13234

Studies on the Cultivation of *Sp. gallinarum*.

I. J. KLIGLER AND DVORA KAPLAN.

From the Department of Hygiene and Bacteriology, Hebrew University, Jerusalem.

Since Noguchi's[1] classic experiments on the cultivation of spirochetes a number of workers have attempted to discover the conditions favorable to primary cultivation and continued subculture of this group of organisms. Most workers have failed to cultivate *Tr. pallida*, whereas several have reported successful results with the genus Spironema.[2-5] The shortcomings of the various media hitherto proposed are twofold: they are complicated and hence contain many variables; other workers have not always been able to repeat the cultivation with the same degree of success.

In connection with studies on fowl spirochetosis we have undertaken a systematic study of the nutritive requirements of this organism, studying each component in turn in order to establish to what extent it is required and, if required, its optimum concentration. The results of these experiments are summarized in this note.

The test of the suitability of a medium of given composition consisted in ascertaining by ultramicroscopic examination the density of growth and the viability of the spirochetes and also the ease with which passages could be carried on when minimal numbers of organisms (1 to 10 or 1 to 20 fields) were inoculated.

The medium finally obtained, which in our hands has given constant and repeatable results consists of the following ingredients:

1.	Basic salt solution*	1000.00 ml
	Proteose Difco peptone	4.00 g
	Dextrose	1.00 "
	Sodium lactate	3.00 "

[1] Noguchi, H., *J. Exp. Med.*, 1912, **16**, 199.

[2] Kligler, I. J., and Robertson, O. H., *J. Exp. Med.*, 1922, **35**, 303.

[3] Marchoux, E., and Chorine, V., *Compt. rend. Soc. de Biol.*, 1931, **106**, 1125.

[4] Galloway, J. A., *Compt. rend. Soc. de Biol.*, 1926, **93**, 1074.

[5] Landauer, E., *Compt. rend. Acad. de Sc.*, 1931, **193**, 301.

* The salt solution was made up as follows:

NaCl	5.00 g
Na_2HPO_4	2.50 "
KH_2PO_4	0.25 "
$MgCl_2$	0.30 "
H_2O	1000 ml

Thioglycollic acid **0.001 g**

2. **Before inoculation we add to each 10 cc of this medium 1.0 ml rabbit serum and 0.6 ml of sedimented chicken red cells resuspended in an equal volume of the basic salt solution.**

Salt Mixture. The basic salt mixture can be either Tyrode solution or the special salt mixture given below in which the bicarbonate is replaced by phosphate, which is a more satisfactory buffer. To the salt or Tyrode solution traces of iron and manganese salts were added. The phosphates were added in the proportion necessary to give a pH 7.7-7.8.

Aerobiosis. From the work of Kligler and Robertson[2] and Marchoux and Chorine,[3] it was apparent that these organisms are aerobic but require a lower reduction potential for initial growth than do bacteria. At the outset we were able to show that 1 mg ascorbic acid per 10 cc of medium had a favorable effect on growth. Since, however, ascorbic acid is readily oxidized we subsequently used thioglycollic acid in the concentration given above with equally good results. It is possible to use twice the amount indicated but higher concentrations have an inhibitive effect.

pH. The optimal reaction for initial growth is pH 7.6-7.7. The end reaction in media in which good growth was obtained is about pH 6.8. Growth is good also in the same medium with an initial pH 7.3, but it is less abundant. At reaction of pH 6.6 the organisms remain viable but no growth occurs. The limiting zone of reaction is, therefore, quite narrow.

Nitrogen Requirements. In the various reports in the literature there are conflicting views regarding the need of peptone. Galloway[4] did not use peptone. Marchoux found that 0.2% Witte peptone improved growth in Galloway's medium. We tested Witte, Difco and Difco Proteose peptone, in various concentrations. The results showed that best growth was obtained in media containing 0.4% proteose peptone; 0.6% gave the same results; 0.8% gave somewhat poorer growth. A really inhibiting effect of peptone was not noted. These results suggest that a certain optimal concentration of the required amino-acids is essential. The amino-acids required by this organism is now being investigated.

