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The Influence of Recent Growth History on the Phenotype of *Escherichia coli*

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SUMMARY

E. coli, strain 300U, was grown for 450 generations in 6 different media. Each line was then subcultured into each of the 6 media, and allowed to grow for about 12 generations. Measurements were made of the growth rates and of the differential rates of induced β-galactosidase synthesis. Samples were also taken from each culture for investigation of the soluble protein profiles determined by polyacrylamide gel electrophoresis. The growth rates and the rates of synthesis of β-galactosidase were, as expected, highly dependent on the medium in which the cells were growing at the time of sampling. Both parameters also showed some influence of the medium in which the cells had previously been grown; this was more pronounced with β-galactosidase synthesis than with growth rate. Of the 26 soluble protein components separated by gel electrophoresis, 9 were highly dependent on the current medium, with insignificant effects of the previous growth conditions; 4 were significantly affected by the previous medium but were not influenced by the current medium; 3 were influenced both by current and earlier growth conditions; and 10 components showed no variation. In further experiments, the

kinetics of labelled phenylalanine incorporation into soluble proteins were studied by pulse-labelling during growth upshifts and downshifts. The responses of the various protein components to the growth shift were kinetically variable. Some responded immediately to the growth shift, while in others the response was delayed. In other cases, a series of oscillations were observed after an initial response. A final category showed no response to the growth shift. The experiments continued for one or two generations after the shift, and the proportional incorporation of phenylalanine into the different bands was still changing at that time. It is concluded that a long period of adaptation is required for cells to reach a new equilibrium after a growth shift. This period appears to last for at least 12 generations.

INTRODUCTION

The bacterial phenotype is known to be strongly influenced by the growth medium. This has been demonstrated both for the broad spectrum of soluble proteins (Moses & Wild, 1969), and for individual proteins as in the secondary regulation of inducible enzyme synthesis by catabolite repression (Magasanik, 1961). Some phenotypic changes occur rapidly when the growth medium is altered. In Escherichia coli, for example, the rate of synthesis of inducible enzymes subject to catabolite repression falls markedly within a few minutes when the medium is enriched (Moses & Prevost, 1966; Tyler & Magasanik, 1967). Some spontaneous escape from this intense transient repression subsequently occurs at periods up to an hour or more after enrichment.

An attempt has been made to correlate this transient repression response with fluctuations of the intracellular concentrations of catabolites (Prevost & Moses, 1967).

Other responses occur more slowly. Yudkin (1969) has reported that growth of several strains of E. coli in rich medium results in an increased susceptibility to glucose repression of β-galactosidase synthesis when the cells were returned to minimal medium. He found that the increased susceptibility persisted for at least several cell generations. Ungar & Moses (197) found in growth shifts with E. coli that the increase in the differential rate of ribosome synthesis lagged behind the increase in the growth rate. After passing through a maximum more than an hour after the growth rate increase, the differential synthesis of ribosomal protein declined to a lower rate.

We have studied the phenomenon of prolonged adaptation in cells which have been grown for several hundred doublings in one medium before being transferred to another. Three sets of parameters have been investigated: the mass doubling times, the differential rates of β-galactosidase synthesis, and the distribution of components among the soluble cell proteins. While these parameters were found to depend largely on the current growth conditions, we obtained evidence for some influence of the growth history.

Further studies have been performed on the pattern of labelled amino acid incorporation into soluble proteins during growth shifts. Labelling of some protein components responded immediately to changes in the growth medium, while other responses were delayed; the cells continued to adjust to the new medium more than one generation after the growth shift.

METHODS

Bacterial strains. Two strains of *E. coli* were employed: 300U (lac $i^+z^+y^-$) from Dr. J. Monod, and C600-1 (lac $i^+z^+y^-$) from Dr. A. B. Pardee.

Media. Minimal medium 63 (Pardee & Prestidge, 1961), containing thiamine, was supplemented as follows: (a) 33 mM-Na acetate; (b) 17 mM-Na succinate; (c) 22 mM-glycerol; (d) 11 mM-glucose; (e) 17 mM-Na succinate + 0.1% (w/v) casein hydrolysate; (f) 11 mM-glucose + 0.1% (w/v) casein hydrolysate. Before this work started, the stock cultures of both strains had been maintained for several years on glycerol-minimal agar.

Growth conditions. Cultures were grown aerobically at 37° with agitation. For successive subcultures, 0.05 ml. of the previous culture was added to 50 ml. of fresh medium. This provided for about 10 mass doublings in each subculture.

When required for experimental study, 0.05 ml. portions of the appropriate fully-grown culture were transferred to 50 ml. of the desired media and incubated overnight. The cultures were then diluted into the same media, and were used during exponential growth about 2 mass doublings later. Growth was followed by measuring E_{650} in a 1 cm. cuvette, using a Beckman DK-2 double-beam spectrophotometer. It has been shown that E_{650} is directly proportional to the concentration of bacterial protein in the suspension (Moses & Prevost, 1966).

Induction and measurement of β-galactosidase. β-Galactosidase synthesis was induced by adding 0.5 mM-isopropyl thiogalactoside to growing cells. Samples of the suspension were assayed for enzyme activity as described previously (Palmer & Moses, 1968).

Soluble protein profiles. These were determined using polyacrylamide gels as described by Moses & Sharp (1970). Measurement of protein in solution (Lowry, Rosebrough , Farr & Randall, 1951) after lysis and removal of cell debris showed that about 80% of the cellular protein was soluble.

Labelling of soluble protein. Samples (10 ml.) of the culture were mixed with 1.1 x 10^{-5} M-L-[G-^{14}C] phenylalanine (specific radioactivity 55 mCi./mmole). After incubation at 37° for 5 min., 1 ml. of chloramphenicol solution (2.5 mg./ml.) was added. The soluble proteins were separated on polyacrylamide gel as before. The gels were dried by being placed on a pad of filter paper in a frame with a porous base. The gel was covered by a sheet of impervious plastic film (Saran Wrap, Dow Chemical Co., Midland, Michigan, U.S.A.) clamped tightly over the gel. Suction was applied to the lower portion of the porous plate, and the gels were allowed to dry at room temp. for several days (Fairbanks, Levinthal & Reeder, 1965). The dried gel remained firmly stuck to the top sheet of filter paper on the pad. Drying sometimes resulted in cracking and contraction of the gel slab; such damaged gels were discarded.

Radioautograms of the dried gels were obtained by pressing them against Kodak Single-Coated Blue-Sensitive Medical X-ray film for periods up to 2 months. After development, the intensities of the film blackening were measured with a Gel-Scan scanner connected to a Servogor recorder (Gelman Instrument Co., Ann Arbor, Michigan, U.S.A.).

Labelling of total protein. This was measured by the accumulation of trichloroacetic acid-precipitable radioactivity following the addition of L-[G-^{14}C]phenylalanine to the culture. Preparation of the samples

for radioactivity measurements used the techniques described by Moses & Prevost (1966).

Chemicals and radiochemicals. L-[G-^{14}C]Phenylalanine was obtained from New England Nuclear Corp., Boston, Msss., U.S.A. Isopropyl-thiogalactoside was from Calbiochem., Los Angeles, Calif., U.S.A.

RESULTS

Growth rates

Measurements were made of the rates of growth (as mass doublings/hr.) of cells maintained for about 450 generations in each of the 6 media, each line then being tested in each of the 6 media. The results are presented in Table 1, which includes an analysis of variance. As expected, the influence of the current medium in which the cells

[Insert Table 1 near here]

were growing (indicated as "present experimental medium") was highly significant in determining growth rates. However, the previous medium, in which the cells had been grown for 450 generations (indicated as "historical medium"), also appeared to exert some influence. There was a slight tendency for the highest current growth rates in acetate, succinate and glycerol media to be exhibited by cultures previously grown in media containing the same carbon source.

Differential rates of β-galactosidase synthesis

The rate of β-galactosidase synthesis is governed both by the primary induction-repression control mechanism, and by the secondary regulation afforded by catabolite repression (Magasanik, 1961). The magnitude of the latter effect is related, in turn, to the physiological state of the cells. Comparative measurements of the rates

of synthesis thus provide an indication of the similarity of metabolic state between different cultures.

Table 2 presents the differential rates of enzyme synthesis in the same cultures as those reported in Table 1; analysis of variance of these measurements in included in Table 2. More variation was

[Insert Table 2 near here]

observed here than among the growth rates. Current media were again highly significant in determining these rates. The effect of historical medium was significant at the 0.05-0.01 level. Cells previously grown in acetate, or in succinate plus casein hydrolysate, showed relatively low differential rates of β-galactosidase synthesis in five out of six current media; cells earlier grown in glucose showed high rates in four out of six current media.

