Rates and duration of hair growth in the albino rat

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INTRODUCTION

Hair growth is a cyclic phenomenon, with alternate phases of activity and rest. In rats and mice, new activity spreads as a wave in a characteristic course over the body regions (Collins, 1918; Dry, 1926, 1928) and the timing of the first appearance of successive waves on the venter, is highly predictable in young animals (Dry, 1926; Butcher, 1934; Dieke, 1947; Mohn, 1958; Johnson, 1958). In the rat, this seemingly stable system has served as a basis for studying the effects of many different hormone, pharmacological, carcinogenic and other treatments, providing information concerning both the action of these agencies and the nature of the hair growth cycle itself (see review by Ebling, 1964).

An essential part of several such inquiries has been the measurement of rates of growth in length of the hairs. Due to the limitations of the methods employed, and in some cases to a lack of familiarity with the coat of the rat and its hair growth cycle, the results obtained so far show little agreement, and the present work is a fresh attempt to define the normal pattern of hair growth rates and duration in the rat, using the more accurate techniques made available by the development of autoradiography.

The efficiency of autoradiography as a method of marking growing fibres at short, precisely determined intervals, by the injection of [35S]cystine, has already been demonstrated (Rougeot, 1959; Downes & Lyne, 1961). The isotope incorporated into the fibre at the time of each injection produces an image when exposed to a suitable photographic emulsion; a rate of length growth can be derived from the distance between successive images, while a count of the images registered on the fibre indicates the duration of its growth.

A great advantage of the autoradiographic method is the fact that each individual fibre is preserved intact, which allows the comparison of different fibre-types, where these occur. The pelage of the rat consists of four main fibre-types (shown in Fig. 1) on the general body region (Dry, 1928), and the starting point for the present inquiry was to determine whether any change in the rate of length growth is associated with the constrictions which occur along the length of the zigzag type. An initial experiment to solve this particular problem also provided information of a more general nature, on both rates and duration of hair growth in all four fibre-types, and a more extensive experiment was designed to confirm and amplify these first observations.

METHODS

For the preliminary study, a male and a female Sprague–Dawley albino rat, about 3 months old, were plucked on corresponding sites on the body, situated midway between head and tail and at dorso-lateral level. No natural growth wave was

present on these regions. Daily injections of [^{35}S]cystine were administered from 5 days after plucking until fibre growth on the experimental areas had ceased.

The second group of rats comprised four litter-mates of the same Sprague–Dawley strain, two males and two females, average body weight 0·3 kg. Areas approximately 1·5 cm square on the mid-dorsum, mid-venter and dorso-lateral regions, midway along the body, were plucked at 58 days old. The growth of fibres initiated, identified as P1, was plucked for autoradiography at 96 days. This second epilation

Fig. 1. The fibre-types of the rat coat (after Dry, 1928). Projected images of fibres from the mid-dorsum. ×7. Left to right: monotrich, awl, auchene and zigzag.

initiated a second generation of fibres, P2, subsequently plucked at 154 days, and the third generation, P3, was sampled at 218 days. In this way, three successive generations of fibres were available for comparison from precisely the same areas of skin.

The progress of the natural waves of hair growth (named by Dry (1926) G1, G2, G3, etc.) was revealed by dyeing the coat with Inecto Rapid black hair dye. The G3 wave was already established on the venter at 58 days; areas adjacent to the plucked sites were sampled as their fibres ceased to grow. In addition, fibres were collected from the flank towards the base of the tail, where the monotrichs appear to be characteristically longer than elsewhere on the body; this region will be referred to as 'left rump' in the results. The G4 wave was present on the venter by

117 d, but made slow progress and had not reached the mid-dorsal sites when the injections were discontinued. Injections were given daily from the 58th to the 215th day of age.

Autoradiography

The injections of DL-[^{35}S]cystine were given at 24 h intervals ($\pm$ 10 min), and administered subcutaneously in the shoulder or abdominal regions. The standard dose was 0·01 mc; double doses were given occasionally for dating purposes.

