THE FUNCTION OF LAG IN BACTERIAL CULTURES

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The period of lag, or the latent period through which a freshly inoculated bacterial culture passes before entering its period of rapid growth, has given rise to much speculation. A general résumé of the subject, and of the various views held concerning its nature, is unnecessary here since it has been discussed at length by several previous investigators. An excellent review has been given by Chesney (1916) in a comparatively recent paper. Although some of the earlier investigators considered the lag to be the fault of the culture medium, the works of Barber (1908), Penfold (1914), and Chesney (1916)—in which it has been amply demonstrated that transplants made from rapidly growing bacteria give no lag—show definitely that the period of reproductive latency is due to a change in the bacterial cells themselves. This has been clearly brought out by Chesney, who has shown that lag occurs when transplants are made immediately after the period of logarithmic growth is past. These and other facts led Chesney to the conclusion, which is the generally accepted one at the present time, that lag is the expression of an injury received by the cells in their previous environment.

While such an explanation in all probability is substantially correct from a practical point of view, biologically viewed it is perhaps not entirely adequate. The growth curve of bacterial cultures, of individual animals (Robertson, 1908), or plants (Reed and Holland, 1919, and others), of colonies of fruit flies (Pearl, 1921), and of human populations (Pearl and Reed, 1920), are all of the same nature, and are apparently controlled by the

same biologic laws. It is perhaps not fair to say that the animal which has stopped growing, or the population which has ceased to increase, has suffered an "injury."

Certain observations have led us to view the period of lag in bacterial cultures from a somewhat different angle. We have reported elsewhere (1922) results which indicate physiological differences between young and mature bacterial cells. It has been shown that the cells of rapidly growing cultures are more sensitive to certain harmful agents than are mature cells. For example, an exposure of one hour in a dilute solution of sodium

TABLE 1

Bacteria per cubic centimeter

TIME AFTER INOCULATION	EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 3	
	Original culture	After one hour in 5 per cent NaCl	Original culture	After one hour in 5 per cent NaCl	Original culture	After one hour in 5 per cent NaCl
hours						
0	55,500	66,000	96,000	82,500	70,500	70,500
1	51,500	55,500	80,500	60,500	75,500	62,000
1¼					68,000	57,500
1½	58,000	36,000	90,500	41,000	72,000	42,000
1¾					89,000	37,000
2	83,000	29,500	143,000	33,000	108,000	40,500
2¼					158,000	50,500
2½	106,000	21,500	255,000	16,500		

chloride in distilled water, while causing little or no injury to cells of an old culture of *Bacterium coli*, will cause a marked mortality among the cells from a rapidly growing culture of the same organism.

The hypothesis presented itself, therefore, that since old cells and rapidly growing or young cells are physiologically different, old cells would have to assume the characteristics of young cells before the culture could enter the phase of rapid growth. In other words, during the latent period the mature cells undergo a rejuvenescence and become fitted for reproduction.

In table 1 data are given from three different experiments bearing on this point. Transfers were made from cultures of *Bacterium coli*, which had been grown in 1 per cent pepton one

week at laboratory temperature, to fresh 1 per cent pepton with a reaction of pH 7.0 which had previously been warmed to 37°C. This newly transferred culture was then incubated at 37°C. Immediately after inoculation plate counts were made on the culture and at the same time 1 cc. of this culture was transferred to a 5 per cent salt solution, which was held at 20°C. At the end of one hour plates were made of the salt solution and the results calculated to the 1 cc. of the broth culture added. This procedure was repeated at stated intervals, as shown in table 1. In testing the resistance of the cells to salt solution 1 cc. from the culture was suspended in 100 cc. of the 5 per cent NaCl.

The results given in table 1 show that immediately after inoculation from an old culture, and for a period of an hour or so thereafter, the cells are not sensitive to the action of the 5 per cent NaCl solution, but after about two hours, when active reproduction is taking place, there is marked destruction of the cells when subjected to the same treatment. This is as was to be expected from our earlier work, but the point of the present paper is brought out by an examination of the results obtained when the cultures were one and one-half hours old, just before the end of the lag period. No measurable increase had taken place in the cultures at this period, but at the same time the salt treatment indicated a destruction of the cells from these cultures to a degree well beyond the probable experimental error. In other words, the mature cells from the old cultures assumed the characteristics of young cells before reproduction began. This fact, we believe, justifies the view that during the lag period the old cells undergo a biologic rejuvenescence which fits them for reproduction.

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