SOME OBSERVATIONS ON THE BIO-CHEMICAL CHARACTERISTICS OF BACILLI OF THE GAERTNER-PARATYPHOID-HOG CHOLERA GROUP.

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Introduction.

This paper is the result first of all of a systematic attempt to classify the very numerous cultures stocked in the Bureau of Microbiology, which were, by preliminary tests, found classifiable under the "Gaertner" group of coliform bacilli, that is to say, bacilli which are closely allied to the normal inhabitants of the intestine of man, and other animals, the colon bacilli, but which though forming gas from various carbohydrates as do these colon organisms, yet differ notably by their failure to ferment lactose and saccharose and by their action on milk, and which have very special relationships with certain definite pathological conditions in men and animals.

For the purpose of this classification I have made use, in the present contribution, entirely of the biochemical tests which attempt to orientate an organism by means of its mode of action on a series of test chemical substances familiarly known as the "sugars."

In this paper I have shown that organisms of the Gaertner group recovered from cases of paratyphoid fever—from cases of food poisoning—from cases of yellow fever in man

and from certain diseases in animals, notably from swine fever, show certain well defined biochemical attributes which demark them from most other coliform bacilli, but which, with one exception, are of little use in separating types in the group. The notable exception is that of the organism found associated with swine disease; this is distinctly marked out from the rest of the group by its apparently absolute inability to ferment arabinose, a pentose sugar readily attacked by all other members of the group.

The biochemical work in this paper has been repeated frequently, and twenty-two tests have been employed in the attempt to differentiate the group. Much of the work done here is substantially in agreement with previous workers, but, as far as I am aware, has never before been attempted by one author on such a large number of organisms and with such a large number of tests.

The use of arabinose as a differentiating agent between the swine bacilli and the remainder has not so far been noted, although at least one authority had he gone a little further must have made the discovery.

The rest of the paper is made up by a review of certain organisms similar to, but differentiable from, the true Gaertner-Paratyphoid-Food poisoning or Swine disease strains. These are especially interesting, as the reactions given by these organisms are in most cases very close to the true type, and might, unless especial care be taken, be mistaken for the true type.

A note is made of one true to type Gaertner organism isolated from the blood of a case of erysipelas, from which streptococci were also found. Also appended are the biochemical reactions of certain organisms of very different type, but which, resembling in some few respects biochemically, and also in their pathogenic relationship,

the Gaertner type, have been in the past classified in that group.

It would be advantageous if some common name were definitely agreed upon to describe the organisms now under discussion. Even the above triple title does not truly include all members of the category to be dealt with. As Gaertner was the first to describe an organism of this group, I will, in the rest of this paper, as I have upon previous occasions,[1] use the term "Gaertner Group" (or "Type") as a collective expression to describe bacilli of this order including b. paratyphosus, b. enteritidis of food poisoning, b. danysz (rat virus), b. icteroides, and b. hog cholera.

Organisms of the Gaertner group are fairly widely distributed in nature especially in association with certain pathological conditions in men and animals. That first found was isolated by Gaertner[2] from the flesh of a cow, eating of which had caused fifty-three cases of gastro-enteritis, with one death. Thereafter similar organisms have been detected, not only in a great number of food-poisoning epidemics, but in numerous other situations presently to be referred to. Nowadays it is recognised that there exists a group of bacilli morphologically similar to the bacillus typhosus (Eberth Gaffky), which have, however, many of the characteristics of bacillus coli communis, but which can be sharply enough separated from either of these, and which in themselves form a well-defined group allied by a number of characteristic properties, even if showing amongst themselves certain differences. Since the discovery of the first member of the group, they have been isolated from an ever-widening range of sources.

History of the Gaertner Group.

It is not my intention here to go fully into the past history of the Gaertner group, or to attempt to give a

complete list of the situations in which organisms belonging to it have been found, but the following short summary will give a general idea of both these sides of the question.

After the discovery in 1888 by Gaertner, very numerous observers found closely similar, if not identical, bacilli in connection with numerous outbreaks of food poisoning. Recent work bearing on the subject of food poisoning associated with a Gaertner type organism has been done by Durham (1898–1899),[3] Delepine (1903),[4] Pottevin (1905),[5] Morgan (1905),[6] MacConkey (1906),[7] Savage and Gunson (1908),[8] Bainbridge (1909, 1911),[9] MacWeeney (1910).[10] Ostertag's Handbook of Meat Inspection[11] gives a good resumé of the earlier epidemics and work done up to 1896. A convenient name for such food poisoning bacilli is bacillus enteritidis.

