

tween *B. paratyphosus* A, *B. paratyphosus* B, *B. paratyphosus* C; between *B. dysenteriae* Shiga, *B. dysenteriae* Flexner, *B. dysenteriae* Y (Hiss and Russell); between *B. ceylonensis* A and *B. ceylonensis* B.

B. paratyphosus B produces acidity (and often gas) in wheat, ginger, potato, sago; *B. paratyphosus* A does not touch wheat, ginger or potato; it touches sago, rice and tapioca; *B. paratyphosus* C does not touch any starch the first 3 weeks, then may produce acidity in tapioca; *B. dysenteriae* Shiga does not touch potato or ginger; Flexner produces acidity in both; Y does not touch them; Duval does not touch them. It is interesting to note that a strain received from a well known laboratory marked Flexner did not touch potato or ginger; further investigation showed this organism to be inert on maltose and to be highly agglutinated by Y serum. *B. ceylonensis* B produces acidity in ginger, corn, potato, rice; *B. ceylonensis* A produces acidity in sago, tapioca, wheat.

If further investigation should prove that all strains of these various organisms have permanent starch reactions, this might be an additional means for differentiating them.

Addendum. It is well known that different starches differ microscopically, their granules being of different appearance. They differ also in all probability biologically and biochemically, as some starches are fermented by certain microorganisms, other starches by other organisms. The testing of a given starch by means of various organisms assist in the identification of that particular starch.

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"Symbiotic Fermentation Phenomenon," Its Use in Differentiation of Microorganisms and Identification of Carbon Compounds.

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The phenomenon called symbiotic fermentation may be defined as follows: "Two microorganisms neither of which alone produces fermentation with gas in certain carbohydrates may do so when living in symbiosis or when artificially mixed." I noted the phenomenon in 1904 and 1905¹; I observed that ordinary baker's yeast in Ceylon and England, as a rule, consisted of two or more organisms

(saccharomyces and bacilli) living in symbiosis, and that baker's yeast *in toto* gas-fermented a larger number of sugars than any of the isolated organisms. In recent years I have used principally pathogenic bacilli. The phenomenon takes place using only certain organisms, one of which apparently must be capable of producing acidity (never gas) in certain carbohydrates.

Examples: I. *B. typhosus* alone does not produce gas in maltose (acidity only); *B. morgani* alone does not produce gas in that sugar (neither acidity nor gas). The mixture *B. typhosus* + *B. morgani* will produce gas.

II. *Staphylococcus aureus* (Ross Institute strain) alone does not produce gas in maltose (acidity only); *B. morgani* alone does not produce gas (neither acidity nor gas); the mixture *Staphylococcus aureus* + *B. morgani* will produce gas.

Liquid media. The phenomenon is easily put in evidence by using the test tubes containing a Durham's fermentation tube or saccharomyters. It is essential that the liquid media (maltose, mannitol, etc.) should be made with sugar-free peptone water, not broth, which as well known usually contains a small amount of glucose.

Solid media. The phenomenon is very evident when solid media are used. The solid sugar media (maltose-agar, mannitol-agar, etc.) are prepared in the usual way, but in the preparation of the agar, sugar-free peptone water must be used, not broth, because broth as a rule contains a little glucose.

When the phenomenon is positive a large number of gas bubbles will be present.

According to the very recent work of Schutze² and Harde,³ the phenomenon is due to one of the organisms causing formation of substances which are gas-fermented by the other. In the case of *B. typhosus* + *B. morgani* producing gas in maltose, this is apparently due to the formation of formic acid from maltose by *B. typhosus*; formic acid is gas fermented by *B. morgani*.

I have found the symbiotic fermentation phenomenon useful in

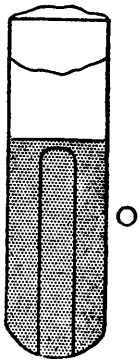
TABLE I.
Differentiation between B. dysenteriae Shiga, B. dysenteriae Flexner, B. dysenteriae Y (Hiss and Russell) by means of their symbiotic fermentation with B. morgani.

	Mannitol	Maltose
Shiga + Morgani	0	G
Flexner + Morgani	G	G
Y + Morgani	G	0

Differentiation between Shiga, Flexner and "Y" by means of the symbiotic fermentation phenomenon.

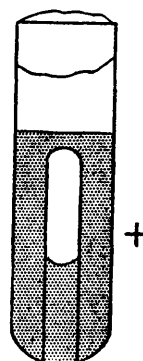
SHIGA.