Soluble protein profiles

The protein profiles of cells in the same experiment as that reported in Tables 1 and 2 were studied. Samples of each culture were removed into chloramphenicol during exponential growth when the cultures contained 90 μg. bacterial protein/ml. The heights of 26 peaks in absorbance scans for each profile were measured; the height of each peak was expressed as a percentage of the sum of all the peak heights from that profile. Comparison of the areas under each peak as percentages of the sum of the areas under all the peaks was less satisfactory than a comparison of peak heights. This was due largely to doubt as to the boundary between one area and the next. With measurement of peak heights, uncertainties rarely occurred and were always satisfactorily resolved. In some stained cases peaks

on the tracing were not visible as distinct entities although bands on the stained gel were visible by eye; it was nevertheless simple to determine the positions of such apparently missing components by reference to their neighbours. The extinction at that position was then taken as applying to the apparently missing component. In some profiles, in which the boundaries between adjacent areas were clearly discernible, assessment of the contribution of each peak to the total using the peak heights was compared with its contribution using areas. The differences between the two methods were trivial. Fig. 1 shows a typical protein profile with each peak identified by an index number.

[Insert Fig. 1 near here]

The magnitudes of each designated peak, from each of the 36 experimental cultures, were compared by analysis of variance. Four types of behaviour were observed and typical analyses are shown in Tables 3-6: (a) Significant influence of current medium, with weak or insignificant effects of historical medium: Peaks 2, 3, 4, 6, 7,

[Insert Tables 3-6 near here]

9, 14, 15, 23 (Table 3); (b) Significant influence of historical medium, with weak or insignificant effects of current medium: Peaks 17, 18, 21, 26 (Table 4); (c) Significant influences of both historical and current media: Peaks 1, 12, 16 (Table 5); (d) Significant influences of neither historical nor current medium (i.e., magnitudes constant): Peaks 5, 8, 10, 11, 13, 19, 20, 22, 24, 25 (Table 6). A summary of the conclusions for all 26 peaks is given in Table 7.

[Insert Table 7 near here]

This shows the mean percentage contribution of each peak, as well as the coefficients of variation, and the probabilities for the current and historical media.

Change of protein profiles during growth shifts

Moses & Wild (1969) showed that some aspects of the protein profiles are dependent upon the current growth medium. The data in Table 7, while confirming this conclusion, indicate a significant residual effect of a previous growth medium (lasting for at least 12 mass doublings) on the relative proportions of some components. It was of interest to examine kinetically the changes in the protein profiles during growth shifts.

Labelling with L-[G-^{14}C]phenylalanine for 5 min. pulses, prior to blocking further protein synthesis with chloramphenicol, was chosen as a means of measuring the rates of synthesis of proteins contributing to the bands of the profile. All experiments were designed so that, even at the highest cell densities employed, no more than 50% of the labelled amino acid would be incorporated into the protein during the 5 min. pulse period.

Labelled protein profiles during growth on succinate

It was necessary to insure that any changes in the incorporation pattern during a growth shift were really dependent on the shift, and were not simply a function of increasing density of the culture. Cells growing in succinate-minimal medium were therefore pulsed with labelled phenylalanine at intervals during growth, the culture density increasing from 21 μg. bacterial protein/ml. at the beginning of the sequence to 288 μg. protein/ml. at the end. Exponential

growth (at a rate of 1.0 mass doubling/hr.) was achieved when the cell density reached 34 μg. protein/ml.

A typical scan tracing of a radioautogram from this series is shown in Fig. 2. Variations in the contributions to the total made

[Insert Fig. 2 near here]

by the various protein bands were slight. Fig. 3 illustrates the percentages of three peaks as a function of growth. Peak 5 showed

[Insert Fig. 3 near here]

essentially no change throughout exponential growth, and similar patterns were obtained with peaks 2, 3, 7, 8, 9, 11, 12, 14, 15, 16, and 17. Peak 13 showed a slow proportional rise with increasing age of the culture, and this was also observed with peak 10. Peak 1 exhibited a maximum midway through growth; peak 4 showed a slight maximum early in growth, while some increase in prominence in peak 6 was seen near the end.

Labelled protein profiles during shift from glycerol to glycerol plus glucose

Addition of glucose to cells growing in glycerol-minimal medium abruptly increased their rate of growth from 1.07 to 1.50 doublings/hr. (Fig. 4). A number of samples were pulsed with labelled phenylalanine

[Insert Fig. 4 near here]

before and after the growth shift. Fig. 5 shows a scan tracing of a radioautogram from this experiment. The addition of glucose produced

[Insert Fig. 5 near here]

a wide variety of responses in the labelling patterns. These are presented in Fig. 6: (i) little or no change in relative proportions

[Insert Fig. 6 near here]

(peaks 10, 17, 24); (ii) marked change in relative amount within 5 min. of adding glucose, with no return to the original level (peaks 3, 4, 11. 18); (iii) marked change within 5 min. with a subsequent return towards the original level (peaks 8, 19, 20, 23); (iv) marked change more than 5 min. after adding glucose (peaks 12, 15, 16, 21); (v) rapid change, possibly followed by oscillations (peaks 5, 6, 9); (vi) both initial and delayed changes (peaks 7, 13, 14, 22). The cells thus responded to the addition of glucose by a series of alterations in the rates of phenylalanine incorporation into the various protein components. The responses were spread throughout the 80 min. period of study following glucose additions; during that time the cells had undergone about 2 mass doublings.

For the most part, no complete separation was obtained between adjacent peaks in the radioautogram scans. The possibility must therefore be considered that where 2 adjacent peaks showed similar responses to the growth shift, a change in the measured height of one peak influenced that of the other. For this reason, there is some doubt of the independent behaviour of peaks 10, 11, and 12, and of peaks 13 and 14, and possibly 15. However, in many cases adjacent peaks clearly did not influence one another. For example, peaks 3 and 4 behaved independently, although peak 4 appeared as a shoulder on peak 3. Peaks 4 and 5, 5 and 6, 6 and 7, 8 and 9, 12 and 13, 15 and 16, 16 and 17, 17 and 18, 20 and 21, 21 and 22, and 23 and 24 are further instances of adjacent peaks acting independently. In some cases, such as peaks 7 and 8, two adjacent peaks behaved similarly although the separation between them was more complete than usual.

Labelled protein profiles during shift from glucose to glycerol

Cells of strain C600-1, previously grown in glucose-minimal medium, were inoculated into glycerol-minimal medium containing a limited amount of glucose. Growth proceeded at a rate of 1.43 doublings/hr. until a cell density of 47 μg. bacterial protein/ml. was reached. An abrupt cessation of growth then occurred. Growth resumed 22 min. later at a rate of 1.13 doublings/hr. (Fig. 7). We conclude that in the first phase of growth only glucose was used as carbon source, second phase utilizing glycerol. Samples for

[Insert Fig. 7 near here]

pulse-labelling with L-[G-^{14}C]phenylalanine were taken at intervals during the growth downshift. A radioautogram scan tracing from one of the samples is shown in Fig. 8.

[Insert Fig. 8 near here]

As in the case of the growth upshift reported in Figs. 4-6, a number of responses to the downshift were seen; these are presented in Fig. 9. They can be divided into seven categories: (i) little

[Insert Fig. 9 near here]

or no change in relative proportions (peaks 10, 11, 12, 14, 18, 22, 23, 24, 25); (ii) marked fall in proportion during the interruption of growth, with a return towards the original level during the period of growth on glycerol (peaks 15, 16, 17); (iii) marked rise during the interruption of growth, with a later return towards the original level (peaks 4, 5); (iv) change in relative proportion only during the glycerol period (peaks 13, 19, 21); (v) change when growth was absent, with a return to the original state when growth was resumed (peak 9); (vi) change occurring late in the

period of interrupted growth (peak 6); (vii) oscillations in relative proportion beginning when growth was absent and continuing into the period of growth on glycerol (peaks 1, 2, 3, 7, 8, 20).

The time required for the cells to adjust completely to glycerol as a carbon source was thus protracted. By the end of the experiment (60 min. and 1 mass doubling after growth started on glycerol) the relative rates at which ^{14}C was being incorporated into the various protein fractions were still changing. In both the growth upshift and downshift experiments there was evidence of changes occurring consecutively: while the rate of ^{14}C incorporation into some fractions was immediately influenced by change of the carbon source, in other cases the responses were delayed for varying periods of time.

Several upshift and downshift experiments were performed. Although they differed in detail, the general conclusions drawn above were applicable to all of them.

DISCUSSION

Bacterial cells possess a wide range of adaptive capabilities. They respond specifically to the presence or absence of a variety of substrates and metabolites in the medium by the induction or repression of specific enzyme synthesis. They are subject to control of enzyme activity by the intracellular levels of effectors. Presumably intracellular effectors also control the rates of synthesis of many proteins. The bacterial cell may thus be regarded as a complex interlinked network of reactions and control mechanisms.