Organisms have also been found in association with pathological conditions, other than food poisoning, in man, which have ever been relegated to the Gaertner group.

Nocard[12] as early as 1892 found organisms associated with a disease in parrots communicable to man, known as Psittacosis, which also belong to the Gaertner group. Gilbert (1895)[13] noted the association with various morbid conditions of bacilli akin to b. coli communis, but showed that some of them differed in particulars which we now know are characteristic broadly speaking of the Gaertner group. The work of Achard and Bensuade (1896),[14] Widal and Nobencourt (1897),[15] still further showed the relationship of Gaertner type organisms to disease in man, but it was unquestionably Gwyn (1898),[16] who first recovered an organism definitely Gaertner type from a case clinically typhoid fever. In his case the Widal reaction was negative to b. typhosus down as low as $\frac{1}{1}$, whereas the isolated organism was agglutinated up to $\frac{1}{200}$. The carefully worked out data of this case form the real starting point

of the modern conceptions of the relationship of such organism to cases of paratyphoid fever, and one sometimes is inclined to resent the way German authors especially, pass over this American's work in favour of Schottmuller's first contribution. The work of Cushing (1900)[17] and Schottmuller (1900 and 1901),[18] Libman (1902),[19] Longcope (1902),[20] Johnston (1902),[21] Hewlett (1902),[22] Boycott (1906),[23] Ruge and Rogge (1908),[24] Bainbridge (1911,)[25] as well as that of other authors have clearly shown that in certain generally mild cases clinically enteric fever there can be recovered bacilli of two types which are generally known as paratyphosus A and B, the latter of which is, culturally, practically identical with Gaertner's original B. enteritidis.

In 1897 Sanarelli[26] isolated from a large number of cases of yellow fever, a bacillus subsequently shown by Reid and Carroll[27] to belong to this group, and though subsequent investigations have not confirmed his original opinion that it was the *cause* of this disease, it seems well enough established that its frequent association with the disease is substantially correct.

Another important situation in which Gaertner type bacilli are found is in association with "hog cholera" now conclusively shown by Dorset, Bolton and McBryde[28] and others, to be caused by a filter passer. Here it is almost certainly to be reckoned as playing a very important, though subsidiary part in the causation of the disease.

A filter passer caused disease of guinea pigs has also been shown by Petrie and O'Brien,[29] O'Brien,[30] to be associated with bacilli of the Gaertner group.

Normal guinea pigs, MacConkey (1906),[31] mice and occasionally normal pigs, Savage (1906-7),[32] have been shown to harbour similar bacilli. Savage also found a Gaertner type organism in a healthy calf.

Members of the group have been found in normal stools, Castellani (1910),[33] food stuffs, Mullens (1904),[34] and Savage (1906-7), and certainly in one case in a water supply, May (1911).[35]

The organism found by Thomassen (1897)[36] in calves suffering from nephritis and cystitis probably also belongs to this order.

General Characteristics of the Gaertner Group.

These organisms all belong to the great colon family by virtue of their morphology, staining reactions, nature of growth on agar and their failure to liquefy gelatin or peptonise milk.[1] Certain of their biological attributes are now generally recognised, and the following description will, I think, be an accurate enough presentation of present day views on the characteristics of organisms certainly able to be included in the group. They all agree with bacillus coli in morphology and staining reactions. They may or may not be motile. They, like b. coli, do not liquefy gelatin or peptonise milk: and they differ from b. coli, in not fermenting lactose, not clotting milk, and in producing little or no indol; and from bacillus typhosus in producing gas on glucose. I think it is recognised also that cane-sugar should not be attacked, and that acid and gas should be produced on mannit, *i.e.*, that such non-mannit fermenting organisms as Morgan's No. 1, and such saccharose fermenters as are fairly commonly found in fæces should be relegated to quite different categories.

The object of the paper is to enquire into the bio-chemical characteristics of organisms agreeing with the above description. The two principal methods used to identify and classify Gaertner type organisms are the agglutination method, including also Pfeiffer's test and the absorption

[1] Not obviously though the "clearing" referred to later may be of this nature.

method, and the bio-chemical method. Without discussing the value or otherwise of the first procedure, I intend to confine myself here to the consideration of the latter.

The history of the use of the changes produced by organisms on various chemical substances is a long one, and I do not pretend to give it more than a brief consideration, but before proceeding to my own work I wish to refer to the finding by certain observers who have specially entered into the matter.

Silberschmidt (1895)[37] noted that b. hog cholera fermented glucose with the formation of acid and gas and produced no indol.

