assumed that the high-weight group (+) contained the human growth hormone gene and that the low-weight group (-) did not. Therefore, mice of the (+) group were considered to be transgenic, although the presence of the growth hormone gene was not tested, and those of the (-) group were to be non-transgenic. Observed differences in 12-week body weights between the (+) and (-) littermates were considerable (Table 1), so the assumption probably was correct. Male and female mice each shows a ratio in weight of (+) to (-) group of about 1.6.

METHODS

Growth curves for body weight and tail length versus age for each mouse was described by a multiphasic function (Koops, 1986):

$$y_t = \sum_{i=1}^{n} \{a_i[1 + \tanh(b_i(t - c_i))]\} \qquad (1)$$

with the first derivative:

$$y'_t = \sum_{i=1}^{n} \{a_i b_i[1 - \tanh(b_i(t - c_i))]\} \qquad (2)$$

where y_t is the observed weight or length at week t; and y'_t is gain in weight or length; n is the number of growth phases; tanh is the hyperbolic tangent; and for each phase i, parameter a_i is half asymptotic value (g or cm), b_i is growth rate relative to a_i (weeks^{-1}) and c_i is the age (weeks) at maximum growth $(a_i b_i)$.

Table 2. Residual standard deviations for mean body weights and for mean tail lengths after fitting the multiphasic growth function[1] by number of phases (n).

		Body weight			Tail length	
Sex	Group	n=1	n=2	n=3	n=1	n=2
Female	(+)	2.502	.629	.365	.138	.077
	(-)	1.822	.492	.325	.138	.060
Male	(+)	2.812	.756	.345	.134	.099
	(-)	2.168	.345	.261	.148	.073

[1] $y_t = \sum_{i=1}^{n} \{a_i[1 + \tanh(b_i(t - c_i))]\}$

The measurement period in this study (3 to 26 weeks) was not comparable to that in Koops *et al.* (1987) (0 to 8 weeks); thus no previous estimate of the number of growth phases for body weight during the first 26 weeks was available. Therefore, the multiphasic function (1) was fitted to the mean growth curve for each of the four groups for body weight and tail length using the DUD nonlinear regression method (PROC NLIN; SAS Institute Inc., 1985).

Residual standard deviations after fitting (1) for $n = 1$, 2 and 3 for mean weights and for $n = 1$ and 2 for mean tail lengths of each group are in Table 2. Number of phases greater than 3 for weight and 2 for length resulted in estimates that did not converge. It was not possible to fit the triphasic function for body weights of all individuals, nor to fit the diphasic for tail length to all individual curves, because of short during fluctuations and lack of precision in measurements (body weight was recorded in g and tail length in 0.1 cm). Therefore a diphasic model was chosen for body weight and also for tail length, except that one parameter (b_1) was held constant for tail length. Because the earliest phase for tail length was before weaning, outside the period of measurement, b_1 was fixed at 0.50, which was equal to the estimate of b_1 in the diphasic function for the total mean curve. Predicted curves for the diphasic function are in Figure 1 for mean body weights and in Figure 2 for mean tail lengths. Because no individual information was available before weaning, one point was added to the data for either body weight and tail length so as to fix the value of the function to zero or close to zero at time of conception ($y_t = 0$ at time $t = -3$ (assuming gestation length to be about 3 weeks)).

Estimates from function (1) and values for body weights and tail lengths at 3, 7, 12 and 26 weeks of age were considered to be characteristic of individual animals and were subjected to the following statistical linear model:

$$(par_m)_{ijkl} = \mu+sx_i+gr_j+(sx*gr)_{ij}+d_{k:i}+(gr*d)_{j*k:i}+ind_{l:i*j*k}$$

where $(par_m)_{ijkl}$ is estimate m for animal ijkl, μ is an overall mean, sx is the effect of sex i (1 = female, 2 = male), gr is the effect of group j (1 = (+), 2 = (-)), d is the effect of dam or litter k within sexes (k=1,..,5) and ind is the effect of individual l within sex*dam*group combinations (l=1,2).

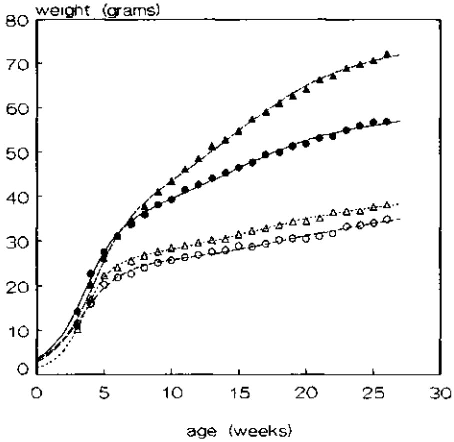

Figure 1. Mean body weights fitted by a diphasic function. (circles, females; triangles, males; closed, transgenic; open, non-transgenic)

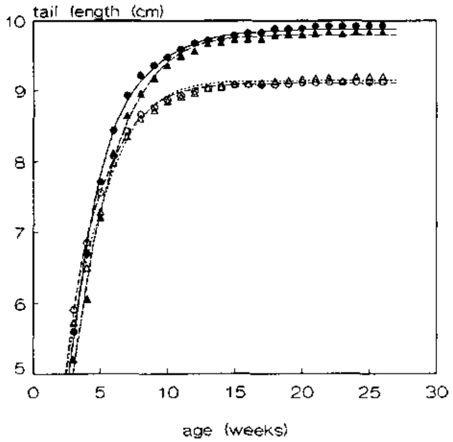

Figure 2. Mean tail lengths fitted by a diphasic function. (For legend, see Figure 1.)

Although some estimates did not meet the assumption of homogeneous variance required for tests of significance, the F-test served as an indicator for significance of effects in the model. The effect of sex was tested by the effect of dam within sex. The effect of group by the d*gr interaction and all other effects by the effect of individual. Focus of tests should be on the effect of transgenic group (+) vs (-), because the effect of sex can be influenced by the dam (within sex). Tests of significance for group are not relevant for observed body weights, because animals were selected on 12-week body weight.

RESULTS AND DISCUSSION

Means and tests of significance for body weights (Table 3-I) and for tail lengths (Table 3-II), are given at 3, 7, 12 and 26 weeks of age. For transgenic females, mean weights at weeks 3, 7, 12 and 26 were, respectively, 1.23, 1.48, 1.59 and 1.63 times higher than mean weights for their (-) littermates. For males these ratios were 1.11, 1.36, 1.64 and 1.90, indicating that growth for females was in a more constant ratio to (-) littermates than for males, from week 12 onwards, and that males grew faster than females to 26 weeks.

Table 3. Means of (+) and (-) females and males, tests of significance for effects of sex, group and dam on body weight and tail length at different ages and on estimates of parameters; and standard deviations (s_e) between littermates within dams.

	Means				Significance[2]					s_e
	Females		Males		effects[3]					
	(+)	(-)	(+)	(-)	sx	gr	sx*gr	d	gr*d	ind
I. *Body weight*										
week 3	14.20	11.50	11.30	10.20	*	**	**	**	ns	.71
week 7	33.70	22.70	34.80	25.60	ns	**	ns	**	*	1.82
week 12	42.60	26.80	48.50	29.60	*	**	ns	**	*	2.03
week 26	56.80	34.80	72.20	38.10	**	**	**	*	ns	5.63
II. *Tail length*										
week 3	5.60	5.91	5.21	5.73	ns	**	ns	**	ns	.20
week 7	8.93	8.42	8.65	8.35	ns	**	ns	**	ns	.24
week 12	9.68	8.99	9.56	8.97	ns	**	ns	**	ns	.27
week 26	9.93	9.12	9.84	9.20	ns	**	ns	**	ns	.26
III. *Body weight*[1]										
a_1	15.65	11.58	15.78	12.21	ns	**	ns	*	ns	1.21
b_1	.42	.40	.39	.54	ns	*	**	**	ns	.07
c_1	3.58	3.25	4.19	3.48	**	**	**	**	ns	.15
a_2	13.71	9.01	21.69	8.89	*	**	**	ns	ns	4.46
b_2	.11	.08	.11	.11	ns	*	ns	ns	ns	.02
c_2	14.18	19.65	14.58	16.84	ns	*	ns	*	*	2.88
IV. *Tail length*[1] (b_1 fixed to: .5)										
a_1	2.98	3.30	2.29	2.75	*	*	ns	**	*	.23
c_1	1.90	1.59	1.54	1.32	ns	ns	ns	**	*	.29
a_2	1.95	1.25	2.61	1.82	*	**	ns	**	**	.20
b_2	.25	.28	.26	.27	ns	ns	ns	*	ns	.03
c_2	4.85	5.26	4.75	4.75	ns	ns	ns	ns	ns	.40

[1] $y_t = a_1 (1 + \tanh(b_1(t - c_1))) + a_2(1 + \tanh(b_2(t - c_2)))$

[2] ns P>.05; * P<.05; ** P<.01.

[3] For explanation of names of effects, variables and parameters see text.

The transgenic effect at week 3 was 23% for females but only 11% for males (see Figure 1 and Table 3-I).

For tail lengths, the ratios of (+) to (-) mice at 3, 7, 12 and 26 weeks were .95, 1.06, 1.08 and 1.09 for females, and .91, 1.04, 1.07 and 1.07 for males.