In a number of specific instances it has been shown that alteration of the rates of inducible or repressible enzyme synthesis occurs rapidly when effectors are added to the medium or removed from it (Pardee & Prestidge, 1961; Branscomb & Stuart, 1968; Kepes, 1963). The magnitude of longer-term changes in the bacterial phenotype is more difficult to assess. The work reported in this paper has been directed towards determining the length of the adaptation period when bacterial cells are subjected to a marked change in their external environment. We have measured parameters which provide information at three levels of precision.

The rate of growth under various conditions is a very rough indication of the physiological state of the cells. With minor exceptions, as noted in the Results section, prolonged culture of cells in one growth medium did not affect their growth rates in the same or other media.

Analysis of the soluble protein profiles, which account for about 80% of the total cell protein, provided more detailed information. Protein profiles of systems as complex as the whole bacterial cell suffer, however, from two drawbacks. E. coli is believed to contain, at any one time, several hundred different types of protein, yet no more than 30-50 discrete bands were visible on a polyacrylamide gel separation of the soluble components. Each band was thus composed of an unknown number of different components which were grouped solely on the basis of their rates of migration in the gel system used. In no case have we identified a specific component of any of the bands. The bands themselves were only partially

discrete and the possibility exists of the height of one peak in a tracing being influenced by the heights of others close to it.

In spite of these drawbacks many distinctions can be drawn between cells in different growth media, and residual effects of recent growth histories have also been detected. Thus, cultures of the same bacterial strain, deriving from different cultural conditions, and growing in the same medium for at least 12 mass doublings, remained appreciably different when their soluble protein profiles were compared. Cohn & Horibata (1959) showed with the lactose system of E. coli that preinduced and non-preinduced cells of the same strain, inoculated into identical media containing appropriate concentrations of glucose and inducer, exhibited different biosynthetic rates for β-galactosidase. The differences between the two cultures could be maintained for many generations. Our results, using the whole of the soluble protein profile, suggest that maintenance phenomena are widespread, and that complete adjustment of a cell to new cultural conditions takes many generations to achieve.

The shorter-term experiments using pulse-labelling with L-[G-^{14}C] phenylalanine also showed that adaptation to a different growth substrate was not complete in 1 or 2 generations. With a number of protein bands there was a suggestion of a series of oscillations in the rates of labelling which started at the time of the growth shift and continued at least for 1 or 2 generations. Our data are not sufficiently extensive to observe damping of the oscillations, though we presume that eventually this would occur.

Maaløe & Kjeldgaard (1966) have described a number of delayed responses to growth shifts. Studies by Moses & Prevost (1966) and

by Paigen (1966) have shown a transient repression and subsequent escape in the synthesis of β-galactosidase and other inducible enzymes during growth shifts. Ungar & Moses (197) found that the rate of ribosome synthesis did not respond immediately to enrichment of the growth medium, and that in their system a period of more than 2 hr. was required for the establishment of the new rate of ribosome synthesis characteristic of the enriched medium. They also showed that overshoot of ribosome synthesis occurred, but did not carry on their experiments long enough to see whether oscillations developed. It is thus clear that a growth shift results in a protracted period of readjustment, the full implications of which may not become manifest for many generations.

Measurements of the rates of β-galactosidase synthesis yield more precise information about one cellular component. They suggest considerable metabolic variations, both in cells currently in different media and in those with different histories. The differential rate of β-galactosidase synthesis is known to be affected by the nutritional state of the cell (Magasanik, 1961). While the molecular origins of this variation have not yet been elucidated, it appears likely at the present time that they are in some way related to the intracellular levels of specific controlling metabolites. Another example of the effect of recent growth history on the rate of β-galactosidase synthesis has recently been reported by Yudkin (1969).

The prolonged period required for full adaptation to a new growth medium, taken together with the delayed response exhibited by some parameters, and the suggestion of oscillatory behaviour of

others, suggests that bacterial cells respond to a complex series of metabolic signals in balancing their overall composition. Some of these are initiated early in the growth shift. Kinetic tracer experiments in E. coli have shown rapid changes in the levels of some intermediary metabolites when the medium is enriched (Prevost & Moses, 1967). The rapid response of the growth rate to enrichment also indicates that the balance of intermediary metabolism undergoes considerable alteration within a short period. Our present results suggest, however, that not all changes occur quickly and there is no rapid attainment of a new steady state. We take this to mean that a considerable period is required for all the interrelated reactions of metabolism, dependent as they are on metabolite levels, on intracellular enzyme concentrations, and on enzyme activities, to achieve the new equilibrium characteristic of growth after the shift.

One aspect of our study which remains unanswered is whether, in our serial subcultures in different media, we produced cells of differing phenotype which nevertheless retained an identical genotype, or whether different genotypes were selected. Dean & Hinshelwood (1966) have considered the question of adaptation versus selection as the predominant factor accounting for the long lag period sometimes observed when bacteria are presented with a new growth substrate. They concluded in the cases of Aerobacter aerogenes presented with D-arabanose, and E. coli supplied with D-arabanose, lactose or dulcitol, that selection is not a significant factor.

In the experiments reported in this paper it might be argued that had selection occurred, resulting in various sub-strains

possessing different growth properties, the growth rates on a particular substrate would be highest in the sub-strain grown through many subcultures on that substrate. In 3 cases there was a slight effect in this direction; in the 3 other cases there was a slight effect in the opposite direction (Table 1). While these individual growth rate differences have not been rigorously investigated, such selection as might have occurred appeared to have no significant effect on the results.

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Table 1. Growth rates and analysis of variance

Strain 300U was grown for 450 generations in each of the 6 media listed below. Each batch of cells was then grown in each of the 6 media, and the growth rates after 10-12 generations are recorded as doublings/hr. A statistical analysis of the growth data is presented.

Present experimental medium.....	acetate	succinate	glycerol	glucose	succinate + casein hydrolysate	glucose + casein hydrolysate
Historical medium						
acetate	0.62	0.71	1.08	1.20	1.34	1.63
succinate	0.49	0.90	1.15	1.30	1.40	1.88
glycerol	0.59	0.81	1.22	1.20	1.28	1.88
glucose	0.55	0.74	1.06	1.15	1.32	1.70
succinate + casein hydrolysate	0.33	0.82	0.95	1.23	1.27	1.61
glucose + casein hydrolysate	0.54	0.86	1.03	1.15	1.48	1.56

Overall mean and coefficient of variation = 1.11 ± 35.5

		Sum of Squares (SS)	Degrees of Freedom (N)	Mean Square (SS/N)	Variance Ratio	Probability
Main effects:	historical medium	0.0905	5	0.0181	2.560	0.05
	present medium	5.2003	5	1.0401	147.1	<0.001
First order interaction (error)		0.1768	25	0.0071		

Table 2. Differential rates of β-galactosidase synthesis and analysis of variance.

Details as for Table 1. The cells were induced with 0.5 mM-isopropyl-thiogalactoside during exponential growth. Differential rates of β-galactosidase synthesis are recorded as enzyme units/mg. of bacterial protein. A statistical analysis of the data is presented.

Present experimental medium.....	acetate	succinate	glycerol	glucose	succinate + casein hydrolysate	glucose + casein hydrolysate
Historical medium						
acetate	10900	7640	5970	2130	8000	2130
succinate	12300	8090	9960	3070	9020	2360
glycerol	10400	8710	6890	2730	8800	1490
glucose	12200	13000	10000	3400	11900	1760
succinate + casein hydrolysate	13600	5220	3690	1010	8670	1210
glucose + casein hydrolysate	13500	9240	6890	2730	9690	2000

Overall mean and coefficient of variation = 6950 ± 58.3

		Sum of Squares (SS)	Degrees of Freedom (N)	Mean Square (SS/N)	Variance Ratio	Probability
Main effects:	historical medium	37836956	5	7567391	4.077	0.05 - 0.01
	present medium	490312023	5	98062404	52.83	<0.001
First order interaction (error)		46402744	25	1856109		

Table 3. Magnitude of peak 4 and analysis of variance

Details as for Table 1. The heights of peak 4 are expressed as percentages of the sums of the peak heights for each protein profile. A statistical analysis of the data is presented.