Widal and Nobencourt (1897)[15] describe the organism they isolated from a thyroid abscess as a gram negative bacillus, non-gelatin liquefier, pathogenic for guinea pigs and mice. On glucose and mannit acid and gas were produced, lactose and saccharose were unaffected.

Thomassen's (1897)[36] nephritis organism was almost certainly Gaertner type to judge by the agglutination results. He describes it as an actively motile organism resembling b. typhosus, not liquefying gelatin, growing in an "invisible" manner on potato. Milk was not coagulated up to three weeks. Glucose was fermented, acid and slight gas being produced—no action occurred on lactose. It produced a slight trace of indol and was very virulent.

Gwyn (1898)[16] describes in some detail the organism he recovered from the blood of a case clinically typhoid. This is the first "paratyphoid" on record. It was a gram negative flagellated organism showing on gelatin "blue" colonies. It produced acid and gas on glucose, mannit and levulose (slight action on saccharose).[1] No action occurred on lactose. Milk showed "cameleonage," being first acid then neutral within ten days.

[1] This is not confirmed by later observers, and was probably due to traces of glucose in the saccharose used.

Durham (1898)[3] gives the bio-chemical characteristics of various Gaertner type cultures. He found, using 1% peptone water solutions of the "sugars," that all gave acid and gas on glucose, mannit, levulose, maltose, dextrin, and no action on lactose, cane sugar, starch, and inulin. On litmus whey, acid was followed by alkalinity. He states that with 2% peptone and 1% of either mannit or glucose, the initial acidity is followed by alkalinity.

Schottmüller (1900)[38] describes an organism from a case of enteric-like illness and differentiates it principally by serum reactions and gas formation.

Cushing (1900)[17] found a bacillus, "bacillus 'O'," from an abscess following a case of ? paratyphoid fever. Glucose was fermented and "cameleonage" and liberation of the fat (clearing) of the litmus milk occurred. The organism was actively motile. Indol was not produced in peptone, but a trace occurred in sugar free broth. He quotes a similar finding in a post-typhoidal rib abscess by Blumer.

Durham (1900)[39] in attempting to classify the colon-typhoid group by means of various sugars, etc., gives some considerable attention to the Gaertner type organisms. He distinguishes :—

I. A true Gaertner type in which he includes bs. Gaertner, Moorseele, aertryck, hog cholera, typhimurium, psittacosis and morbif bovis, etc. These he describes as forming acid and gas in glucose, and none in lactose and cane sugar. They give preliminary acidity followed by alkalinity in litmus whey.

II. A type including b. Gwyn and b. "O" Cushing. These he found, while giving abundant acid in glucose, gave free gas only under certain circumstances. Lactose was not affected.

III. A group consisting of organisms which showed a "colon-like" instead of a typhoid like morphology. Some of these gave acid but no gas on lactose not affecting saccharose. Others gave no action on lactose. The milk whey reaction differed from that of Group I. Sometimes milk was clotted.

Schottmüller (1901) describes two paratyphoid organisms and differentiates an A. and B. type. The type A. paracolon according to him rendered the milk slightly and permanently acid, while the B. type produced eventual alkalinity.

Longcope (1902),[20] describing two paratyphoid organisms isolated by him from the blood of two cases like enteric with negative Widals, found that one of these produced eventual alkalinity on milk while the other showed merely a gradual return to neutrality. Both organisms produced acid and gas on glucose and mannit, and no reaction on lactose and saccharose. Indol was negative.

Hewlett (1902),[22] describing the paratyphoid organism "Noonan," isolated from the blood of a case, notes cameleonage, acid and gas on glucose and no action on lactose and saccharose.

Libman (1902)[19] gives the reactions of a paratyphoid organism he himself recovered from the blood, bile, urine and spleen of a rather anomalous typhoid-like case: acid and gas were found on glucose and mannit, no action occurring on lactose or cane sugar. Milk was transitorily acidified and later alkali was produced.

Johnston (1902)[21] isolated from the blood of two case paratyphoid organisms, these he tested with numerous other strains, and agrees with the differentiation into A. and B. types.

Pottevin (1905)[5] gives a careful description of a Gaertner type organism isolated from ham. On glucose, mannit, maltose and galactose, acid and gas were produced, acid only on glycerine, and no action on erythrit, lactose or saccharose. The organism was pathogenic to guinea pigs and certain other laboratory animals.