These results indicated that (+) females and males had shorter tails at 3 weeks than their (-) littermates. By 7 weeks, however, this difference changed in favor of the (+) mice. Tail length of (+) mice at 26 weeks averaged about 8% longer than that of (-) littermates. The mean growth pattern of tail length is in Figure 2.

Figures 1 and 2 show different growth patterns. At weaning, tail length is more than 50% of mature length, whereas body weight is about 33% of mature weight for (-) mice and 15% for (+) mice.

Monophasic growth functions will fail to fit body weight-age curves in this case, because apparently there is no asymptote in these data. For tail length-age curves there is an obvious asymptote, so that application of a monophasic function would be possible. Results of fitting mean growth curves with the multiphasic function (1) show that at least a diphasic function is needed (Table 2). The choice of whether to use a diphasic function for individual curves was discussed earlier.

For body weights, means and tests of significance for parameters of the diphasic function are in Table 3-III. For weight gained during first phase ($2a_1$), the ratio of (+) mice to their (-) littermates was 1.35 for females and 1.30 for males. For weight gained during second phase ($2a_2$), this ratio was 1.52 for females and 2.44 for males. A large part of the growth of (+) mice took place in the second phase, especially in males.

The age at maximum gain in the first phase is significantly later for (+) mice than for their (-) littermates: for females .33 weeks later and for males .71 weeks later. On the contrary, age at maximum gain in the second phase was earlier for (+) mice; for females 5.47 weeks earlier; and for males 2.26 weeks earlier. Because estimates of c_2 had large standard errors, these differences were less significant than in the first phase.

Differences in b_1 and b_2 were small, not consistent over sexes and seemed to be less related to transgenic group.

Results of the diphasic function fitted to tail length are in Table 3-IV.

For gain in tail length during first phase ($2a_1$), the ratio for (+) mice to their (-) littermates was .90 for females and .83 for males. This agrees with the conclusion based on the observed tail length at 3 weeks. For gain during second phase ($2a_2$), the ratio for (+) mice to (-) littermates was 1.56 for females and 1.43 for males. Differences in growth pattern of tail length caused by transgeneity were reflected mostly in a_1 and a_2; differences in c_1, b_2 and c_2 were not significant.

Using estimates of the multiphasic function (1), gain in weight and length can be shown with function (2). The gain curve is in Figure 3 for body weight and in Figure 4 for tail length. These figures show that gain in body weight continued to week 26, whereas gain in tail length decreased to near zero by week 15. This phenomenon supports the multiphasic growth theory that some parts of the body cease growing while others continue to grow.

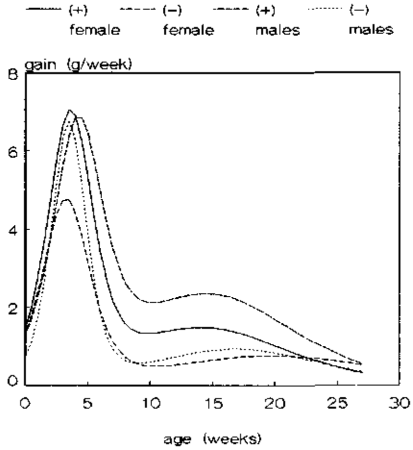

Figure 3. Weekly gain in body weight by sex and group, fitted by a diphasic function.

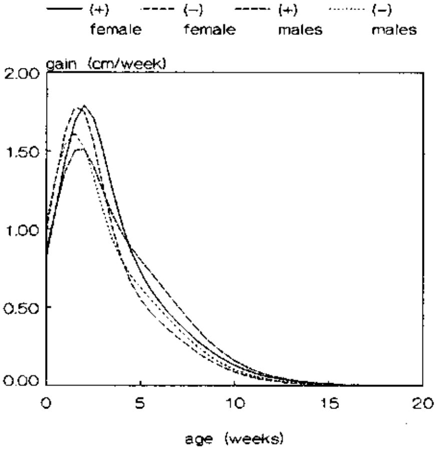

Figure 4. Weekly gain in tail length, by sex and group, fitted by a diphasic function.

Maximum weight gain is at about 4 weeks (Figure 3), which agrees with the age of the most important phase as found by Koops *et al.* (1987) and with the age at the inflection point in the logistic analysis of Eisen (1976). Results before 3 weeks have to be considered cautiously because there were no individual observations. No comparisons of the second phase were available in literature because most growth experiments with mice end at 8 weeks of age.

Maximum weight gain in second phase (a_2b_2) was 2.39 g/week for (+) males and 1.50 g/week for (+) females. For (-) mice, maximum weight gain was .97 g/week for males and .72 g/week for females. Differences in weekly weight gain between (+) and (-) mice are shown in Figure 5 by phase and sex. A large portion of weight gain for transgenic males and females was attained in the second phase. Maximum differences in the first phase appeared 1 to 2 weeks later for males than for females.

Weekly gain in length (Figure 4) did not show a pronounced diphasic pattern. When phase and sex were separated, however, for differences in gain between (+) and (-) mice (Figure 6), the pattern becomes more clear.

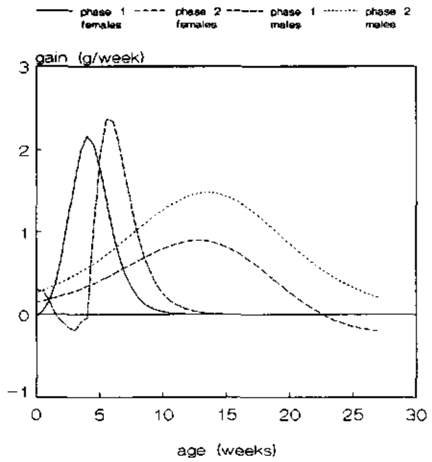

Figure 5. Differences in diphasic functions for weekly gain in weight between (+) and (-) mice, by sex and phases.

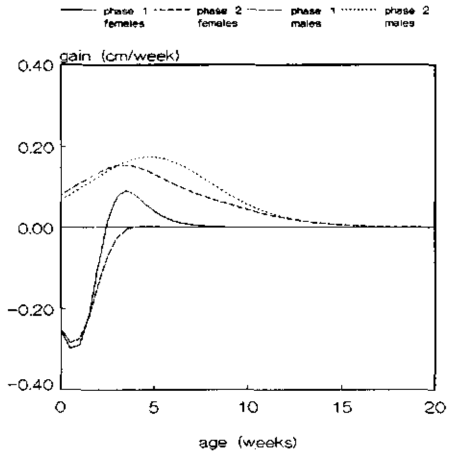

Figure 6. Difference between diphasic functions of tail length between (+) and (-) mice, by sex and phases.

In first phase, gains for transgenic males and females were less than those for their (-) littermates. The compensatory gain in the second phase resulted in longer tails for (+) mice than for (-) mice at week 15, with only slight differences between sexes. If tail-length growth is assumed to be a measure for bone growth, this result seems to indicate that transgenic mice delay growth of their skeleton.

CONCLUSIONS

With respect to the aim of this study, growth curves of body weight and tail length of transgenic and non-transgenic mice in these data can be described satisfactorily with multiphasic growth functions suggested by Koops (1986). Use of these functions give detailed insight into differences in growth pattern of transgenic and non-transgenic mice.

In these data, transgenic mice at age 26 weeks reached an average body weight of 1.6 (females) to 1.9 (males) times that of their non-transgenic littermates. Using a diphasic growth function, transgenic female and male mice each grew 30% more in the first phase than their non-transgenic littermates, with maximum gain about half a week later. Transgenic females grew 52% and males 144% more in the second phase than non-transgenic mice, with maximum gain 5.5 and 2.3 weeks earlier, respectively.

Applying the diphasic function to tail length, transgenic mice reached significantly shorter tail lengths than non-transgenic mice in the first phase; females were .64 cm shorter and males were .92 cm shorter. Female transgenic mice grew 1.4 cm more and males 1.58 more in the second phase than their non-transgenic littermates. Transgenic mice averaged .7 cm longer tails than their non-transgenic littermates. This means that for transgenic mice tail-length gain in second phase more than compensated for the smaller gain in first phase. This could indicate that transgenic animals delay growth of their skeleton, if tail-length growth is assumed to be a measure of bone growth, which could mean that 'the biological program of growth' in transgenic animals is changed. This can have important consequences if growth of other parts, for instance, organs, is changed too.

Transfer of exogenous growth hormone genes in mice and rats appear to create attractive alternatives to take permanent advantage of higher growth hormone levels in livestock, although possible negative side-effects can be expected. Further experiments are needed to study mechanisms of genetic regulation of growth and to quantify in detail improvement of performance and negative effects accompanying this improvement.

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Chapter 6

MULTIPHASIC GROWTH AND ALLOMETRY

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ABSTRACT

Multiphasic growth functions assign components of body measures to different phases, which has important consequences for morphometric studies. These kind of studies, frequently called "allometric growth studies," have an extra dimension when considering phases. To study relations in growth of different body measures of an individual, the well-known allometric function often is used. It could be concluded that the allometric equation is too extensive a generalization. Complex allometry exists when ages at maximum gain for pairs of body measures are different. Growth functions can be used to quantify and judge these ages. Although simple allometry is not incorporated into the logistic relation of growth of different body measures, it can be used in the same way.

