NOTES

ENHANCEMENT OF SURVIVAL IN VITRO OF *TREPONEMA PALLIDUM* BY ADDITION OF GLUCOSE AND MAGNESIUM

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In the course of experimental studies on media, it was observed that the addition of glucose or magnesium salts to Nelson's basal medium had an enhancing effect on the survival of virulent *Treponema pallidum* in vitro.

TABLE 1

Effect of graded amounts of glucose on survival of virulent Treponema pallidum in Nelson's basal medium

Level of Motility	Incubation Time in Days at 35 C			
	Glucose concn in mg/100 ml			
	0	10	100	200
%				
100	0	0	0	0
50	4	6	8	8
0	9	>10 (7)*	>10 (22)*	>10 (32)*

* Percentage motile on the 10th day.

TABLE 2

Effect of graded amounts of $MgCl_2 \cdot 6H_2O$ on survival of virulent Treponema pallidum in Nelson's basal medium

Incubation Time at 35 C	Motile Treponemes*			
	$MgCl_2 \cdot 6H_2O$ in mg/100 ml			
	0	200	400	600
hr	%	%	%	%
0	100	100	99	99
93	59	91	88	81
142	9	83	73	70
191	0	71	67	27
270		15	18	0

* Each figure is a report of the arithmetic mean of 6 counts (2 counts from each tube set up in triplicate).

Test organisms (virulent Nichols' strain) were harvested from rabbit testicle syphilomas by shaking slices of the infected tissue in the basal medium in an atmosphere of 5 per cent carbon dioxide and 95 per cent nitrogen. The basal medium was that recommended by Nelson and Diesendruck (J. Immunol., **66,** 667, 1951) with the exception that it contained double the specified quantity of sodium thioglycolate.

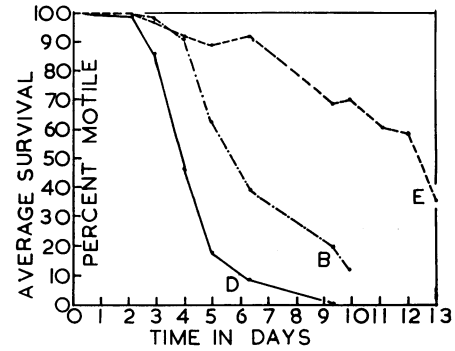

Figure 1. Comparison of the survival in vitro of virulent *Treponema pallidum* in liquid media at 35 C. *Curve D:* in Nelson's basal medium; *curve B:* in *D* plus 200 mg per cent $MgCl_2 \cdot 6H_2O$; *curve E:* in *D* plus 200 mg per cent $MgCl_2 \cdot 6H_2O$ and 100 mg per cent glucose; *average survival:* the arithmetic mean of 6 counts (2 counts from each tube set up in triplicate).

Gross cellular debris was removed by centrifugation and the supernatant was mixed with each experimental test medium in the ratio of 1 to 19. All test mixtures were incubated at 35 C in an atmosphere of 5 per cent carbon dioxide and 95 per cent nitrogen, and counts were made at intervals by dark-field examination to determine the percentage of motile organisms in each mixture.

The results condensed in table 1 indicate that glucose exerted an enhancing effect on survival

when added to Nelson's basal medium. Generally this effect was most apparent after 4 days of incubation at a glucose concentration of 100 to 200 mg per 100 ml.

Data on the effect of $MgCl_2 \cdot 6H_2O$, as recorded briefly in table 2, indicate that the most beneficial effect on survival was obtained at a concentration of 200 to 400 mg per 100 ml. Since similar findings were obtained with $MgSO_4 \cdot 7H_2O$, and the chloride ion was already present in the basal medium, the magnesium ion was considered the significant ingredient. On the basis of results with magnesium chloride, the optimal concentration of the magnesium ion was found to be 25 to 50 mg per 100 ml.

The results obtained when both glucose and magnesium chloride were added are plotted in figure 1. Over 90 per cent of the organisms remained motile for 6 to 7 days. An additive effect amounting to a threefold increase in survival time at 35 C was observed.

Further studies on the relationship between the survival time of the spirochaetes and the composition of the test media are in progress.

ISOLATION OF STAPHYLOCOCCI BY REPLICA PLATING[1]

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Lederberg and Lederberg (J. Bacteriol., **63,** 399, 1952) introduced replica plating for the selection and study of bacterial mutants. Wiseman and Sarles (J. Bacteriol., **71,** 480, 1956) employed the technique for screening of coliforms from intestinal flora. Simultaneous observation of numerous colonies from the same sample and their respective characteristics on several indicator or selective media is the primary feature of the technique. This feature offers three important advantages over usual methods for isolation of staphylococci. First, selection may be made on the basis of 6 to 12 in vitro characteristics rather than the usual colonial appearance, hemolysis, and mannitol fermentation. Second, time-consuming subculture with the hazards of variation and erratic results is eliminated. Third, many more samples can be processed.

Solid media were employed in the isolation, selection and study of staphylococci from the nares of a large number of animals and human beings. Samples were taken from within the nares with cotton applicators. These were placed in beef-infusion broth (Difco) with 7.5 per cent NaCl and incubated for 6 to 8 hr at 37 C. Streaks were made for isolated colonies on mannitol-salt agar (Difco). After 24 hr incubation, colonies on mannitol-salt agar were transferred by replica plating to fibrinogen agar, rabbit, sheep and human blood agar and staphylococcus agar no. 110 (Difco). In our hands the Lederberg technique was more versatile when the replica plating device was manipulated in ink-stamp fashion. Mannitol-salt agar provided a selective medium and information on mannitol fermentation as well as colonial characteristics. Fibrinogen agar provided a basis for determination of coagulase and fibrinolysin. The various hemolysins were observed on the blood agars. Pigment production and gelatin utilization were assessed on staphylococcus agar no. 110.

Fibrinogen agar was composed of 21.5 g of bovine fibrinogen (Nutritional Biochemicals, fraction I) in 471.5 ml physiological saline, 28.5 ml rabbit plasma, 12.5 g dehydrated beef-infusion broth (Difco), 1.75 g NaCl, and 500 ml distilled water. Other media were of standard composition.

Use of this technique means that rather than pick a colony of staphylococcus from a primary isolation plate to represent "the culture" from an individual, we are able to assay all the colonies on the primary isolation plate for a large number of characteristics before isolating a culture or cultures. It was not uncommon to isolate from 3 to 5 strains, each different from the other in at least one characteristic, from a sample taken from an individual. This technique

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