Mannitol



Shiga + Morgan

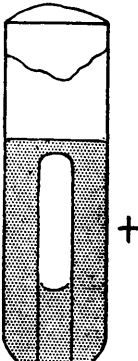
Maltose



Shiga + Morgan

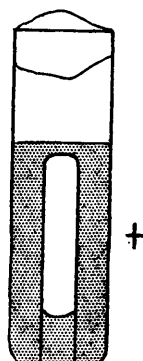
FLEXNER.

Mannitol



Flexner + Morgan

Maltose

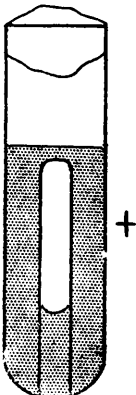


Flexner + Morgan

“Y”

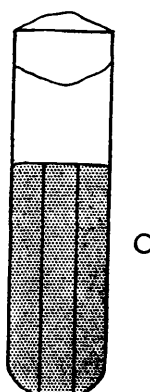
(Hiss-Russell)

Mannitol



“Y” + Morgan

Maltose



“Y” + Morgan

the differentiation of certain microorganisms, for instance, organisms of the dysentery group. *Shiga* may be differentiated from *Flexner* and *Y* in this way: *Shiga* + *Morgani* will not produce gas in mannitol; *Flexner* + *Morgani* will produce gas; *Y* + *Morgani* will produce gas. *Flexner* may be differentiated from *Y* in this way: *Flexner* + *Morgani* produces gas in maltose, *Y* + *Morgani* does not produce gas in that sugar.

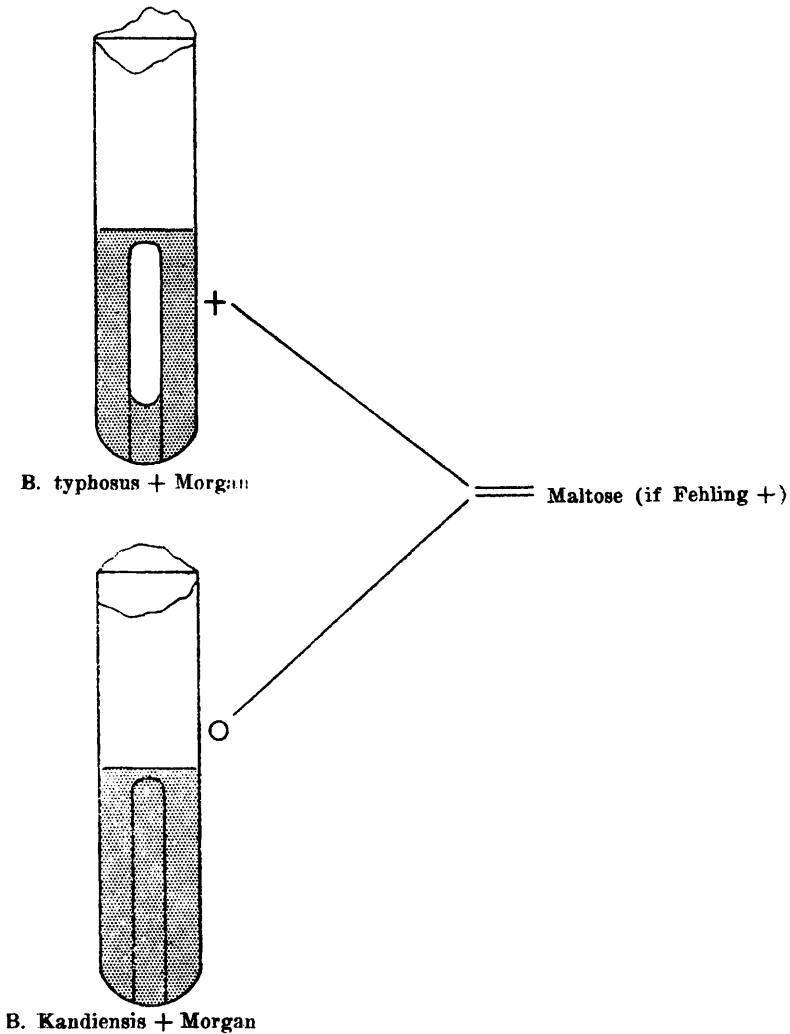
It may be said in general that the results of the symbiotic fermentation correspond to a great extent to the results obtainable with simple acid fermentation, *viz.*, there will be gas in those carbohydrates in which the organism to be identified will produce—alone—simple acidity; the presence of gas, however, is much more striking than simple acid fermentation, and moreover, it may in certain cases become evident very rapidly while the simple production of acid may be very slow. There has been a great deal of discussion on the point whether *B. dysenteriac Shiga-Kruse* produces acidity in maltose. In the older textbooks it is often described as giving acidity in that sugar; in the modern textbooks, as a rule, as not giving acidity. I have found that the symbiosis *Shiga* + *Morgani* produces gas in maltose, though in small amount, and therefore it is practically certain that *Shiga* must produce acidity in that sugar.