A method is described and demonstrated to relate body weight and tail length growth in mice. For these data, the two phases of tail length and the two phases of body weight differed in age at maximum gain. Gain in second phase of body weight seemed to be unrelated to other components. The method seems to be a suitable extension to study growth in different body measures or components, if used in combination with multiphasic growth functions.

INTRODUCTION

Use of multiphasic growth functions was suggested by Koops (1986) and discussed and demonstrated for body-weight and body-length growth curves of different species of animals (Koops *et al.*, 1987; Grossman and Koops, 1988; and Koops and Grossman, 1989a and b). The multiphasic growth function can be used as an alternative to well-known single-phase growth functions, such as Johnson-Schumacher, Michaelis-Menten and Gompertz functions (Koops and Grossman, 1989a). Theory of multiphasic growth assumes increase in body weight, or in other measures of body growth, to be a result of more than one growth impulse, or growth phase, caused by internal or external factors (Koops, 1989). In the absence of external influences, the multiphasic function assigns growth of components of weight, or of other body measures, to different phases (Koops, 1989), which has important consequences for morphometric studies. These studies, frequently called 'allometric growth studies', have an extra dimension when phases are considered.

The relation between different measures of body growth as well as the relation between different components of growth produced by different phases has to be studied. Koops and Grossman (1989b) fitted a diphasic growth function to body-weight and tail-length curves of 20 transgenic (somatotropin) mice and 20 non-transgenic littermates. Comparison of growth relations on different levels is now possible: between phases within one measure (body weight or tail length), between measures within one animal (body weight and tail length), and between animals within groups (transgenic and non-transgenic).

The objective of this study is to compare relations of growth of different body measures using multiphasic growth functions. Data of Koops and Grossman (1989b) are used to illustrate the problem and the analysis.

ALLOMETRIC GROWTH

Since its introduction, the allometric growth equation (Huxley, 1924) has been used widely to study growth of different measures of the body, relative to each other or to the whole body. The allometric equation assumes a linear relationship between the logarithmic transformed body measures Y_1 and Y_2:

$$\ln Y_1 = \ln \alpha + \beta \ln Y_2 \qquad (1)$$

where α is a constant and β is the growth coefficient. Transformed to the original scale, this equation becomes:

$$Y_1 = \alpha Y_2^{\beta} \qquad (2)$$

The log-linear equation (1) is the integral of an equation in which there is a constant ratio between specific growth rates of two components, Y_1 and Y_2:

$$Y_1'/Y_1 = \beta Y_2'/Y_2 \qquad (3)$$

This method works well, in general, but in many studies it has been reported that two different body measures showed a nonlinear relation on a logarithmic scale. In some studies, the relation appears curvilinear and in other cases it appears as a disjointed line with 'breakpoints'.

Laird (1965) associated the allometric equation with the Gompertz growth curve and showed that deviations from linearity are caused by differences in the time (age) scale. To solve the problem of nonlinearity between body measures, different approaches have been used. For curvilinearity, quadratic or even cubic terms were added to the log-linear equation (Walstra, 1980). If breakpoints exist, the allometric relation can be applied to each linear part of the curve (Lilja *et al*. 1985).

The allometric relation was extended to the multivariate case by Jolicoeur (1963). Turner (1978) associated multivariate allometry with conservative and non-conservative Lotka-Volterra equations. Lebeau *et al*. (1986) presented a modified von Bertalanffy or Pütter growth model as a tool to make the relation of allometry and growth curves meaningful. Recently, Jolicoeur and Pirlot (1988) extended the work of Lebeau *et al*. (1986) by introducing the term *complex allometry* to indicate that differences in time scale disturb *simple allometry*.

ASYMPTOTIC GROWTH FUNCTIONS AND ALLOMETRY

Lebeau *et al*. (1986) and Jolicoeur and Pirlot (1988) used a three-parameter modification of the Pütter growth curve to describe different measures of body growth. The p-variate allometric relation, including time, was:

$$[1-(Y_1/A_1)^{1/C_1}]^{D_1} = \dots = [1-(Y_p/A_p)^{1/C_p}]^{D_p} = e^{-t} \qquad (4)$$

where Y_i = body measure i ($i = 1,\dots,p$); A_i = asymptote for body measure i; C_i and D_i are parameters, with D_i related to age and C_i to the body measure; and e^{-t} = negative exponential of age (t), from the moment at which development starts. If $D = D_1 = \dots = D_p$, then *simple allometry* is assumed and equation (4) can be written as:

$$(Y_1/A_1)^{1/C_1} = \dots = (Y_p/A_p)^{1/C_p} = [1-e^{-t/D}] \qquad (5)$$

Without the time term, this equation is equal to the p-variate allometric model given by Jolicoeur (1963). The growth coefficient ß relating Y_1 to Y_2, for example, is C_1/C_2.

If values for D are different, then *complex allometry* is assumed, which is the same phenomenon discussed by Laird (1965), based on the Gompertz curve. In this case, the p-variate allometric relation could be written as:

$$[K_1/B_1\{\ln(A_1/Y_1)\}]^{1/K_1} = \ldots = [K_p/B_p\{\ln(A_p/Y_p)\}]^{1/K_p} = e^{-t} \tag{6}$$

where Y_i, A_i and e^{-t} are as defined in (4), K_i is a parameter related to age, and B_i is a parameter related to body measure. For K's not equal, there is complex allometry; and for equal K's, the relation is simple allometric:

$$1/B_1\{\ln(A_1/Y_1)\} = \ldots = 1/B_p\{\ln(A_p/Y_p)\} = e^{-Kt} \ . \tag{7}$$

The growth coefficient β relating Y_1 to Y_2, for example, is now B_1/B_2.

In case of the logistic function, the p-variate allometric relation is different (Lumer, 1937):

$$\{1/H_1(A_1/Y_1-1)\}^{G_1} = \ldots = \{1/H_p(A_p/Y_p-1)\}^{G_p} = e^{-t} \tag{8}$$

where G_i is a parameter related to age and parameter H_i is related to body measure. Other quantities are as in (4) and (6). In contrast with the Pütter and Gompertz functions, (8) does not reduce to simple allometry for equal G's or for equal H's. Lumer (1937) showed that simple allometry can be assumed if Y is small relative to A because (A/Y-1) is approximately equal to A/Y:

$$\{H_1(Y_1/A_1)\}^{1/G_1} = \ldots = \{H_p(Y_p/A_p)\}^{1/G_p} = e^{-t} \tag{9}$$

The growth coefficient β relating Y_1 to Y_2, for example, is then G_1/G_2. If G's are equal, $\beta = 1$ and the relation is *isometric*, which is a special case of simple allometry.

The relation of asymptotic growth functions to allometry is clear for most of functions. If one parameter is assumed to be equal for all p variates, simple allometry will result. Then, if a second parameter is assumed to be equal for all p variates, the relation will be isometric and the growth coefficient β is 1. The logistic function is an exception, because it only reduces to simple allometry for small values of Y relative to A, without the assumption of equal parameters.

The allometric equation seems to be too extensive a generalization of growth relations. In many cases, time (age) should be included in the comparison of growth of one body component to another.

MULTIPHASIC GROWTH FUNCTION AND ALLOMETRY

The multiphasic growth function (Koops, 1986) is based on a summation of n logistic growth functions, which is written in hyperbolic tangent form as:

$$Y_t = \sum_{i=1}^{n} \{a_i \ [1 + \tanh(b_i \ (t - c_i))]\} \tag{10}$$

where Y_t is the observation of a body measurement at age t, n is number of growth phases, tanh is the hyperbolic tangent; and for each phase i: a_i is half asymptotic value, b_i is growth rate relative to a_i; and c_i is age at maximum gain (a_ib_i). Because 96.4% of gain in phase i is within $c_i \pm 2/b_i$, b_i is also a function of duration for that phase (Koops, 1989).

Body measure at age t can be partitioned into components according to n phases:

$$Y_{it} = y_{i1t} + y_{i2t} + \ \ldots \ + y_{int} \tag{11}$$

In this case, it is impossible to study allometric relations between y's directly, because Y is observed and the y's are estimates. Therefore, comparisons must be made indirectly with parameter estimates of the multiphasic function. Each component (y) is a logistic growth function and, therefore, the internal allometric relation of n components of a given body measure is:

$$c_1+1/b_1\tanh^{-1}(y_1/a_1-1) = \ldots = c_n+1/b_n\tanh^{-1}(y_n/a_n-1) = t \tag{12}$$

For n = 2, if y_1 is expressed in terms of y_2, (12) leads to:

$$y_1 = a_1[1 + \tanh\{b_1(c_2-c_1) + b_1/b_2 \ \tanh^{-1}(y_2/a_2-1)\}] \tag{13}$$

If $c_2 - c_1 = 0$ and $b_1/b_2 = 1$, growth of the two parts is isometric; if only $c_2 - c_1 = 0$, the relation is close to simple allometry with growth coefficient $ß = b_1/b_2$. If c_1 is not equal to c_2, however, the ratio b_1/b_2 has less meaning because the phases are shifted in time. Therefore, (13) contains two interesting relations between parameters for each phase: differences between c's, i.e., differences between ages at maximum gain, and the ratio of b's, i.e., the ratio of the duration of phase for the two components.

