THE INFLUENCE OF CARBOHYDRATES ON THE CULTIVATION OF SPIROCHETES.

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While a newly isolated bacterium would usually, as a matter of bionomic routine, be tested soon for its power of fermentation of various carbohydrates, nothing systematic has been done in this direction with the group of organisms classed as spirochetes. The lack of investigation along this line may be partly due to the technical difficulties which still surround the cultivation of these organisms, and probably also to the limited number of workers in the field.

The present paper is a report of experiments which deal with the influence of starch and sugars upon pure cultures of various spirochetes. Attention has been directed to the fermentation phenomena as well as to the effect upon the growth and morphology of the organisms, since we are mindful of the fact that the addition of certain of these substances to some bacterial cultures may bring about almost incredible involution of forms[1] or sometimes induce spore formation in a bacterium which is otherwise not sporiferous.[1,2,3]

EXPERIMENTAL.

Method.

For the present work, the following substances were tested: amygdalin, arabinose, beerwort, dextrin, galactose, glycogen, glucose, inulin, lactose, levulose, maltose, mannite, raffinose, saccharose, and

[1] For example, Noguchi noticed that the colon bacillus undergoes striking morphological changes when cultivated in beerwort, producing gigantic pleomorphic forms of various shapes. Some of the large specimens measured about 30 μ in length and 2 or 3 μ in width in the swollen portion.

[2] Noguchi, H., Sporulation of the Group of *Bacillus aerogenes capsulatus*, *Proc. N. Y. Path. Soc.*, 1907, vii, 196.

[3] Fitzgerald, M. P., The Induction of Sporulation in the Bacilli Belonging to the *Aerogenes capsulatus* Group, *J. Path. and Bacteriol.*, 1911, xv, 147.

starch. These fifteen carbohydrates were added to the tubes containing the usual medium (equal parts of ascitic fluid and bouillon with a piece of fresh tissue, a total volume of 10 cc. to each tube) in a proportion of 1 : 100. Inoculations were then made into the media with each of the seven strains of *Treponema pallidum* and one each of *Treponema calligyrum*, *Treponema microdentium*, *Treponema mucosum*, and *Spirochæta refringens*. The culture tubes were covered with a layer of sterile paraffin oil and placed in an incubator at 36°C. Examinations of the spirochetes were made from time to time, for a period of 3 months with the first generation and of 2 months with the second generation.

Results of Experiments.

It was soon found that none of the spirochetes employed for the present study produced gas in the presence of these carbohydrates, and even after many weeks there was no turbidity or precipitate in any of the cultures which would indicate that the acid produced, if any, was sufficient in amount to cause coagulation of the proteins of the media. No difference in appearance could be discerned between the cultures in the sugar media and those in the sugar-free media, save the slight opalescence in the cultures of the *microdentium* in the glycogen and glucose media. When examined under the dark-field microscope, all the spirochetes were found to have grown vigorously in all the sugar media except those containing amygdalin, glycogen, glucose, and lactose. In the glycogen medium, the *microdentium* grew for several days, but quickly degenerated, though it multiplied abundantly in the beerwort medium. Glucose medium induced good growth of all the spirochetes in the first generation, but *microdentium* and some *pallidum* strains failed to grow when transferred to a new medium containing the same sugar. The effects of glycogen and glucose are similar. *Treponema mucosum*, which is similar to *Treponema microdentium* in morphology and odor-producing property, seems to be distinguished from the latter by its indifference to the addition of glucose to the medium. In Table I the results of the dark-field examination of the cultures are given as recorded after 3 weeks for the first generation and 4 weeks for the second.

The morphological features of the spirochetes were not appreciably influenced, except that the terminal appendages[4] were much more in evidence in the specimens grown in a sugar medium than in the sugar-free control cultures. In the culture of *microdentium*, the spirochetes showed numerous refractile spherical bodies, laterally attached, similar to those first described by Noguchi in the specimens of the *pallidum* cultures. Since the life of *Treponema microdentium* in sugar media is shorter than in a sugar-free medium, that is, since it grows earlier and degenerates earlier, the observed phenomenon may have an intimate connection with a process of degeneration such as plasmoptysis, where the minute granules do not represent a resistant form (spore) of the spirochetes, for they succumb to the degeneration as do the spirochetes themselves.

