8. R. CAVANNA, E. B. CHAIN, C. G. HEDÉN, B. MALMGREN, N. A. ELIASSON, E. HAMMARSTEN, S. ÅQVIST. — **Interrelation of protein and polynucleotide syntheses in Escherichia coli.**

Summary. — E. coli has been cultivated in volumes up to 1000 litres on a synthetic medium and in broth.

The amounts per cell of PNA, DNA and protein and the rates of cell fission showed no correlation. The agreement between this finding and the results obtained with regenerating liver was discussed.

The amounts of PNA and of protein per cell increased both in the lag and log phase.

The amount of DNA per cell was on the whole constant, showing an undulating run. In spite of this the incorporation of ^{15}N was very rapid particularly during the lag phase, indicating an active breakdown occurring simultaneously with a synthesis.

The low molecular compounds, related to nucleic acids, contributed only a few per cent of the polynucleotides.

The lipids increased in amount per cell during the lag phase and earliest log phase and showed high figures for ^{15}N.

The nitrogen incorporation rates in PNA, DNA and amino acids were highest in the lag phase.

The results are discussed.

This investigation was supported by the Knut and Alice Wallenbergs Stiftelse and The Rockefeller Foundation.

Riassunto. — L'E. coli è stato coltivato in quantità variabili fino a 1000 l., sia in terreno sintetico che in brodo.

Non si osservò correlazione tra le quantità per cellula di PNA, DNA e proteine e l'andamento della divisione cellulare. Viene discusso l'accordo tra questi risultati e quelli ottenuti con fegato in fase di rigenerazione.

Le quantità per cellula di PNA e di proteina aumentarono sia nella fase di latenza che in quella logaritmica.

In complesso la quantità per cellula di DNA si mantenne costante, con un andamento ondulato. Ciò nonostante l'incorporazione dell'^{15}N fu molto rapida, particolarmente durante la fase di latenza; e questo indicherebbe che simultaneamente a una sintesi aveva luogo un attivo processo di demolizione.

I composti a basso peso molecolare, correlati con gli acidi nucleici, costituivano soltanto una piccola percentuale dei polinucleotidi.

Durante la fase di latenza e all'inizio di quella logaritmica si ebbe

un aumento della quantità per cellula di lipidi, nei quali si riscontrò un elevato contenuto di ^{15}N.

La velocità di incorporazione dell'azoto nel PNA, DNA e negli aminoacidi era massima nella fase di latenza.

Vengono discussi i risultati.

Questa ricerca è stata sostenuta dalla Fondazione Knut e Alice Wallenberg e dalla Fondazione Rockefeller.

Résumé. — On a cultivé l'E. coli en milieu synthétique et en bouillon, en volumes allant jusqu'à 1000 l..

On n'a pas observé de corrélation entre les quantités de PNA, DNA et protéines par cellule et les taux de division cellulaire. On discute l'accord entre ces résultats et ceux obtenus en travaillant sur du foie en régénération.

Les quantités par cellule de PNA et de protéines augmentaient et dans la phase de latence et dans celle logarithmique.

La quantité de DNA par cellule était dans l'ensemble constante, la courbe affectant de légères ondulations. Néanmoins l'incorporation de ^{15}N se faisait trés rapidement, particulièrement pendant la phase de latence, ce qui indiquerait que simultanément à une synthèse se produisait un processus actif de démolition.

Les composés de poids moléculaire peu élevé corrélés avec les acides nucléiques ne représentaient qu'un faible pourcentage des polynucléotides.

Il y eut au cours de la phase de latence et au début de la phase logarithmique une augmentation de la quantité par cellule de lipides, dont le contenu en ^{15}N était élevé.

La vitesse d'incorporation de l'azote dans le PNA, le DNA et les acides aminés était la plus élevée au cours de la phase de latence.

On discute les résultats.

Cette recherche a pu être effectuée grâce á l'aide de la Fondation Knut et Alice Wallenberg et de la Fondation Rockefeller.

Zusammenfassung. — E. coli wurde in verschiedenen Mengen bis 1000 l., auf synthetischen Boden und in Bouillon kultiviert.

Der Gehalt an PNA, DNA und Protein per Zelle zeigte keine Abhängigkeit von der Spaltungsgeschwindigkeit der Zellen.

Die Uebereinstimmung zwischen diesen Resultaten und denen mit Leber in der Regenerationsphase erhaltenen wurde diskutiert. Der PNA- und Protein-Gehalt per Zelle erhöhte sich in der Latenz- sowie in der logaritmischen Phase.

Der DNA Gehalt per Zelle blieb im allgemeinen konstant und zeigte einen wellenförmigen Verlauf. Trotzdem ging der Einbau des ^{15}N, besonders in der Latenzphase, sehr schnell vor sich, was auf einen aktive Abbau und gleichseitige Synthese hinweist.

Die niedermolekularen Substanzen, die mit Nukleinsaüre in Beziehung stehen, stellten nur einen kleinen Prozentsatz der Polynucleotide dar.

Der Gehalt per Zelle an Lipiden, welche eine hohe Konzentrazion von ^{15}N zeigten, nahm während der Latenz- und am Anfang der logaritmischen Phase zu.

Die Einbaugeschwindigkeit des Stickstoffes in PNA, DNA und Aminosaüren war am höchsten während der Latenzphase.

Die Ergebnisse wurden diskutiert.

Diese Untersuchung wurde von der Knut und Alice Wallenbergs Stiftung und von der Rockefeller Foundation unterstützt.

Friedrich Mieschers original discovery around 1870 of the simultaneous formation of protein and polynucleotide has been followed by a great number of investigations with a view to finding some correlation between the syntheses of polynucleotides and proteins.

During the course of investigations carried out at the biochemical department of Karolinska Institutet on the incorporation of nitrogen (^{15}N-glycine) into the polynucleotides and proteins in regenerating liver the possible connection between those two groups of substances was investigated ([11]). Their maxima of isotope incorporation were found to be separated in time, but the exact correlation between the amount of ribose nucleic acid (PNA) per dry weight and the regeneration rate seemed to be somewhat doubtful. In a later study ([1]) no positive correlation was obtained between the rate of protein synthesis and amount of PNA, (fig. 4). Neither was the incorporation of ^{15}N directly proportional to the growth rate (fig. 5).

Organs like the liver present certain difficulties. The cells are not homogenous from the functional and morphological point of view, and the production of plasma proteins makes it impossible to account for all protein produced during a certain period of time. Also that organ does not seem to be well suited for inhibition experiments. To eliminate these diffculties we turned to a microorganism, *Escherichia coli*.

The importance of the nucleic acids in microorganisms has been emphasized by many research workers. The early literature has been

reviewed by Boivin 1942 ([2]), by Chargaff ([8]) and by Stacey 1947 ([30]). Boivin and Vendrely ([3]) pointed out that the ratio nucleic acid/protein increased when bacteria were transferred from a deficient to a rich medium. However, they did not find any relation between the bacterial content of nucleic acid and the multiplication capacity of the cells. Malmgren and Hedén ([23]), in their studies using the ultraviolet-microspectrographic technique, found that the content of absorbing substance (2570 Å) in the cells from different bacterial types increased manifold during the lag phase of bacterial growth. Also for the following generations there is no positive correlation between the amount of this substance and the rate of fission of the individual bacterium. However, the amount of absorbing substance, calculated as PNA newly formed during the generation was definitely related to the generation time. This was, however, not the case for the first generation including the lag phase. In part similar results were later obtained by different authors using chemical methods (Levy, Skutsch and Schade ([22]), Morse and Carter ([25]), Gros, Beljanski, Macheboeuf and Grunbach ([16]), Mitchell and Noyle ([24]), estimating the « total nucleotide » by means of a direct spectrophotometric method and the desoxyribonucleic acid (DNA) by a chemical method concluded that it is the PNA synthesis which accelerates most during the phase of accelerated cell growth. In 1950 Caldwell and Hinshelwood ([6]) and Caldwell, Mackor and Hinshelwood ([7]) in experiments on *B. lactis aerogenes* grown in different types of media could show that the DNA content of individual cells remained nearly constant despite pronounced variations in cell size. The PNA on the other hand showed marked variations, and the results suggested a close relation between the rate at which the cells could grow and the rate of synthesis of this nucleic acid; a hyperbolic relationship between the mean generation time and the PNA content could also be established. Similar results were reported by Price ([27]), who concluded that these results may indicate a relationship between protein synthesis and PNA synthesis under the experimental conditions in question. Gale and Folkes ([13, 14]) found in studies on Staph. aureus that the rate of protein synthesis was directly related to the nucleic acid content of the cells.

