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A simple polynomial model of the growth of the gastro-intestinal tract in the mouse embryo

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INTRODUCTION

In previous papers we presented data on the growth in volume of the intestine and stomach in mouse embryos from 11·5 days after fertilization to birth (Sbarbati, 1979) and on the growth in volume of the epithelium and mesenchyme of the intestine during the same period (Sbarbati & Strackee, 1980).

We used exponential functions to test a growth model (Goedbloed, 1972) based on exponential phases separated by breaks. The conclusion drawn from the testing of Goedbloed's model was that the growth curves of the gastro-intestinal tract are continuous in time (Sbarbati, 1979). A given set of data can often be described by several regression equations, the choice of function depending on the investigator's purpose and biological knowledge of the process under study. Our next step was to attempt a new description of the same growth data under the assumption that the growth processes in question are continuous in time and summable and that it should be possible to introduce the beginning of the growth process in the growth equation as an additional parameter.

The use of summable functions to fit growth data is expected to be useful for investigation of the growth characteristics of one organ, those of its components being known. We estimated (Sbarbati & Strackee, 1980) the volumes of the two layers, i.e. the epithelium and the mesenchyme, whose sum is the volume of the whole intestine. The exponential function is a useful tool for the study of single processes but does not permit the expression of properties such as summation, because summing of exponential functions leads to a composite exponential function.

We chose a simple polynomial function to re-describe the growth data already published (Sbarbati & Strackee, 1980) as well as new data on the growth in the volume of the epithelium and mesenchyme of the stomach during the same embryonic period. Particular attention is paid in this report to the fact that the polynomial description and the exponential description lead to different interpretations of the temporal characteristics.

MATERIALS AND METHODS

Estimations of the volume of the epithelium and of the mesenchyme of the stomach

The investigation was performed on a series of 27 mouse embryos of the CPB-S strain of a known developmental stage (Goedbloed, 1972), aged between 11·6 days after fertilization and birth. The material was serially sectioned at a constant

thickness of 10 μm. Four of the 31 embryos originally used (Sbarbati, 1979) were discarded because of poor preservation.

For practical purposes, the material was divided into two groups: 7 embryos younger and 20 embryos older than 13·1 days. The procedure was based on the point-counting method (Weibel, 1963) and was used to estimate the volume of the intestinal epithelium (V_{EI}) and mesenchyme (V_{MI}) (Sbarbati & Strackee, 1980). It was also applied to estimate the volume of the gastric epithelium (V_{ES}) and mesenchyme (V_{MS}). Of the material available (the number of the cross sections made of the stomach ranged from 60 in the youngest to 350 in the oldest embryo), 30 systematically chosen cross sections of the stomach were used for the analysis (Sbarbati, 1979). In the former group at a low magnification (e.g. 100 times), even the largest cross section of the stomach was contained in the field and the demarcation between epithelium and mesenchyme (Sbarbati & Strackee, 1980) was easily seen. The cross sections of the older group required a much higher magnification (e.g. 400 times) to make the tissue components distinguishable. It was therefore necessary to infer the volumes from samples in the cross sections themselves. A sample size of 30 fields was required by the level of accuracy chosen and the procedures used for sampling and volume estimation were similar to those employed for the epithelium and mesenchyme of the intestine (Sbarbati & Strackee, 1980).

A simple polynomial model of growth

For this model, use was made of the data on the growth in the volume of the epithelium and mesenchyme of the stomach as well as of the epithelium and mesenchyme of the intestine (Sbarbati & Strackee, 1980).

Model representation

The estimates of the growth in volume of the epithelium and mesenchyme of the stomach were first obtained by using an extended exponential growth curve. Polynomial regression analysis was applied to logarithmically transformed data, the degree of polynomial employed being the highest for which the highest coefficient was significant at least at the 95 % confidence level (F. test) (Sbarbati, 1979).

