# STUDIES ON THE EVOLUTION OF NUCLEOPROTEIN FRACTIONS OF THE CYTOPLASM DURING THE GROWTH OF A CULTURE OF *POLYTOMELLA COECA*

## II. RATE OF SYNTHESIS OF NUCLEIC ACID, STUDIED BY MEANS OF LABELLED PHOSPHATE

by

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The preceding paper* was devoted to a study of the increase of ribonucleic acid (RNA) and proteins during the exponential and slowing down phases of growth of cultures of the colourless flagellate, *Polytomella coeca*. The present work outlines the results of a similar study bearing on the phase of sudden acceleration of growth, induced by the addition of phosphate to a culture previously limited by the impoverishment of the medium in this ion. We have attempted this time to study the rate of synthesis of RNA with the aid of $^{32}P$ labelled phosphate, the previous studies concerning solely the quantitative variations of this substance.

### METHODS

Methods of measurement of RNA and protein N were the same as in the preceding work. The technique used for the determination of specific radioactivity (counts per minute per 100 $\gamma$ P) of the RNA P is a modification of that previously described[1]. The value of the measurements depending on the strict elimination of impurities, the P of which may display a radioactivity quite different from that of the nucleic P, it may perhaps be useful to outline it here[2].

The whole *Polytomella* cells or the nucleoprotein fractions separated by differential centrifugation were treated with 10% trichloracetic acid and the precipitate washed 3 times with 5% trichloracetic acid, 3 times with alcohol and 2 times with ether. The resulting dried powder was finely ground and delipidated 3 times for three minutes in alcohol-ether according to the method of SCHNEIDER[3]. It was then heated at 110° C for 30 minutes in the presence of 25% $NH_4OH$ in sealed tubes in an autoclave. Excess $NH_3$ was then eliminated under vacuum (final pH 8). Following centrifugation, the solution of nucleotides obtained was chromatographed on Whatman paper 4, using water-saturated phenol as solvent (ascending chromatogram). The chromatogram obtained was dried and the zone occupied by the RNA nucleotides ($R_F$: 0.37-0.87) was eluted in distilled water at 100° C for 30 minutes. The resulting solution was evaporated after addition of a small quantity of NaCl, the purpose of which is to terminate with an almost constant quantity of powder. The determination of the radioactivity of this powder, spread in a very fine layer, is carried out with a counter having a mica window. This powder was then incinerated with perchloric acid and its P content determined by the method of KÜTTNER[4].

Proof that the area eluted is that occupied by the nucleotides is based on the following facts. After elimination of the phenol with ether, the chromatogram shows, in the zone to be eluted, spots strongly absorbing at 2537 A., the $R_F$ values of which correspond to those of the four nucleotides

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of RNA chromatographed at the same time. Furthermore, if the nucleic acid of the *Polytomella* utilised was marked with $^{32}P$, a zone of intense radioactivity appeared in the area eluted. Finally, the elution liquid showed a positive reaction to pentose with orcinol and contained an organic P fraction of which 50% was hydrolysed by *N* HCl at 100° C in one hour (P of purine nucleotides).

The efficiency of the method utilised for the separation of the RNA nucleotides from the orthophosphate liberated by the action of $NH_4OH$ on the phosphoproteins or eluted from the proteins upon which it was adsorbed, may be demonstrated by the chromatography of a mixture of non-labelled nucleotides to which is added $^{32}P$ labelled orthophosphate. The radioactive region now moves less rapidly than the region occupied by the nucleotides and is separated from it entirely.

The chromatography of nucleotides could be effected with advantage by the aid of a solvent which is non-absorbing in the U.V., such as propanol. The trials we made did not enable us to give up the phenol; the use of other solvents did not permit a complete separation of orthophosphate from the nucleotides.

## RESULTS

The culture is seeded in a medium resembling the normal one (*cf.* preceding paper), but where the phosphate ion concentration is reduced 50 times. It is easy to follow the exhaustion of the medium in phosphate, if radioactive phosphate is added at the moment of seeding. Measurements of the radioactivity of unit volumes of the medium, freed from cells by centrifugation, show (Fig. 1) that the rate of growth decreases when the cells cease to fix new quantities of phosphate. At this point, the phosphate concentration of the medium is about 1/350 of that in a normal medium. Cell multiplication then continues at a decreasing rate. During this period of slowing growth, the RNA content of the cells decreases to 1/10 of its value during the exponential phase

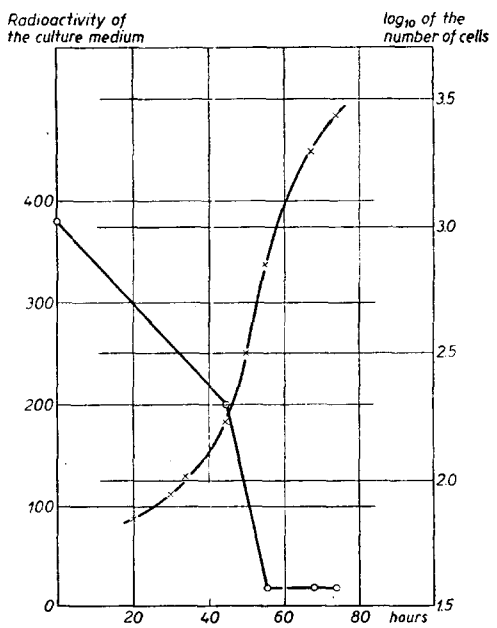


Fig. 1. Growth of a culture of *Polytomella* limited by the utilisation of phosphate (The quantities of phosphate of the medium are given in arbitrary radioactivity units) —x——x— Number of cells; —o——o— Radioactivity of the culture medium

