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Changes in Glycogen and Nitrogen-containing Compounds in *Escherichia coli B* during Growth in Deficient Media

II. Phosphorus and Sulphur Starvation

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The glycogen per dry weight and litre of culture did not increase during the starvation phase in phosphorus-starved cultures to the same extent as in nitrogen-starved ones. An addition of phosphate to the phosphorus-starved cultures caused an immediate increase in the rate of glycogen synthesis, followed by a decrease when the rate of synthesis of nitrogen-containing compounds increased.

Some basic mechanism involving the syntheses of both nitrogen-containing compounds and glycogen seemed to be affected during the starvation phase in sulphur-deficient cultures.

In a preceding article [1] experiments have been described which deal with the chemical composition of *Escherichia coli B* starved of the nitrogen or carbon sources. In this paper the observations have been extended to cells starved of the phosphorus or sulphur sources. As in the experiments previously described, the changes in the chemical composition of the cells after addition of the limiting factor was also investigated.

METHODS

The media used in these experiments were modified from Friedlein's salt-sodium lactate medium [2]. In the experiments with the phosphorus source as the limiting factor, the initial concentration of phosphate (KH_2PO_4) was 7.4×10^{-2} M. Instead of the phosphate buffer, 2×10^{-2} M tris(hydroxymethyl)-aminomethane was used. Growth curves of cultures in non-limited media were not affected by this concentration of "tris" buffer. The medium was made 1.3×10^{-2} M with regard to the potassium concentration by adding KCl. After sterilisation, the pH of the medium was 7.0. In the experiments in which the sulphur source was the limiting factor, the initial concentration of the sodium sulphate was 7.0×10^{-5} M. Instead of magnesium sulphate, magnesium chloride was added.

After a certain period of starvation, an amount of the limiting factor was added to the cultures in order to give them the same concentration as in the complete medium. In the event of phosphorus starvation, the phosphate buffer of the Friedlein medium was added. To the sulphur-starved culture, sodium sulphate was added.

The cultivation techniques and chemical methods were the same as those previously described [1].

RESULTS

Phosphorus starvation. (Expts. E and F, Table 1). The duration of the lag phase was about the same as in the complete medium. A starvation phase, characterized by an almost constant cell concentration, followed after an initial multiplication period. The dry weight of cells during the starvation phase was in expt. E 165 mg, and in expt. F 185 mg per litre of culture. After the addition of the limiting factor, a difference occurred in the behaviour of the two cultures. In expt. E there followed a phase of rapid cell division during the first 30 min. after the addition, whereupon a cell division lag of about one hour's duration was noted, followed by a further cell division period. The dry weight of the cells per litre of culture increased independantly.

In expt. F the cell division rate was low during the first hour after the addition of the limiting factor. Then there was a period of more rapid cell division lasting for about two hours before the culture reached the stationary phase.

The glycogen in per cent of the dry weight did not reach the same high values as in nitrogen-starved cultures [1]. These values varied between 1 and

Table 1. Phosphorus starvation.

Expt.	Time after inoculation, min.	Cells per ml × 10^{-6}	Dry weight of cells per litre of culture (mg)	Glycogen-glucose per litre of culture (mg)	Protein-N per litre of culture (mg)	Cold TCA-N per litre of culture (mg)	Hot TCA-N per litre of culture (mg)
E	0	1.0					
	90	1.2					
	120	2.5					
	160	3.6					
	185	3.7	137.4	1.4	13.7	0.8	4.5
	250	4.3	165.4	2.4	16.7	1.3	4.7
	250	addition of phosphate					
	255	5.9	172.2	5.0	16.0	1.2	4.9
	265	7.8	190.4	4.7	18.6	1.2	5.6
	280	9.6	201.3	5.2	19.1	1.2	6.3
	300	10.3	225.8	4.3	21.7	1.5	7.4
	325	10.6	287.2	3.7	28.4	2.3	9.8
	370	12	379.7	4.1	37.4	3.2	12.3
	430	18	490.3	4.5	49.7	2.8	15.1
	540	21	625.6	5.5	59.9	4.4	19.0
F	0	0.9					
	90	1.0					
	150	2.2					
	240	5.0	132.0	5.0	12.4	0.9	4.0
	270	7.4					
	300	7.4					
	345	7.4	184.8	11.6	16.4	1.4	4.1
	345	addition of phosphate					
	350	7.4	184.2	12.9	16.0	1.3	4.2
	360	7.8	183.4	13.9	15.6	1.3	4.3
	375	7.8	202.2	15.6	16.7	1.5	5.8
	395	8.7	232.3	15.3	19.2	1.8	6.5
	420	9.8	265.0	11.4	22.6	2.1	7.8
	525	22.5	413.8	10.3	37.4	3.4	12.5

Table 2. Sulphur starvation.