In general, one defines the specific growth rate as the derivative of the log-function (Sbarbati, 1979). For exponential growth, one has:

$$\lambda(t) = \frac{d}{dt} \ln V(t) = \frac{d}{dt} \ln Ae^{\lambda t} \tag{1}$$

We also tried to describe our growth data with a different type of function that is invariant under summation. At the same time, we wanted to introduce a different parameter, namely the beginning of the growth process. This led to polynomials with the form

$$V(t) = b(t-t_0)^m.$$

The parameters in this function are b, t_0, and m, t_0 being the beginning of the specific process. This form has, however, a slight drawback: summing of two of these expressions only leads to an identical form of the polynomial for equal values of m and t_0.

For the specific growth rate, one has:

$$\lambda(t) = \frac{d}{dt} \ln V(t) = \frac{m}{t-t_0}. \tag{2}$$

At $t = t_0$ the value of λ becomes infinite.

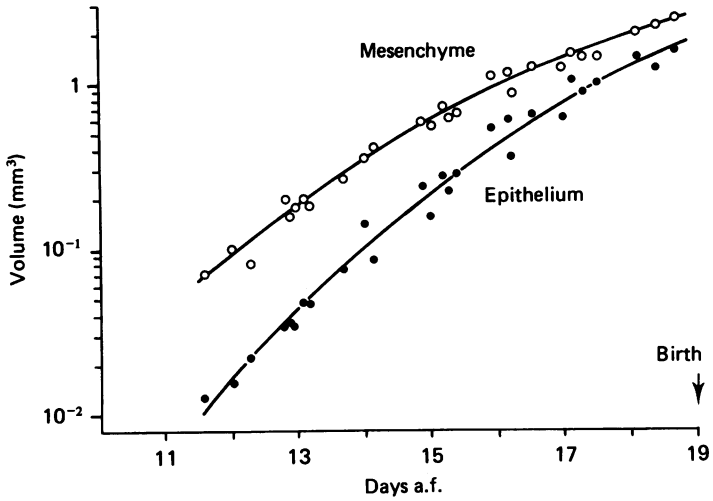

Fig. 1. Volume growth of the epithelium and mesenchyme of the stomach, on a log scale, plotted against time. At any given time the specific growth rate is the derivative of the log function. a.f., after fertilization.

The data were fitted with use of the lowest integer m value, according to the residual sum of squares, and the mean values, the standard deviation and the 95 % confidence limits of b and t_0 were computed using an iteration procedure.

A linear representation of the results was obtained plotting $\sqrt[m]{V(t)}$ against $b(t-t_0)$.

RESULTS

Figure 1 shows the growth curves plotted on a log data scale of the epithelium and mesenchyme of the stomach separately. The data were accurately fitted ($r^2 = 0{\cdot}99$) by the equations:

$$\ln V_{ES}(t) = -23{\cdot}13 + 2{\cdot}164\,t - 0{\cdot}048\,14\,t^2, \quad (3)$$

$$\ln V_{MS}(t) = -16{\cdot}35 + 1{\cdot}605\,t - 0{\cdot}036\,64\,t^2. \quad (4)$$

Only an additional quadratic term was needed for an accurate fit, and this term also provided for the slowing down of the specific growth rates. According to this model, at the beginning of the observations (11·6 days) the volume of the epithelium doubles every 16 hours and that of the mesenchyme every 23 hours. Just before birth, these volume-doubling times are 38 and 53 hours respectively. Between day 11·6 and day 18·7, the volume of the epithelium increases 157-fold and that of the mesenchyme 35-fold.