In the present paper the results of Malmgren and Hedén ([23]) were substantiated and it could also be shown in four to six hours experiments on *E. coli* that there was no direct proportionality between amounts of PNA, DNA or of protein per cell and the rate of cell fission. The amounts of PNA and protein rose already during the lag phase and the isotope figures for the incorporation rates of nitrogen in PNA, DNA and protein were not proportional to the rate of cell fission in any phase.

EXPERIMENTAL.

Strain and culture medium. Dry cultures of the same strain of E. coli B, that had previously been used by HEDÉN ([19]) for phage work, were used to start all experiments.

The main substrate, called FSA below [for details cf. ([19])] was FRIEDLEIN'S ([12]) synthetic medium containing 1% sodium lactate as the carbon source and 1% ammonium chloride as the nitrogen source. The salts and the bulk of the medium were sterilized separately because otherwise toxic ions from the stainless steel were dissolved. Their effect on the growth is demonstrated in experiment A, where the complete medium was sterilized in the tank. The amount of heavy metals which reacted with ammonium sulphide went down from $2.5 \cdot 10^{-5}$ to around 10^{-6} molarity when the salts were sterilized separately in glass flasks. One experiment (J) was carried out in broth (pH 7.2,1% Witte peptone, 0.5% argentine meat extract and 0.3% NaCl, filtered through coarse paper after boiling for 10 min., subsequently sterilized at 120° C for 20 min.). With this medium foaming had to be controlled with a silicone (Dow-Corning's antifoam). The growth temperature was 37° C in all experiments.

In the experiments A, B and C, which were carried out in Rome, the dry stock culture was suspended in a few drops of FSA and used to inoculate 25 ml of the same medium in an Erlenmeyer flask. After 20 hours growth on a rotating turntable the culture was transferred to 37 liters of the synthetic medium in a 90 liter stainless steel tank using vortex aeration ([26]). After another 20 hours the bacteria were harvested in a sterile Sharples centrifuge, transferred with the aid of about 300 ml of physiological saline to a Waring blender, which was then run for 45 seconds at low speed and at high speed for only a few seconds to ensure that the resulting suspension was completely homogenous. After counting the bacteria in the suspension, enough of this was added to 1000 liters of ^{15}N-marked FSA in a 3000 liter stainless steel tank to give a density of about 6.10^7 bact./ml. The remaining suspension was analyzed as the zero hour sample. The growth, promoted by vortex aeration and stirring was followed at close intervals by counting the bacteria in the phase-contrast microscope.

In the experiments D, F, G, and J, which were carried out in Stockholm, a first preculture was effected on agar-slopes and a second in a 5 liter Erlenmeyer flask containing 3 liters of medium. It was aerated (about 0.5 liters of air/liter of medium/min) through a glass tube ending in a sintered glass disc. After 20 hours of growth the culture was

Table 1a. - The available nitrogen source in the substrate was labeled to 4.07 atom per cent excess ^{15}N. All figures given are expressed in atom per cent excess ^{15}N.

Series		*A*					*B*						*C*						
Time of growth in minutes		60	120	180	240	360	60	120	180	240	360	480	30	90	150	210	270	330	390
Number of cells x 10^6 per ml.		63		68	89	184	54	50	85	140	400	1030	57	55	107	175	330	655	725
DNA	Guanine	.60	1.10	1.52		2.60	.81	1.46	2.14			3.39	1.54	1.54	2.29	2.68	2.72	3.40	3.65
DNA	Adenine	.58	.98	1.41		2.59	.77	1.48	2.17	2.45		3.53	1.42	1.62	2 19	2.73	2.77	3.49	3.70
DNA	Cytosine	.48	.92	1.44		2.60	.71	1.48	1.81	2.91		3.29	1.09	1.50	2.40	2.55	2.85		
DNA	Thymine	.55	1.00	1.03		2.40	.77	1.25	2.01	2.54		3.44	1.40	1.39	2.32	2.04		3.05	3.58
PNA	Guanine	1.85	2.23	2.56		2.98	1.03	1.82	2.52	2.81		3.49	1.38	1.79	2.49	2.64	3.18	3.57	3.64
PNA	Adenine	1.77	2.20	2.57		3.23	1.08	1.88	2.61	2.88		3.00	1.51	1.95	2.60	2.90	3.19	3.60	3.75
PNA	Cytosine	1.83	2.26	2.54		3.15	1.08	1.96	2.30	2.60			1.48	1.67	2.67	3.01	3 21	3.41	3.75
PNA	Uracil	1.68	2.43	2.73		2.81	1.18	1.95	2.57	2.76		3.54	1.34	1.95	2.57	2.95		3.50	3.75
Protein	Leucine			1.46			1.19	1.79				3.71	1.32						3.28
Protein	Isoleucine			1.46			1.16	1.84				3.50	1.31						3.14
Protein	Phenylalanine	.42	.72	1.51		2.44	1.10	1.76		2.65			1.35		2.65				4.03
Protein	Valine	.48	.94	1.51			1.10	1.80		2.79		3.76	1.65		2.54				3.64
Protein	Methionine	.35	.87	1.59	1.67	2.68	.95	1.75		2.59		3.64			2.46				3.64
Protein	Tyrosine	.42	.87	1.44		2.68	1.02	1.74		2.65		3.66	1.50						
Protein	Proline	.47	.79	1.35	1.99	2.13	1.08	1.77		2.75		3.64	1.46		2.38				3.62
Protein	Glutamic acid	.48	.94	1.47	1.66	2.37	1.15	1.81		2.70		3.68	1.42		2.64				3.73
Protein	Alanine	.50	.90	1.49	1.98	2.58	1.11	1.82		2.95		3.87	1.53		2.65				3.84
Protein	Threonine	.47	.95	1.48		2.63	1.13	1.80		2.90		3.56							
Protein	Aspartic acid	.48	.95	1.51		2.44	1.16	1.88		2.96		3.79							
Protein	Serine	.44	.92	1.46		2.59	1.12	1.78		2.89		3.66	1.59		2.48				3.58
Protein	Glycine	.33	.85	1.52	1.79	2.42	1.09	1.77		2.85		3.73	1.59		2.55				3.69
Protein	Arginine	.45	.94	1.44		2.53	1.14			2.82		3.68	1.90						3.81
Protein	Lysine	.47	.91	1.53	1.67	2.56	1.06	1.73		2.80		3.64	1.60		2.59				3.81
Protein	Whole protein	.47	.94	1.48	1 89	2.53	1.11	1.78	2.48	2.68	3.23	3.74	1.62	1.92	2.58	3.04	3.37		3.74
Lipid													2.13	2.85	3.25	3.46	3.63	3.81	3.77

transferred to 75 liters of FSA in a 100 liter stainless steel tank ([20]). The bacteria grown in this run were centrifuged, resuspended as described above and used for inoculating 700 liters of the synthetic medium in a 1000 liter tank similar to the 100 liter vessel.