As may be seen from Table I, the addition of glycogen and glucose had a decidedly unfavorable effect upon the cultures of *Treponema calligyrum*, *Treponema microdentium*, *Treponema mucosum*, and some strains of the *pallidum*. *Treponema mucosum* grew most luxuriantly in all media except in that containing glycogen, and its uniform length, regular curves, and energetic motility indicate that this organism finds an ideal medium when these substances are added.

The reactions of the cultures varied from almost neutral to distinctly acid when tested with litmus paper. The acidity was strongest in the *microdentium* culture in glycogen and glucose media, and somewhat weaker in the media containing galactose, lactose, maltose, and inulin. Only a faint acidity was found in any of the cultures of spirochetes in sugar media except in glycogen and glucose media, where some of them produced a distinct acidity. In the control cultures without any carbohydrate, the reaction of the *microdentium* only was faintly acid, all the rest being practically neutral. By titrating the total acidity of 10 cc. of the fluid culture, it was found that the highest acidity as represented in the glycogen and glucose cultures of the *microdentium* was 0.1 N 4.8 cc., while in the cultures showing a weaker acidity, it was between 0.1 N 2 cc. to 0.1 N 3.2 cc. The ciphers for the control cultures varied from 0.1 N 0.8 cc. to 0.1 N 2 cc. Leaving a more exact qualitative and quantitative determination

[4] Noguchi, A Method for the Pure Cultivation of Pathogenic *Treponema pallidum* (*Spirochæta pallida*), *J. Exp. Med.*, 1911, xiv, 99.

TABLE I.

	Generation.	*T. pallidum.*							*T. calligyrum.*	*S. refringens.*	*T. microdentium.*	*T. mucosum.*
		McD.	No. 11	R.	C_2.	B29	B30	Z. A.				
Amygdalin.	1st.	+++	+	++	+++	+++	++	+++	++	+++	++	++
	2nd.	++	−	++	++	++	+	+	++	++	−	+
Arabinose.	1st.	+++	+++	+++	+++	+++	+++	+++	+++	+++	++g	+++
	2nd.	++	+++	+++	+++	++++	+++	+++	+++	+++	++	+++
Beerwort.	1st.	+++	+++	+++	+++	+++*c*	+++	+++	+++*c*	+++*c*	+++g	+++
	2nd.	+++	+++	+++	+++	+++	+++	+++	++	+++	++g	++
Dextrin.	1st.	+++	++	++	+++	+++	+++	++	+++	+++	+++	+++
	2nd.	+g	++	+	++	++	+++*c*	+++	++	+*c*	+g	+++
Galactose.	1st.	+	++	+++	+++	++	+++	+++*c*	++	++	+g	++
	2nd.	+++	+++	+++	+++	++	++	++	++	++	++	+++
Glycogen.	1st.	+++*c*	−	+++	++	++	++	++	−	+++	−	+
	2nd.	+	−	++	+	−	++	++	+	+	−	−
Glucose.	1st.	++*c*	+++	++	++	+++*c*	+++	+++*c*	+++*c*	+++*c*	++g	+++
	2nd.	++	−	+	+	++	+	−	+	++	−	++
Inulin.	1st.	+++	+++	+++	+++	+++*c*	+++	+++*c*	++	+++	++g	+++
	2nd.	+++	+++	+++	+++	+++	+++	+++	+++	+++	+g	+++
Lactose.	1st.	++	++	++	++	+	++*c*	+++	++	+++*c*	++g	+++
	2nd.	+++	+++	+++	+++	+++	+++*c*	+++	+++	+++	+g	+++
Levulose.	1st.	+++	+++	+++	+++	+++*c*	+++	+++*c*	+++	+++	+++	+++
	2nd.	+++	+++	+++	+++	++++	+++	+++	++	++	++g	+++
Maltose.	1st.	+++*c*	+++	+++	+++	+++*c*	+++	++	+++	+++	+++	+++
	2nd.	+++	+++	+++	+++	+++	+++	+++	++	+++	+	+++
Mannite.	1st.	+++	+++	+++	+++	+++*c*	+++	+++*c*	+++	+++*c*	++g	+++
	2nd.	+++	+++	+++	+++*c*	+++*c*	+++	+++	+++	+++	++g	+++
Raffinose.	1st.	++*c*	++	++	+++	+++*c*	+++	+++*c*	++	+++	++g	+++
	2nd.	++*c*	+++	+++	+++	+++	++	++++	+++	+++	+++	+++
Saccharose.	1st.	+++	+++	++	+++*c*	+++	+++	+++	+++	+++	+++g	+++
	2nd.	++++	+++	++	+++	+++	+++	+++	+++	+++	++	+++
Starch.	1st.	+++	+++	+++	+++	+++	+++	++++	+++	+++	++g	++++
	2nd.	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++