Figure 2 shows the data of the epithelium of the stomach (ES) and that of the intestine (EI), plotted on a $\sqrt[m]{V(t)}$ scale:

$$V_{ES}(t) = (0{\cdot}172 \times 10^{-3})(t-8{\cdot}8)^4, \quad (5)$$

$$V_{EI}(t) = (0{\cdot}154 \times 10^{-4})(t-8{\cdot}8)^6. \quad (6)$$

Figure 3 shows the corresponding results for the mesenchyne:

$$V_{MS}(t) = (0{\cdot}250 \times 10^{-2})(t-8{\cdot}8)^3, \quad (7)$$

$$V_{MI}(t) = (0{\cdot}661 \times 10^{-3})(t-8{\cdot}2)^4. \quad (8)$$

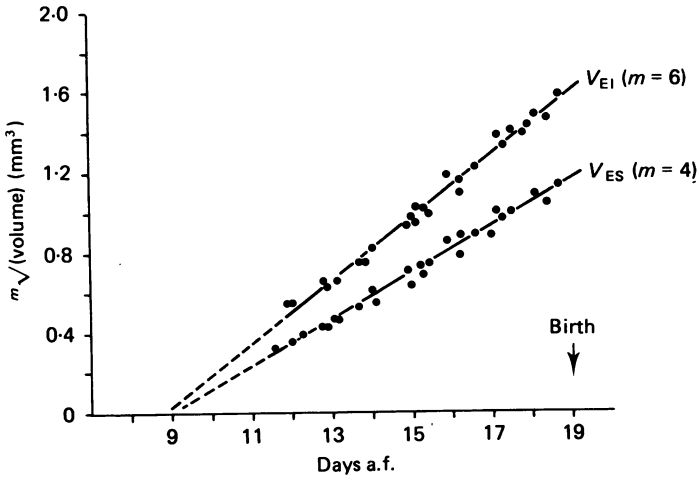

Fig. 2. Growth curves of the intestinal epithelium (plotted on a $\sqrt[6]{[V(t)]}$ scale) and of the gastric epithelium ($\sqrt[4]{[V(t)]}$ scale).

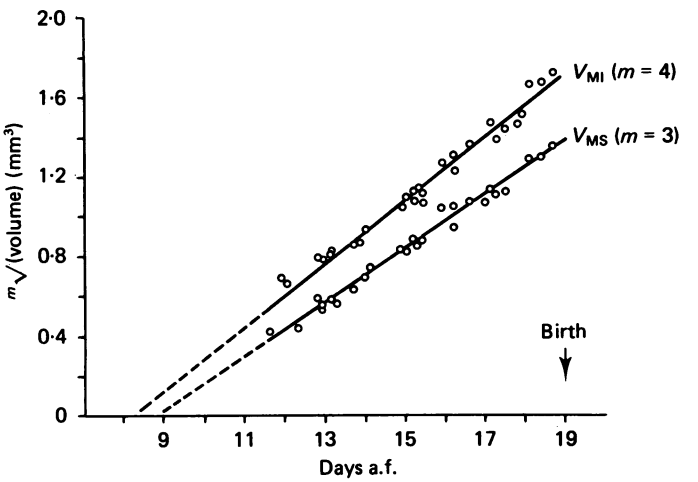

Fig. 3. Growth curves of the intestinal mesenchyme (plotted on a $\sqrt[4]{[V(t)]}$ scale) and of the gastric mesenchyme ($\sqrt[3]{[V(t)]}$ scale).

Table 1 gives the standard deviation and the 95 % confidence limits for b and t_0, and Figure 4 shows the temporal characteristics of growth given by the exponential and the polynomial descriptions.

DISCUSSION

The findings on the growth of the stomach, expressed as increase of the volume, confirm the results obtained for the intestine: the epithelium grows faster than the mesenchyme and the retardation rate of the former exceeds that of the latter. In roughly the same period, the relative increase in volume is much greater for the epithelium of the intestine (700-fold) than for that of the stomach (157-fold). However the relative increases in the volume of the mesenchyme of the intestine (40-fold) is similar to that of the gastric mesenchyme (35-fold).