Sampling of cells for analysis. Samples of 50 to 250 liters were drawn off at intervals varying between 45 to 70 minutes, cooled to + 2-3° C within a couple of minutes and stored at nearly 0° C until the bacteria could be spun down in Sharples centrifuges.

The collected cells were washed and centrifuged at 0° C once with physiological saline and once with water. They were then freeze-dried and from the homogenized dry material samples were taken for counting the number of cells, determination of dry weight (in vacuo, 90° C, P_2O_5), extraction of lipids and polynucleotides and preparation of the raw protein fraction.

Analysis. In the experiments on isotope incorporation (series A, B and C) the cells were vibrated for nine days (100 amplitudes of 1 mm per second) in a stoppered glass tube in the presence of glass-powder, glass beads (6 mm in diameter) and absolute ethanol. This treatment effected a satisfactory mechanical fragmentation of the cells. The ethanol was decanted after centrifugation and the further extraction of lipids was accomplished by boiling twice with ethanol-ethylether (3+2) for two hours each time. The ethanol-ether extracts were mixed and evaporated *in vacuo* and the residue extracted with chloroform. A small amount of material not soluble in chloroform was added to the material which remained after the lipid extraction. The lipids in one experiment (C) were then analyzed for ^{15}N (table 1-a). The material after lipid extraction was air-dried and submitted to polynucleotide extraction according to the method of HAMMARSTEN ([17]). The remaining material was treated with 5% trichloroacetic acid (TCA) once at 0° C to wash it free of salt and twice at 90° C for 10 minutes to remove eventual traces of polynucleotides not extracted during the previous operation. The solid ethanol-ethyl-ether-dried residue has been considered as protein.

The polynucleotides were separated into PNA and DNA and further degraded to obtain their individual nitrogenous bases. These were analysed for their content of ^{15}N all according to methods previously published ([9, 10, 17, 28, 31]). The protein residue was hydrolysed and the individual amino acids isolated and their content of ^{15}N determined ([32]).

No determinations of quantitative amounts of the isolated compounds

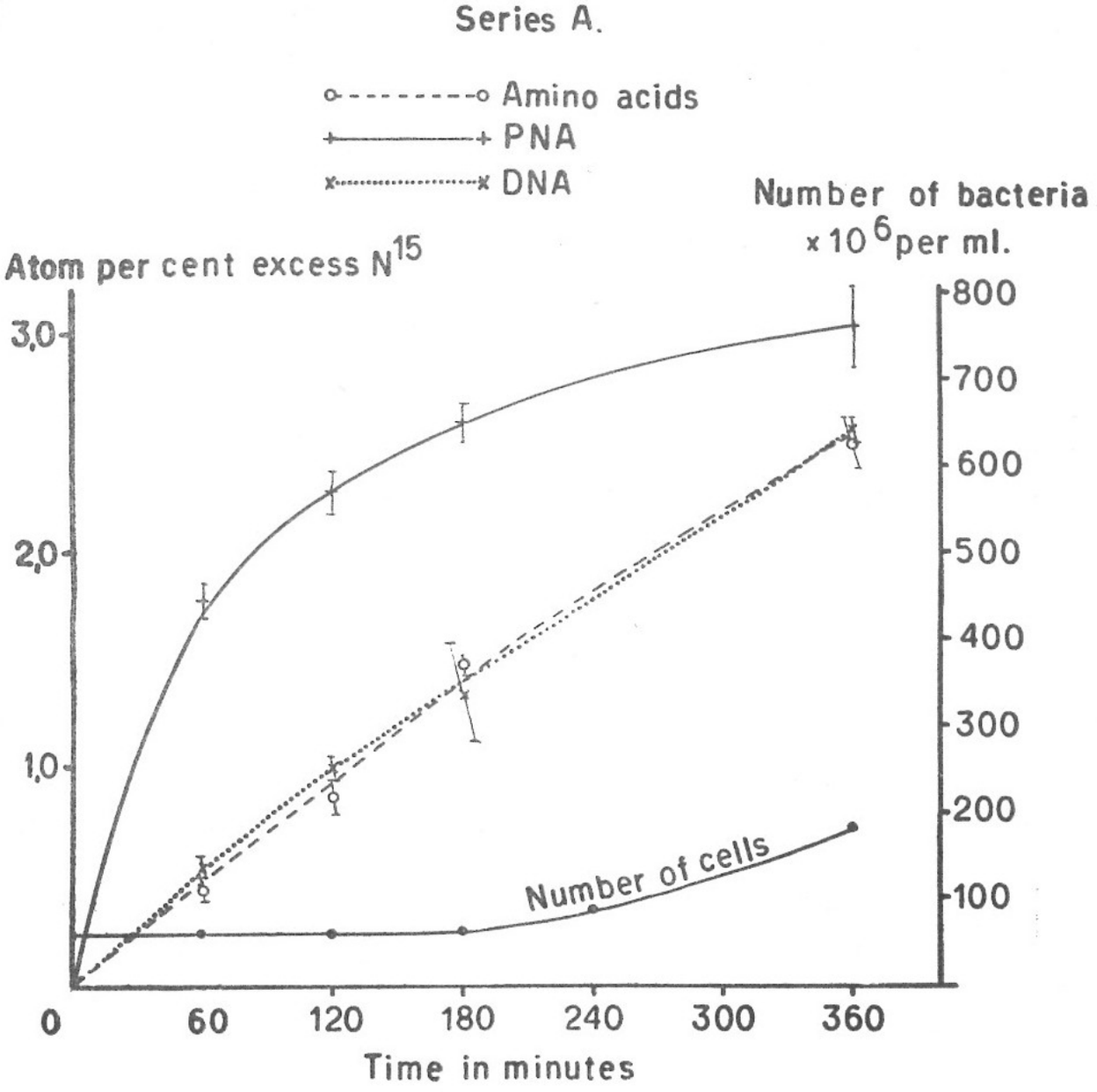

Figure 1. - Growth curve and incorporation curve of ^{15}N into protein, PNA and DNA in series A.

could be made in the series A, B and C (fig. 1, 2, 3), because the bacteria were too heavily contaminated with iron to ensure quantitative extraction of the polynucleotides.

In the series D, F, G and J no tracer was used. To eliminate as far as possible all sources of metal, the bottom bearings in the Sharples centrifuges (self lubricating metal bearings) were now substituted with non metal (antifriction) bearings. The tanks, the heat exchanger etc. were also submitted to rigorous rinsing and the iron contamination in the preparations was then too low to interfere with the quantitative extraction of the polynucleotides. In the series just mentioned fragmentation of the cells was also carried out in a somewhat different way,

namely with the aid of minute glass beads [cf KING and ALEXANDER ([21])]. An amount of dry bacteria (0.5 gram) was mixed with 10 ml of pyrex *ballotini* (0.28 mm in diameter) and vibrated in a specially adapted « Microid flask shaker » in the presence of ethanol-water (3 + 7) at —15° C for 12 to 24 hours. The vibration was carried out in a thick walled pyrex tube fitted with a ground joint. All subsequent operations could be made without removing the solids from this tube. After fragmentation the material was freeze-dried and then extracted twice with boiling ethanol-ethylether (3 + 2) for two hours to remove lipids. The lipid extract and the remains in the tube were further treated in the same manner as described for the tracer series.