Fig. 2. Composite print from autoradiographs of fibres of each type, in same order as in Fig. 1, and from the mid-dorsum. × 5. Some of the faintest images at the tips of the fibres have not been reproduced.

The plucked fibres were sorted into fibre-types and laid out on glass plates, on which a dilute gelatine solution had been allowed to set slightly. When the gelatine was dry, an Ilfex X-ray film was pressed firmly over each plate for the exposure period. The differences in diameter between the fibre-types necessitated different exposure periods, but times as short as 3 days sufficed for the largest fibres. Films were developed in ID 19, and projected in a photographic enlarger at × 4 (× 10 for zigzags). Tracings were made with a sharp 2H pencil and the distance between the most distal parts of successive images was measured with a finely graduated milli-metre rule. Counts of the injections registered on each fibre were made under a

× 20 binocular microscope. Where possible, autoradiographs of 200 fibres of each type were prepared from each site at each sampling, but the natural frequency of the monotrichs (1–2 % of the total) did not permit such totals to be achieved; also only smaller numbers of fibres were available from the venter. All the autoradiographs were included in counts of growth duration, while groups of twenty-five fibres were used to prepare mean growth-rate curves for each fibre-type at each sampling. The growth between the first and second injection was plotted as day 1, and so on. To avoid confusion in the latter part of the mean curves, when some fibres cease to grow before others, each curve was discontinued when less than fifteen of the twenty-five fibres contributed to the mean.

RESULTS

The large volume of data amassed in the two experiments cannot be reported in full here. Instead, the specific points to be made will be illustrated by selected examples.
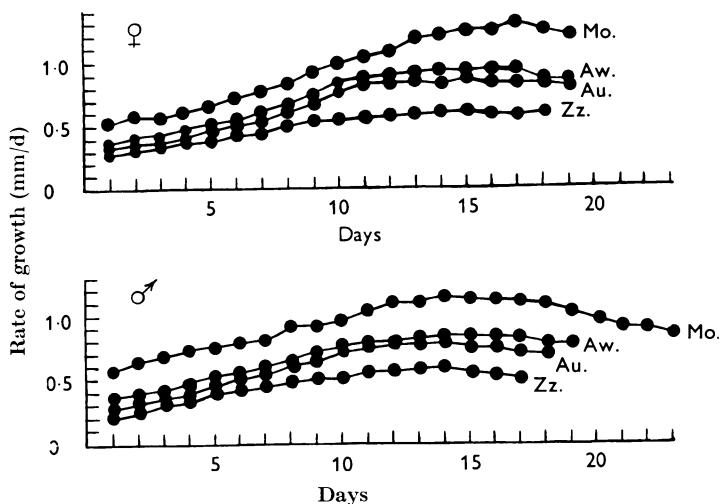

Fig. 3. Specimen curves of mean daily length growth of the fibre-types from the first group of two rats. Dorso-lateral region. Each curve represents twenty-five fibres. Symbols for fibre-types: Mo., monotrichs; Aw., awls; Au., auchenes; Zz., zigzags.

(1) Rates of growth in length

Specimen curves of fibre-length growth rates from the two animals in the first group are given in Fig. 3. Considerable differences occur between the first-types, which are arranged with the monotrichs achieving the highest rate of growth, followed by the awls, auchenes and zigzags, in that order. Equally apparent is a pattern of length growth common to all four fibre-types. The growth rates are not constant, and the pattern of the plotted values resembles a very shallow sigmoid growth curve; a phase of increasing rate is followed by a phase when a maximum rate is reached and maintained, after which the rate tends to fall towards the end

of fibre growth. The curves from the second group of rats follow the same basic shape, irrespective of body region, sex and age at sampling, and have the same arrangement of the fibre-types. Specimen curves are illustrated in Fig. 4.