In (12) the same expression is found for components of a multiphasic model as for cases of different measures. Consequently, comparison of growth patterns of dimensions of size *measured* or *estimated* on an individual is possible by comparing c's and b's derived from equation (10). This will be illustrated for growth data of mice.

ILLUSTRATION

Material

Body weights and lengths of mice used in this study are from Koops and Grossman (1989b). Forty mice (20 females and 20 males) were progeny from a male that carried the human growth hormone (somatotropin) gene and was mated random bred NMRI females. It is expected that some fraction of the offspring will be a carrier for the human growth hormone gene.

From weaning at 3 weeks of age, each individual mouse was weighed and its tail length was measured weekly. At week 12, ten litters were chosen such that five litters of four females and five of four males could be selected. Each litter included two animals at a high and two at a normal body-weight level. These animals were weighed and measured weekly till week 26. It is assumed that the high group (indicated as (+)) carries the human growth hormone gene; the normal group is indicted as (-).

Statistical methods

Data for individual body weights and tail lengths at week 12 for the 40 selected mice (Koops and Grossman, 1989b) were fitted by equation (10) (with n=2) using the DUD nonlinear regression method (PROC NLIN; SAS Institute Inc., 1985). Parameter estimates and residual standard deviations are in Table 1.

Table 1. Means of parameter estimates for a diphasic function[1] for females and males and for (+) and (-) groups, fitted to individual body measures, and standard deviations (s_e) between littermates within dams for body weight and tail length (Koops and Grossman, 1989b).

Parameter	Body weight					Tail length				
	Females		Males			Females		Males		
	(+)	(-)	(+)	(-)	s_e	(+)	(-)	(+)	(-)	s_e
a_1	15.65	11.58	15.78	12.21	1.21	2.98	3.30	2.29	2.75	.230
b_1	.42	.40	.39	.54	.07	(fixed: .50)				
c_1	3.58	3.25	4.19	3.48	.15	1.90	1.59	1.54	1.32	.287
a_2	13.71	9.01	21.69	8.89	4.46	1.95	1.25	2.61	1.82	.202
b_2	.11	.08	.11	.11	.02	.25	.28	.26	.27	.028
c_2	14.18	19.65	14.58	16.84	2.88	4.85	5.26	4.75	4.75	.396

[1] $y_t = a_1(1 + \tanh(b_1(t - c_1))) + a_2(1 + \tanh(b_2(t - c_2)))$

Two different body measures, weight and length, and because a diphasic function was used, two estimates of phases are available for each individual mouse. Parameters have to be compared for phases 1 and 2 of body weight and for phases 1 and 2 of tail length. The pairwise difference of parameters c and the ratios of b's were calculated and subjected to the following statistical linear model:

$$z_{ijkl} = \mu + s_i + g_j + (s*g)_{ij} + d_{k:i} + (g*d)_{jk:i} + ind_{l:ijk}$$

where z_{ijkl} is difference between c's or ratios of b's for mouse ijkl, μ is overall mean, s is the effect of sex i (i=1 (female), i=2 (male)), g is the effect of transgeneity group j (j=1 (+), j=2 (-)), d is the effect of dam or litter k within sexes (k=1,..,5) and ind is individual l within sex*dam*group combinations (l=1,2).

Although some parameters did not meet the assumption of homogeneous variance, as required for tests of significance, the F-test was used for an indication of the importance of effects in the model: μ (only for differences in c) and sex effect were compared with dam within sex, group with the g*d interaction, and all other effects with ind.

Results and discussion

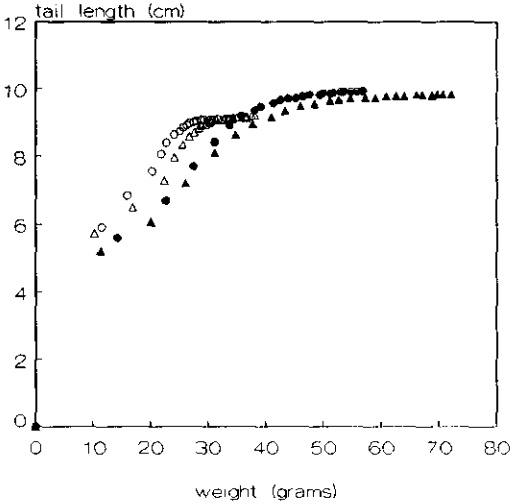

Figure 1. Tail length as function of body weight. (circles, females; triangles, males; closed, (+) group; open, (-) group.)

In Figure 1, the relation between mean tail length and mean body weight is shown for each of the four groups. From this figure, we see that no simple allometry exists. For each group, body weight continues to increase while tail length has already reached an asymptote. In case of simple allometry there should be an increase in both directions, without asymptotes. Results for the analysis of differences in c's and ratios of b's are in Table 2. Overall differences (μ) in c are highly significant, which means that no pair of body measures meets the condition for simple allometry. Body measures closest to this condition are phase 2 for tail length and phase 1 for body weight. The second phase for body weight seems to be independent of body measures because distance between age at maximum gain for this measure and others is about 10 weeks or more. Group effects (g) on all (except for combination L1 with W1) differences in c are highly significant.

As mentioned before, if c's are different, then ratios of b's are almost meaningless in relation to simple allometry. Duration of a growth phase, however, is defined as 4/b (Koops, 1989) so that the ratio of b's is the same as the ratio of durations; i.e., b_1/b_2 is the ratio of duration 2 to duration 1. From Table 2.II, the second phase of body weight had longer duration compared with other durations, except for combination L2, W1. Large group differences for b ratios are concentrated within length and within weight; b ratios for length were higher for (+) group; b ratios for weight were higher for (-) group.

Table 2. Means of (+) and (-) groups for females and males; tests of significance for effects of sex, group and dam on differences between c's and ratios of b's for phases in body weight-tail length relation; and standard deviations (s_e) between littermates within dams.

Measure		Means					Significance[1]						
			Females		Males		Effects[2]						
i1	i2	μ	(+)	(-)	(+)	(-)	μ	s	g	s*g	d	g*d	s_e
I. $c_{i2}-c_{i1}$													
L1[3]	L2	3.31	2.95	3.67	3.21	3.43	**	ns	**	ns	**	ns	.39
W1	W2	12.69	10.61	16.40	10.38	13.37	**	ns	**	ns	*	*	2.88
L1	W1	2.04	1.67	1.66	2.66	2.16	**	**	ns	ns	*	*	.37
L1	W2	14.73	12.28	18.06	13.04	15.52	**	ns	**	ns	*	*	3.00
L2	W1	-1.28	-1.27	-2.01	-0.55	-1.27	**	**	**	ns	*	ns	.41
L2	W2	11.41	9.33	14.39	9.83	12.10	**	ns	**	ns	*	*	2.99
II. b_{i1}/b_{i2}													
L1	L2	1.94	2.05	1.85	1.98	1.88	-	ns	**	ns	**	ns	.22
W1	W2	4.45	3.94	4.74	3.60	5.50	-	ns	**	ns	**	ns	.89
L1	W1	1.22	1.27	1.31	1.34	.94	-	ns	*	**	**	ns	.19
L1	W2	5.12	4.70	5.99	4.68	5.09	-	ns	*	ns	ns	ns	.24
L2	W1	.63	.61	.72	.69	.51	-	ns	ns	**	ns	ns	.12
L2	W2	2.72	2.32	3.37	2.40	2.80	-	ns	*	ns	ns	ns	.57

[1] - not tested; ns $P>.05$; * $P<.05$; ** $P<.01$

[2] See text for statistical model, names of variables and effects.

[3] L1 means: first phase of tail length; W2: second phase of weight, etc.

Koops (1989) concluded that phases of the multiphasic function contain body components based on similar ages where gain is maximum (or inflection points). Consequently, no simple allometry will exist between phases within the same body measure. This is supported by results in Table 2, phases within length (L1 and L2) and phases within weight (W1 and W2) differ considerably in differences of c's and ratios of b's. Therefore, the discussion will not concentrate on relations within measures, but on phases crossed over measures.