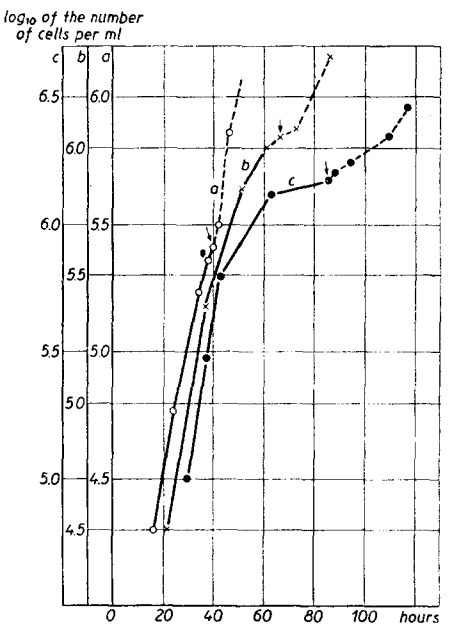


Fig. 2. Effect of addition of phosphate to cultures limited by the utilisation of phosphate, at various stages of slowing down of growth (The addition is indicated by an arrow)

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of growth. At various times during loss of RNA by the cells and their diminishing growth rate, the normal phosphate concentration is suddenly re-established by the addition to the medium of a very small volume of a concentrated phosphate solution. The rate of growth of the culture then increases markedly after a delay, which is longer, when the rate of growth has been most depressed (Fig. 2).

A. In a first experiment, cells were collected at various times following addition of phosphate (time 0 on the graphs) and dispersed in $M/200$ phosphate buffer at pH 7.3, the extract being then separated into two fractions by centrifugation for 10 minutes at 13,000 $g$. The RNA and protein N were measured in each fraction and transformed into the corresponding values for 100 ml of culture. An illustration of the results obtained is shown in Fig. 3 and 4 which are representative of an experiment where the addition of concentrated phosphate was made at the moment when the rate of growth (measured photometrically) was reduced to 1% of its value at the exponential phase.

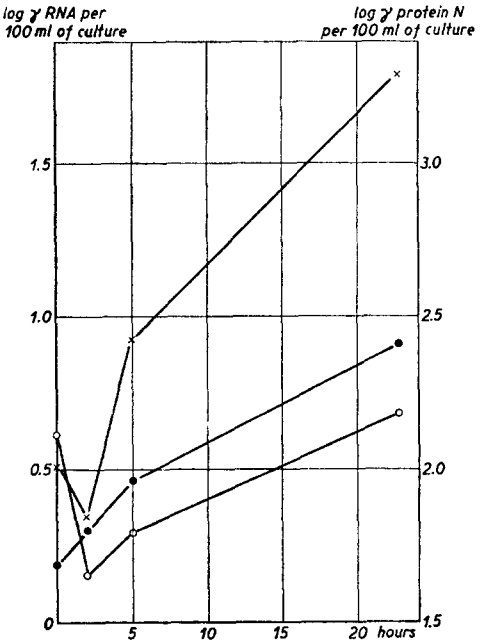


Fig. 3. Effect of addition of phosphate on the evolution of the RNA of the two fractions separated by centrifugation for 10 minutes at 13,000 $g$ (Addition of phosphate at the time 0)
—●——●— Log total protein N;
—×——×— Log RNA of supernatant liquid;
—o——o— LogRNA of pellet

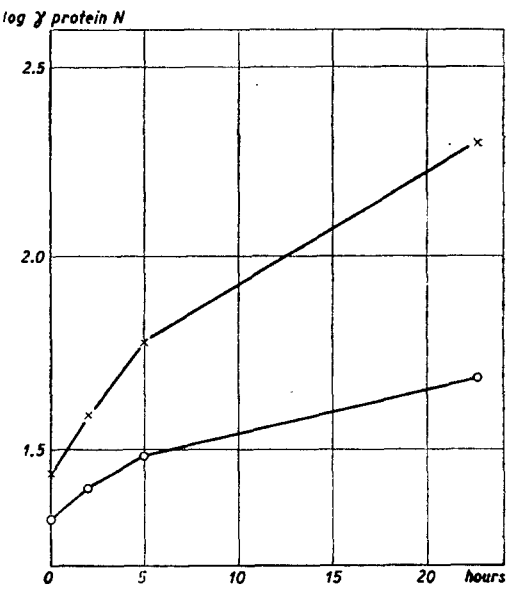


Fig. 4. Effect of addition of phosphate on the evolution of the protein N of the two fractions separated by centrifugation for 10 minutes at 13,000 $g$ (Addition of phosphate at the time 0)
—×——×— Protein N of the supernatant liquid;
—o——o— Protein N of the pellet

The most striking fact noted is a sudden decrease in the RNA of the supernatant liquid and pellet during the first two hours following the addition of the phosphate. This initial decrease in the quantity of RNA per unit volume of culture is followed by an increase, the speed of which in the supernatant fraction surpasses that found in the pellet to such an extent that the ratio of RNA of supernatant liquid to RNA of the

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pellet passes from 0.8 to 12.7 between 0 hours and 22 hours following addition of the phosphate.