The curves given by the constricted fibres, auchenes and zigzags are uninterrupted by any features which might correspond with the position of the constrictions. Since
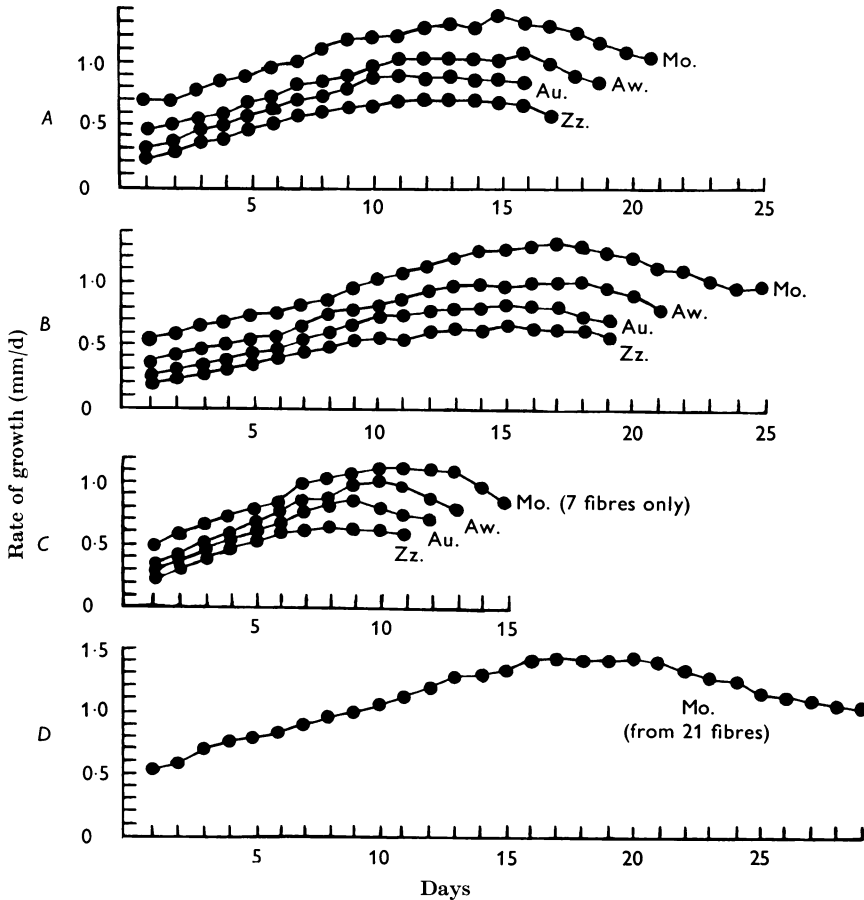

Fig. 4. Specimen curves of mean daily length growth of the fibre-types in four samples from a male rat in the second group. Each curve represents twenty-five fibres except where indicated. *A*, P 1 fibres from mid-dorsum; *B*, P 3 fibres from the mid-dorsum; *C*, P 1 fibres from mid-venter; *D*, G 3 fibres from left rump. Symbols for fibre-types as in Fig. 1.

such features might be obscured in the drawing up of mean curves from many fibres, single fibres were plotted, as shown in Fig. 5, where the position of each constriction is indicated by an arrow. There is only occasional coincidence of minor peaks with the constrictions, and curves from the unconstricted fibre-types (Fig. 5*A*) show a similar level of day-to-day variation. The results from the second group of rats were similar in this respect.