Present experimental medium.....	acetate	succinate	glycerol	glucose	succinate + casein hydrolysate	glucose + casein hydrolysate
Historical medium						
acetate	6.4	3.8	3.8	4.0	5.6	3.6
succinate	6.6	4.9	4.3	3.8	5.6	3.0
glycerol	6.2	5.8	4.1	3.4	5.6	3.8
glucose	7.9	5.8	4.1	3.5	5.4	3.2
succinate + casein hydrolysate	6.0	5.2	4.2	3.0	5.3	3.8
glucose + casein hydrolysate	6.6	4.6	4.3	3.4	4.1	3.4

Overall mean and coefficient of variation = 4.67 ± 26.2

		Sum of Squares (SS)	Degrees of Freedom (N)	Mean Square (SS/N)	Variance Ratio	Probability
Main effects:	historical medium	1.3181	5	0.2636	0.957	>0.2
	present medium	43.9914	5	8.7983	31.94	<0.001
First order interaction (error)		6.8869	25	0.2755		

Table 4. Magnitude of peak 18 and analysis of variance

Details as for Table 1. The heights of peak 18 are expressed as percentages of the sums of the peak heights for each protein profile. A statistical analysis of the data is presented.

Present experimental medium.....	acetate	succinate	glycerol	glucose	succinate + casein hydrolysate	glucose + casein hydrolysate
Historical medium						
acetate	8.4	6.3	7.3	7.5	7.9	7.8
succinate	7.8	7.3	7.0	6.7	8.0	7.8
glycerol	6.4	6.8	7.0	6.7	5.5	6.4
glucose	8.1	7.3	7.6	6.8	7.2	7.0
succinate + casein hydrolysate	6.9	7.6	6.6	7.4	7.6	8.8
glucose + casein hydrolysate	5.5	5.8	5.7	6.3	5.5	6.2

Overall mean and coefficient of variation = 7.01 ± 12.3

		Sum of Squares (SS)	Degrees of Freedom (N)	Mean Square (SS/N)	Variance Ratio	Probability
Main effects:	historical medium	16.1706	5	3.2341	11.2	<0.001
	present medium	2.5806	5	0.5161	1.78	0.2 - 0.05
First order interaction (error)		7.2344	25	0.2894		

Table 5. Magnitude of peak 16 and analysis of variance

Details as for Table 1. The heights of peak 16 are expressed as percentages of the sums of the peak heights for each protein profile. A statistical analysis of the data is presented.

Present experimental medium.....	acetate	succinate	glycerol	glucose	succinate + casein hydrolysate	glucose + casein hydrolysate
Historical medium						
acetate	7.6	7.0	7.5	9.2	9.9	11.2
succinate	7.1	8.0	9.8	7.1	10.9	9.7
glycerol	6.0	6.9	7.9	7.6	7.4	9.0
glucose	7.9	8.3	10.5	9.6	9.8	10.1
succinate + casein hydrolysate	6.5	8.2	8.9	9.2	9.3	11.9
glucose + casein hydrolysate	5.3	5.6	6.5	6.4	6.7	8.3

Overall mean and coefficient of variation = 8.30 ± 20.1

		Sum of Squares (SS)	Degrees of Freedom (N)	Mean Square (SS/N)	Variance Ratio	Probability
Main effects:	historical medium	35.0231	5	7.0046	8.37	<0.001
	present medium	41.0214	5	8.2043	9.80	<0.001
First order interaction (error)		20.9252	25	0.8370		

Table 6. Magnitude of peak 19 and analysis of variance

Details as for Table 1. The heights of peak 19 are expressed as percentages of the sums of the peak heights for each protein profile. A statistical analysis of the data is presented.

Present experimental medium.....	acetate	succinate	glycerol	glucose	succinate + casein hydrolysate	glucose + casein hydrolysate
Historical medium						
acetate	4.0	4.4	4.4	4.2	4.5	4.6
succinate	4.6	4.7	4.6	3.6	4.7	5.4
glycerol	4.6	4.6	4.4	4.9	4.2	5.0
glucose	4.9	5.0	5.0	4.5	4.6	4.5
succinate + casein hydrolysate	4.5	4.1	4.4	4.9	4.9	4.6
glucose + casein hydrolysate	3.9	4.4	4.2	4.5	4.9	4.8

Overall mean and coefficient of variation = 4.56 ± 7.8

		Sum of Squares (SS)	Degrees of Freedom (N)	Mean Square (SS/N)	Variance Ratio	Probability
Main effects:	historical medium	0.5822	5	0.1164	0.929	>0.2
	present medium	0.6722	5	0.1344	1.07	>0.2
First order interaction (error)		3.1345	25	0.1254		

Table 7. Summary of statistical evaluation of 26 peaks

Peak No.	Overall values			Probabilities	
	Mean	S.D.	Coefficient of variation*	Present experimental medium	Historical medium
1.	3.66	0.761	20.8	<0.001	<0.001
2.	4.58	0.850	18.5	<0.001	0.001 - 0.01
3.	3.60	0.627	17.4	<0.001	0.001 - 0.01
4.	4.67	1.221	26.2	<0.001	>0.2
5.	3.48	0.724	20.8	>0.2	>0.2
6.	3.28	0.618	18.8	<0.001	0.01 - 0.05
7.	3.54	0.608	17.2	<0.001	0.001 - 0.01
8.	2.71	0.476	17.6	>0.2	0.01 - 0.05
9.	5.00	1.442	28.8	<0.001	0.2
10.	3.22	0.478	14.8	0.05 - 0.2	0.05
11.	3.63	0.368	10.1	0.01 - 0.05	>0.2
12.	4.41	0.967	21.9	<0.001	<0.001
13.	3.70	0.545	14.8	0.001 - 0.01	0.001 - 0.01
14.	3.91	0.875	22.4	<0.001	0.01 - 0.05
15.	4.30	0.565	13.1	<0.001	0.01 - 0.05
16.	8.30	1.664	20.1	<0.001	<0.001
17.	6.97	1.386	19.9	0.01 - 0.05	<0.001
18.	7.01	0.862	12.3	0.05 - 0.2	<0.001
19.	4.56	0.354	7.8	>0.2	>0.2
20.	3.27	0.529	16.2	>0.2	>0.2
21.	2.73	0.674	24.7	0.001 - 0.01	<0.001
22.	2.83	0.653	23.0	0.05 - 0.2	0.01 - 0.05
23.	2.62	0.748	28.6	<0.001	0.001 - 0.01
24.	1.44	0.653	45.4	0.01 - 0.05	0.01 - 0.05
25.	1.34	0.614	45.8	0.05 - 0.2	0.001 - 0.01
26.	1.37	0.523	38.2	0.01	<0.001

*Coefficient of variation is S.D. expressed as % of mean.

CAPTIONS FOR FIGURES

Fig. 1. Densitometric tracing of typical gel electrophoretic separation of soluble proteins from experiments with strain 300U reported in Tables 1-7. The index numbers of the peaks correspond to those of Tables 3-7. Experimental details as in Table 1.

Fig. 2. Densitometric tracing of radioautogram taken from electrophoretogram of labelled soluble proteins from cells of strain C600-1 growing in succinate. Index numbers correspond to those of Fig. 3.

Fig. 3. Kinetic behaviour of soluble protein components from cells of strain C600-1 growing in succinate. Index numbers correspond to those in Fig. 2. At each sampling time, the extent of labelling in each peak is expressed as a % of the sum of the label in all the peaks from that sample.

Fig. 4. Kinetics of growth of strain C600-1 during glycerol to glycerol + glucose shift. Arrow indicates time glucose was added.

Fig. 5. Densitometric tracing of radioautogram taken from electrophoretogram of labelled soluble protein from cells of strain C600-1 during growth upshift reported in Fig. 4. Index numbers correspond to those in Fig. 6.

Fig. 6. Kinetic behaviour of soluble protein components from cells of strain C600-1 during growth upshift reported in Figs. 4 and 5. Index numbers correspond to those in Fig. 5. Arrows indicate time of adding glucose. At each sampling time, the extent of labelling in each peak is expressed as a % of the sum of the label from all the peaks from that sample.

Fig. 7. Kinetics of growth of strain C600-1 with limiting glucose and excess glycerol.

CAPTIONS FOR FIGURES (CONTINUED)

Fig. 8. Densitometric tracing of radioautogram taken from electrophoretogram of labelled soluble proteins from cells of strain C600-1 during growth downshift reported in Fig. 7. Index numbers correspond to those in Fig. 9.

Fig. 9. Kinetic behaviour of soluble protein components from cells of strain C600-1 during growth downshift reported in Figs. 7 and 8. Index numbers correspond to those in Fig. 8. Period bracketed by arrows indicates the time of no growth, as shown in Fig. 7. At each sampling time, the extent of labelling in each peak is expressed as a % of the sum of the label from all the peaks in that sample.