These modifications of the quantities of RNA present in the two fractions are accompanied by an increase in the quantity of proteins which takes place regularly, without appearing in the least to be influenced by the variations in the quantity of RNA, and from the very beginning of the phenomenon involves the proteins of the supernatant liquid as well as those of the pellet. The proteins of the supernatant liquid increase more rapidly than the proteins of the pellet, the difference between the two rates being however less marked than in the case of the RNA.

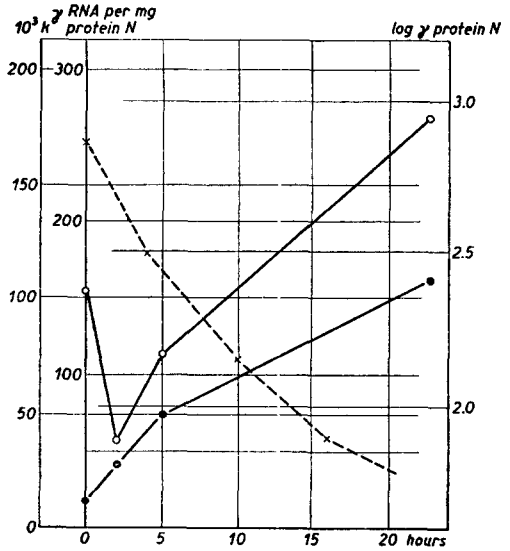


Fig. 5. Evolution of the quantity of RNA per mg protein N following addition of phosphate at the time 0
—●——●— Log $Q$ ($\gamma$ protein N per 100 ml of culture);
---×------×--- $k$ (rate of increase of protein N);
—o——o— $\gamma$ RNA per mg protein N

If, on the same graph (Fig. 5), we plot the rate of increase of protein, $k$, as defined in the preceding work, and the quantities of RNA per mg protein N, it is readily seen that there is no simple relation between the two series of results; the rate of protein increase is highest when the quantity of RNA decreases; this rate of increase then decreases progressively while the quantity of RNA increases.

To no better extent then, than in the results described in the previous work, do we find the existence of a linear relation between the rate of protein synthesis and the quantity of RNA per mg protein N, as was expected[5]. It is readily shown in the same manner that no like relationship exists between the RNA of the pellet or that of the supernatant liquid, considered separately, and the rate of synthesis of proteins.

At the moment, we are unable to supply an adequate interpretation of the initial decrease of the RNA. Possibly it is used up, in the same way as other substrates as a result of the marked increase in respiration provoked by the addition of phosphate. When phosphate is added to the culture at periods progressively closer to the end of the exponential phase of growth, the initial decrease in the quantity of RNA per unit volume becomes less and less marked, finally disappearing altogether. Possibly a more rapid acceleration of the rate of synthesis of RNA suffices in these cases to compensate for the degradation.

The numerical results supplied by the preceding experiment were calculated per unit volume of culture and not per cell. The number of cells increases rapidly following addition of phosphate, as is found by direct count as well as by measurement of the "extinction coefficient" of the culture photometrically; but it is not known whether cell multiplication and increase in the quantity of protein per unit volume of culture take place at the same speed.

As we have already had occasion to report briefly[1], the *Polytomella* cells, removed

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while growth is markedly slowed down as a result of the impoverishment of the medium in P and colored in smears following ZENKER fixation, fix only traces of toluidine blue both in the cytoplasm and in the nucleolus. Following addition of phosphate, a further diminution takes place in the quantity of toluidine blue fixed during 2 and 5 hours. Subsequently, and paralleling the increase of RNA measured, the quantity of toluidine blue fixed by each cell increases considerably. The dye is first fixed on the nucleolus and a narrow cytoplasmic region surrounding the nucleus. The coloration then spreads progressively to the whole of the cytoplasm while remaining most intense in the perinuclear area.

It is most probable that the staining observed is due to RNA, which is very abundant in the cell, and not to the protein-bound metaphosphate which would not have been extracted by the acid fixative utilised. In effect, the protein-bound metaphosphate P (measured by the method of WIAME[6]) represents only $^1/_6$ of the nucleic P in a cell undergoing active growth. Furthermore the blue colour observed did not have the metachromatic characteristic of metaphosphate, in the cells carrying on a rapid synthesis of RNA[6].

These histochemical observations also showed that the large majority of the cells follow the same physiological path and that what we observed on an entire culture applies as well to each cell taken individually. The variations of RNA in relation to the proteins described up to this point, do not therefore appear to result from the fact that the cultures may contain proportions of cells, variable from one moment to another, in physiologically different states.

B. The impression we have come to, as a result of the study of the growth of a culture in a complete medium (cf. preceding work), is that the ribonucleoprotein particles may grow or become degraded at rates differing with their speed of sedimentation. This idea is confirmed by the fact that the relation existing between the RNA of the supernatant liquid and of the pellet, separated at 13,000 *g* for 10 minutes, varies enormously during the sudden growth provoked by the addition of phosphate. We have sought to render this more precise by studying the evolution of the quantity of RNA of 5 fractions separated by successive centrifugations at increasing speeds and periods of time, on the same extract.

The "efficiency" $e$ of those centrifugations

$$e = \frac{\text{gravitational field} \cdot \text{time of centrifugation}}{\text{distance covered by particles}}$$

is given in Table I. The pellets obtained successively are denoted by letters A to D; E is the supernatant liquid obtained at the end of the operation. The centrifuges utilised being of the "angle" type, the values are given for the top and bottom of the tubes. For the calculation of $e$, the gravitational field is expressed in *g*, the time in minutes and the distance in cm.

