A COMPARISON OF THE AMOUNTS OF DEOXYRIBONUCLEIC ACID IN THE NUCLEI OF PROTEUS P18 AND DIFFERENT FORMS OF L PHASE DERIVED FROM IT.

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We analysed the amounts of nucleic acids (NA) in Proteus P18 and in the L forms of different sizes which derive from these. Two types of L forms were studied, one stable, the other reversible. The Proteus and the L forms were both cultivated in the same hypertonic medium: broth-horse serum described previously (Mandel, Terranova and Sensenbrenner 1957). The stable L forms from the Tulasne stock culture were grown in the hypertonic medium; this allowed us to establish the kinetics of their proliferation in parallel to that of Proteus. Thus, the duration of the lag phase, of exponential growth and the beginning of the stationary phase could be defined. The reversible L forms were obtained first on a solid medium: agar-horse serum, according to the technique of Dienes (1953), then grown in a hypertonic medium. The growth curve of the stable L forms and of Proteus

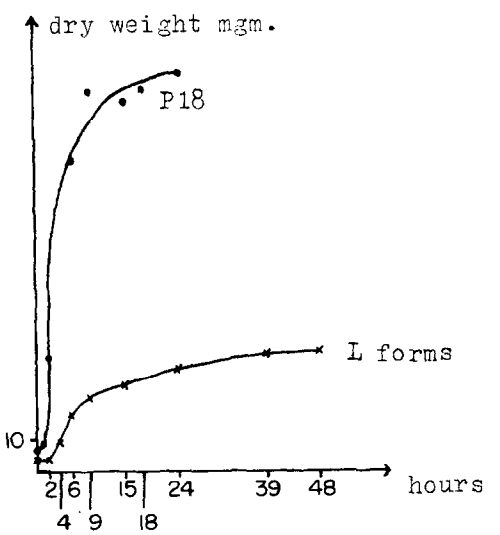

Fig. 1 Growth curve of the stable L forms and of Proteus P18.

P18 indicated that the stationary phase corresponding to 24 hours for Proteus corresponds approximately to 48 hours for the stable L forms (Fig. 1). After this delay the cultures were studied. Because of the reversibility of the unfixed L forms, the time limit for their culture was 24 hours.

After the periods mentioned above, the cultures of Proteus and of L forms were centrifuged, washed twice with 0.9% sodium chloride which leaves the L forms unaltered. After freeze-drying, lipid extraction according to Folch (1951) and removal of the acid-soluble by trichloracetic acid, the residue underwent alkaline hydrolysis and ribo- and deoxyribonucleic acid fractions were separated according to Schmidt and Thannhauser (1945). In these fractions ribose was measured by the orcinol technique according to Mejbaum (1939) and deoxyribose by the method of Dische (1930) modified by Burton (1956).

The L forms were fractioned according to the method described previously (Mandel, Terranova, and Sensenbrenner 1957) giving a fraction 1 of large sized bodies (4.5-11.4 μ), a fraction 2 of medium sized bodies (2.2-7.6 μ), a fraction 3 of small sized bodies (1.2-3.8 μ) and a fraction 4 at the limit of visibility or even filtrable. The bacteria and the bodies of fractions 1, 2 and 3 were counted in a Thoma cell using phase contrast microscopy. As to Proteus which often contains more than one nucleus, the number of nuclei was determined according to Robinow (1944).

The results of these experiments are recorded in the Table. For the stable L forms which more readily yield a heavy culture, the assays were carried out on the three fractions. For the reversible L forms measurements were made only on the third fraction which was most abundant and which seemed most interesting in view of previous results (Mandel, Feo, Sensenbrenner and Terranova 1959).

Let us point out first that Proteus in the stationary phase contains 69.2% of elements with one nucleus, 20.6% with two nuclei, 6.7% with three nuclei, and 3.5% with four nuclei, a total of 150 nuclei for 100 bacteria; this allowed us to evaluate the quantity of DNA P (deoxyribonucleic acid phosphorus) per nucleus of 1.13×10^{-9} μg.

Table

Nucleic acid phosphorus per bacteria and L forms of different sizes

	Proteus P18 24h µg/bacteria	Stable L forms 48 h			Reversible L forms 24h
		°Fr. 1 µg/body	°Fr. 2 µg/body	°Fr. 3 µg/body	°Fr. 3 µg/body
No. Exp.†	5	3	3	3	3
RNA.P.					
Mean	5.3×10^{-9} ±0.77*	260×10^{-9}	9×10^{-9}	11×10^{-9}	6.84×10^{-9}
extreme values		240-280	8-11	9-12.9	4.66-9.35
DNA.P.					
Mean	1.7×10^{-9} ±0.2*	127×10^{-9}	3×10^{-9}	0.56×10^{-9}	0.57×10^{-9}
extreme values		120-130	2-4	0.5-0.68	0.48-0.64

*Standard error was calculated only in the case of five experiments
°Fr.: fraction
No.Exp.†: number of experiments

Number of Roux-bottles, containing 120 ml of nutrient medium, used per experiment: 3 bottles for P18, 9 bottles for L forms.

We may infer from this table that the large L forms have 112 times more DNA, the medium size 2.66, fraction 3 nearly half of the bacterial nucleus DNA. But fraction 3 was metabolically more active in the stable forms and this suggests that it is the proliferating form of the L forms. In any case, as is proved by our experiments with the reversible forms, the elements of this fraction give normal bacteria.

In the light of these results, it seems that the minimal quantity of DNA necessary to give one bacterium is approximately half that of a nucleus of a Proteus in the stationary phase. Two possibilities are therefore to be considered. Either the L forms of the third fraction contain the haploid quantity of bacterial DNA, or it represents all of the genetic DNA and the adult bacteria would possess an additional DNA having a metabolic function. However, the existence of such DNA, assumed by some authors, is still to be proved.

Summary. The small forms (1.2-3.8 μ) of the L phase of Proteus contain per unit half the DNA of the adult bacteria nucleus. The possibilities to be considered are: either the L forms contain the haploid quantity of bacterial DNA or the genetic DNA, which in the normal bacterial nucleus is accompanied by DNA having a metabolic role.

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