Running title: Effect of growth history on *E. coli* phenotype

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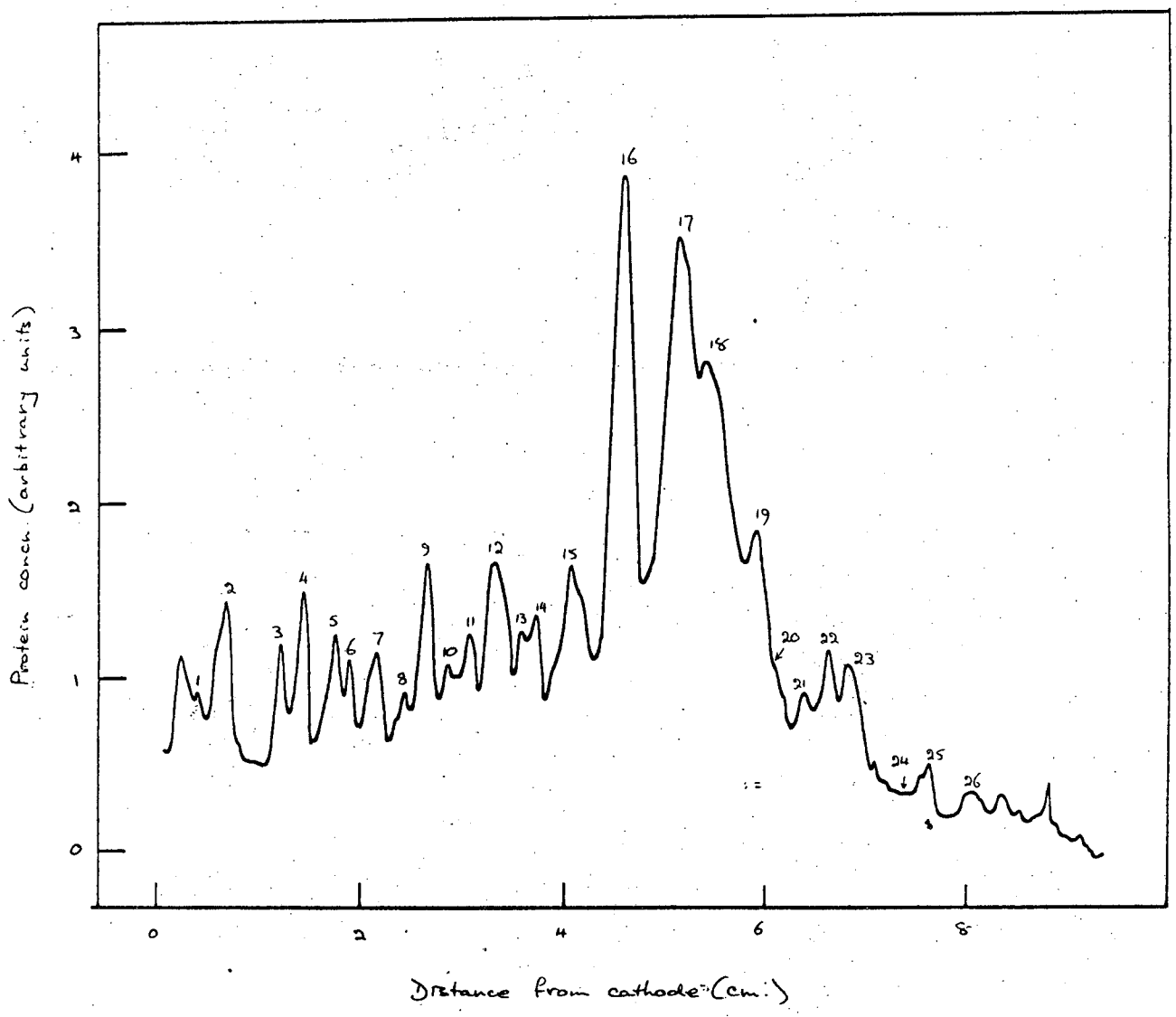
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Distance from cathode (cm.)
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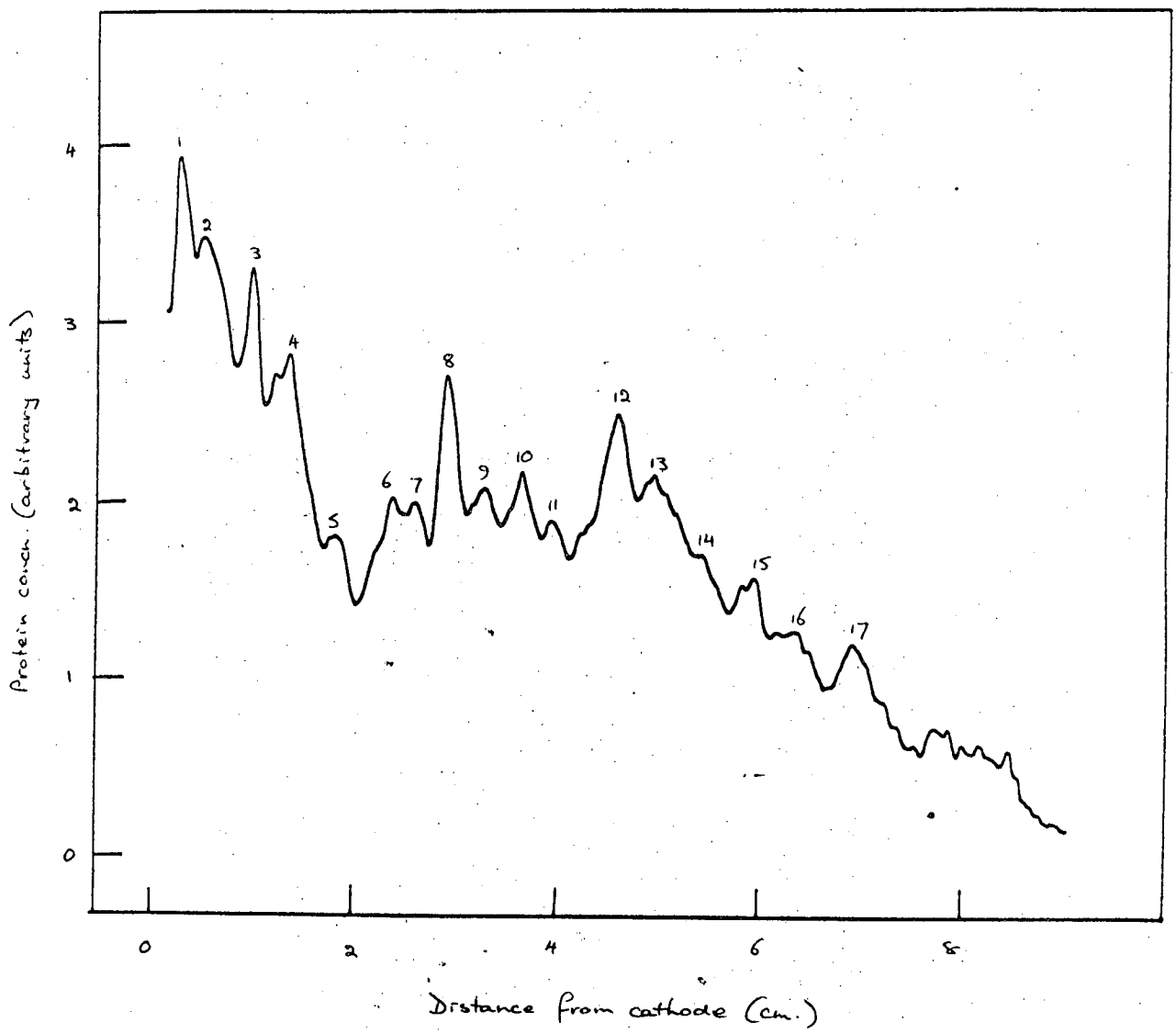
1
2
3
4
0
2
4
6
8
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26

Fig. 1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0
1
2
3
4
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
0
2
4
6
8
Distance from cathode (cm.)

