mine[3], when tested under similar conditions, proved to be practically inert in the present system.

Attempts to grow *A. fischeri* in a 1 per cent peptone medium (freed by 'Norit *A*' treatment of its spermine content) devoid of sodium chloride but supplemented with spermine, were unsuccessful. Preliminary experiments, however, indicate that spermine exerts a definite growth furthering effect in combination with osmotically inadequate concentrations of sodium chloride (0·5 per cent).

The mechanism whereby spermine in these relatively minute amounts imitates the osmotic effects of salts is now being explored. A possible interplay with nucleic acids is suggested by the pronounced cationic characteristics of the compound and also by some reported interactions of apparent biological significance[4,5]. It may be concluded, therefore, that the present observations appear to bear out in a rather striking fashion the previously postulated role of spermine as a factor influencing cellular permeability[1,2]. Furthermore, some preliminary findings with a number of additional organisms suggest that the 'osmomimetic' function of this rather ubiquitous polyamine[6] may be of much wider applicability than hitherto anticipated.

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Phagocytosis of *Escherichia coli* Protoplasts

This communication reports the results of experiments on phagocytosis of the bacterial protoplasts prepared from *Escherichia coli* B, and on phagocytosis of bacterial vegetative forms obtained from these protoplasts by reversion.

The protoplasts were prepared by Lederberg's penicillin method[1]. Before each experiment the protoplasts were washed free of saccharose with physiological saline.

Both protoplasts and vegetative forms of *E. coli* were used for phagocytic tests made with horse leucocytes. The phagocytic reactions were carried out in various media, namely, in fresh undiluted serum, in fresh diluted (1 : 10) serum, in undiluted serum inactivated for half an hour at 56° C., and in inactivated diluted serum (1 : 10). The phagocytic indices were thus determined, both in the presence and in the absence of thermolabile components of complement. Such experiments afford an opportunity for discovering possible differences in the degree of phagocytosis of normal vegetative forms and of protoplasts, depending on the presence or absence of complement and fresh serum. The diluting of serum was necessary to avoid inhibition of the degree of phagocytosis. Such inhibition occurs in the phagocytic reaction with *E. coli*.

The phagocytic indices were determined by the method of Skurski *et al.*[2] without prior sensitization; the time of phagocytosis was 20 min. Comparison of the phagocytic indices afforded a measure of the phagocytosis of both vegetative forms and protoplasts. According to Wright's definition[3], the phagocytic index is the average number of bacterial cells phagocytized by one leucocyte (100 leucocytes were counted in each preparation).

The results are presented in Table 1. They show the existence of large differences in the degree of phagocytosis, the phagocytic indices obtained with protoplasts being significantly lower than those obtained with mother cells. Photomicrographs showed that protoplasts within leucocytes (phagocytized protoplasts) were swollen, whereas the shape of protoplasts outside the leucocytes were similar to those in control preparations.

Table 1. Phagocytosis of Protoplasts and Normal Forms of *E. coli* B, and of *E. coli* B Cells obtained by Reversion of Protoplasts

Phagocytized cells	Phagocytic indices			
	Normal serum		Inactivated serum	
	Undiluted	Diluted 1 : 10	Undiluted	Diluted 1 : 10
E. coli B (normal forms)	12·0	19·3	23·2	13·9
Protoplasts	1·9	5·0	9·5	4·7
Protoplasts after 6 hr. incubation	11·0	22·0	24·4	10·0
Protoplasts after 24 hr. incubation	10·0	19·0	23·3	9·5

Lederberg[1] reports that in favourable conditions reversion of protoplasts to the mother forms may occur. We have investigated this phenomenon in relation to phagocytosis. Reversion occurred in broth at 37° C. It is evident from Table 1 that after 6 hr. incubation of the protoplasts at 37° C. in broth, the phagocytic indices returned to the values obtained in control tests with mother cells. The differences in the values of the phagocytic indices of vegetative forms and of protoplasts were independent of the medium in which the phagocytic reaction occurred.

The protoplasts were much less readily phagocytized both in the presence of fresh serum (containing all the active components of complement), and in inactivated serum (containing inactivated thermolabile components of complement).

According to Park and Strominger[4], penicillin inhibits the biosynthesis of bacterial cell walls giving the protoplasts. The present communication indicates that the removal of cell walls leads to significant inhibition of the phagocytic reaction.

Prolongation of incubation times up to 24 hr. failed to change the phagocytic indices. Typical vegetative forms were observed after incubation for 24 hr., whereas after 6 hr. incubation both vegetative forms and protoplasts appear. Neither reversion nor phagocytic indices changes were observed with protoplasts stored at 2° C. in physiological saline and 20 per cent saccharose for several days.

This investigation is being continued, and the details will be published elsewhere.

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