As indicated by Table I, the centrifugations were such that their efficiencies are in approximately geometrical progression. They therefore constitute a rational exploration of the distribution of the particles as a function of their speed of sedimentation.

If we compare the quantities of RNA contained in each fraction at the various stages of the sudden growth "triggered" by the addition of phosphate, in terms of unit

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TABLE I

EFFICIENCY OF THE CENTRIFUGATIONS USED TO SEPARATE THE FRACTIONS A TO E

| Fraction | Part of the tube | Gravitational field, in $g$ | Efficiency $\log_{10} e$ |
|---|---|---|---|
| A | top | 490 | 3.329 |
| | bottom | 795 | 3.538 |
| B | top | 5400 | 4.372 |
| | bottom | 8750 | 4.580 |
| C | top | 23900 | 5.378 |
| | bottom | 40000 | 5.602 |
| D | top | 23900 | 6.155 |
| | bottom | 40000 | 6.380 |

TABLE II

INCREASE OF RNA OF THE FRACTIONS A TO E

| Fractions | RNA 7 h / RNA 5 h | RNA 9 h / RNA 5 h |
|---|---|---|
| A | 1.57 | 2.00 |
| B | 1.60 | 3.90 |
| C | 1.19 | 2.34 |
| D | 9.00 | 19.5 |
| E | 4.10 | 4.60 |

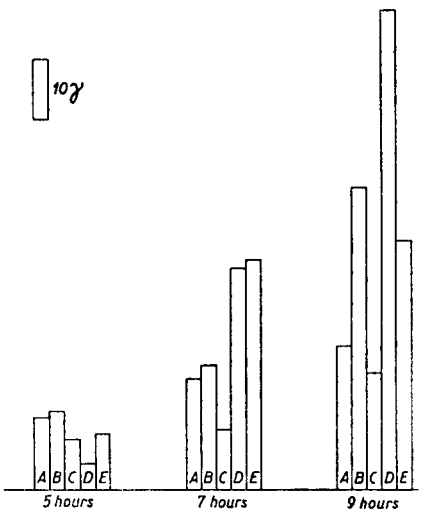


Fig. 6. Quantities of RNA found in the fractions A to E separated from the cells of 100 ml of culture, 5, 7 and 9 hours following addition of phosphate

volumes of culture (Fig. 6), it is seen that the rate of increase of the RNA of the various fractions is markedly different (Table II).

The fractions B and D show a more rapid increase than the fractions A, C and E between which they are interspersed and the fraction C increases less rapidly than B and D; none of these five fractions can therefore be considered as made up exclusively of the same particles as one of its neighbours. We should therefore conclude that the *Polytomella* contain at least 5 categories of ribonucleoprotein particles differing physiologically in their rate of increase. It should be noted as well that the fractions B and D are also distinctly different in so far as their chemical composition is concerned (*cf.* preceding work).

This result was obtained by a series of trials during which we varied the efficiency of our successive centrifugations until a series of centrifugations was obtained which showed as clearly as possible the differences between the rates of increase of the various fractions. Naturally, each fraction necessarily contains particles of various speeds of sedimentation; it could hardly be otherwise with this type of fractionation. What we believe to be shown is simply that each fraction contains a sufficient proportion of characteristic particles so that its behaviour is different from that of other fractions,

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and it is possible that a different plan of fractionation might result in other types of fractions just as characteristic.

We are therefore led, as was CHANTRENNE[7], to doubt the existence in the cell of merely two types of particles, the mitochondria and the microsomes[8]. Our results do not however confirm the provisional hypothesis of CHANTRENNE according to which there exist in the cell particles of all sizes, the smallest of which serve as nuclei for the synthesis of larger and larger particles, with a progressively greater content of varied enzymes. If such a theory were correct, we would expect the rate of increase of the particles to vary in a regular manner, from the smallest to the largest, in the same way as the other properties exhibited by them. The net discordant increase of fractions B and D leads us rather to the idea that the various types of particles grow independently of each other, each at its own characteristic speed. This independence of the various types of particles is furthermore confirmed by the experiments described in the following section.

C. A culture of *Polytomella* is made in a medium containing a quantity of $^{32}P$ labelled phosphate sufficiently small so that growth stops when the number of cells is about half the number of those present, at the end of growth, in a medium containing a large excess of phosphate. Non-labelled phosphate is added in large excess while growth is still rapid, the exponential phase having however been passed. As in the experiments previously described, this addition provokes an acceleration of the rate of synthesis of proteins and of the frequency of divisions, and the cell content of RNA decreases for 2 to 3 hours, and then increases rapidly. In the present experiment, the culture is followed until a new limiting factor comes into play: a deficiency in oxygen concentration. The slowing down in growth which is then produced is always accompanied by a diminution of the RNA content of the cells.

The evolution of RNA (per cell or per unit volume of culture) thus displays three characteristic phases following the addition of an excess of phosphate:

a. A phase of sudden decrease, of short duration (2 to 5 hours) followed by a return to normal.

b. A phase of rapid synthesis during which the increase of RNA follows approximately the cellular multiplication.

c. A phase of decrease which appears when the cellular multiplication decreases.