Fig. 2

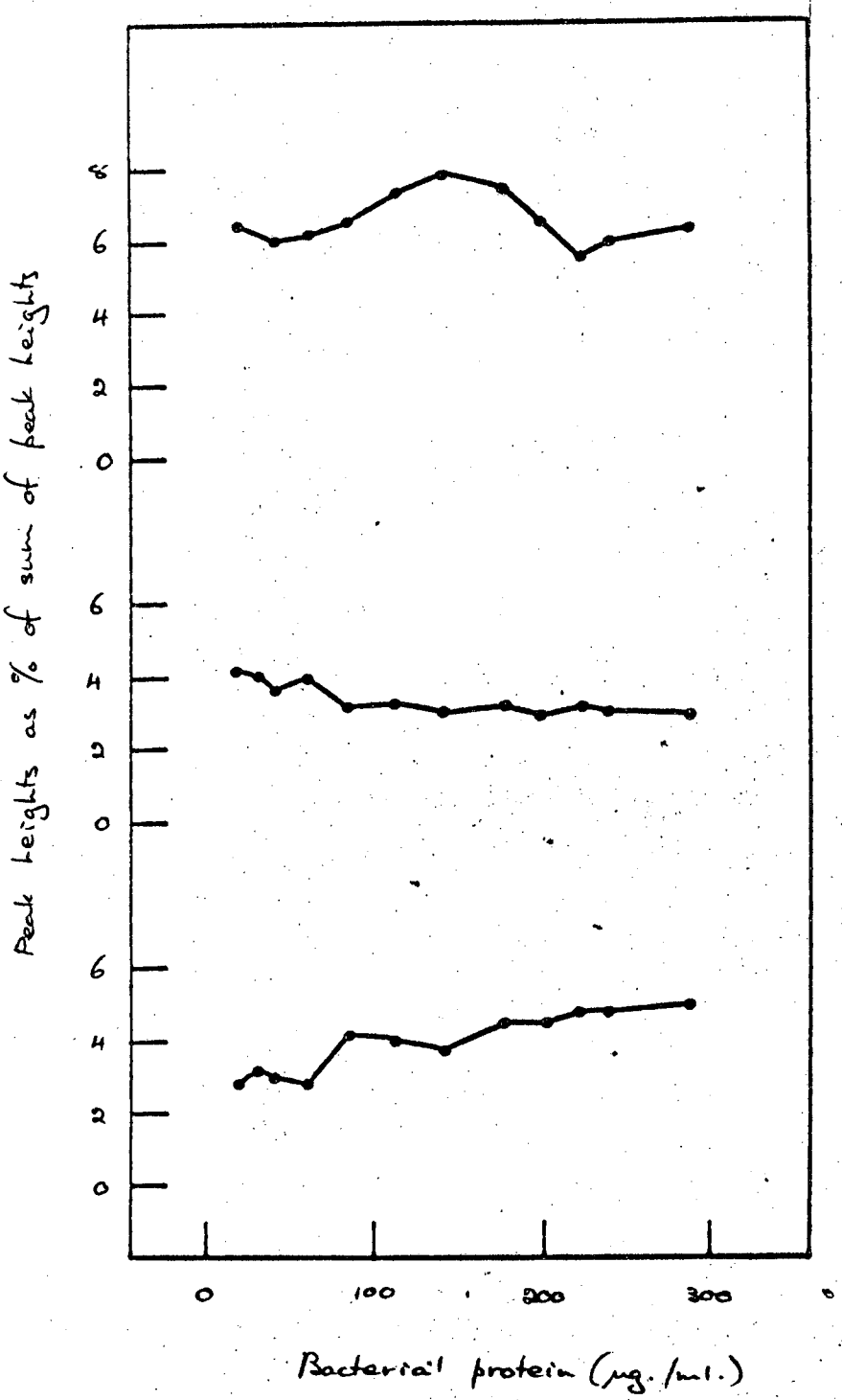

Peak heights as % of sum of peak heights
8
6
4
2
0
6
4
2
0
6
4
2
0
0
100
200
300
Bacterial protein (µg./ml.)

Fig. 3

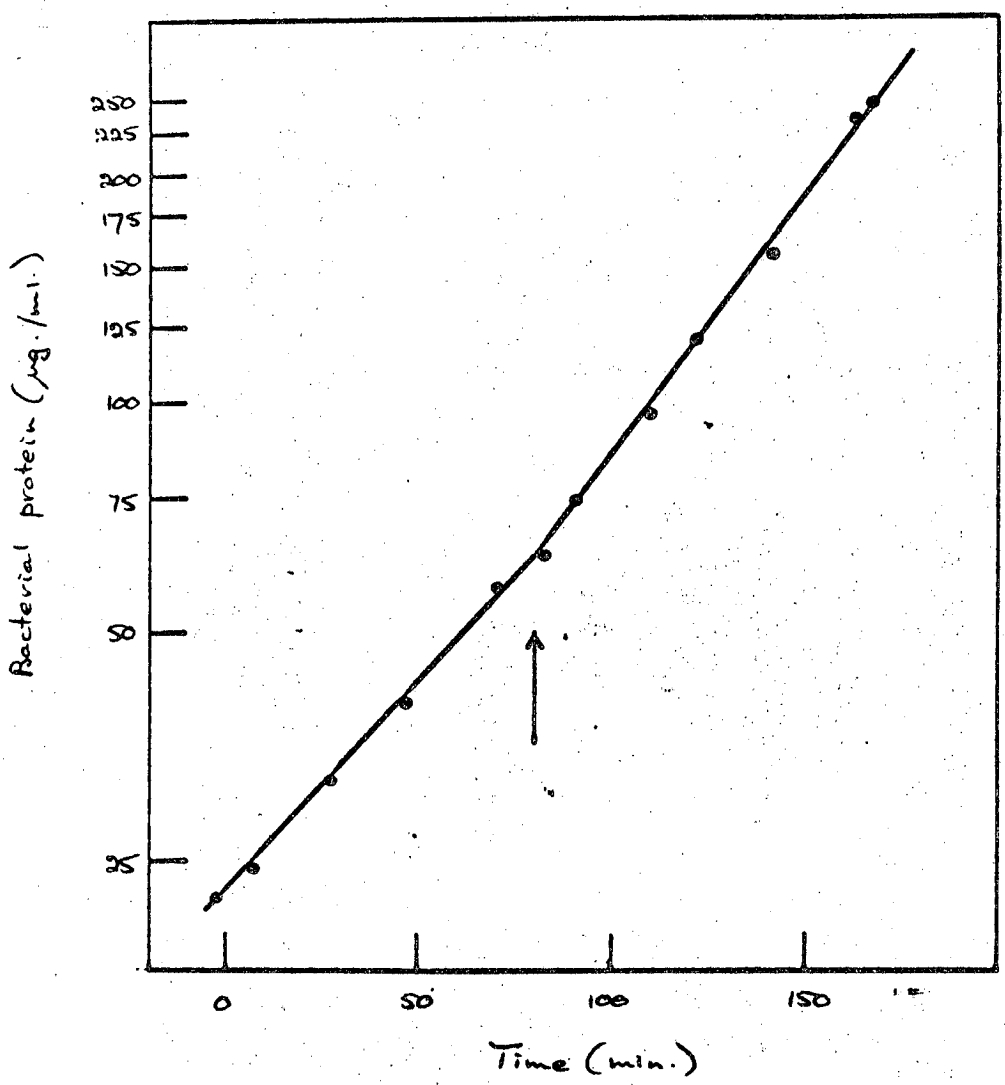
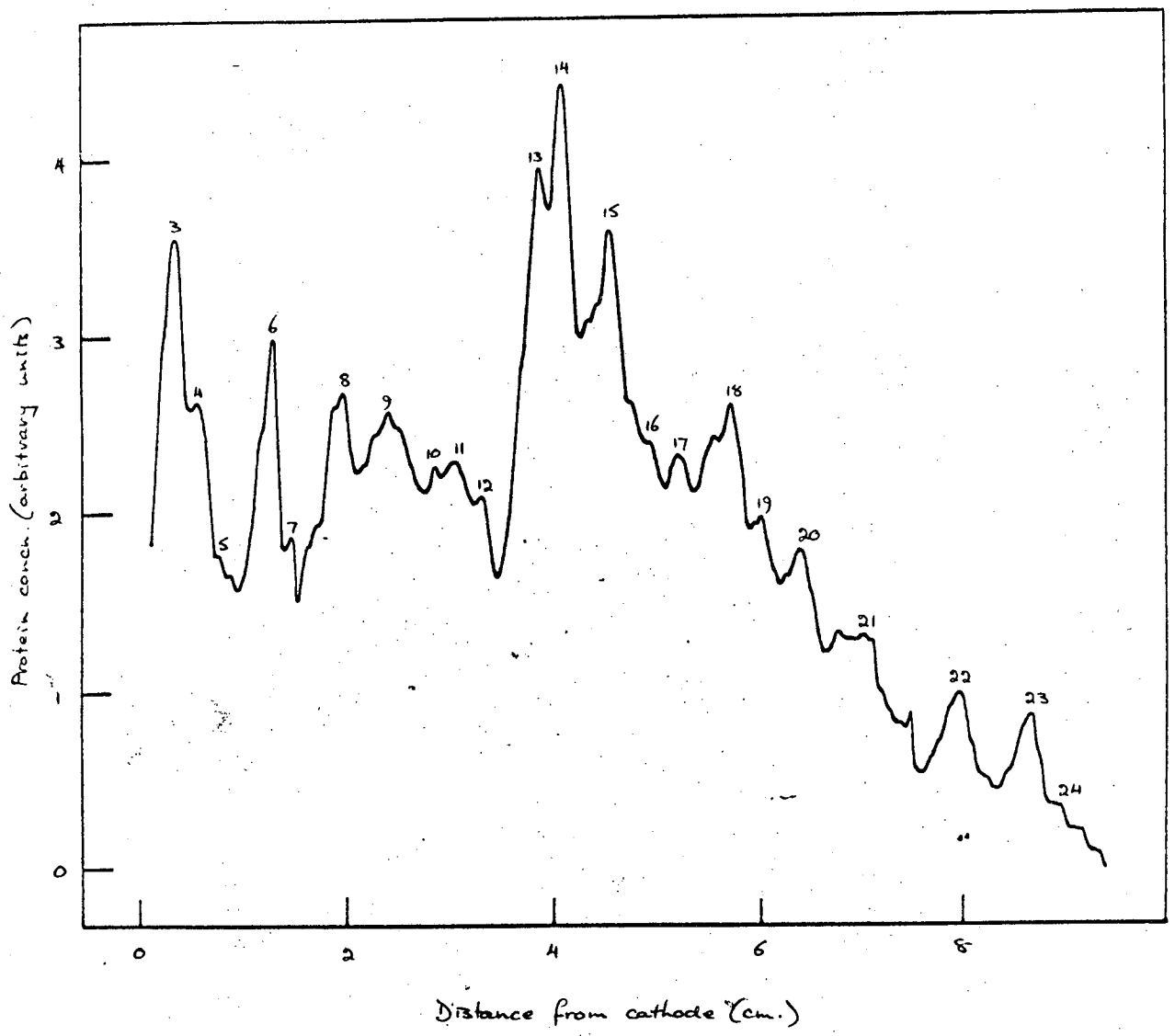