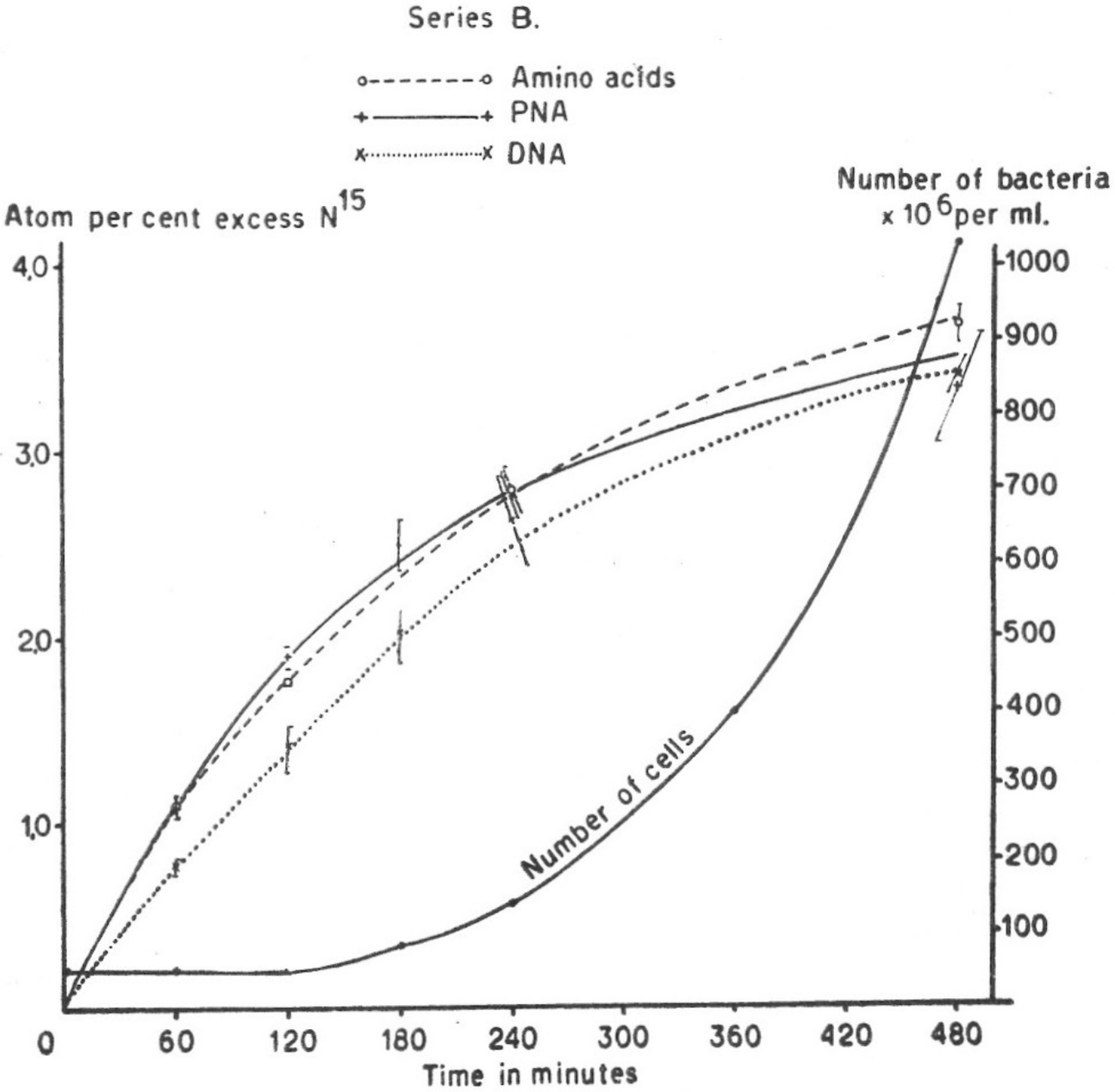

Figure 2. - Growth curve and incorporation curve of ^{15}N into protein, PNA and DNA in series B.

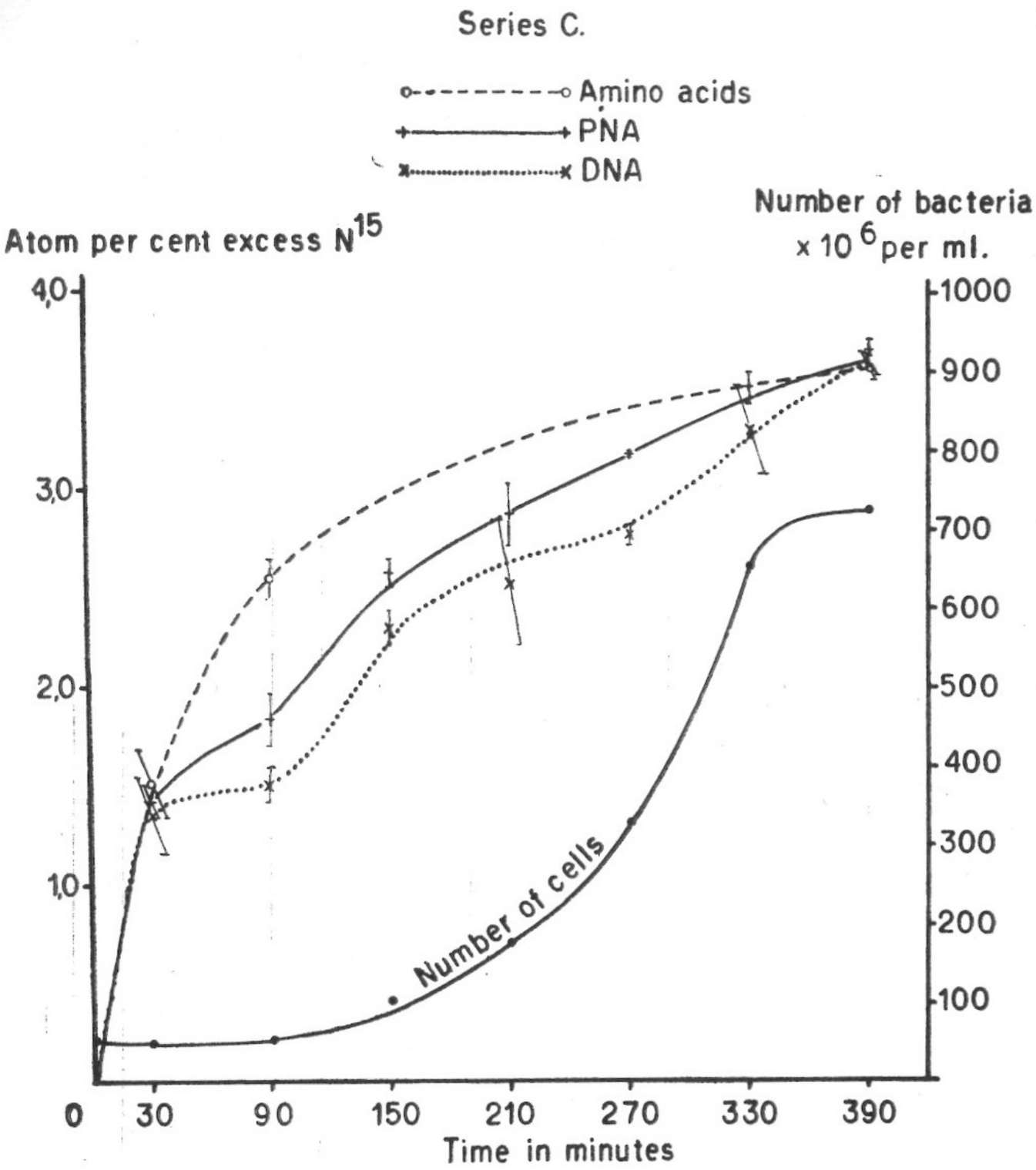

Figure 3. - Growth curve and incorporation curve of ^{15}N into protein, PNA and DNA in series C.