The various fractions of RNA do not follow a parallel evolution. We have separated only two fractions by centrifugation at 40,000 r.p.m. for 10 minutes: the pellet and the supernatant liquid. During the phase a. and the phase c., the quantity of RNA present in the supernatant liquid always varies much more rapidly than the quantity present in the pellet. During the phase b., the evolution of the two fractions runs practically parallel.

As the culture was seeded in a medium containing $^{32}P$ labelled phosphate, all the cellular constituents containing P will have the same specific radioactivity (counts per minute per 100 $\gamma$ P) at the moment of the addition of the excess of non-labelled P. From the moment of addition of non-labelled P in excess, the growth of the culture is accompanied by a diminution of the specific radioactivity of the P of all its constituents.

The rate of diminution of specific radioactivity for each of them depends in principle:

1. on the speed with which the synthesis of new molecules takes place and, eventually, the degradation of already existing molecules;

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2. on the specific radioactivity of the P of the precursor from which the synthesis is effected; this radioactivity itself decreases continuously;

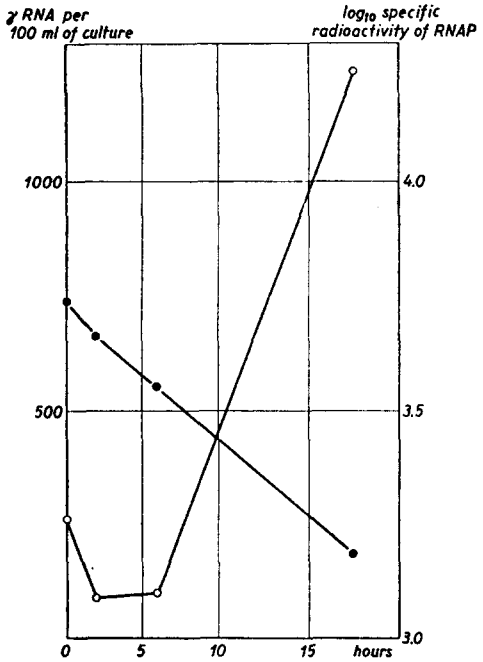


Fig. 7. Modifications undergone by the quantity of RNA present in 100 ml of culture and the specific radioactivity of RNAP following addition of phosphate at the time o

—o——o— Quantity of RNA

—●——●— Specific radioactivity

3. on the speed of eventual equilibrium reactions by which the phosphate groups of the substance under study exchange with those of other substances or with free phosphate ions.

The variations in observed radioactivity will therefore be exactly the inverse of those which we would have obtained by adding an excess of $P^{32}$ labelled phosphate to a culture, the initial phase of which would take place in the presence of non-labelled phosphate. This type of procedure has the following advantages:

a. it is possible to study the evolution of the specific radioactivity of various constituents over a large range of cellular generations,

b. the specific radioactivities to be determined are high from the very beginning of the experiment,

c. the danger of contamination of the substances studied, by $^{32}P$ from the medium, is negligible,

d. the quantities of $^{32}P$ used are small enough so that their radioactivity does not affect the normal functioning of the cells.

To illustrate the results obtained, consider a graph (Fig. 7) on which are plotted the logarithms of the specific radioactivities of the RNAP and the quantities of RNA per unit volume of culture found at various times, an excess of phosphate having been added at time o to a culture prepared as outlined above. As soon as the phosphate has been added, the specific radioactivity of the nucleic P begins to decrease with a speed which is at first constant (6 to 12 hours) and then becomes slower. This decrease of the specific radioactivity of the nucleic P continues regularly, notwithstanding that the quantity of nucleic acid per unit volume decreases 64% during the two hours which follow the addition of the excess of phosphate, remains practically constant for the following two hours, and finally increases rapidly.

Fig. 8 shows us the analogous behaviours involving this time separately the RNA of the

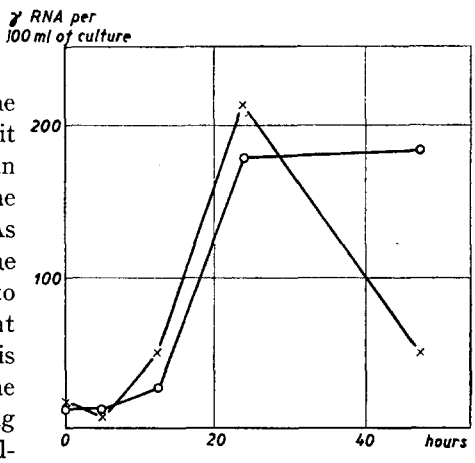


Fig. 8. Modifications undergone by the quantity of RNA of the two fractions separated by centrifugation for 10 minutes at 40,000 r.p.m. following addition of phosphate at the time o

—×——×— RNA of supernatant liquid;

—o——o— RNA of pellet

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pellet and the RNA of the supernatant separated during a centrifugation of 10 minutes at 40,000 r.p.m. Immediately following addition of the non-labelled phosphate, the quantity of RNA of the supernatant liquid decreases 42%, while the quantity of RNA of the pellet remains constant. A similar phenomenon appears between 24 h. and 48 h. following the addition. Once again, the RNA of the supernatant liquid decreases 78%, while the RNA of the pellet remains constant. These independent variations of the quantity of RNA of the two fractions involves no modification of the rate of decrease of the specific radioactivity, which varies regularly and in a parallel manner in both (Fig. 9).