Table 1. (*e*) *estimate*, (*s*) *standard deviation and* (*c*) 95 % *confidence limits*

	b (in mm^3)	t_0 (in days)
$V_{ES}(t)$	(*e*) $0{\cdot}17\times10^{-3}$	(*e*) 8·8
	(*s*) $0{\cdot}35\times10^{-4}$	(*s*) 0·45
	(*c*) $0{\cdot}99\times10^{-4}/0{\cdot}24\times10^{-3}$	(*c*) 7·9/9·7
$V_{EI}(t)$	(*e*) $0{\cdot}15\times10^{-4}$	(*e*) 8·8
	(*s*) $0{\cdot}48\times10^{-5}$	(*s*) 0·48
	(*c*) $0{\cdot}54\times10^{-5}/0{\cdot}25\times10^{-4}$	(*c*) 7·8/9·8
$V_{MS}(t)$	(*e*) $0{\cdot}25\times10^{-2}$	(*e*) 8·8
	(*s*) $0{\cdot}25\times10^{-3}$	(*s*) 0·28
	(*c*) $0{\cdot}19\times10^{-2}/0{\cdot}302\times10^{-2}$	(*c*) 8·2/9·4
$V_{MI}(t)$	(*e*) $0{\cdot}66\times10^{-3}$	(*e*) 8·2
	(*s*) $0{\cdot}15\times10^{-3}$	(*s*) 0·53
	(*c*) $0{\cdot}35\times10^{-3}/0{\cdot}97\times10^{-3}$	(*c*) 7·1/9·3

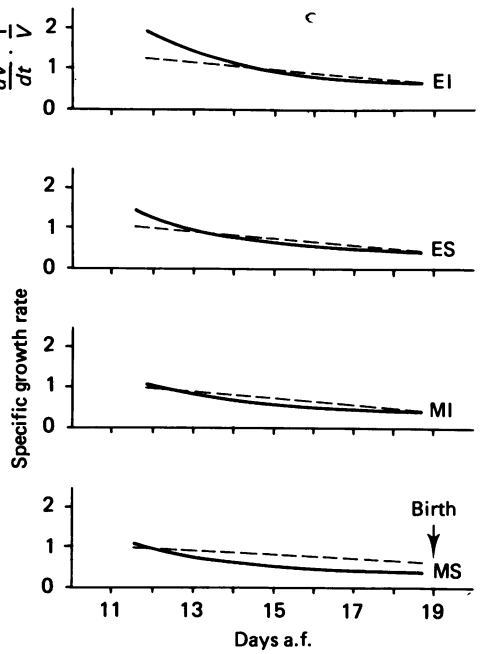

Fig. 4. The specific rates of the volume growth of the epithelium and mesenchyme of the stomach and intestine, as obtained with the exponential (dashed line, equation (1) in the text) and the polynomial (solid line, equation (2) in the text) descriptions.

About 7·5 days after fertilization the anlage of the foregut appears as a group of about 40 endodermal cells originating from the head-process mesoderm (Poelmann, 1980) and situated posterior to the developing heart rudiment (Theiler, 1972). The onset of the formation of the endoderm, which coincides with that of the epithelium of the intestinal tube, can be assumed to occur in this period. Between 7·5 and 8 days after fertilization mesenchyme originating from the lateral-plate mesoderm surrounds the definitive endoderm (Poelmann, personal communication), which can be considered the onset of the formation of the intestinal mesenchyme. Not until 8·5 days

(Pleeging, 1975) or 9 days (Theiler, 1972) is the first anlage of the midgut, from which a large part of the intestinal tube will develop, observed.

Our results are in good agreement with these biological considerations. The values of t_0 provided by the polynomial fit of the data, with their 95 % confidence limits, cover a period in which, according to our knowledge of the process, growth most probably begins.

The usefulness of the polynomial model lies in its simplicity and flexibility: with only three parameters it gives an accurate description of the process and it also gives on a transformed data scale, a linear fit of the data and permits extrapolation to the starting point of growth. From a purely statistical point of view, the regression curve provides only a description of the interrelation between the variables within the limited time range of the observations. However, extrapolation is justified because it is based on adequate knowledge of the growth process.