Carbohydrate Requirements. Galloway[4] and Marchoux and Chorine[3] found that glycogen was essential for growth and the latter authors claim that 0.1% glucose inhibits growth. We have not been able to confirm either of these findings. Our results show that a combination of lactate and glucose is most favorable for growth, that in the presence of lactate higher concentrations of glucose up to

0.2% have no unfavorable effect and that in their presence glycogen proved an indifferent substance. It is questionable, therefore, whether the favorable effect of glycogen noted by the authors mentioned above is due to its function as a nutrient. As already noted the reaction of the medium changes during growth to the acid side although the amount of sugar consumed is apparently very small. The carbohydrate metabolism is now being studied.

Serum. Various sera were tested—chicken, rabbit, guinea pig, sheep, cow and rat. Rabbit serum gave the best results, guinea pig and cow, poorest. In the absence of serum, even though blood is added, there is no, or only slight, growth. Growth improves with increasing amounts of serum until 10.0% is reached. The factor in the serum essential for growth has not yet been established, but experiments made thus far suggest that it is heat labile. Serum heated at 86-90°C for half an hour is unfavorable for growth; initial growth is poor and viability of short duration. Inactivation of serum (56°C for half an hour) favors moderate initial growth in the absence of blood although the organisms are short lived; in active serum growth is sparse unless blood is added.

Blood Cells. Chicken red cells are essential for good growth and viability. The optimum amount is 0.6 cc of a 50% cell suspension to 10 cc of medium. Rabbit blood cells are also good, but less satisfactory than those of chicken. Transplants failed rapidly when red cells were excluded. It still remains to determine whether there is some factor in the red cell other than the hemoglobin that plays a rôle in growth.

Accessory Substances. Since growth in this, as in other media, does not approximate that of bacteria we tested the effect of the addition of accessory substances. To 10 cc of medium were added 30 γ and 3 γ of thiamine, 3 γ and 30 γ of riboflavin, 1.5 and 0.3 mg β-alanine singly and in combination without noticeable effect. Glutamine and pimelic acid were also without influence.

Liquid Paraffine. A layer of 1 to 2 cm of paraffine was found by all authors to be essential. The rôle of the paraffine is most probably that of regulator of the gas exchange and the maintenance of an optimum balance, because equally good growth was obtained when paraffine was eliminated and the tubes closed with rubber stoppers.

Conclusion. On the medium described above we have been able to cultivate *Sp. gallinarum* without difficulty. The cultures grow best at 37°C, but growth occurs also at 30°C and at 39°C; at 30°C growth is slower but the viability better than at 37°C, at 39°C the

reverse is the case. The cultures reach optimum growth density in 4 to 5 days according to the size of the inoculum. If transferred to the ice box at this point they remain active for 10 days to 2 weeks. Transplants have been made as late as the 15th day of culture. One strain has been carried through 19 generations during a period of 150 days. The 18th subculture of this strain was still fully virulent for chickens and caused fatal infections.

13235

Action of Sulfamido Compounds upon *M. lysodeikticus* and Lytic and Bactericidal Activities of Lysozyme.

ERWIN NETER.

From the Bacteriological Laboratories of the Children's Hospital and the Department of Pathology and Bacteriology, University of Buffalo, School of Medicine, Buffalo, N. Y.

In contrast to many antiseptics used in surgical practice, sulfanilamide and its derivatives do not interfere with the lytic action of bacteriophage. In view of the fact that lysozyme has certain similarities to bacteriophage, experiments were carried out to determine what effects these chemotherapeutic substances have upon the lytic and bactericidal activity of lysozyme. Furthermore, the effects of sulfanilamide upon the growth of *M. lysodeikticus* were studied. Finally, experiments were done to determine whether or not lysozyme and sulfamido compounds act synergistically, as do sulfamido compounds and other anti-microbial agents, *e. g.*, immune serums, bacteriophage and certain disinfectants.

As a source of lysozyme egg white powder* was used. A strain of *M. lysodeikticus* was obtained through the courtesy of Dr. Marshall L. Snyder, Ann Arbor, Michigan. Brain heart infusion broth (Difco) was used as culture medium. Physiological saline solution and citric acid-disodium phosphate buffer, as recommended for blood cultures by Nelson,[1] were also employed.

The strain of *M. lysodeikticus* was grown on plain agar slants at 37°C. Suspensions were prepared either in physiological saline solution, distilled water or buffer solution.

---

* The egg white powder was kindly supplied by Stein, Hall & Company, Inc., New York, New York.

1 Nelson, C. I., *J. Infect. Dis.*, 1940, **66**, 113.