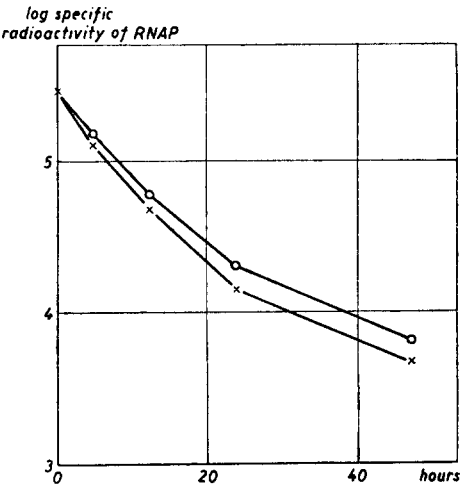


Fig. 9. Same experiment as in Fig. 8. Modifications undergone by the specific radioactivity of RNAP
—×——×— RNAP of supernatant liquid;
—o——o— RNAP of pellet

Such results appear at first sight rather difficult to interpret. We would expect, normally, that the specific radioactivity of the RNA would be dependent at each instant on the ratio between the number of newly-formed molecules and the number of old molecules, *i.e.*, that it would decrease as rapidly as the quantity of RNA would become smaller, or as the rate of increase of RNA goes up. On the contrary, however, the speed with which the specific radioactivity decreases is entirely independent of the variations undergone by the quantities of RNA present.

It appears to us that two hypotheses may account for these observations:

1. The rate of renewal of RNA molecules or of the phosphate groups of their constituent nucleotides is sufficiently high so that the specific radioactivity of the RNA remains always independent of quantitative variations which it undergoes.

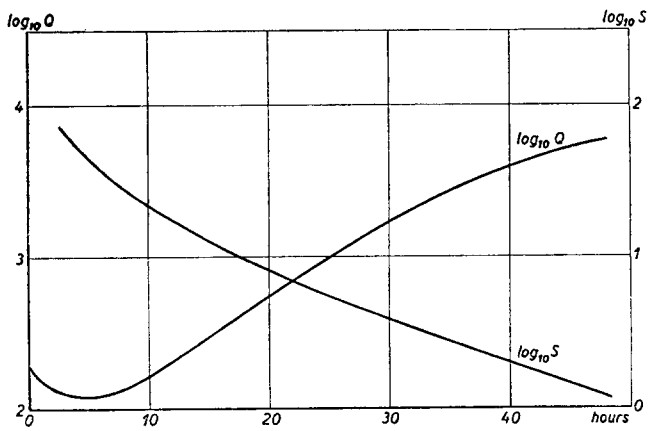


Fig. 10. Evolution of the quantity $Q$ of RNA per 100 ml of culture and the specific radioactivity $S$ of the intracellular orthophosphate following addition of phosphate at the time 0 (The values obtained serve as the basis for the calculation, the results of which are given in Fig. 11)

2. The quantity of RNA synthesized at any moment is proportional to the quantity of RNA present. The rate with which the specific radioactivity of the RNA evolves is then independent of the quantity of RNA present.

The first hypothesis is somewhat difficult to adopt, if we recall that the rate of renewal of RNA molecules has been shown to be very low for all cases studied up to the present (mammalian tissue, embryos, etc.). We have nevertheless sought to verify it by studying the evolution of the specific

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radioactivity of the P of the RNA and of the inorganic P on a *Polytomella* culture identical to the preceding ones. The measurements were made at a time during which there was no important variation in the RNA content of the cells (Fig. 10). We know

$Q(t)$: the quantity of RNAP in $\gamma$, as a function of the time,

$S(t)$: the specific radioactivity of the inorganic P in counts per minute per $\gamma$, as a function of the time.

Let $N(t)$ be the total radioactivity of the RNAP in counts/min (obtained by multiplying the specific radioactivity by the quantity of RNAP). If we suppose:

1. that the quantity of RNAP which undergoes the turnover during the time $dt$ is $TQ.dt$,

2. that all the P which enters into RNA has radioactivity $S(t)$, we then have that:

$$dN(t) = \frac{dQ(t)}{dt} S(t)dt + TQ(t)S(t)dt - TN(t)dt$$

The curves of Fig. 11 represent the solutions of this equation corresponding to various values of $T$ and to $N$ (11 h.) = 9,050 counts/min (value found experimentally). The points marked are the experimental values of $N$ at 11, 24 and 48 hours. The graph shows that:

a. In the case of the hypothesis where the variation of the radioactivity of the RNAP is due solely to the synthesis of new molecules of RNA, the radioactivities calculated are superior to the experimental values.

b. It suffices that a rate of renewal of RNAP of 3 to 6% per hour add its effect to the variation due to the sole phenomenon of the synthesis of new molecules to make the calculated values inferior to the experimental ones.

c. As soon as the rate of renewal rises above these values, the calculated curves move rapidly away from the region of the graph containing the experimental points.