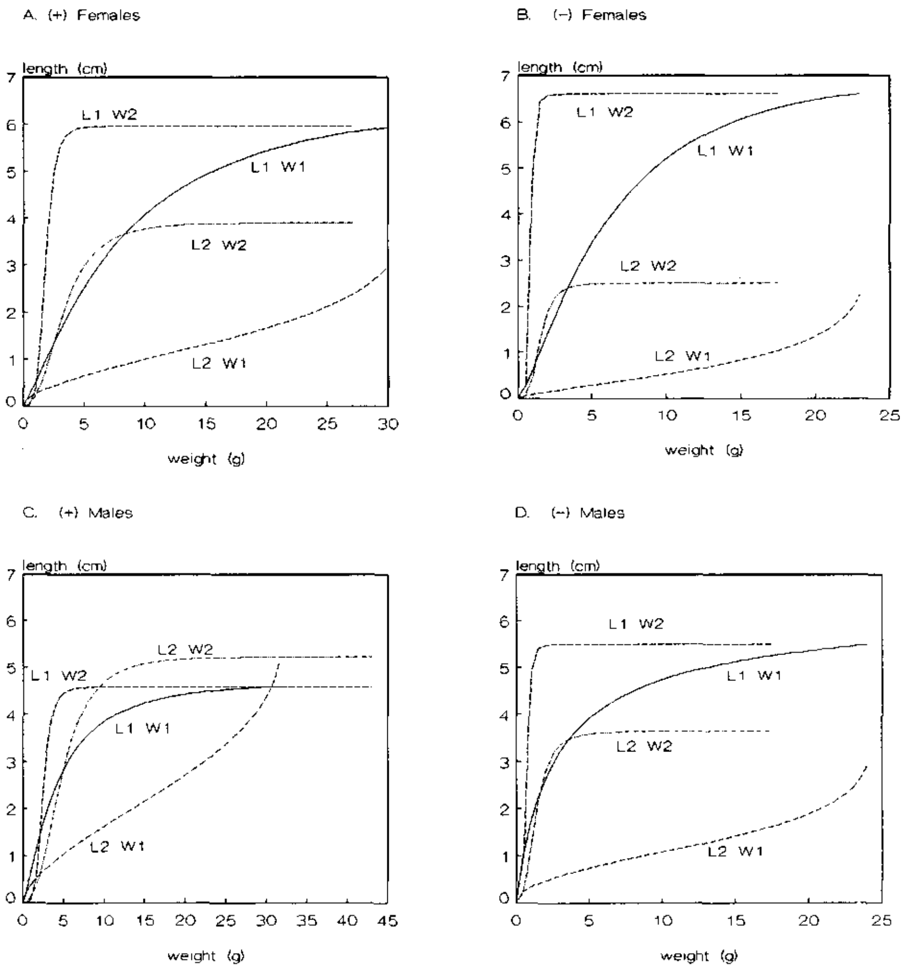

Figure 2. Phases estimated by a diphasic function for tail length and body weight, related by sex and group. (L1 is phase 1 of tail length, etc.)

Pairwise values were calculated according to equation (13) for each sex and group to show how phases in tail length are related to phases in body weight (Figure 2 (A, B, C and D)).

It is clear that weight gain in second phase is almost independent of gain in tail length for each sex and group; most of the gain in weight of the second phase is on constant levels (asymptotes) of the two length phases.

Figure 2 shows for all sex and group combinations, that gain in weight in the first phase is related mostly to gain in length in second phase. This relation is the only combination with gain in both directions over the whole period, and tends to be asymptotic in the weight direction. All other combinations are asymptotically in the direction of tail length.

CONCLUSIONS

To study relations between growth of different body measures of an individual animal, the well-known allometric function is used often. Lebeau *et al.* (1986) and Jolicoeur and Pirlot (1988) have shown that simple allometry exists only when maximum gain of each measure is at the same age. Jolicoeur and Pirlot used a modified Pütter growth function to demonstrate this in (cross-sectional) brain and body weights of rats. Laird (1965) showed the same phenomenon using the Gompertz growth function. The logistic growth function cannot incorporate the simple allometric relation, but is approximately simple allometric at low levels of Y relative to the asymptotic value.

It could be concluded that simple allometry is a very special case of the relations between body measures, if it is based on growth functions. The allometric equation seems to be too extensive a generalization.

Although simple allometry is not incorporated in the logistic function of growth of different body measures, it can be used in the way that Lebeau *et al.* (1986) did. This logistic function is the basis for the multiphasic growth function presented by Koops (1986). The multiphasic function has the opportunity to relate growth in different phases to each other or to growth in phases of other body measures. Koops (1989) showed that phases of the multiphasic function contain groups of body components with similar age at maximum gain. Given this information, no simple allometry will exist between phases within a same measure.

A method is worked out and demonstrated for growth in mice for body weight and tail length (data from Koops and Grossman, 1989b). The method is based on comparing phases of the multiphasic growth functions of components by means of the age at maximum gain and duration of phases. For these data, the two phases within tail length and the two phases within body weight differed in age at maximum gain, which supports the conclusion of Koops (1989) mentioned before. Gain in second phase of body weight seemed to be unrelated to other components. For most relations, there was an asymptotic behavior to tail length, whereas only the relation first phase of body weight to second phase of tail length had any indication of tending to be asymptotic to weight. This method seems to be a suitable extension to study growth of different body measures if multiphasic growth functions are used. Especially in the field of body composition research, it seems to be attractive to use the multiphasic growth function in combination with the method presented here.

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GENERAL DISCUSSION AND CONCLUSIONS

On the use of multiphasic growth functions

Mathematical functions to describe growth in body size are used extensively in animal production research. Many different growth functions are available for the biologist. Because criteria to select the 'best' function are arbitrary, personal preference and experience of the researcher often are the basis for selecting a function. Comparison of results for different growth functions is difficult because each function has a unique set of properties. Most of the well known growth functions, such as Gompertz, logistic or the von Bertalanffy function, have three parameters. More recent functions, such as Richards or generalized logistic, with four or more parameters are suggested for use as a general function. In practice these functions are difficult to fit because of high correlations between parameters.

An undesirable aspect of use of three-parameter growth functions is the existence of trends in the residuals. In statistical terms these residuals are explained by autocorrelation, assuming random residuals and using the correct growth function. Based upon these principles, stochastic growth models were developed (Sandland and McGilchrist, 1979). From a biological point of view, systematic deviations can be an indication for rejecting the model. This dilemma was motivation for a study of growth literature to look for biological evidences in explaining systematic residuals.

Results of studying the growth literature led to the conclusion that in animal and human growth data, 'growth cycles' or 'growth phases' could be detected. Each phase is defined as a period of growth, which includes an exponential increase until maximum gain, followed by an exponential decrease. This approach was recommended first by Robertson (1923) and discussed extensively by Courtis (1937) and Zucker *et al.* (1941). Since that time, this approach has been accepted completely in the domain of human growth, but not in animal growth. Recently, Peil and Helwin (1981) suggested applying a 'multiphasic' growth function to animal growth, but they used the function only for human height growth data. They suggested that phases should be a result of what they called the 'biological program of the growth process.'

The phenomenon of multiphasic growth in animals was also mentioned by Gall and Kyle (1968) and Eisen (1976) for mice. They called it the 'diphasic nature' of growth in mice, and Eisen (1976) even suggested analyzing the curve in parts.

The literature gave evidence for a 'multiphasic nature' of growth curves in man and animals. Therefore, the main objective of this study is formulated as: investigation of the use of a multiphasic growth function (Koops, 1986) for animal growth curves, based upon the existence of multiphasic growth.

Application of a growth function has many aspects. This investigation was focussed mainly on practical aspects. No attention was given to statistical and computational methods, because many good working nonlinear regression techniques are available in statistical computer packages. Judging goodness of fit, however, is only possible with statistical measures, such as residual standard deviations and measures of autocorrelation.

In practical terms, it can be concluded that application of the function appeared to be successful. Compared with usual single-phase growth functions, systematic trends in residuals disappeared, in many cases, and predicted values were close to observations, as shown in Chapter 1, 2 and 5. The statistical goodness of fit approximates results obtained by using high degree polynomials. The use of polynomials (Grizzle and Allen, 1969) has been promulgated because of favorable statistical properties; however, it answers only the question 'are curves probably different?' and gives no biologically interpretable parameters. There are some similarities in the polynomial and the multiphasic approach. In the use of polynomials it has to be decided which degree fits best, and in the multiphasic approach the number of detectable phases has to be determined. The degree of polynomial and the number of phases depends on the number of observations. This may be one of the weak points of the method because it includes the risk of over-prediction. The essential difference in the two approaches is that higher degrees in the polynomial method represent curves of different form without any biological meaning, whereas each new phase of the multiphasic function is of a fixed form and is based on a growth curve pattern. Therefore, in case of over-prediction or extrapolation outside the observation period, the polynomial method can give nonsense results, but the multiphasic function still gives realistic results.

The multiphasic function can be applied in different circumstances (Chapter 3), with easy interpretable parameters. One of the most interesting properties of the function is the relation of the phases determined by the function to the existing phases determined on biological grounds. In Chapter 3, it was shown that body components with similar ages (inflection point) where gain is maximum were grouped together into one phase. This is easy to understand, because the sum of components with equal growth patterns located at the same point of inflection are impossible to separate.

In Chapter 4, simulated data of four single-phase functions that differed primarily in shape were fitted by multiphasic functions. Results for simulated and for actual data showed that the multiphasic function was a reasonable alternative to single-phase functions. This is an important result, because if the multiphasic function is used, it is not necessary to select the 'best' single-phase function. Discussions related to the ability of single-phase functions to fit growth curves of different species or different body measurements can be avoided by using the multiphasic function as a general growth function. In Chapter 5, it was shown that body weight as well as tail length of individual mice could be fitted by a diphasic function. If single-phase functions were used, for instance, weight should be fitted best by the Johnson-Schumacher function and length by the Gompertz function. In such cases, not only is the use of one general function an advantage, but it offers also the opportunity to compare growth phases of different body measurements. This is demonstrated in Chapter 6 for phases of body weight and tail length of mice. It was shown how multiple phases could be incorporated into allometric growth comparisons. Results in Chapter 3 and 6 indicate that application of the multiphasic growth function can give significant support to morphometric or allometric studies.