The experiments on this subject will be given *in extenso* in another paper. In this paper I may say that the phenomenon may be made use of in the identification and differentiation of certain Fehling reducing sugars. For instance, a Fehling-reducing sugar which is gas-fermented by *B. typhosus* + *Morgani* and not by *B. kandiensis* + *Morgani* is probably maltose. The explanation is as follows: The symbiosis *Typhosus* + *Morgani* and the symbiosis *Kandiensis* + *Morgani* produce gas in the same Fehling-reducing sugars, except maltose, in which *Typhosus* + *Morgani* produces gas and *Kandiensis* + *Morgani* does not. If a Fehling-reducing sugar, therefore, is gas fermented by *Typhosus* + *Morgani* and not by *Kandiensis* + *Morgani* the inference is that it is maltose. For the identification of maltose and other common carbohydrates, however, the mycological method I devised in Ceylon, and later worked out in England with Dr. F. E. Taylor, is much simpler¹:

TABLE II.
Showing action of the symbioses *B. typhosus* + *B. morgani* and *B. kandiensis* + *B. morgani* on the principal Fehling reducing carbohydrates.

	Glucose	Levulose	Maltose	Galactose	Lactose	Pentose
<i>Typhosus</i> + <i>Morgani</i>	G	G	G	G	0	0
<i>Kandiensis</i> + <i>Morgani</i>	G	G	0	G	0	0

Identification of maltose by means of the symbiotic fermentation phenomenon.



Recently I have found that the symbiotic phenomenon is useful in assisting in the identification of erythritol. This substance, which as well known is obtained from certain lichens, such as *Rocella tinctoria*, is a polyhedric alcohol $C_4H_6(OH)_4$ or $CH_2OH.(CHOH)_2.CH_2OH$. It is not fermented with production of gas by any organism I have tried; it is fermented however with production of gas by the symbiosis *B. kandiensis* + *B. morgani*, while it is not fermented by the symbiosis *B. typhosus* + *B. morgani*.

Of course the finding that a given substance is gas fermented by the symbiosis *B. kandiensis* + *B. morgani* and not by the symbiosis

B. kandiensis + *B. typhosus* is not sufficient to come to the conclusion that we are dealing with erythrite, but if other characteristics of erythrite are present, it may help in the identification.

The formula :

$$\begin{cases} B. kandiensis + B. morgani \dots\dots G \\ B. typhosus + B. morgani \dots\dots 0 \end{cases}$$

may indicate several other carbon compounds apart from erythrite, principally adonitol, isodulcitol, inositol. To exclude adonitol the symbiosis *B. coli* + *B. morgani* may be used; if gas is not produced it is not adonitol; to exclude isodulcitol and inositol the simple fermentation with *B. paratyphosus* B may be used; if there is no production of gas the substance is neither inositol nor isodulcitol.

This is a preliminary report.

¹ Castellani, A., and Taylor, F. E., *J. Am. Med. Assn.*, 1926, lxxxvi, 523-527. Castellani, A., *J. of Trop. Med.*, 1926, lxxx, 217-226; *J. Am. Med. Assn.*, 1926, lxxxvii, 15-22; *Proc. Soc. Exp. Biol. and Med.*, 1926, xxiii, 481; *Brit. Med. J.*, 1925, ii, 734; (1904) Meetings Ceylon Branch B. M. A. (yeast).

Fiallos, J. M., *J. Trop. Med.*, 1925, xxviii, 426-428.

² Shütze, H.—Communication by letter.

³ Harde, H.—Communication by letter.

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The use of Ionized Gas Electric Lamps as Recorders in Measuring the Velocity of the Pulse Wave.*

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Bramwell¹ and his co-workers have devised an accurate method for measuring the velocity of the pulse wave, and have made valuable studies of the factors influencing it. They have shown that the two principal factors causing increased velocity are inelasticity of the arterial walls and a high diastolic blood pressure. That is, there is an organic and a functional cause for increased velocity. The greatest need for such a method is to differentiate between the two. It seems that this might be done by observing the effect on pulse wave velocity of reduction of diastolic pressure by means of drugs. No such study has been made, due to frequent accidents with the

* Aided by a grant from the Schwartz Research Fund.