(2) *Duration of growth*

The mean values for duration of hair growth in the second experiment are summarized in Table 1. Again the fibre-types give different results, the monotrichs growing for a longer time than the awls, auchenes and zigzags. Comparison of the results from different samples indicates that the duration of growth is influenced by several factors, including body region, sex, and from the evidence of P1–3 on the mid-dorsum, the age at sampling.
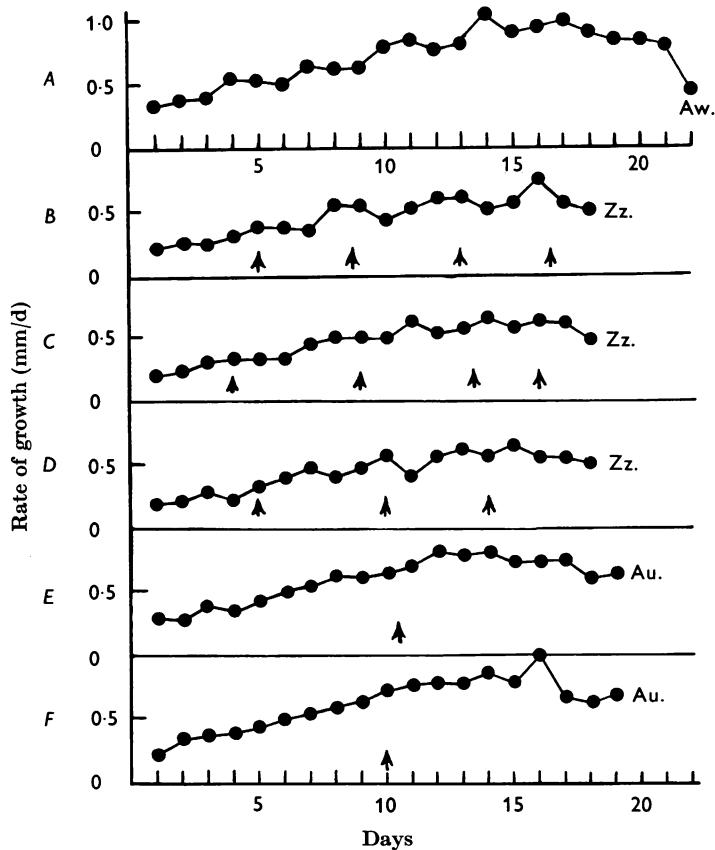

Fig. 5. Specimen growth-rate curves of individual fibres from a male rat in the first group. The position of a constriction is indicated by an arrow. *A*, Awl; B-D, zigzags; E, F, auchenes.

Table 2 compares the effect of rate and duration of growth on the final mean length of the hairs in three samples of monotrichs. There are differences in length between the samples, between the sexes in each sample, and between the two males, and parallel differences of sample, sex and individual are apparent in the values for duration of growth. On the other hand, the growth rates are approximately the same in all cases.

Similarly, we can compare the growth of fibres on the mid-dorsal and mid-ventral

regions. The hairs from the venter reach about two-thirds of the length of those on the dorsum, and Table 1 shows that the difference in duration of growth between the two regions is of the same order, while the growth-rate curves (Fig. 4*A*, *C*) suggest no difference in the mean rate of length increase. (The slightly lower maximum reached by the monotrichs on the venter is of doubtful significance, since only seven fibres contributed to this curve.)

Table 1. *Summary of values for duration of hair growth, as indicated by number of daily injections recorded per fibre*

(The figures given are pooled mean values from the second group of four rats, except for the venter, where means from only two rats are included.)

Origin of sample	Monotrichs	Awls	Auchenes	Zigzags
P1, mid-dorsum	23·8	20·3	19·8	18·5
P2, mid-dorsum	24·9	21·6	19·2	18·5
P3, mid-dorsum	26·1	22·9	20·7	19·8
G3, mid-dorsum	24·7	22·1	20·7	19·1
G3, left rump	28·0	—	—	—
P1, mid-venter	16·4	14·3	14·1	13·0
Average number of fibres contributing to mean in each rat	28	164	169	172

Table 2. *Relation of rate and duration of growth to fibre length*

Sample	Male A	Male B	Female A	Female B
	(A) Mean length (mm)			
P1, mid-dorsum	22·0	23·4	21·5	20·6
P3, mid-dorsum	24·0	25·6	23·5	23·2
G3, left rump	25·6	30·5	23·3	25·2
	(B) Mean daily growth rates (mm/day)			
P1, mid-dorsum	1·01	1·06	0·99	0·98
P3, mid-dorsum	0·95	1·05	1·02	1·00
G3, left rump	1·02	1·04	0·94	0·96
	(C) Mean duration of growth (injections registered per fibre)			
P1, mid-dorsum	24·1	24·6	23·5	23·1
P3, mid-dorsum	27·0	27·3	25·1	25·1
G3, left rump	27·8	29·1	27·3	27·8

All the fibres are monotrichs. The lengths in section A, were measured from tracings of auto-radiographs, and neglect growth before the first, and after the last injection; this underestimates the true length, hence the discrepancy with the other 2 sets of figures B and C. Mean growth rates were calculated from the growth-rate curves and represent the mean rate of growth between first and last injection marks.