Fields for application

Although a great deal of investigation on growth curves is based on mice, there is no reason to believe that the multiphasic growth function should behave very differently in other species of animals. Grossman and Koops (1988) applied the multiphasic growth function successfully to chickens, and it has been applied to pigs, in Chapters 4 and 5, and to cows in Chapter 4.

Reasons for using mice to study growth data are: mice are held in a stable environment so that external (environmental) influences can expected to be small, and the multiphasic pattern of growth seems to be most pronounced in mice.

One large field of application is in research that requires a mathematical description of growth curves. Success in using this function, however, depends on variability of measurements, frequency of measurements, and length of the measurement period relative to lifetime. As shown in Chapter 3, in some situations, a few (but precise) number of observations can give very good results.

During the last century, a large number of selection experiments were conducted to change the form of the growth curve of different species of animals. Body weights on a fixed age were used as selection criterion. Most of the results of such selection showed no change in form of the curve, but only a change in mature weight (Taylor, 1985). If growth phases are taken into account it should be possible to select for weight in different phases; for instance, high weight in the first phase and low weight in the second phase, which is a type of antagonistic selection. Probably this will result in a change in shape of the growth curve. Further research in changing growth curves based on the existence of multiphasic growth, is recommended.

A more specific field of application is opened by the relation of the multiphasic growth function to body components. As shown in Chapters 3 and 6, phases seemed to group together those body components with similar age at maximum gain (or at the inflection point). Body components such as fat and skin, especially, have maximum gain at later ages than other components, as shown in the analysis of pig data in Chapter 3. This is confirmed by Walstra (1980) and Ferrell and Cornelius (1983) for pigs, by Loewer *et al.* (1983) for beef animals, and by Leenstra *et al.* (1986) for broilers. If one phase is primarily fat growth, multiphasic analysis of growth has the opportunity to examine fat-adjusted weight in living animals.

In evaluation of animal growth, Taylor (1985) has advocated the use of 'genetic size-scaling.' This scaling method is based on the assumption that each genotype has its own inherent genetic size factor operating throughout growth. Mature body weight (A) in kg is suggested as the genetic size factor. Time and age variables (in days) should be treated as being proportional to $A^{.27}$ and cumulated growth variables as being proportional to A. Growth curves of mammals, transformed according to these rules, were similar (Taylor ,1985). Mature weight, however, is fluctuating and therefore almost undeterminable. Referring to the mice data in Chapters 5 and 6, tail length showed an excellent asymptotic response, but at week 26 body weight was still increasing. The A could be estimated over a wide range; depending on how A is determined, it can be body weight at the age when tail length reached its asymptote, or the highest body weight. Values used for mature weight are always rough guesses. Although Taylor defined mature body weight precisely, there is still need for a better description in order to estimate the genetic factor more accurately. A weakness in his definition of mature weight is the degree of fatness at maturity, which ranges from 15 to 25% chemical body fat. An important remark, in the context of this study, made by Taylor (1985) at the end of the paragraph defining A, is: '... estimates of A should be based on all available information.' It is obvious, based on the previous discussion of multiple phases and body components, that results of the multiphasic function can provide more information and a better definition of adult size and, therefore, can probably provide better estimates for the genetic size-factor.

Conclusions

1. The existence of more than one phase in growth curves of men and animals is supported by biological explanations in literature.
2. Application of the multiphasic growth function provides detailed insight into growth patterns of body weight or other body measures, for individuals or groups of individuals.
3. Use of the multiphasic function requires frequent measurements during a relatively long period of life; this improves discrimination of the phases.
4. The multiphasic function is applicable in circumstances where phases are caused by systematic external influences as well as in cases where phases are the result of internal factors.
5. In each phase of multiphasic growth, body components that have maximum gain at a similar age will be grouped together, if external influences are negligible.

6. In cases where detection of multiple phases in the growth curve is not the main objective, use of the multiphasic function is still an attractive alternative to single-phase functions. If the multiphasic function is modified according to circumstances in which growth took place, then parameters are less correlated than those of single-phase functions.
7. Use of the multiphasic function in 'allometric growth studies' leads to an extra dimension of comparison. In addition to comparing growth of different body measures, it is possible to compare growth for different phases. In light of conclusion 5, phases within one body measure will show 'complex allometric' relations.
8. Application of the multiphasic growth function can make an important contribution in determining stage of physiological maturity, which is especially of interest when using the 'genetic scaling rules' defined by Taylor (1985).

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SUMMARY

The central theme of this thesis is the mathematical analysis of growth in animals, based on the theory of multiphasic growth. Growth in biological terms is related to increase in size and shape. This increase is determined by internal (genetical) and external (environmental) factors. Well known mathematical functions, used in studies to quantify growth in size from birth to maturity, assume growth to be a result of one growth phase. Over the course of time, body weight or other body measures first show an exponential increase, followed by a decreasing increase. For size-age relationships, this results in a S-shaped growth curve.

Multiphasic growth theory states that the total growth curve is a result of a summation of many smaller S-curves. Each cell or group of cells has its own genetically determined growth potential, with different ages where gain is maximum. The total growth curve reflects an average growth pattern. The number of detectable phases depends on frequency and variability of the measurements.

Multiphasic, or multicyclic, growth theory was a subject of discussion in growth literature from 1900 to 1945. In the Introduction of this thesis, a review of this literature is given. Multiphasic growth functions have been widely accepted to describe human growth. In the description of animal growth, however, single-phase (or monophasic) functions have been used, although in the extensive literature of animal growth studies there is sufficient evidence for the existence of more than one growth phase.

The main objective of this study was to investigate the application of a multiphasic growth function to quantify animal growth. Investigation includes: application of the multiphasic function for different growth data (mean and individual curves) under different circumstances (internal and external influences), comparison of a multiple-phase function to single-phase functions and consequences on morphometric growth studies of assuming multiphasic growth.

Application of a multiphasic growth function is demonstrated in Chapter 1, with four data sets taken from literature. The multiphasic growth function used was a summation of n logistic growth functions. Human height growth curves of this type are known as "double logistic" (n=2) or "triple logistic" (n=3) growth curves. When applied to the human height curve and to

pika, mice and rabbit weight curves, the fit of the multiphasic growth function was superior to the monophasic model in terms of smaller residual variances and absence of autocorrelation of residuals. For pika weights, two phases could be distinguished and for the other data sets, three phases.

Application of a multiphasic function to individual weights is tested in Chapter 2. Growth curves of mean body weights were compared to those of individual weights, when fitted to data of male and female mice using monophasic and triphasic growth (logistic) functions. Because of the large variability in individual weights, it was necessary to set bounds on some parameters. Goodness-of-fit criteria suggested that the triphasic function, with smaller and less correlated residuals, described the data better than the monophasic function. For the triphasic function, residual variances were larger when fitting curves for individual weights than for mean weights. Means of parameters for the triphasic function were higher for individual weights than for mean weights. Differences in parameter estimates between curves within sex were small. Parameters were similar for males and females in the first phase of growth. For the second and the third phase, however, asymptotic weight was higher for males than for females. It could be concluded that the triphasic function was able to describe accurately individual weights of male and female mice.

In Chapter 3, a multiphasic growth function is applied to problems of growth in different circumstances. Seasonal influence on growth in length of Northsea herring is an example of an external factor causing phases of growth. By modifying the multiphasic function slightly, length growth was described. The most important internal factor causing phases of growth is the difference in growth patterns of body components. The multiphasic function was modified and applied to growth of body components in pigs. Growth of total dry matter was analyzed with a diphasic function, and growth of fat and fat-free components were each analyzed with a monophasic function. Results for total dry matter showed clearly that parameter estimates of the diphasic function for the two distinguishable phases were related closely to parameter estimates of a monophasic function for each of the two components. In a second illustration on growth in pigs, also a relation between growth phases of the function and growth of different body components could be shown. By restricting parameters of the general multiphasic function, or treating some parameters as constants, growth functions can be constructed that have parameters that are easy to interpret.

The relation of the multiple-phase to the single-phase approach for describing growth curves is studied in Chapter 4. A multiple-phase growth function is compared with four single-phase growth functions. From a general five-parameter function, four functions were selected to achieve maximal differences in shape: the Johnson-Schumacher, Michaelis-Menten, Gompertz and Logistic function. The multiphasic function was fitted to the simulated data from each of the four functions. Body weights of a cow and a boar were analyzed with four single-phase functions and with the multiphasic function.

Results of simulation showed that a triphasic function satisfactorily described simulated data from the four functions, in terms of smaller residual standard deviation and absence of systematic deviations in residuals. It could be concluded, therefore, that single-phase functions, with early inflection points, show relatively high fractions of the asymptotic value in the first phase. Using a single-phase function, cow and boar data could be described best with the Johnson-Schumacher function. Using a diphasic function for each data set, systematic deviations were eliminated and residual standard deviation was no larger than when using the Johnson-Schumacher function for the two data sets. This comparison showed that a multiple-phase function is a reasonable alternative to a single-phase function. An important advantage of a multiple-phase over a single-phase function is not having to select the 'best' single-phase function. Problems of having to estimate a larger number of parameters for a multiple-phase function than for a single-phase function can be overcome because parameters for a multiple-phase function are less correlated than those for a single-phase function.