The chloroform soluble lipids were combusted and the phosphorus content determined. The polynucleotides were separated into PNA and DNA in the usual way and the amount of each based on phosphorus determinations. To check these estimations, the amounts of PNA and DNA were also determined with a colorimetric method measuring their sugar content ([4]). In some cases the amount of PNA was also calculated

from the figures for uridylic acid, cytidylic acid, guanine and adenine separated from degraded PNA according to the method of Cohn ([9]). A comparison between the figures obtained on the basis of phosphorus and sugar determinations showed a good agreement ([5]). In the cases where Cohn's method was used the agreement amounted to 90-95 per cent isolated as pyrimidine mononucleotides and purines.

To prove the completeness of the polynucleotide extraction with the method used, the undissolved material after the extraction was

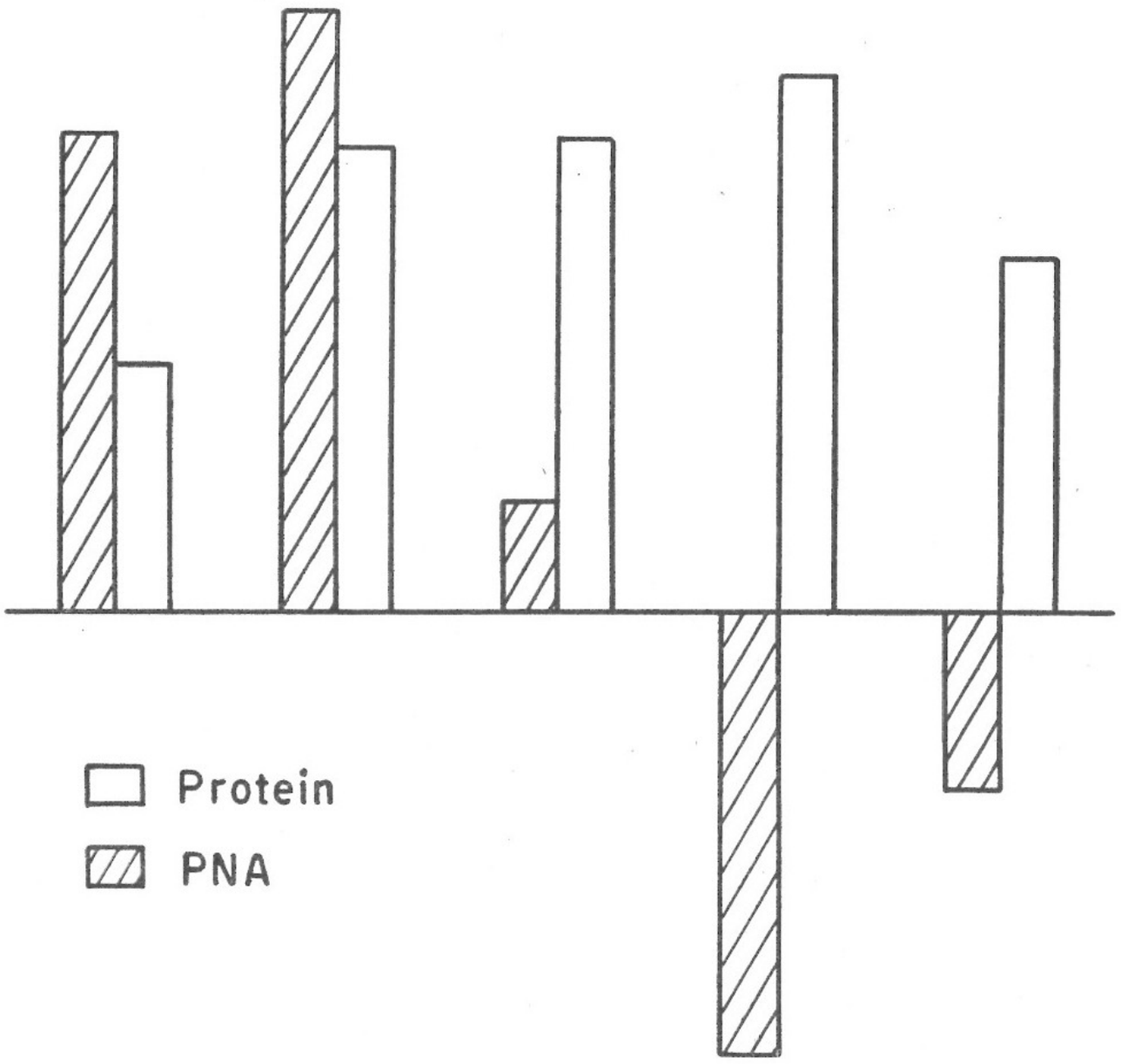

Figure 4. - Relative figures of the changes of growth rate in 10 hourly intervals (expressed as $\log_{10}$ of the amount of protein nitrogen formed) and the changes of PNA-phosphorus per nitrogen during the same period in regenerating rat liver after partial hepatectomy. The different columns correspond, from left to right, to time periods after partial hepatectomy of 0-20, 20-30, 30-40, 40-60, 60-80 hours.

washed with 5% TCA at 0° C until salt free and the remaining phosphorus determined in a hot TCA extract of th. residue. The same check was also made according to the method of GRAFF-MACULLA ([15]) determining the amount of purines. The results based on phosphorus determinations in the TCA extract gave higher figures than the GRAFF-MACULLA estimations, and the HAMMARSTEN method can be assumed to isolate at least 90-95 per cent of the polynucleotides present.

In the series D, F, G and J the amounts of TCA insoluble residue was quantitatively determined by submitting the material directly after lipid extraction to treatment with TCA according to SCHNEIDER ([29]) and determining the nitrogen content in the whole residue.

The nitrogenous « bases », nucleosides and nucleotides that are not precipitated as copper compounds in the HAMMARSTEN method, were approximately determined by their light absorbtion. For brevity they were called the « low fraction ».

Table 1b. - Mean values and standard deviation of atom per cent excess ^{15}N for the sums of amino acids and of nitrogenous compounds in polynucleotides.

Series	*Time of growth in minutes*	*Amino acids*	*PNA*	*DNA*
A	60	.44 ± .05	1.78 ± .08	.55 ± .05
	120	.87 ± .08	2.28 ± .10	1.00 ± .06
	180	1.48 ± .05	2.60 ± .09	1.35 ± .22
	240	1.79 ± .04		
	360	2.50 ± .12	3.04 ± .19	2.57 ± .05
B	60	1.10 ± .06	1.09 ± .06	.77 ± .04
	120	1.77 ± .02	1.90 ± .06	1.42 ± .11
	180		2.50 ± .14	2.03 ± .16
	240	2.79 ± .12	2.76 ± .12	2.63 ± .24
	480	3.68 ± .09	3.34 ± .30	3,41 ± .10
C	30	1.52 ± .17	1.43 ± .08	1.36 ± .19
	90		1.84 ± .13	1.51 ± .09
	150	2.55 ± .10	2.58 ± .07	2.30 ± .09
	210		2.88 ± .16	2.53 ± .32
	270		3.19 ± .01	2.78 ± .05
	330		3.52 ± .08	3.31 ± .23
	390	3.63 ± .08	3.72 ± .05	3.64 ± .06

Table 2. - Quantitative changes of cells and cell components during different runs of the non isotopic series.