DISCUSSION

(1) *Accuracy of method*

The technique of fibre autoradiography represents a considerable advance over the methods previously available. Rates of length growth of animal hairs have usually been measured by clipping the fibres at intervals and calculating a mean growth rate from the measured fragments. Such a method is best suited to growth over a prolonged period, where short-term fluctuations in growth rate are not

expected; it has been used most effectively with wool fibres and human hair. In most types of animal hairs, however, the growing period is short, an irregular pattern of length growth may be present, and it becomes important to have information on the growth rates at the tips of the fibres. These cases require very frequent measurements, for which the autoradiographic method is ideal. It has the further advantages that different fibre-types can be compared, as can different parts of the same fibre, and that all the fibres growing on the animal are marked simultaneously, permitting a more comprehensive series of measurements than was hitherto possible.

Downes & Lyne (1961) claimed a standard error of $\pm 2\%$ in their measurements of wool-fibre growth rates after injections of isotope at 4-day intervals. With daily injections in the rat such a degree of accuracy could not be achieved, and the greater range of values obtained between fibre-types within each sample (0·2–1·3 mm/d) makes a precise estimate of the error involved in the present results less meaningful. However, the average error arising from the measurement of tracings of projected autoradiographs was estimated to be of the order $\pm 5\%$. This error would be increased by incorrect location of the injection images (particularly at the tips and other fine parts of the fibres, where the images are less intense) but reduced by the drawing up of curves representing groups of fibres.

The main source of error in the values given for duration of growth is, again, failure to recognize the fainter images at the fibre tips. The total number of injections registered on each fibre approximates to the period of growth in days, since growth before the first, and after the last injection is not recorded. This margin of error is thought to lie within that involved in previous estimates of duration of growth derived from histological studies, where no separation of fibre-types was made, and much smaller samples of the fibre populations will have been examined (Butcher, 1934; Montagna, 1956; Johnson, 1958).

These reservations having been taken into account, the results reported here are believed to represent the most accurate assessment yet achieved of length growth rates and duration for the hair of the rat.

One final question arises: has the continued injection of radiocystine itself interfered with the normal pattern of hair growth? First, there was no evidence of radiation damage to the skin or hair follicles. The premature shedding of hair fibres after low doses of X-rays, reported by Montagna & Chase (1956), and others, constitutes a shortening of the period of hair growth, and the only relevant feature of the present experiments was a progressively *increased* duration of hair growth, attributed to the increasing age of the animals. Secondly, while sulphur-containing amino acids have been shown to increase wool growth when given per abomasum (Reis & Schinckel, 1963), the results obtained here from the rat give no reason to suppose that the much lower dosages used (total 0·02 g per rat over 150 days) have produced any comparable effect. In particular, no overall increase in the rate of length growth of the hair was apparent in the latter part of the experiments.

(2) *Rates of length growth*

The pattern of length growth revealed here is not that suggested by previous authors. The growth rate is not constant, as was claimed by Fraser (1951), who

measured mouse hairs. Again, the maximum rate is clearly achieved towards the end of the growing period, rather than initially, as indicated by Johnson (1958). Fraser's measurements dealt only with the middle length of the hairs, avoiding tips and basal ends, but the significant differences shown by different fibre-types in his data are confirmed in the present study. The disagreement between the results shown here and those of Johnson partly arises because the latter did not distinguish fibre-types, the fibres examined being presumably a mixture of monotrichs, awls and auchenes. The tip of the fibre appears to take at least 2–3 days to grow from the base of the follicle to the skin surface (Chase *et al.* 1951; Johnson, 1958), a distance of 1–2 mm, and the much lower initial growth rates indicated in Figs. 3–5 would seem to be more in agreement with this observation than the values of over 1 mm per day given by Johnson.