Morgan (1905)[40] investigated the bio-chemical reactions of a number of Gaertner type organisms (b. Gaertner, b. aertryck, b. Moorseele, b. Hanstedt, by Breslaviensis, b. morbif bovis, b. Gunther, b. Abel, b. Renfleth, b. typhi murium, b. psittacosis, b. hog cholera Theo. Smith, b. hog cholera Evans, b. paratyphosus Schottmüller A, b. paratyphosus Schottmüller B, b. paratyphosus Brion and Kayser A.

He found that all of these organisms gave acid and gas on glucose and mannit, but no action on lactose and cane-sugar. Paratyphosus A and B, produced indol in five days. Paratyphosus A. produced acidity on litmus milk which was permanent up to a month. B. hog cholera Smith, produced no change on dulcit up to fourteen days. With the exceptions mentioned above, all produced acid and gas on dulcit and "cameleonage" on litmus milk. Morgan says that b. paratyphosus produces alkalinity less rapidly than b. Gaertner, which may do this in forty-eight hours.

Sacquepee and Chevrel (1906)[41] describing the characteristics of various paratyphoid organisms, note that on glucose, levulose, maltose, galactose, acid and gas are produced. Arabinose, dulcit, and mannit are likewise attacked, but less readily, and on arabinose in anaerobic conditions no gas is given off. Glycerin is even less readily attacked. It is not quite clear to me whether they found gas given off on the glycerin.

Boycott (1906)[23] gives the reactions of various Gaertner types: b. paratyphosus Schottmüller A (Brion and Kayser),

b. paratyphosus Schottmüller B., b. Aertryck, b. Gaertner, L.I.P.M., b. Gaertner orig. A. He says that on glucose, levulose, mannit, dulcit, maltose, dextrin, galactose, arabinose, and sorbit, acid and gas are produced, while no action occurs on lactose, cane-sugar, inulin, amygdalin, salicin, raffinose or erythrit. He states that the indol reaction is variable, but more often found in b. paratyphoid and b. aertryck than in b. Gaertner, and says that on two occasions b. paratyphosus Schottmüller A, and also the Brion and Kayser strain, showed strong alkalinity on milk after two months. He gives notes of two atypical paratyphoid organisms which gave strong acidity on milk, acid and gas on salicin, otherwise resembling the Gaertner type organisms.

MacConkey (1906)[7] describing the organism associated with an outbreak of food poisoning at Fulham, isolated from the spleen of a child and the hind limb of a rabbit, says that it corresponded in every way with those of b. enteritidis (Gaertner) group, and notes that acid and gas were produced on glucose, mannose, maltose, arabinose, raffinose, mannit, dulcit, sorbit and dextrin. (He notes reasons for the unreliability of the raffinose test). No action took place on lactose, cane-sugar, adonit, erythrit, inulin.

MacConkey states that b. L. Hume ferments adonit with the production of acid and gas. (A similar organism is later noted by the author).

Savage and Gunsen (1908)[8] describing an outbreak of food poisoning due to infected brawn, discovered a bacillus which they finally agreed to place in the Aertryck branch of the Gaertner group. It produced acid and gas on glucose, mannit, dulcit, and maltose, but showed no action on lactose, saccharose or salicin. Litmus milk was turned acid and later alkaline.

Bainbridge (1909)[9c] finds that b. paratyphoid B., b. Danysz, b. suipestifer, b. Gaertner, and b. typhi murium are culturally indistinguishable, and notes the formation by them of acid and gas on glucose, mannit, dulcit, maltose, galactose, and arabinose, while without action on lactose, saccharose, raffinose, salicin or inulin.

He makes the observation that one strain of suipestifer never produced gas on any media, and concludes on rather inadequate grounds that this was a variant form. In Table IX are shown a number of this type of organism, as tested by myself in this laboratory, from widely different sources. It is evident that the organism whatever be its relation to the Gaertner group is a well defined type.

May (1911)[35] isolated a paratyphoid type organism from water. He found that on glucose, mannit and galactose, acid and gas were produced. On dulcit and maltose, acid only was produced; no action occurred on lactose, saccharose, dextrin or glycerin.

McWeeney (1911)[10] describing the characteristics of food poisoning organisms, after noting the fermentation of glucose and dulcit with the production of acid and gas, and the fact that lactose is not attacked, says that besides showing cameleonage on litmus milk, they gradually "clear up" ordinary milk. This I will refer to later.

Summary of results of the above observers.