250
225
200
175
150
125
100
75
50
25
Bacterial protein (µg./ml.)
0
50
100
150
Time (min.)

Fig. 4


Protein conc. (arbitrary units)
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1
2
3
4
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
0
2
4
6
8
Distance from cathode (cm.)

Fig. 5

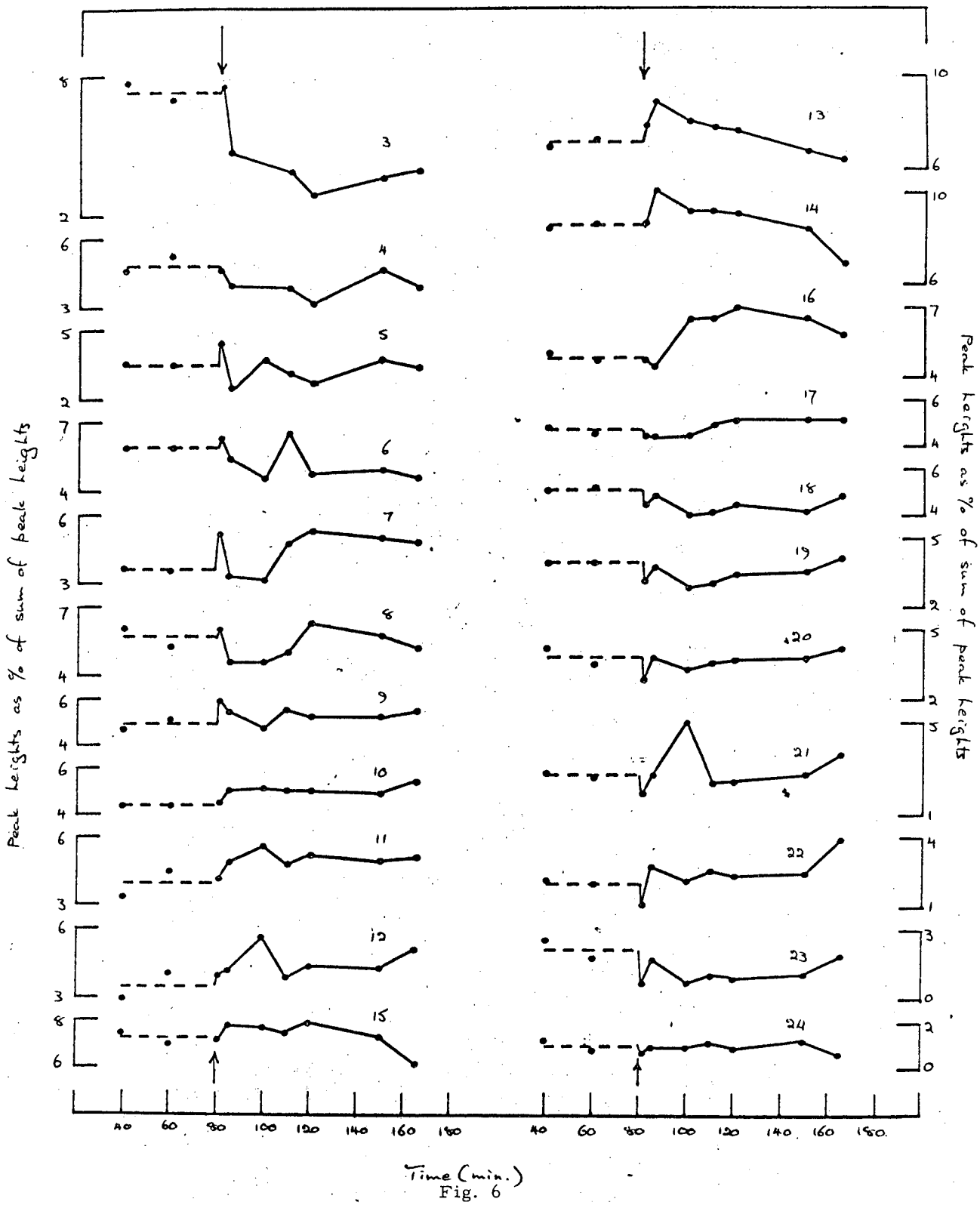
Peak heights as % of sum of peak heights
Peak heights as % of sum of peak heights
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
40
60
80
100
120
140
160
180
Time (min.)

Fig. 6

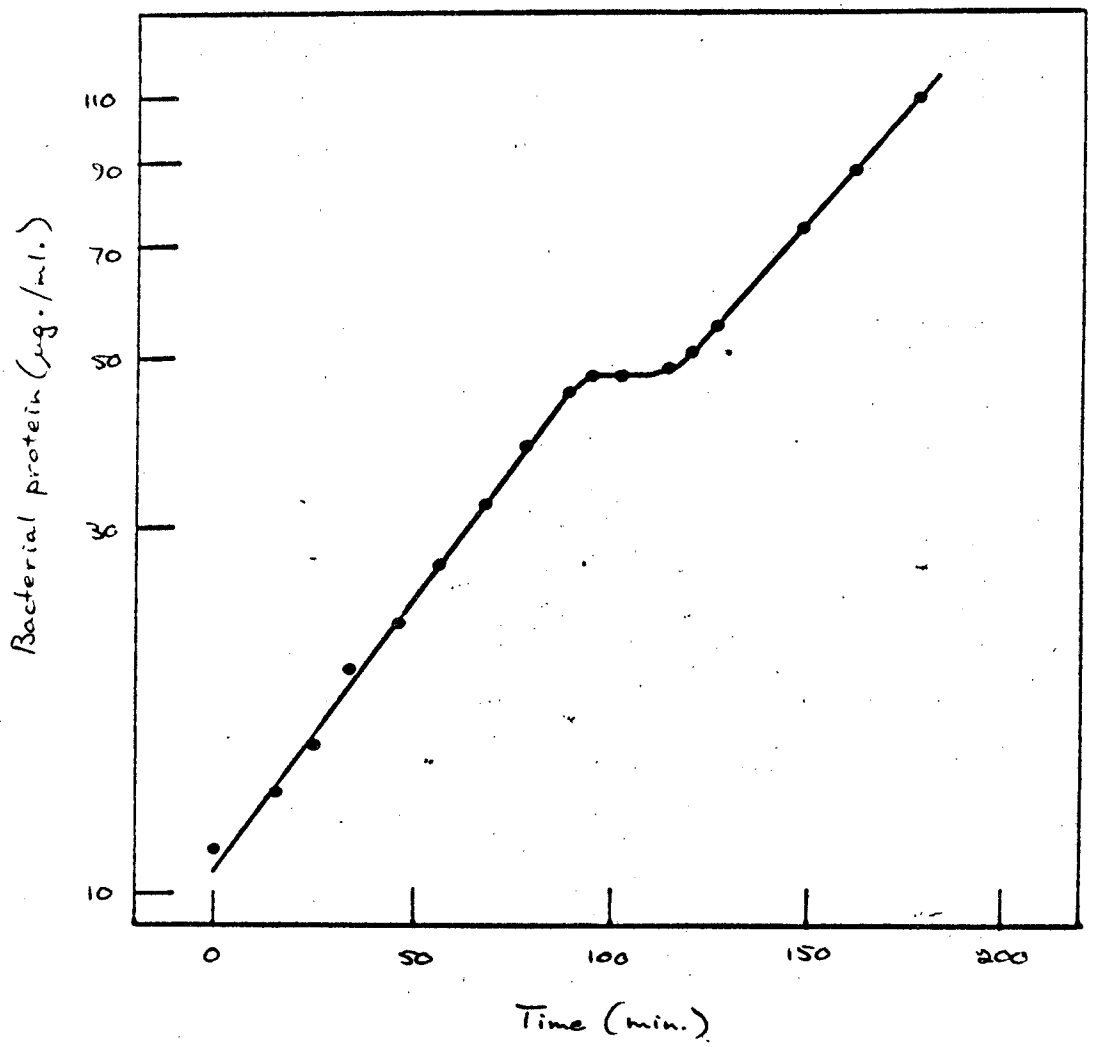
110
90
70
50
30
10
Bacterial protein (μg./ml.)
0
50
100
150
200
Time (min.)