Results obtained by a similar technique of autoradiography with fibres of sheep birth-coats (Side & Rudall, 1964) suggest that the general pattern of length growth reported here may be typical of hair growth outside the order Rodentia, the second phase of the growth period, when the rate is maintained at a maximum, being very extended in fibres of indeterminate growth, like the wool fibre.

There were no detectable changes in the rate of length growth at the constrictions in auchene and zigzag fibres. This completes the picture given by histological techniques (Priestley & Rudall, 1965), which indicated that the dramatic decrease in fibre diameter at each constriction is offset by a complementary increase in the thickness of the inner root sheath, and no change was apparent in the output of the follicle, as judged in this way. Some change in the rate of length growth at each constriction could not, however, be ruled out until now. In the past, reductions in the diameter of other fibres have been thought to indicate a decrease in growth rate— e.g. in the birth-coat fibres of the sheep (Fraser, 1952)—and it is clear that such suggestions must be treated with caution.

One of the most important results of this work is the demonstration of very considerable differences between the fibre-types in rate, and to a lesser extent, duration of growth. The longer fibres, monotrichs and awls, grow at a faster rate *and* for a longer time than the shorter ones, auchenes and zigzags. The findings of differences in rates of growth agrees with the results of Fraser (1951) from the mouse; the longer duration of growth in monotrichs has previously been noted (Dry, 1926; Durward & Rudall, 1958), but the less obvious differences between the other types had so far escaped attention. It has already been mentioned that failure to identify fibre-types appears to have been a source of confusion in previous studies of growth rates, and it should be obvious from the data given here (if not from Dry's meticulous descriptions of the fibres themselves) that this elementary precaution is a first essential in any quantitative experiment dealing with similar aspects of rodent hair growth.

(3) *Duration of growth*

It is at once apparent from Table 1 that the duration of hair growth in the rat is a more variable parameter than has been appreciated by previous authors, who have usually quoted a single figure for the duration of activity in the follicle (which exceeds the duration of hair growth by about 5 days); for example, Montagna (1956)

26 days. Johnson (1958) gave an estimate of only 19 days for follicle activity on the dorsum of Wistar rats (14 days for growth of the fibres), and comparing this figure with those in Table 1 suggests that strain itself may be a factor influencing the duration of growth. It is also of note that some of the mean values quoted here are much higher than any in the literature, indeed, odd fibres from the flank near the base of the tail on one (male) rat registered as many as thirty-six injections.

The marked tendency towards a progressively longer period of growth shown in the three plucked generations P1–3 is thought to reflect the influence of increased age, and the conclusion drawn from the data in Table 2 is that this longer duration of growth, rather than any increase in growth *rate*, is responsible for the increase in fibre length. Although a tendency for fibre length to increase with age is apparent in some previous descriptions of rat hair growth, differences being observed between successive natural generations on the same site (Dry, 1928; Johnson, 1958), it has not been suggested that these differences indicated a longer period of growth.

Johnson's contention that regional differences in hair length result from differences in duration of growth, and her finding of longer fibres in male rats, are supported by the data here, but Table 2 shows clearly that in this case only the duration of growth differed between the sexes, and not the growth rate.

To summarize, the results from these experiments suggest that duration of hair growth in rats is influenced by strain, age, sex, body region and fibre-type, for which due allowance should be made in any experimental approach, and that differences in duration of growth are the major factor in causing differences in hair length, wherever these may occur. The rate of length growth, within each fibre-type, appears to be a much more stable parameter.

SUMMARY

1. The rates and duration of hair growth in the albino rat have been studied by autoradiography of the fibres after daily injection of [^{35}S]cystine.

2. Although there are considerable differences between the four main fibre-types, they show a common pattern of length growth rate which is uninterrupted at the constrictions in auchene and zigzag types.

3. Within each fibre-type differences in the duration of hair growth, rather than in growth *rate*, appear to account for differences in hair length, which may be associated with strain, sex, age, body region.

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