Glucose,	tested by	all observers		...	...	acid and gas
Lactose,	,,	,,	,,	...	...	nil
Mannit	,,	14	,,	...	...	acid and gas
Saccharose	,,	12	,,	...	...	nil
	,,	Gwyn		...	...	slight action
Maltose	,,	7	,,	...	...	acid and gas
	,,	May	,,	...	...	acid alone
Dulcit	,,	7	,,	...	...	acid and gas

,, MacConkey for hog cholera Smith, nil.

Galactose	tested by	5 observers	... ...	acid and gas
Inulin	,,	5 ,,	... ...	nil
Levulose	,,	5 ,,	... ...	acid and gas
Raffinose	,,	3 ,,	... ...	nil
	,,	MacConkey	... ...	acid and gas
Dextrin	,,	3 observers	... ...	acid and gas
	,,	May	... ...	nil
Arabinose	,,	3 observers	... ...	acid and gas
Salicin	,,	3 ,,	... ...	nil
Erythrit	,,	3 ,,	... ...	nil
Sorbit	,,	2 ,,	... ...	acid and gas
Amygdalin	,,	1 ,,	... ...	nil
Glycerin	,,	1 ,,	... ...	slight action

Litmus milk, or litmus whey, was used by the majority of the above observers and the results are in agreement. Acidity is always produced followed quickly by alkalinity in one group. In the other group the acidity is either permanent or much more slowly replaced by alkali. The indol reaction seems to have given rather variable results.

Present Investigations.

The present investigation is firstly the outcome of an inquiry into the bio-chemical properties as tested by the various media detailed in tabular form of the Gaertner type organisms stocked in the Bureau, which have either come into our possession as "standard types" from various European laboratories, or been collected from various sources as part of the work of this department. Later in this communication I give the results of the bio-chemical findings in certain organisms isolated under my direction from samples of normal human faeces, food stuffs, etc., which approximately belong to the Gaertner type. Finally I conclude with some findings in connection with certain cultures of the anaerogene class which was found in our stock labelled incorrectly.

Technique.—For all "sugar" tests 1% litmus peptone water containing the various sugars ½% strength was used, except in the case of glucose, mannit, lactose and cane-sugar, where 1% strength was substituted. The solutions were put up in test tubes with Durham's gas collecting tubes. All sugars are from Merck, except saccharose, which is ordinary brewers' crystals.

The results following are made up from the notes of nearly a year's work, during which time the majority of the tests have been applied at least twice.

In Table I. are displayed the bio-chemical reactions of thirteen cultures from European laboratories labelled para-typhosus or paracolon. This table incidentally shows the media used and the general method adopted.

The reactions of the various "sugar" media are identical in quality, though variable to some extent in quantity. Thus there is some variation in the time taken to ferment dulcit, some cultures giving acid and gas on this sugar in twenty-four hours, others taking several days. But as considerable variations occurred in individual tests of certain organisms, it was not thought advantageous to rely much on this characteristic. All, however, are able to ferment dulcit within four days.

A similar but less marked irregularity is found with maltose, galactose and arabinose. As regards morphology, motility, and growth on agar, I do not wish to dwell, except to say that I have found no useful distinguishing properties by these tests.

Litmus milk is usually regarded as a valuable means of separating two types of b. paratyphosus, A and B. Type A being said to give slight permanent acidity, Type B, "Cameleonage" or acid followed by alkaline reaction; but it will be seen that there are several variations in the type

Table I.—*Showing the reactions of 14 Paratyphoid and Paracolon Cultures from European Laboratories.*