Since m depends on λ and t_0, (see equation 1), only processes characterized by the same specific growth rate and the same starting point can be described by the same value of m, and only in that case does the sum give a polynomial of the same form.

In our case the growth processes have different rates within the period under study. Since each process has a different m value, the polynomial that describes the growth in the volume of the whole organ does not have the same form as the polynomials that describe the growth of the epithelium and mesenchyme separately. The value of m is a direct expression of the relative increase in volume, i.e. of the specific growth rate. The layer showing the largest increase in volume, which is the epithelium of the intestine, demands the highest power of m, followed by the epithelium of the stomach and by the mesenchyme of the intestine and stomach.

In some cases an even better fit was obtained by using fractional values of m.

The fact that at $t = t_0$ the growth rate becomes infinite seems surprising. However, the absolute onset of growth, when the volume is equal to zero, is in our opinion an abstraction; at any instant $t_0 + \Delta t$ there is a finite volume and growth rate.

It is interesting that the specific growth rates given by the polynomial model do not slow down with a constant rate of decrease as in the exponential model. They seem to reach an exponential retardation which is typical for tissues in rapid growth, such as fetuses (Laird, 1966) and tumours (Laird, 1969; Gratton, Appleton & Alwiswasy, 1978), whose growth curves are usually fitted by S-shaped Gompertz functions. On this basis its seems justified to assume that the retardation of the growth rate given by our simple polynomial model represents the properties of the embryonic growth better than that given by the other model. Sbarbati (1979) argued that the curves of the volume growth of the intestine and stomach could represent the initial segment of S-shaped curves before the inflection point. The results discussed in the present paper support this conjecture and might explain the purely exponential growth of the whole intestine (Sbarbati, 1979). Studies on tumoral (Laird, 1969) and fetal growth (Laird, 1966) have shown that if the measured values lie early with respect to the inflection point, the decay of the specific growth rate is statistically not different from zero, i.e. the growth approximates a simple exponential growth.

Two functions were used to describe the volume data, and perhaps an accurate fit could be obtained by others with the same numbers of parameters or more (Gratton *et al.* 1978). We should like to point out that the choice of the functions to be used to describe experimental data should be based on a sound biological hypothesis, and it should be possible to test conclusions against data. There is sometimes

misunderstanding about the fact that the use of a particular function depends on the investigator's purpose and that the results must be seen in that light. Mathematics is a language which uses quantities and symbols instead of words but just as for words in the natural language, the choice of the quantities and symbols depends on what one wants to express. Therefore, expressions such as 'true description of the data' do not make any sense. A mathematical model of a natural process can be adequate or not, never true or false. We can only say that the 'least erroneous description' (Gratton *et al.* 1978) of the data is the one which, in agreement with biological considerations, is not rejected by the statistical test at the level of confidence chosen.

SUMMARY

The growth in volume of the epithelium and mesenchyme of the intestine and stomach was estimated in mouse embryos aged between 11·6 days after fertilization and birth, by means of a morphometric technique.

The findings on the growth in volume of the stomach confirm the results obtained for the intestine: the epithelium grows faster than the mesenchyme and the retardation rate of the former exceeds that of the latter. In roughly the same period, the relative increase in volume is much greater for the epithelium of the intestine than for that of the stomach. However, the relative increase in volume of the mesenchyme of the intestine is similar to that of the gastric mesenchyme.

A set of data can often be described by several regression equations, the choice of the function depending on the investigator's purpose and knowledge of the process under study. The growth data were first fitted by exponential and then by polynomial functions. Both fits permit an accurate linear representation of the results, but only the polynomial description permits the expression of properties like summation and the introduction of the beginning of the growth process into the growth equation as an additional parameter.

Furthermore, while the specific growth rates given by the exponential model slow down with a constant rate of decrease, those given by the polynomial model seem to reach an exponential retardation. This is in accordance with other growth concepts formulated in the past.

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