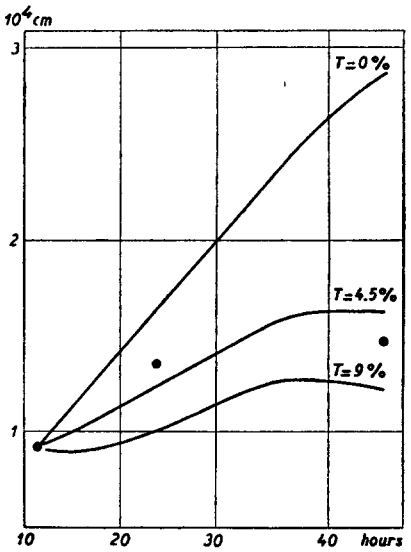


Fig. 11. Results of the calculation of the values $N$ of the radioactivity corresponding to the total quantity of RNAP contained in 100 ml of culture, assuming that the renewal of the RNA molecules is 0, 4.5 and 9% per hour. The points shown on the graph represent the experimental data found 11, 24 and 48 hours following addition of phosphate

Furthermore, a turnover rate of RNAP, sufficient as to make its specific radioactivity independent of the quantitative changes of RNA, would make it constantly very nearly equal to that of inorganic P. But, on the contrary, the specific radioactivity of the inorganic P decreases much more rapidly than that of the nucleic P following addition of excess phosphate, and remains largely inferior to it during the duration of the experiments.

Having thus discarded the hypothesis of a rapid renewal of RNA molecules, it is difficult to avoid the second hypothesis formulated above: the quantity of RNA synthesized at any instant of time is proportional to the quantity of RNA present.

## DISCUSSION

1. The hypothesis to which we have just been led would appear unjustified if we

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did not know that the RNA forms part of organites of diverse complexity and dimensions, of which the largest or mitochondria resemble the vegetal plasts endowed with genetic continuity, while the smallest particles resemble viruses by their poverty in enzymes and their small dimensions. It should be noted too that experiments to prove an "autocatalytic" multiplication of ribonucleoprotein particles within the cytoplasm have already been attempted[9]. This idea tallies with a good deal of recent research on the genetic continuity of various cytoplasmic particles (cf. the kinetosomes of LWOFF, the kappa particles of SONNEBORN, etc.). If we admit such an "autocatalytic" multiplication of the various types of ribonucleoprotein particles, then the fact that the quantity of RNA synthesized at any instant is proportional to the quantity already present is endowed with a very simple explanation.

What we postulate then, in effect, is that the number of particles of each kind present at a given time is the factor which limits the rate of synthesis of similar particles. This hypothesis does not in any way prejudge the detailed mechanism of multiplication of these particles. It is possible that each, after having undergone an increase in size, divides as most likely happens in the case of the mitochondria, and as most certainly does happen in the case of the kinetosomes of ciliates[10]. But it is by no means excluded that we may be dealing with a much more complex mechanism, bearing some resemblance to phage multiplication, and involving the rupture of the particle into smaller units, the multiplication of these latter and their ultimate regroupment[11].

This last representation does not however appear the most likely. As indicated by Fig. 9, the specific radioactivity of the RNA diminishes almost simultaneously in the two fractions separated by 10 minutes ultracentrifugation at 40,000 r.p.m., as soon as an excess of non-labelled phosphate is added to the cultures. If the synthesis were effected by the multiplication of the smaller particles serving as a core during the formation of larger particles or aggregating to form these larger particles[7], we would expect the specific radioactivity of the smaller particles to diminish rapidly, while that of the larger particles would not start decreasing before a marked time lag.

The fact that the specific radioactivity of the RNA of both fractions decreases as soon as the non-labelled phosphate has been added therefore agrees with the idea of independent multiplication of small and large particles, which has already received support from the finding that the specific radioactivity of the RNA evolved, in each of the two fractions, independently of the quantities of RNA present at each instant of time.

2. The hypothesis to which we are led by our last experiments now enable us to attempt an interpretation of the results outlined in this and in the preceding work, taken as a whole.

While in the case of yeast, according to the results of CALDWELL, MACKOR, AND HINSHELWOOD[5], the quantity of RNA per mg protein N is proportional to the reciprocal of the mean generation time, *i.e.*, most likely to the rate of protein synthesis, no such relationship has been found tenable in the course of our research, as shown by the numerous examples given above. It should be recalled that ABRAMS, HAMMARSTEN, REICHARD, AND SPERBER[12] had already reported the same type of disagreement.

There can, however, be no question at the present time of abandonning the idea of a relationship between the RNA content of a cell and the rate of protein synthesis by that cell. Recent cytochemical literature provides constant confirmation of such a relationship and the abundance of RNA in all micro-organisms in rapid growth is one of the arguments in its favour.

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These difficulties can however be easily solved if we note the following points:

a. In most cases where we did not observe a relationship between the RNA per mg protein N and the rate of protein synthesis, the behaviour of the various fractions of RNA was entirely different, depending upon whether the RNA linked to the smaller particles increases much more rapidly than that which forms part of the larger particles, or whether this same fraction undergoes a large decrease while the RNA tied to the larger particles continues to increase.

b. Based upon the results obtained above with labelled phosphate, we have been led to represent the nucleoprotein particles of each type as multiplying themselves autocatalytically in a manner independent of the multiplication of other types of particles.

c. The discordant behaviour of the RNA of the various fractions only appears clearly when the cells become submitted to important modifications of their physiological state, resulting, *e.g.*, from a sudden change of the phosphate content of the medium, or the progressive decrease of its oxygen content. On the contrary, during exponential phase of growth, the RNA of the various fractions always increases with very similar rates.