Application of the multiphasic growth function to body weights and tail lengths of mice is studied in Chapter 5, in cases where large, genetically determined differences in size exist between littermates. Mice were progeny of one male that carried the human growth hormone gene (somatotropin) and random bred NMRI females. At week 12, ten litters, with at least four females or four males, were chosen. Within each litter, four females or four males were selected, two with highest and two with lowest body weight. Mice with highest body weight were considered to be transgenic. Although this was not tested biologically, differences in body weight were considerable and the assumption probably was correct. Body weight and tail length of these 40 mice were measured about weekly from week 3 to 26. Female transgenic mice reached

26-week body weight that averaged 1.6 times that of their non-transgenic littermates; for males, this ratio was 1.9. A diphasic growth function was used either for body weight and for tail length with marked results, especially for tail length. In the first phase, transgenic females had .64 cm shorter tails and transgenic males had .92 cm shorter tails than non-transgenic littermates. In second phase, transgenic females grew 1.4 cm and males 1.58 cm more than non-transgenic littermates. Body weight differences in each phase were in favor of transgenic mice. Multiphasic growth functions fitted data for body weight and tail length satisfactorily and provided clearer insight into differences in growth patterns of transgenic and non-transgenic mice.

Body weights and tail lengths of these same mice were used in Chapter 6 to study consequences on morphometric studies of assuming multiphasic growth. These types of studies, frequently indicated as 'allometric growth studies', will have an extra dimension when phases are taken into consideration. Multiphasic growth functions are based on assigning weight or other body measures to different phases. The well-known allometric function is used most often to study relations in growth of different body dimensions of an individual. Complex allometry exists when age at maximum gain is shifted on the age scale. Growth functions can used to estimate these ages. In this chapter, the literature on this subject is reviewed. By using multiphasic functions to describe growth of different body measures, it is possible to relate the growth in different phases. In mice data, it could be shown that the second phase of tail length was related the strongest to the first phase of body weight. Gain in second phase of body weight seemed to be unrelated to other body measures. Multiphasic growth analysis provides a suitable extension to study relations of growth in different body dimensions.

Findings in these investigations can be summarized in the following conclusions:

1. The existence of more than one phase in growth curves of humans and animals is supported by biological explanations in the literature.
2. Application of the multiphasic growth function provides detailed insight into growth patterns of body weight or other body measures, for individuals or groups of individuals.

3. Use of the multiphasic function requires frequent measurements during a relatively long period of life; this improves discrimination of the phases.
4. The multiphasic function is applicable in circumstances where phases are caused by systematic external influences and in cases where phases are the result of internal factors.
5. In each phase of the multiphasic function, body components that have maximum gain at a similar age will be grouped together, if external influences are negligible.
6. In cases where detection of multiple phases in the growth curve is not the main objective, use of a multiphasic function is an attractive alternative to single-phasic functions. If a multiphasic function is modified according to circumstances in which growth took place, then parameters are less correlated then those of single-phase functions.
7. Use of a multiphasic function in "allometric growth studies" leads to an extra dimension for comparison. In addition to comparing growth of different body measures, it is possible to compare growth of different phases. In light of conclusion 5, phases within one body measure will show "complex allometric" relations.
8. Application of the multiphasic growth function can make an important contribution in determining stage of physiological maturity, which is especially of interest when using the "genetic scaling rules" defined by Taylor.

SAMENVATTING

Het centrale thema in dit proefschrift is de analyse van groei bij dieren, gebaseerd op de theorie van meerfasengroei. Groei in biologische termen is gerelateerd aan de toename in maat en formaat. Deze toename wordt bepaald door interne (genetische) en externe (milieu) factoren. Bekende wiskundige groeifuncties die worden gebruikt om het verloop in groei te kunnen kwantificeren van geboorte tot volwassenheid, veronderstellen deze groei als resultaat van één groeifase. Het verloop van lichaamsgewicht of -maten in de tijd laat eerst een exponentiële toename zien, gevolgd door een toename die exponentiëel afneemt. Voor de relatie lichaamsmaat-leeftijd resulteert dit in een S-vormige curve. De meerfasengroeitheorie veronderstelt dat deze curve de resultante is van een groot aantal kleine S-curven. Elke cel of groep van cellen heeft een eigen genetisch bepaalde groeipotentie, met maximale groei op verschillende leeftijden. De totaal groeicurve reflecteert een gemiddeld groeipatroon. Het aantal aantoonbare groeifasen hangt af van de frequentie van meten en de variabiliteit van het kenmerk.

In de literatuur over groei vond in de periode van ongeveer 1900 tot 1945 een uitgebreide discussie plaats over de meerfasengroei. In de introductie van dit proefschrift is deze literatuur bediscussiëerd. Wiskundige functies die groei beschrijven als resultaat van meerfasengroei zijn volledig geaccepteerd in de beschrijving van groei bij de mens. Bij de beschrijving van groei bij dieren wordt echter vrijwel altijd gebruik gemaakt van enkelfasige functies. De uitgebreide literatuur over groei bij dieren bevat echter vele indicaties die in de richting wijzen van het bestaan van meer dan één groeifase.

Het hoofddoel van dit onderzoek is na te gaan of toepassing van een wiskundige groeifunctie, gebaseerd op meerfasengroei, mogelijkheden biedt in het kwantificeren van groei bij dieren. Het onderzoek omvat o.a.: toepassing van de functie op verschillende groeigegevens (gemiddelde en individuele curven) onder verschillende omstandigheden (interne en externe invloeden); vergelijking met enkelfasige groeifuncties en de consequenties van de aanname van meerfasengroei voor het morfometrisch of allometrisch groeionderzoek.

Toepassing van een meerfasengroeifunctie is gedemonstreerd in Hoofdstuk 1, met vier gegevenssets overgenomen uit de literatuur. De groeicurve die werd gebruikt is de som van n logistische functies. Curven van lichaamslengte

bij de mens zijn bekend als "double logistic" (n=2) en "triple logistic" (n=3) groeicurven. De aanpassing van de meerfasengroeicurve, toegepast op lichaamslengte bij de mens en lichaamsgewicht bij pika's, muizen en konijnen was beter dan die van het enkelfasige model in termen van restvarianties en afwezigheid van autocorrelatie van de residuen. In het gewichtsverloop van de pika's konden twee fasen en in de andere gegevenssets drie fasen worden onderscheiden.

Of de meerfasengroeifunctie in staat is ook individuele curven goed te beschrijven, is onderzocht in Hoofdstuk 2. Groeicurven van muizen op basis van gemiddelde lichaamsgewichten werden vergeleken met curven van individuele gewichten. Aanpassing aan de gegevens van mannelijke en vrouwelijk muizen vond plaats met een enkelfasige (logistisch) en een driefasige groeifunctie. Vanwege de grote variatie in individuele gewichten bleek het noodzakelijk een aantal parameters te begrenzen. Criteria voor de kwaliteit van de aanpassing lieten zien dat de driefasige functie, met kleinere en minder gecorreleerde residuen, de gegevens duidelijk beter beschreef dan de enkelfasige functie. Bij de aanpassing van de driefasige functie aan de individuele gewichten waren de restvarianties groter dan bij de gemiddelde gewichten. De gemiddelde parameters van de driefasige functie bij individuele schattingen waren hoger dan de geschatte parameters van de gemiddelde gewichten. Verschillen in parameterschattingen tussen curven binnen geslachten waren gering. In de eerste groeifase waren de parameterschattingen voor de beide geslachten ongeveer gelijk. In de tweede en derde groeifase was de schatting van het asymptotisch gewicht bij de mannelijke muizen hoger dan bij de vrouwelijke muizen. Uit de resultaten kon worden geconcludeerd dat de driefasige functie het gewichtsverloop van mannelijke en vrouwelijke muizen in dit materiaal nauwkeurig beschreef.

In Hoofdstuk 3 is de meerfasengroeifunctie toegepast op situaties van groei onder verschillende omstandigheden. Seizoensinvloed op lengtegroei van Noordzee-haring is een voorbeeld van een externe factor die groeifasen veroorzaakt. Door een eenvoudige modificatie van de meerfasengroeifunctie bleek de lengtegroei goed beschrijfbaar. De belangrijkste interne factor die aanleiding geeft tot groeifasen is het verschil in groeipatronen van verschillende lichaamscomponenten. Voor toepassing in onderzoek waar lichaamssamenstelling centraal staat, is de algemene meerfasengroeifunctie gemodificeerd en toegepast op groeigegevens van varkens. Groei van de totale droge stof in het lichaam werd geanalyseerd met een tweefasige functie, terwijl groei van vet en van vetvrije droge stof werd geanalyseerd met enkelfasige functies. De resultaten voor de totale droge stof lieten duidelijk zien dat parameters voor de twee te onderscheiden fasen van de tweefasige functie sterke overeenkomst vertoonden met de parameter-schattingen in de enkelfasige functies van de twee componenten. In een tweede illustratie bij groei van varkens kon deze overeenkomst eveneens worden aangetoond. Door in de algemene meerfasenfunctie parameters restricties op te leggen of constant te veronderstellen, in overeenstemming met de gepleegde aannames, bleek het mogelijk handzame groeifuncties te construeren met duidelijk interpreteerbare parameters.