Series	Time of growth in minutes	Number of cells $\times 10^{-6}$ per ml	Average dry weight of the cell mg $\times 10^{-10}$	mg nitrogen per litre in culture medium		Protein nitrogen mg $\times 10^{-12}$ per cell	Polynucleotide phosphorus mg $\times 10^{-12}$ per cell		Relative increase of cell components per unit volume of culture		
				distilled at pH 10	in organic linkage		PNA	DNA	Protein	PNA	DNA
D	0	65	1.60	—	—	20.0	—	.485	1.0	—	1.0
	60	65	2.00	234.6	0.42	22.5	.742	.532	1.1	1.0	1.1
	120	80	1.80	—	0.090	20.1	1.260	.462	1.2	2.1	1.2
	180	143	2.30	231.2	0.024	25.9	1.367	.519	2.9	4.1	2.4
	240	206	2.40	234.4	0.034	27.1	1.855	.526	4.3	7.9	3.4
F	0	65	1.64	—	—	17.6	.727	.752	1.0	1.0	1.0
	70	60	3.03	209.8	4.0	17.6	1.966	.788	—	2.5	1.0
	140	120	2.70	212.6	6.0	23.4	2.736	.841	2.5	6.9	2.1
	210	360	2.75	203.0	6.0	23.5	2.935	.673	7.4	22.3	5.0
	280	830	2.91	183.8	6.0	27.3	3.173	.722	19.8	55.7	12.3
G	0	103	1.81	—	—	17.4	1.267	.865	1.0	1.0	1.0
	70	96	2.90	244.4	0.060	23.4	2.879	.755	1.3	2.1	0.8
	140	185	3.53	244.8	0.066	28.2	4.368	.946	2.9	6.2	2.0
	210	459	4.31	236.8	0.066	39.7	4.890	1.370	10.2	17.2	7.1
	280	1076	3.73	208.6	0.012	35.3	3.623	.831	21.2	29.9	10.0
J	0	60	1.95			21.4	1.377	.575	1.0	1.0	1.0
	45	54	3.82			35.5	5.104	1.070	1.5	3.3	1.7
	135	231	3.89			34.1	6.580	1.112	6.1	18.2	7.4
	180	580	4.07			37.1	6.460	1.208	16.6	45.0	20.1
	360	1000	4.19			39.2	6.318	1.224	30.2	75.8	35.2

Results.

The short cultivation time (4-6 hours) was chosen because the metabolic exchange then seems to go mainly from the medium to the cells. This is proved by the values for nitrogen (table 2) and for light absorbtion in the medium (no increase), and by the fact that the isotope in the medium was not diluted. In those respects the conditions are simpler than those in liver.

The nitrogen incorporation figures from table 1-a, have for simplicity also been given as mean values for the four individual nitrogenous compounds in the nucleic acids and for fifteen amino acids in the protein. These values are given in table 1-b. The sometimes big standard deviations are not due to analytical errors or lack of purity of the compounds. The deviations might indicate biologically significant differences in the

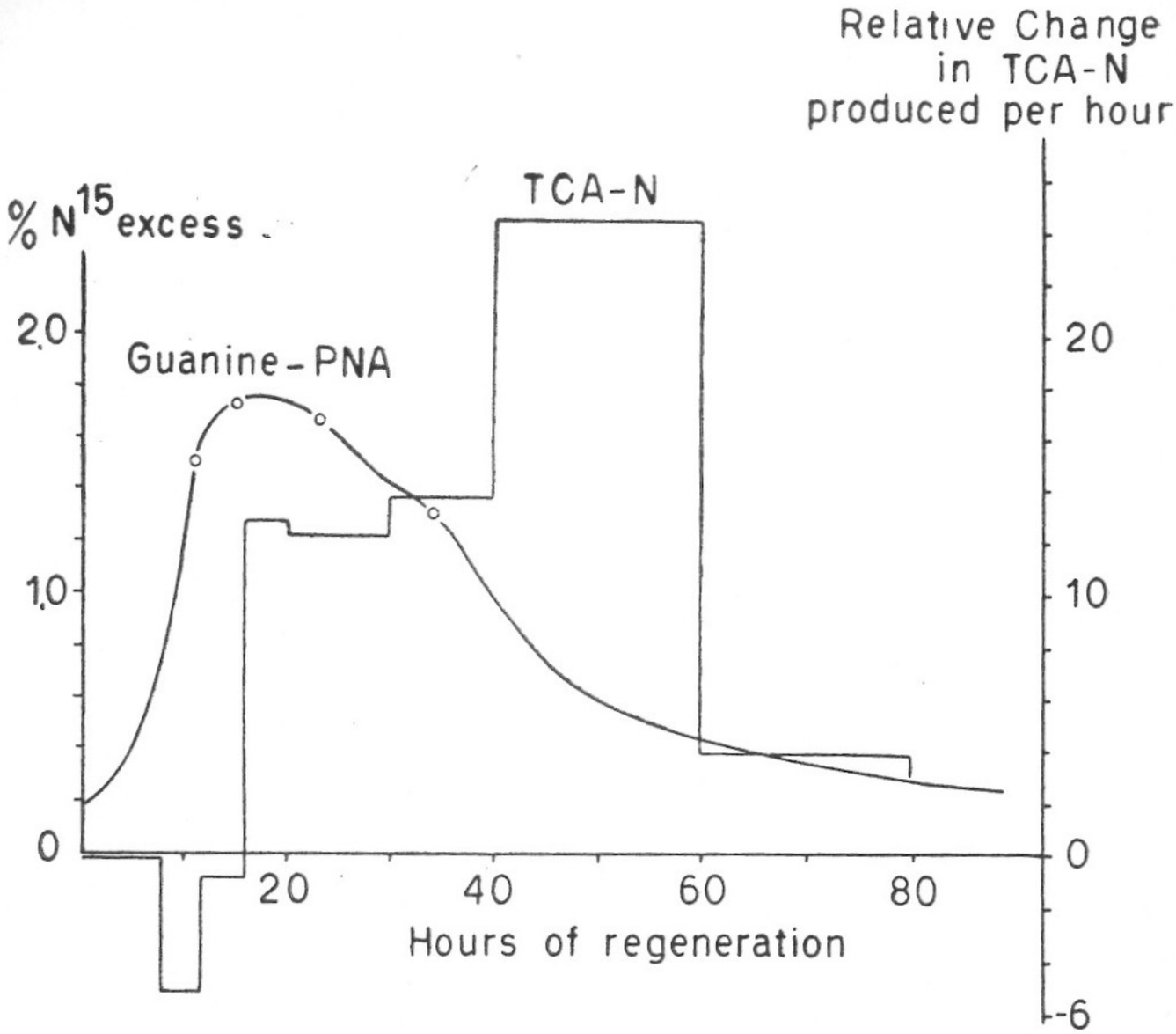

Figure 5. - Comparison of change of TCA nitrogen produced per hour with incorporation of ^{15}N (precursor glycine-^{15}N) into guanine from liver cytoplasmic PNA in regenerating liver.

rate of formation of individual compounds, a fact which should however not preclude the use of the mean values for comparing the rates of cell fission with the rates of nitrogen incorporation.

The rate of nitrogen incorporation was highest in the lag phase where PNA increased in amount per cell, which DNA did not. Although DNA showed the same high incorporation as PNA during the lag phase, the amounts of DNA per cell remained — with characteristic undulations — practically unchanged both during lag and log phase (fig. 6).

The incorporation of ^{15}N into the amino acids was nearly directly proportional to the rate of incorporation into PNA and DNA (table 1-a and 1-b). The components that increased in amounts per cell were PNA and protein. The rise began already in the lag phase when there was no cell proliferation but an increase in cell weight.

Series G.