Taking account of these three remarks and the fact that the results of CALDWELL *et al.*[5] are most likely based on yeast cultures in the exponential phase of growth, it appears that we may venture the hypothesis that the rate of synthesis of proteins is proportional to the quantity of RNA per mg protein N during the exponential phase only, *i.e.*, when all categories of particles are increasing at the same rate, the cells maintaining the same composition at the same time as a constant rate of growth. This simple relation would disappear, however, when, the physiological state of the cells being modified, the various categories of particles (the RNA content of which varies from 1 to 10) show rates of growth or degradation which vary according to the category considered.

The picture to which we would thus be led recalls that of SONNEBORN[13] who recognizes that the physiological conditions under which paramecia are placed may have an effect on the competition of their plasmagenes or on the relationship existing between their rate of duplication and that of the cells which contain them.

It should be noted, finally, that a linear relation between the RNA per mg protein N and the rate of synthesis of proteins could exist during any phase of the evolution of the culture, at the level of the particles and no longer at that of the entire cells. For this to be so, it would suffice that the presence of nucleic acid in a normal particle be as indispensable to its autoduplication as it is in the case of a virus[14].

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SUMMARY

1. The quantity of ribonucleic acid (RNA) present in *Polytomella* cells, the growth of which is limited by the impoverishment of the medium in phosphate, diminishes rapidly on addition of an excess of phosphate to the culture. This diminution of the quantity of RNA is accompanied by a large acceleration in protein synthesis. The initial diminution of the RNA content of the cells is followed by a rapid increase, the rate of which is by far superior to that of protein synthesis. The various nucleoprotein fractions separated by fractional centrifugation increase or degrade at markedly different rates during this evolution.

2. The study of the variations of the specific radioactivity of RNA of cells cultivated in presence

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of $^{32}P$ labelled phosphate and thereafter placed into a medium containing only $^{31}P$, shows that the quantity of RNA synthesized at any moment is proportional to the quantity of RNA present. This law is verified for the total RNA and for the RNA of particulate fractions separated by centrifugation.

3. Immediately upon the addition of the excess phosphate, the specific radioactivity decreases at a rate which is practically the same for the fraction of the smallest particles and for that of the larger ones. The smallest particles do not therefore represent an initial stage of the formation of the larger ones.

4. The significance of these results is discussed.


## RÉSUMÉ

1. La quantité d'acide ribonucléique (RNA) présent dans des cellules de *Polytomella*, dont la croissance est limitée par l'épuisement du milieu en phosphate, diminue rapidement lorsqu'un excès de phosphate est ajouté à la culture. Cette diminution de la quantité de RNA est accompagnée d'une forte accélération de la synthèse des protéines. A la diminution initiale de la teneur des cellules en RNA succède une augmentation rapide, dont la vitesse dépasse largement celle de la synthèse des protéines. Les diverses fractions nucléoprotéiques, séparées par centrifugation fractionnée, s'accroissent ou se dégradent à des vitesses fort différentes au cours de ce processus.

2. L'étude des variations de la radioactivité spécifique du RNA de cellules cultivées en présence de phosphate marqué par le $^{32}P$ et placées ensuite dans un milieu ne contenant que du $^{31}P$, montre que la quantité de RNA synthétisé à chaque instant est proportionnelle à la quantité de RNA présent. Cette loi se vérifie pour le RNA total et pour le RNA de fractions de particules séparées par centrifugation.

3. Dès l'adjonction de l'excès de phosphate, la radioactivité spécifique baisse à une vitesse presque identique dans la fraction constituée par les particules les plus petites et dans celle constituée par les particules les plus grosses. Les particules les plus petites ne représentent donc pas un stade initial de la formation des plus grosses.

4. La signification de ces résultats est discutée.

## ZUSAMMENFASSUNG

1. Die Ribonukleinsäuremenge, die in *Polytomella*-Zellen vorhanden ist, deren Wachstum durch Erschöpfung des Mediums an Phosphat begrenzt ist, nimmt rasch ab, wenn ein Phosphatüberschuss der Kultur zugefügt wird. Diese Abnahme der Ribonukleinsäuremenge wird von einer starken Beschleunigung der Eiweissynthese begleitet. Die anfängliche Abnahme des Ribonukleinsäuregehaltes der Zellen wird von einer schnellen Zunahme gefolgt, deren Geschwindigkeit bei weitem die der Eiweissynthese übertrifft. Die verschiedenen Nukleoproteinfraktionen, die durch fraktionierte Zentrifugation gewonnen werden, nehmen mit sehr verschiedenen Geschwindigkeiten während dieses Vorganges zu oder ab.

2. Die Untersuchung der Variationen der spezifischen Radioaktivität der Ribonukleinsäure von Zellen, die in Anwesenheit von mit $^{32}P$ markiertem Phosphat gezüchtet worden waren und danach in ein Medium gebracht wurden, dass ausschliesslich $^{31}P$ enthielt, zeigt, dass die Ribonukleinsäuremenge, die zu jeder Zeit synthetisiert wird, der vorhandenen Ribonukleinsäuremenge proportional ist. Dieses Gesetz gilt für die totale Ribonukleinsäure und für die Ribonukleinsäure der durch Zentrifugation gewonnenen Teilchenfraktionen.

3. Sofort nach der Zugabe des Phosphatüberschusses nimmt die spezifische Radioaktivität ab mit einer Geschwindigkeit, die praktisch die gleiche für die Fraktion der kleinsten Teilchen und für die der Grösseren ist. Die kleinsten Teilchen stellen also keine anfängliche Stufe der Bildung der Grösseren dar.

4. Die Bedeutung dieser Ergebnisse wird diskutiert.


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