Register Number.	Morph.	Gram.	Gelatine. 21 days.	LITMUS MILK.						All positive results shown occurred before the 4th day unless otherwise marked. All negative results shown were observed up to 3 weeks.																	Labelling of Culture.
				1 day.	3 days.	7 days.	15 days.	21 days.	28 days.	Glucose.	Mannit.	Dulcit.	Lactose.	Saccharose.	Maltose.	Dextrin.	Galactose.	Inulin.	Amygdalin.	Salicin.	Arabinose.	Raffinose.	Sorbit.	Erythrit.	Adonit.	Indol. 7 days.	
1	smb	–	–	a	a	.	.	– ?	.	AG	AG	AG	–	–	AG	–	AG	–	–	–	AG	–	AG	–	–	sl	B. paratyph. Coleman and Buxton Kral, 1906.
2	smb	–	–	a	a	a	a	...	–	AG	AG	AG	–	–	AG	–	AG	–	–	–	AG	–	AG	–	–	sl	B. paratyph. Hewlett Kral 1906.
11	smb	–	–	a	a	alk	ALK	ALK	ALK	AG	AG	AG	–	–	AG	–	AG	–	–	–	AG	–	AG	–	–	tr	B. paratyph. (A) Schottmüller Kral 1906.
3	mb	–	–	a	a	a	–	.	ALK	AG	AG	AG	–	–	AG	–	AG	–	–	–	AG	–	AG	–	–	sl	B, paratyph. (A) Brion and Kayser Kral 1906.
6	smb	-	–	–	a	a	.	– ?	.	AG	AG	AG	–	–	AG	–	AG	–	–	–	AG	–	AG	–	–	tr	B. paratyph. (A) Lentz 1911.
13	smb	–	–	a	a	a	–	.	alk	AG	AG	AG	–	-	AG	–	AG	–	–	–	AG	–	AG	–	–	tr	B. paratyph. (A) Schottmüller L.I.P.M. 1911.
12	smb	–	–	a	a	alk	ALK	.	ALK	AG	AG	AG	–	-	AG	–	AG	–	–	–	AG	–	AG	–	–	tr	B. paratyph. (B) Schottmüller Kral 1906.
42	mb	-	–	a	-	ALK	ALK	.	ALK	AG	AG	AG	–	–	AG	–	AG	-	–	-	AG	-	AG	–	–	tr	B. paratyph. (B) Brion and Kayser Kral 1906.
60	smb	–	–	a ?	-	alk	ALK	.	ALK	AG	AG	AG	–	–	AG	–	AG	–	–	–	AG	–	AG	–	–	tr	B. paratyph. (B) Lentz 1911.
68	mb	–	–	a ?	a	-	ALK	.	ALK	AG	AG	AG	–	–	AG	-	AG	–	–	-	AG	-	AG	–	–	–	B. paratyph. (B) Schottmüller L.I.P.M. 1911.
69	mb	–	–	a ?	a ?	alk	ALK	.	ALK	AG	AG	AG	–	–	AG	–	AG	–	–	–	AG	–	AG	–	–	tr	B. paratyph. (B) McWeeney L.I.P.M. 1911.
4	smb	–	–	a	a	a	–	–	ALK	AG	AG	AG	–	–	AG	-	AG	–	–	–	AG	–	AG	–	–	sl	B. paracoli Allen Kral 1906.
5	mb	–	–	a	a	a	.	ALK	.	AG	AG	AG	–	–	AG	-	AG	–	–	-	AG	-	AG	–	–	tr	B. paracoli Gwyn Kral 1906.
66	smb	–	–	a	alk ?	ALK	ALK	.	ALK	AG	AG	AG	-	–	AG	-	AG	–	–	–	AG	–	AG	–	–	tr	B. paracoli Strong Kral 1906.

Explanation.—mb = motile bacillus; smb = slightly motile bacillus; a = slight acid; AG = acid and gas; alk = slight alkalinity; ALK = marked alikalnity; sl = slight; tr = trace; – = negative or no action.

of action on milk, some showing a long continued slight acidity gradually becoming less, and probably, if left long enough, eventually producing alkalinity, others more rapidly produce strong alkalinity, while between the extreme types are found several varieties.

The indol reaction tested by the nitrite and sulphuric acid method varies considerably, but my own experience shows that this method of testing is unreliable. It has been possible since these tables were drawn up to procure the reagents for the benzaldehyde test.[42] All the Gaertner type organisms referred to in Table I. were tested by it, but *none of them gave positive indol reactions.*

The following cultures were tested in the same manner:

B. *Food Poisoning Type.*

P. 54	Gaertner	Institut Pasteur
P. 55	Gaertner	Kral 1902
P. 53	Gaertner	Kral 1904
P. 29	Gaertner	Lentz 1911
51	Abel	Kral 1906
56	Breslaviensis	Kral
57	Moorseele	Kral
61	Gunther	Kral
65	Hanstedt	Kral 1906
67	Renfleth	Kral 1906
70	Aertryck	L.I.P.M. 1911

C. *Rat Virus Type.*

P 46	Danysz 1904
P 47	Danysz from Danysz Virus Co., 1904
P 72	Danysz from Liverpool Institute 1904
P 48	Danysz from Dr. Danysz 1906
P 44	From "Azoa" (P.D. & Co.) (Bur.) 1907
P 63	From "Azoa" (P.D. & Co.) (Bur.) 1910
P 45	From Ratin (Ratin Virus Co.) (Bur.) 1907

D. *Swine Diseases.*

P 22	Swine fever	L.I.P.M.
P 23	Hog cholera	French
P 24	Hog cholera	Klein
P 25	Swine fever	Gilruth

E. *Miscellaneous.*

P 40	Typhi murium	Ray I.P.
P 49	Psittacosis	I.P.
P 50	Psittacosis	Kral 1902
P 52	Morbif bovis	Kral

As regards the reactions of the food poisoning, rat virus, and miscellaneous types above described, the vast majority show no marked variation from the B. type paratyphoid bacilli shown in the table.