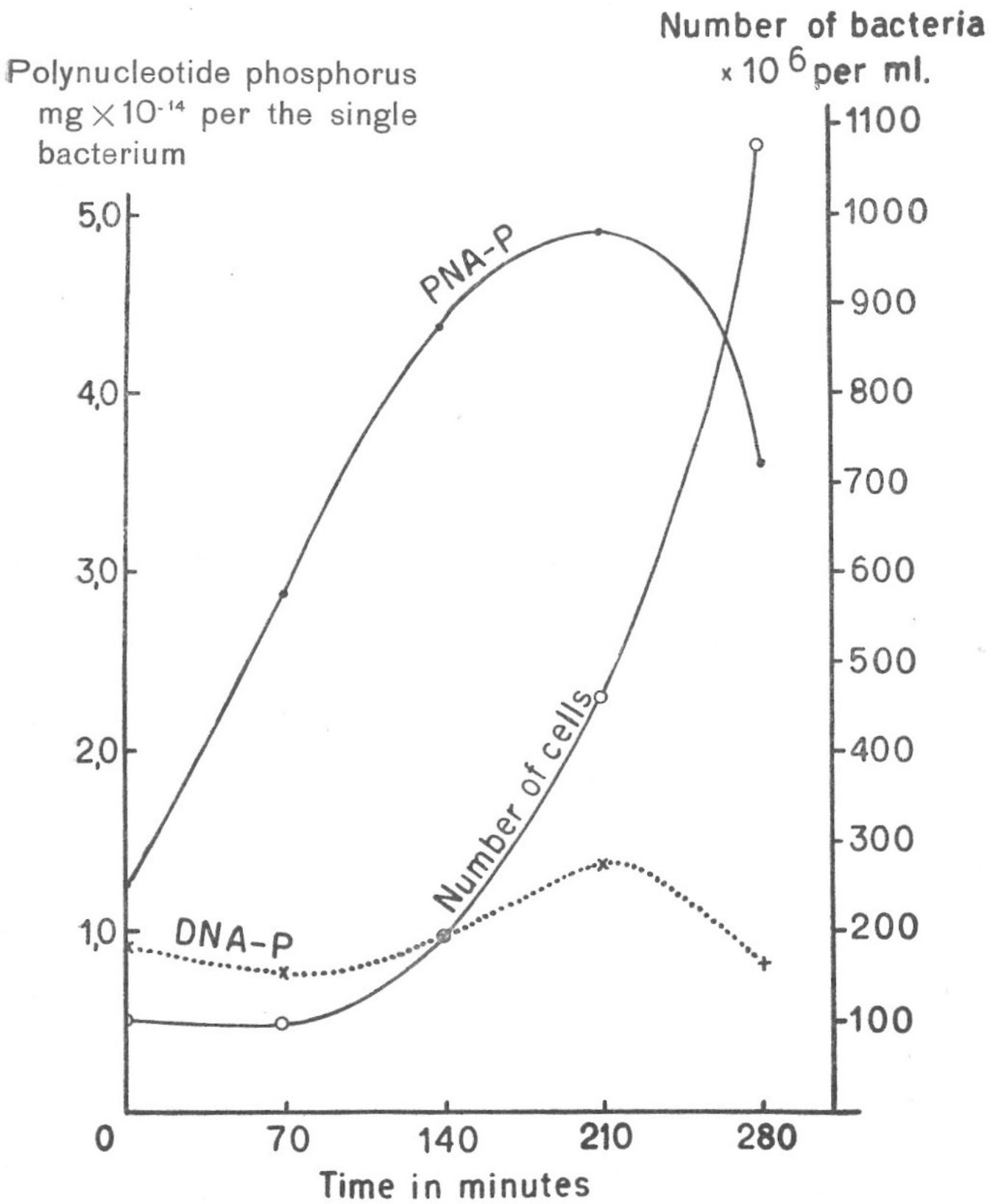

Figure 6. - Changes of DNA-P and PNA-P during the logarithmic growth phase.

The « low fraction » (page 6) amounted only to 4-2.5 per cent of the polynucleotides.

In the lag and early log phase the lipids increase per cell and, as is clearly evident from table 1-a, their isotope incorporation is here more rapid than that of PNA, DNA and protein. The nitrogen in the lipid fraction is very probably to some extent of non-phospholipid caracter because it was much higher than the amount corresponding to phosphorus. This is also the case in liver, where the phospolipids have been found to be heavily contaminated both by the precursor and by other nitrogenous compounds not known to be proper constituents of phospholipids ([33]). The relatively high figure for ^{15}N given in table 1-a can thus not safely be considered as indicating a specially high metabolic activity in the phospholipids.

Discussion.

The results do not demonstrate any constant, positive correlation between the amount of substance or of incorporation of ^{15}N into the different cell constituents and the rate of growth (fig. 7). The results from the study on regenerating liver ([1]) having a bearing on this relation are summarized and exemplified with PNA in fig. 4. They demonstrate

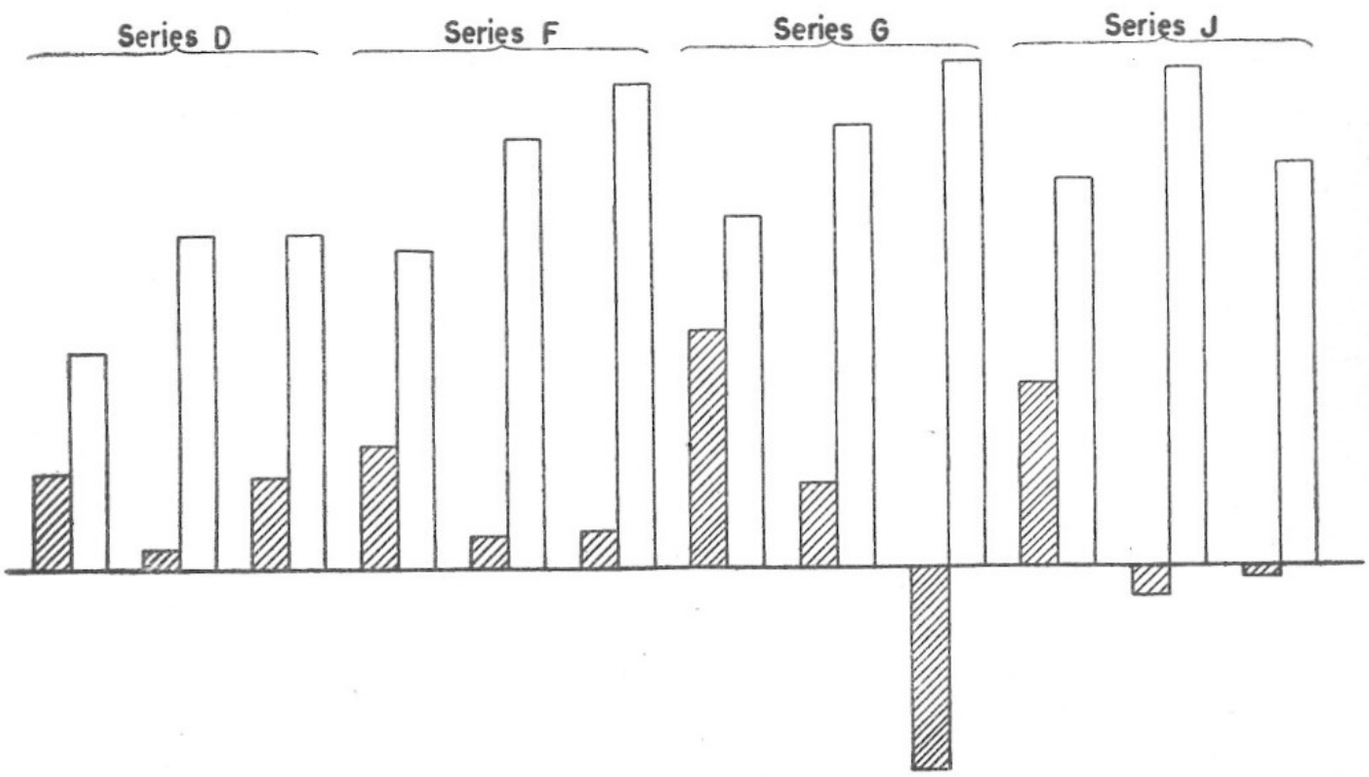

Figure 7. - Relative figures (calculated from Table 2) for the changes of growth rate in 60 minutes intervals (expressed as $\log_{10}$ of the number of cells formed in this period) and PNA-phosphorus (mg. $\times$ 10^{-12} per cell).

a similar absence of correlation, a fact which gives further emphasis to the assumption that there is no immediate, biologically significant connection between the total amount and turnover rates of these compounds and the rate of growth.