Fig. 7

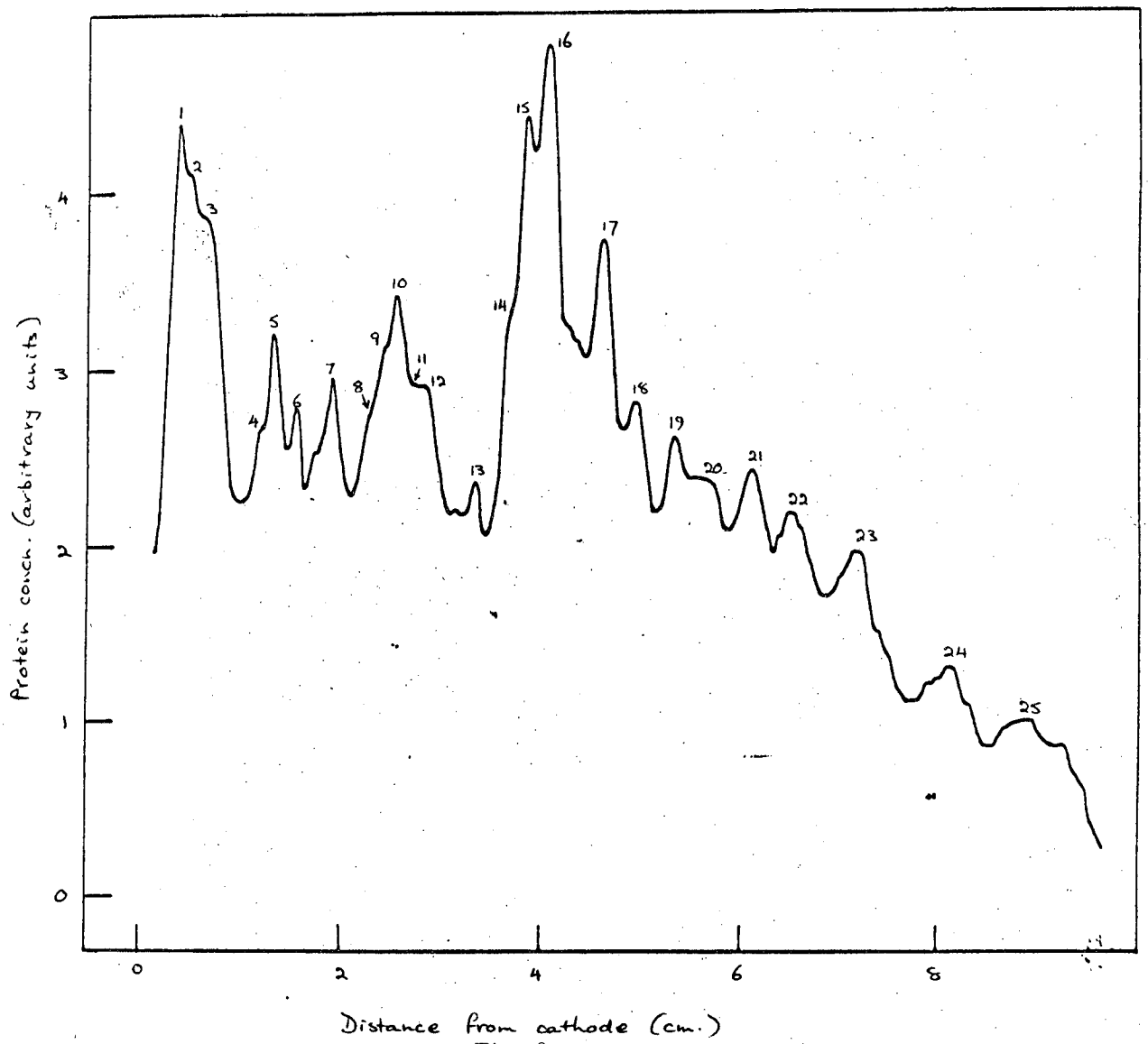
Protein concn. (arbitrary units)
0
1
2
3
4
0
2
4
6
8
Distance from cathode (cm.)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25

Fig. 8

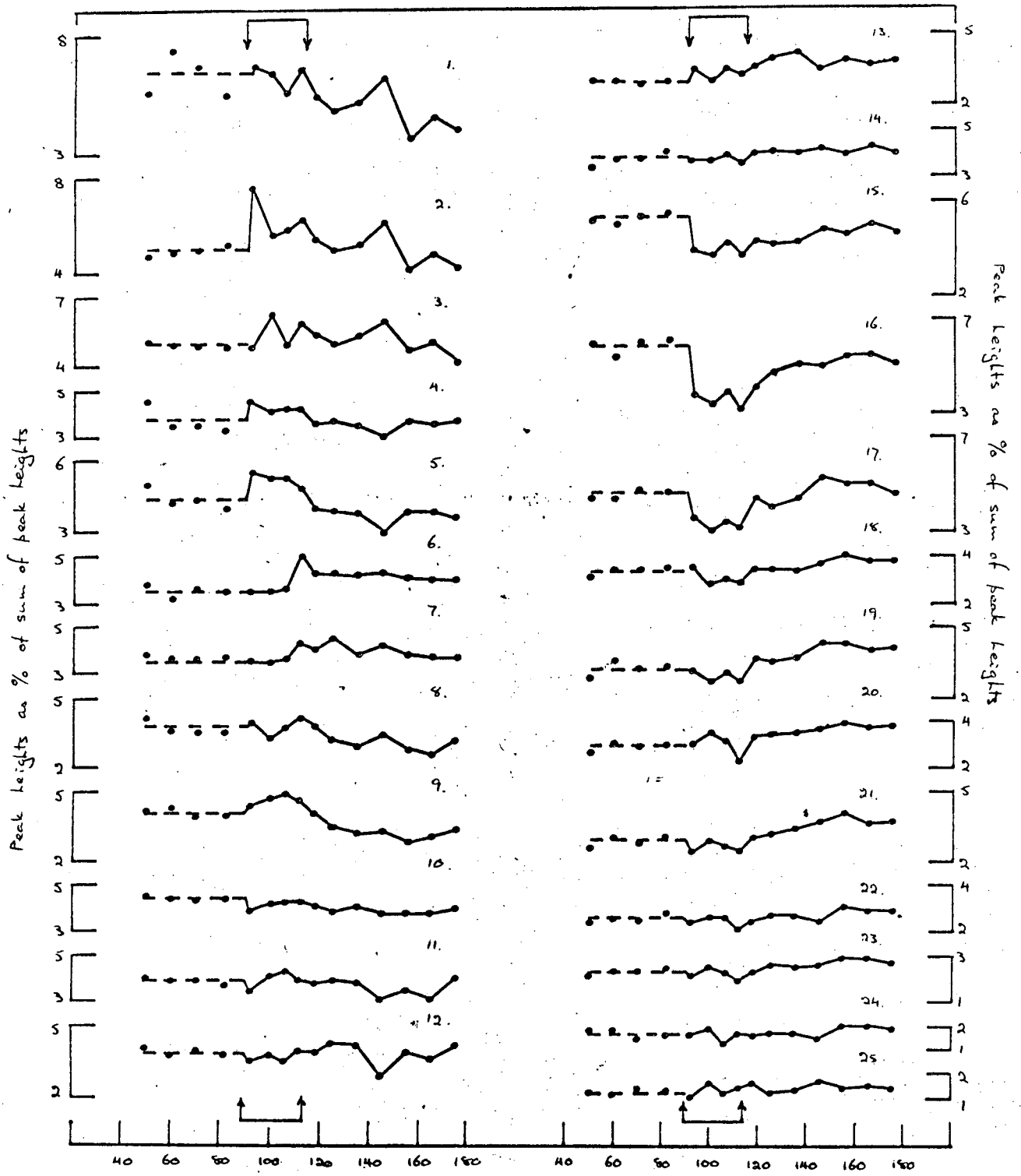
Peak heights as % of sum of peak heights
Peak heights as % of sum of peak heights
Time (min.)
40 60 80 100 120 140 160 180
1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12.
13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25.

Fig. 9

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