Numbers P_{61} (b. Gunther Kral.), P_{47} (b. Danysz from Danysz Virus Co.) gave slight acidity on salicin after a week. Numbers P_{29} (b. Gaertner Lenz), P_5 (b. Abel Kral), P_{65} (b. Hanstedt Kral), P_{67} (b. Renfleth Kral) showed late action on arabinose. This was not affected till after a week (between 7 and 21 days). There were also irregular differences in the amount of motility displayed and in the exact rate of alkalinisation of the litmus milk.

The indol reaction in all cases was negative by the benzaldehyde method though frequently there was shown a trace of colour by the sulphuric and nitrite method.

The swine fever cultures while agreeing with the remainder in all other respects *never affected arabinose during the three weeks period of observation.* Also it may be added none of these four cultures attacked dulcit under four days, one strain taking over a week (between 7 and 21 days).

Table II. shows the biological characteristics of fifteen organisms isolated at the Bureau and provisionally classified

Table II.—*Showing the reactions of 13 locally isolated Gaertner Type Cultures.*

No.	Morph.	Gram.	Gelatine.	LITMUS MILK. 1 day.	3 days.	7 days.	15 days.	21 days.	28 days.	All positive results shown occurred before the fourth day unless otherwise marked. All negative results shown were observed up to three weeks. Glucose.	Mannit.	Dulcit.	Lactose.	Saccharose.	Maltose.	Dextrin.	Galactose.	Inulin.	Amygdalin.	Salicin.	Arabinose.	Raffinose.	Sorbit.	Erythrit.	Adonit.	Indol.	Labelling of Culture.	
P7	smb	–	–	a	a	alk	ALK	ALK	ALK	AG	AG	AG	–	–	AG	–	AG	–	–	–	AG	–	AG	–	–	tr	B. from milk (Bur) 1903.	I.
P28	smb	–	–	a	a	alk	ALK	...	A	AG	AG	–	–	a[7]	AG	–	AG	–	–	ag[7]	AG	–	AG	–	–	tr	B. from corned beef (Bur) 1906.	I.
P58	mb	–	–	a	a	alk	ALK	...	ALK	AG	AG	AG	–	–	AG	–	AG	–	–	a[7]	AG	–	AG	–	–	tr	B. from brawn (Bur) 1909.	I.
P62	mb	–	–	a	–?	alk	ALK	...	ALK	AG	AG	AG	–	–	AG	–	AG	–	–	–	AG	–	AG	–	–	–	B. from case "ptomaine" (Bur) 1911.	I.
P18	smb	–	–	a	–	alk	ALK	ALK	ALK	AG	AG	–	–	–	AG	–	AG	–	–	–	–	–	AG	–	–	tr	B. swine fever (Bur) 1909,	II.
P19	smb	–	–	a	–	alk	ALK	ALK	ALK	AG	AG	–?	–	–	AG	–	AG	–	–	–	–	–	AG	–	–	tr	B. from glands pig (Bur) 1910.	II.
P64	mb	–	–	a	...	...	...	...	...	AG	AG	AG[7]	–	–	AG	–	AG	–	–	–	–	–	AG	–	–	–	B. from glands pig (Bur) 1910.	II.
P21	smb	–	–	a	–?	alk	ALK	...	ALK	AG	AG	AG	–	–	AG	–	AG	–	–	–	–	–	AG	–	–	–	B. swine fever Rookwood (Bur) 1910.	II.
P25	mb	–	–	a	–	alk	ALK	...	ALK	AG	AG	AG[5]	–	–	AG	–	AG	–	–	–	–	–	AG	–	–	tr	B. swine fever Sydney (Bur) 1905.	II.
P26	mb	–	–	a	–	alk	ALK	...	ALK	AG	AG	AG[7]	–	–	AG	–	AG	–	–	–	–	–	AG	–	–	tr	B. swine fever Sydney (Bur).	II.
P43	mb	–	–	a	–	alk	ALK	...	ALK	AG	AG	AG	–	–	AG	–	AG	–	–	–	AG	–	AG	–	–	tr	B. calf Broughton Island (Bur) 1907.	III.
P59	mb	–	–	a	–?	alk	ALK	...	ALK	AG	AG	AG	–	–	AG	–	AG	–	–	–	AG	–	AG	–	–	–	B. ourang outang (Bur) 1910.	III.
P41	mb	–	–	a	a	alk	ALK		ALK	AG	AG	AG	–	–	AG	–	AG	–	–	–	AG	–	AG	–	–	–	B. canary (Bur).	III.