A further comparison with the results on liver is more difficult. In liver the incorporation rates (precursor ^{15}N-glycine) into nucleic acids, exemplified in fig. 5 by the figures for guanine from cytoplasmic PNA and the rate of growth as measured by the increase in protein nitrogen, are not running in parallel. During the peak in the PNA incorporation rate the protein is low and not until later when the incorporation rate goes down does it reach its maximum. There are thus two well separated maxima, but since we can not determine the losses of plasma proteins produced during the regeneration stages, it is difficult to interprete the finding in detail. However available figures on isotope incorporation into plasma proteins during different stages of regeneration ([34]) give no support to the assumption that the observed difference can be eliminated by the formation of proteins leaving the liver.

When the nitrogen incorporation data were compared it was demonstrated ([1, 11, 18]) that the maxima were separated in time, that of nucleic acids preceeding that of protein. With the data so far available it is however not possible to analyze more closely the relation between the incorporation into nucleic acids and proteins and the growth rate. The fact that in regenerating liver the increase in nucleic acid turnover begins earlier than in proteins could however indicate that the nucleic acids have a primary role in an assumed interrelation. This time lag for proteins could not as yet be demonstrated in E. coli, where the cell processes are of course more rapid than in regenerating liver.

The results from the inhibited series A resemble in one respect those described by Levy et al. ([22]) for cobalt inhibition of *B. proteus*. The amounts of PNA in their experiments was unaffected by the inhibition of growth. We found that the turnover in PNA was unaffected whereas the inhibition of the turnover in DNA and protein was very marked. We have been unable to reproduce this inhibition by adding ferriammoniumsulphate to the culture medium.

The results obtained on bacteria certainly do not speak against the assumption that the nucleic acids may function as activating agents for amino acid and/or protein syntheses, but they give very little help in revealing the nature of such an interaction. They would indicate that in a possible activation process one or more reactions are interposed and thus obscure a relationship.

Endeavours to correlate the ^{15}N incorporations into PNA and the generation time did not yield any clearcut results (cf. figs. 8, 9 and 10). To settle this point further experiments are being carried out, especially in continuous cultivation.

If in a diagram incorporation rate (d PNA/dt) against time, the number of bacteria in progressing generations (n, 2n, 4n etc.) is plotted along

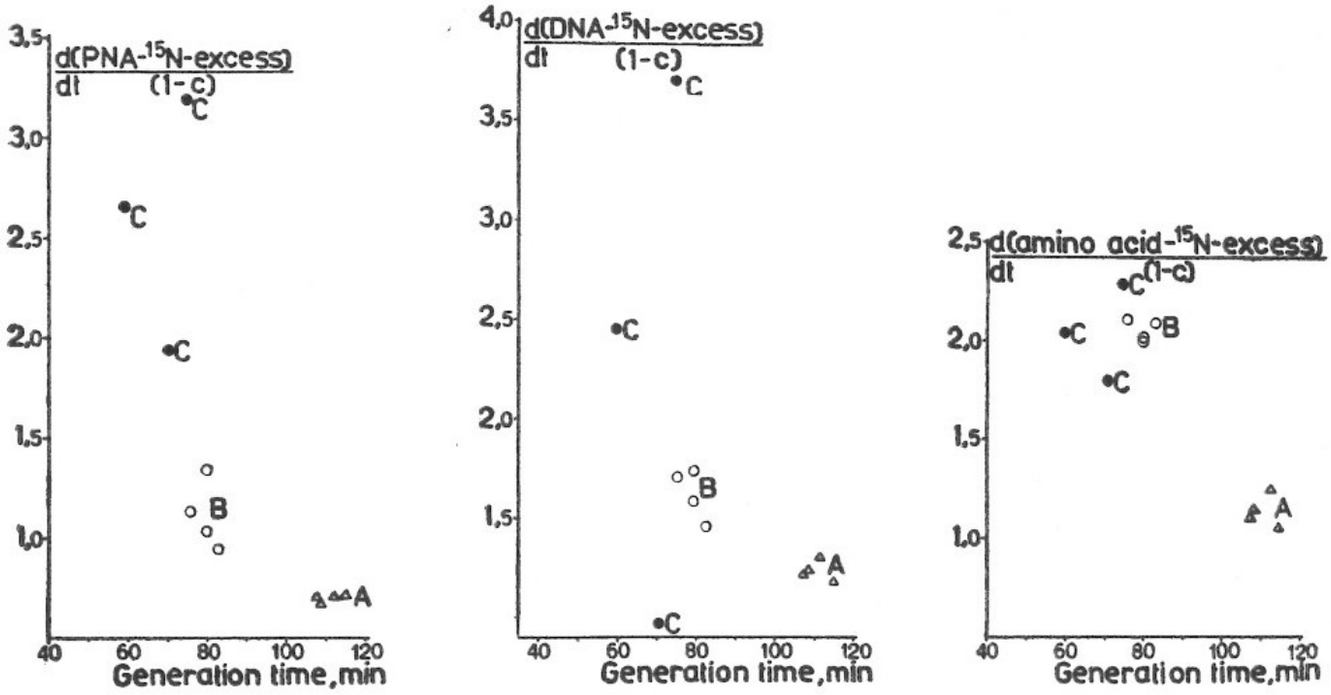

Figure 8, 9 and 10. - Relationship between rates of incorporation of ^{15}N (*d metabolite - ^{15}N excess*) in PNA, DNA and amino acids generation time [*dt(1-c)*].

the time axis, the total number of incorporations occurring during one generation may be estimated by projecting the points 2n, 4n etc. onto the d PNA/dt curve and measuring the surface of the area enclosed by the curve and the projections. If this is done for the first generation (by measuring the area between curve and projection through n and 2n) it is found that the total number of incorporations remains fairly constant in the different experiments (250, 280, 280), independently of the length of the generation time which varied between 155 and 300 minutes.

The points on the d PNA/dt-curve corresponding to the midpoint values of the generations following the first one were corrected on the basis of the following considerations (for which we are greatly indebted to Mr. A. Vestermark). In the expression for the change in excess of ^{15}N in the bacterial components in every particular phase on the growth curve one must consider a factor (1-c) besides the value of the ^{15}N-excess of the medium and the values of the absolute amounts of the bacterial

components. In this factor, c represents that fraction of the ^{15}N-excess of the medium which the ^{15}N-excess of the bacterial components reaches at the time in question. Thus, the change in excess of the bacterial components at the different points on the growth curve is a function of the actual ^{15}N-excess of the component at that point. In order to compare the rates of changes of the different excess values during different generations, the expressions for their time derivatives have to be divided by that value (1-c) which corresponds to the midpoint value of the generation in question.

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