De relatie van de meerfasige tot de enkelfasige benadering in het beschrijven van groeicurven komt aan de orde in Hoofdstuk 4, waarbij een meerfasen groeifunctie is vergeleken met vier bekende enkelfasige groeifuncties. De vier functies werden geselecteerd op basis van een algemene 5-parameter functie, zodanig dat er een maximaal verschil in de vorm van de curven aanwezig was. Geselecteerd werden: de Johnson-Schumacher, Michaelis-Menten, Gompertz en de Logistische functie. De meerfasenfunctie is toegepast op gesimuleerde gegevens voor de vier functies. Verder zijn gewichten van een koe en een varken geanalyseerd met de vier enkelfasige functies en de tweefasige functie. De resultaten van de simulatie lieten zien dat een driefasige functie de gesimuleerde gegevens bevredigend beschreef, in termen van residuele spreiding en afwezigheid van systematische afwijkingen in de residuen. Er kon worden geconcludeerd dat enkelfasige functies met een vroeg buigpunt in de eerste fase relatief hoge fracties van de totale asymptoot vertoonden. De gewichten van de koe en het varken werden door de Johnson-Schumacher, als beste van de vier enkelfasige functies, goed beschreven. Wanneer een tweefasige functie werd gebruikt voor beide gegevenssets, verdwenen de systematische afwijkingen in de residuen en was de residuele spreiding vergelijkbaar of kleiner dan bij de Johnson-Schumacher aanpassing. Deze vergelijking maakte duidelijk dat het meerfasenmodel, op basis van de kwaliteit van de aanpassing, een aantrekkelijk alternatief is ten opzichte van het gebruik van enkelfasige functies. Een bijkomend belangrijk voordeel is dat niet de 'beste' enkelfasige functie geselecteerd behoeft te worden. Problemen die verband houden met een groter aantal te schatten parameters in de meerfasenfunctie zijn niet te verwachten omdat in het algemeen deze parameters onderling veel minder gecorreleerd zijn dan in de enkelfasige functies.

Toepassing van de meerfasenfunctie op individuele gewichten en staartlengtes bij muizen, in een situatie waar grote genetisch bepaalde verschillen bestonden tussen worpgenoten, is bestudeerd in Hoofdstuk 5. Daartoe werden afstammelingen gebruikt van één vader, die drager was van het menselijk groeihormoon-gen (somatotropine) en 'random bred' normale vrouwtjes. Op een leeftijd van 12 weken werden in 10 worpen, waarin minimaal vier vrouwtjes of vier mannetjes voorkwamen, de twee zwaarste en de twee lichtste muizen van het zelfde geslacht geselecteerd. De zware muizen werden als transgeen beschouwd. Hoewel dit niet is getest, waren de verschillen in gewicht dusdanig groot, dat deze aanname waarschijnlijk correct is geweest. Deze 40 muizen zijn wekelijks gemeten en gewogen vanaf 3 weken tot een leeftijd van 26 weken. De vrouwelijke transgene muizen bereikten op week 26 gemiddeld een gewicht dat 1,6 maal zo hoog was dan van de niet-transgene worpgenoten. Voor de mannelijke muizen was deze verhouding 1,9. Er werd voor zowel gewicht als staartlengte een tweefasige functie gebruikt, waarbij vooral de resultaten van staartlengte opvallend waren. Na verloop van de eerste fase hadden de transgene vrouwtjes een 0,64 cm kortere staart en de mannetjes een 0,92 cm kortere staart dan hun worpgenoten. In de tweede fase werd dit verschil ruimschoots goed gemaakt, de staarten van de transgene vrouwtjes groeiden 1,4 cm meer en de mannetjes 1,58 cm meer dan van hun niet-transgene worpgenoten. Voor de toename in gewicht waren de verschillen in de eerste fase reeds in het voordeel van de transgene muizen, in de tweede fase werd dit verschil nog groter. Het verloop in gewicht en staartlengte werd goed beschreven door een tweefasige functie en verschafte daarmee een goed inzicht in het verschil in groeipatroon van transgene en niet-transgene muizen.

De gewichten en staartlengtes van genoemde 40 muizen zijn eveneens gebruikt om in Hoofdstuk 6 de consequenties te bestuderen van de aaname van meerfasengroei voor morfometrische studies. Dergelijke studies, die vaak worden aangeduid met 'allometrische groeistudies', krijgen een extra dimensie wanneer ook groeifasen in de vergelijking worden betrokken. De meerfasengroeifunctie is gebaseerd op het toekennen van de groei van verschillende delen van het gewicht of andere lichaamsmaten aan fasen. Om onderlinge relaties in groei van verschillende dimensies van het lichaam te bestuderen, wordt veelal gebruik gemaakt van de bekende allometrische vergelijking. Wanneer de leeftijd, waarbij de groei maximaal is voor de componenten, verschillend is, bestaat er complexe allometrie. Er kan dan gebruik gemaakt worden van groeifuncties, om de onderlinge relatie te beschrijven. Er is in dit hoofdstuk een overzicht gegeven van de literatuur betreffende dit onderwerp. Wanneer meerfasengroeifuncties worden gebruikt om groei te beschrijven van verschillende kenmerken, is het mogelijk de groei in de verschillende fasen onderling te relateren. In de gewichten en staartlengtes van muizen kon worden aangetoond dat de eerste groeifase van gewicht nauw gerelateerd was aan de tweede fase van staartlengte. De tweede groeifase van gewicht bleek vrijwel onafhankelijk van andere componenten tot stand te komen. De hier beschreven methode lijkt een bruikbare uitbreiding van de methoden die gebruikt worden bij het bestuderen van groei van verschillende dimensies van het lichaam.

De bevindingen in het onderzoek kunnen worden samengevat in de volgende conclusies:

1. Het bestaan van meer dan één groeifase in een groeicurve van mens of dier wordt op biologisch gronden gestaafd door de literatuur.
2. Toepassing van de meerfasengroeifunctie levert een gedetailleerd inzicht in het groeipatroon van gewicht of lichaamsmaten bij toenemende leeftijd van een individu of groepen individuen.
3. Voor toepassing van deze functie is het nodig frequent te meten of te wegen gedurende een relatief lange periode van het leven. Dit verhoogt de mogelijkheid tot het onderscheiden van fasen.
4. De functie is gebleken toepasbaar te zijn in zowel situaties waarin fasen worden veroorzaakt door systematische externe invloeden als in situaties waarin de fasen een gevolg zijn van interne factoren.
5. In situaties waarbij geen sprake is van externe oorzaken die leiden tot fasen, worden in iedere fase van een meerfasengroeifunctie componenten gegroepeerd die maximale groei vertonen op ongeveer dezelfde leeftijd.
6. Ook in situaties waarin het onderscheiden van fasen in de groeicurve niet het direkte doel is, is de meerfasengroeifunctie een aantrekkelijk alternatief ten opzichte van enkelfasige groeifuncties. Bij gebruik van een meerfasenfunctie, die op de juiste manier is aangepast aan de omstandigheden waaronder groei heeft plaats gevonden, zijn de parameters minder onderling gecorreleerd dan in het geval van enkelfasige functies.

7. Het hanteren van de meerfasenfunctie in 'allometrische groei studies' leidt tot een extra dimensie van vergelijking. Naast vergelijking van de groei van kenmerken onderling, kan ook de groei van verschillende fasen worden vergeleken. Gezien het geconcludeerde onder 5, zullen de fasen binnen een kenmerk onderling vrijwel altijd 'complex allometrisch' zijn gerelateerd.
8. Gebruik van de meerfasengroeifunctie kan een belangrijke bijdrage leveren tot een betere bepaling van het stadium van het fysiologisch volwassen zijn, hetgeen met name van belang is bij de toepassing van de 'genetic scaling rules' gedefinieerd door Taylor.

Curriculum vitae

Wiebe Jacob Koops werd op 12 januari 1942 geboren te Elsloo (Friesland). In 1959 behaalde hij het diploma van de Lagere Landbouwschool te Oldeberkoop, in 1961 het diploma van de Middelbare Landbouwschool te Drachten en in 1966 het diploma van de Bijzondere Hogere Landbouwschool te Leeuwarden. Eind 1966 volgde zijn aanstelling als technisch assistent bij de vakgroep Veefokkerij van de toenmalige Landbouwhogeschool. Hij werd ingeschakeld bij het schapenonderzoek en kreeg tevens de opdracht zich te verdiepen in de statistische verwerking van veeteeltkundige onderzoeksgegevens met behulp van de computer. Na het volgen van een aantal statistische cursussen en de nodige ervaring in het wetenschappelijk onderzoek opgedaan te hebben, werd het gehele technische rangenstelsel doorlopen en werd hij in 1982 voorgedragen voor een wetenschappelijke functie. In 1983 volgde zijn benoeming als wetenschappelijk medewerker, met als onderzoeksproject: de wiskundige beschrijving van groei bij landbouwhuisdieren. Sinds 1984 is hij als universitair docent werkzaam bij de vakgroep Veefokkerij van de Landbouwuniversiteit.