In the table the sign − indicates absence of spirochetes; +, less than 100; ++, a number between 100 and 200; +++, more than 200; and ++++, innumerable spirochetes per field under Leitz oc. 3, $\frac{1}{12}$ oil immersion. The letter *c* denotes the presence of long threads or chains of spirochetes, while *g* indicates that there were numerous granular particles due to the degenerated spirochetes.

for another occasion, we believe that the fact has been established that some sugars are attacked to a certain extent by some of the spirochetes employed for the present experiment, and especially by *Treponema microdentium*. Of course, it is impossible from the present experiment to determine how much of the acidity found should be ascribed to the split products of the sugars. While the amount of acidity in the glycogen or glucose media did not produce coagulation of the proteins, nevertheless the early disintegration of the organisms, particularly in the case of *Treponema microdentium* shows the effect of the changes associated with the acid production in the media.

SUMMARY.

Various carbohydrates have been added to the fluid cultures of different strains of spirochetes in order to determine the behavior of the latter toward the carbohydrates. In the present experiment, amygdalin, arabinose, beerwort, dextrin, galactose, glycogen, glucose, inulin, lactose, levulose, maltose, mannite, raffinose, saccharose, and starch were tested with seven strains of *Treponema pallidum* and one strain each of *Treponema calligyrum*, *Treponema microdentium*, *Treponema mucosum*, and *Spirochæta refringens*. The results may be summarized as follows:

1. In the media containing glycogen and glucose, *Treponema microdentium* did not grow as vigorously as in other sugar media, and an earlier degeneration set in. One strain of the *pallidum* and the *calligyrum* and *mucosum* showed a poor growth in the glycogen medium. Similarly, there was little growth in the second transfer of these spirochetes in the glucose medium. The growth of the spirochetes in the media containing carbohydrates other than those just mentioned was generally good, and no difference could be distinguished between these and the control cultures without any carbohydrate. The only phenomenon which might be interpreted as indicating

a favorable influence of these media upon growth was the abundant growth of the *mucosum*, which showed uniform length, regular curves, and active motility somewhat better than in the sugar-free medium.

2. The height of acidity was found in the cultures containing glycogen and glucose in the *microdentium*, amounting to 0.1 N 4.8 cc. for 10 cc. of the fluid culture. In the other sugar media the acidity varied between 0.1 N 2 cc. and 0.1 N 3.2 cc. for the same amount. In the control cultures, the acidity fluctuated from 0.1 N 0.8 cc. to 0.1 N 2 cc. There was no visible alteration in the appearance of the media after the spirochetes had grown for 3 or 4 weeks. In the case of *Treponema microdentium*, a slight opalescence developed in the glycogen and glucose media after several weeks' standing, but there was no precipitation or coagulation of the proteins of the culture media.

3. There was no unusual morphological change in the spirochetes grown in the media containing any of the carbohydrates employed. The only phenomena which should be mentioned are (*a*) the frequent presence of the terminal appendages (or projections) in the *refringens* and in most of the *pallidum* strains, and (*b*) the appearance of minute, refractile spherical bodies along the side of the spirochetes in the *microdentium* cultivated in the glucose or glycogen media. Judging from the earlier degeneration of the species in the above mentioned media, these peculiar bodies may be interpreted as indicating a phase of plasmoptysis associated with the unfavorable surroundings prior to degeneration. Experimental evidence was not found for considering these spherules as a resistant or spore form of the spirochete.

In conclusion I wish to express my indebtedness to Dr. Hideyo Noguchi for his assistance and advice.