Expt.	Time after inoculation, min.	Cells per ml $\times 10^{-8}$	Dry weight of cells per litre of culture (mg)	Glycogen-glucose per litre of culture (mg)	Protein-N per litre of culture (mg)	Cold TCA-N per litre of culture (mg)	Hot TCA-N per litre of culture (mg)
G	0	0.9					
	90	1.1					
	150	2.9					
	210	5.7					
	240	6.0	203.6	1.8	18.9	1.4	6.8
	270	6.7					
	300	7.8	272.7	2.6	25.8	2.1	8.5
	300	addition of sodium sulphate					
	305	9.5	279.9	4.0	26.1	1.5	8.6
	315	10	291.6	3.1	26.9	2.0	9.3
	330	11	309.3	3.0	29.4	2.3	9.6
	350	13	347.0	3.2	32.3	2.5	10.7
	375	15	412.9	3.5	38.5	3.1	13.0
	480	27	652.7	5.1	57.8	4.7	18.7
	660	43					
H	0	1.0					
	135	1.8	66.8	2.3			
	180	3.6	111.9	3.0			
	225	5.0	175.9	3.2			
	225	addition of sodium sulphate					
	230	8.0	187.9	3.9			
	240	8.3	204.0	4.5			
	265	10	259.8	4.4			
	315	19	441.8	6.6			
	375	37	860.0	16.3			

4 % of the dry weight at the beginning of the starvation phase, and increased approximately 1.5 times during the first hour of this phase (four experiments). The addition of phosphate was followed within five minutes by a very rapid increase in the rate of glycogen synthesis. Total glycogen (glycogen per dry weight and litre of culture) reached a maximum in about 30 min. from the addition of the limiting factor. The maximum rate in the glycogen increase observed immediately after the addition coincided with a lag in the synthesis of nitrogen-containing compounds. When the synthesis of these compounds was resumed, the total glycogen decreased.

Sulphur starvation. (Expts. G and H, Table 2). During the starvation phase in the sulphur-starved cultures, the dry weight of cells per litre of culture continued to increase, but at a low rate in comparison with the rate in non-limited cultures. In expt. G the cell concentration increased from 5.7 to 7.8×10^8 cells per ml during the starvation phase, which was interrupted after about one hour by the addition of sodium sulphate. After this addition there was a small increase in glycogen in per cent of the dry weight during the first five minutes, but it is doubtful whether it is significant. In fact, the glycogen in per cent of the dry weight remained in the neighbourhood of the lowest values observed in cultures grown in lactate media. When the limiting factor was added after at least one hour's starvation, the rate of increase in the dry

weight remained at the same low level as during the starvation phase (expt. G). If, however, the addition was made at the moment when the starvation phase was expected to begin (expt. H), the increase in dry weight per litre of culture proceeded at about the same rate as during the logarithmic phase in non-limited cultures, grown in lactate medium.

No significant increase in the absorption between 220 and 310 mμ of the cell-free culture medium was observed during phosphorus or sulphur starvation.

DISCUSSION

In previous experiments it was shown [1] that glycogen accumulated rapidly in cultures of *Escherichia coli B* when the synthesis of nitrogen-containing compounds was prevented by nitrogen starvation.

During the starvation phase in phosphorus-starved cultures, there was a considerable difference in glycogen in per cent of the dry weight between different cultures, but the glycogen values never reached the high values observed in nitrogen-starved cultures. There was, however, a slow increase in total glycogen during the starvation phase. During the lag in the synthesis of nitrogen-containing compounds following the addition of phosphate, there was an immediate increase in the rate of glycogen synthesis, showing that it was a lack of phosphate that caused the comparatively low increase in glycogen during the starvation phase. As in all other cases tested, the decrease in total glycogen that followed coincided with an increased rate of synthesis of nitrogen-containing compounds.

As shown in expt. E (Table 1), a synchronous cell division occurred immediately after the addition of phosphate, *i.e.* the cell count was doubled in about 20 min. This was followed by a cell division lag of about one hour's duration, which was followed in turn by a new cell division period. This result indicates that it may be possible to obtain simultaneous cell divisions by using the phosphate starvation technique.

Sulphur starvation caused a slowing down of the synthesis of nitrogen-containing compounds as well as of the glycogen synthesis (expt. G). The fact that these syntheses proceeded at a relatively slow rate even after the limiting factor had been added, indicates that some basic mechanism was affected. It seems probable that this change in the cells occurred during the starvation phase, since the addition of sulphate at the beginning of this phase rendered possible a synthesis of these substances at the same rate as in non-limited cultures.

REFERENCES

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