The numbers after the reaction letters indicate approximately the date of first sign of action.

as Grertner type. With the doubtful exception P_{28}, which slowly ferments saccharose, they are undoubtedly to be included under that heading. Two cultures P_{28} and P_{58}, slowly and slightly affect salicin.

With regard to Sections I. and III. of this table, no comment is necessary, but Section II. showing the reactions of locally isolated organisms from swine gives confirmatory evidence to the facts mentioned above. *Again none of these "swine fever" organisms ferment arabinose, and again the hesitancy to act upon dulcit is marked.*

It is interesting to note that no other organism out of the cultures tested except those isolated from swine failed to ferment arabinose.

Glycerine and Sodium formate.

I have made some experiments to determine whether the action of Gaertner type organisms on glycerine or sodium formate would be of any value as means of differentiation, but although both of these substances are acted upon by numerous strains the action is slow and uncertain. On glycerine, acid is produced by the majority of the strains tested, generally there is no gas formation apparent, but sometimes also a little is formed. On sodium formate at times no action occurs while at other times gas is produced. The reason for the irregularity of the action of these substances is by no means apparent.

Clearing of Milk.

I have tested the observation that milk is gradually cleared by the Gaertner type cultures and have come to conclusions practically identical with McWeeney. This change cannot be perceived well on the litmus milk media, and so I inoculated with all the European and locally isolated Gaertner type cultures described, plain sterilised milk tubes. At the end of a month the change is clearly perceptible, the great majority of the Gaertner group show

this reaction very definitely. Compared with a non-inoculated tube of milk, or better with a tube clotted by the action of b. coli, there is seen to be a distinct lessening of opacity, but as mentioned before, no obvious peptonisation as is seen in many of the proteus and other gelatin liquefiers. This reaction owing to the time taken to manifest itself, cannot, however, be considered of much practical use.

A few cultures did not show the reaction, but these are, as one might expect, only found amongst those which produce the alkaline reaction slowly, probably if left longer they too would show the change.

This characteristic of the Gaertner group, which is a very fundamental one, has been noticed by comparatively few observers (19, 10). It is probably due to a slow proteolytic action of the bacillus upon the "membranes" of proteid between the fat globules which are liberated and rise to the top.

Conclusions.

1. The bio-chemical characteristics of forty "standard" (type Gaertner) cultures principally from European laboratories have been systematically tested, using a large number of "sugars," milk, and litmus milk.

2. There is a very close similarity in the bio-chemical characteristics of the members of the group though fairly wide individual variations in degree exist.

3. On glucose, mannit, maltose, galactose and sorbit, acid and gas are rapidly produced usually within forty-eight hours, always before five days.

4. On lactose, saccharose, dextrin, inulin, amygdalin, raffinose, adonit and erythrit, no action is produced by any of the strains incubated up to three weeks.

5. On dulcit, acid and gas is produced by all the strains, but the time taken is usually longer than with the other sugars, and is especially long in the hog cholera group.

6. On arabinose, acid and gas is produced by all but the hog cholera type, generally under four days, but certain of the food poisoning group took longer—over a week to do that. *The hog cholera cultures do not attack arabinose under twenty-one days.*

7. Salicin is rendered slightly acid by three cultures; by the rest it is unaffected up to three weeks.

8. The Gaertner group produce on litmus a transient and often very feeble acidity, followed later by a reversal of the process generally shown by marked alkalinity. The subdivision into A and B types is one of degree, and cannot be strictly maintained bio-chemically as linking types are found between the extremes, but may be of interest in tracing the relationship of various strains of Gaertner type, pseudo-Gaertner and other colon bacilli.

9. Ordinary milk is after a month perceptibly cleared by the vast majority of Gaertner type strains. There is a close relationship between the degree of alkali formation and the "clearing."

10. The indol reaction tested on the seventh day in peptone water by the sulphuric acid and nitrite method is variable, but never more than slight. This test is unreliable. No indol can be demonstrated by the benzaldehyde method under identical conditions.

11. Morphology, motility, growth on gelatin agar or potato are of no assistance whatever in the grouping of these organisms.

12. Fourteen stock cultures isolated at the Bureau from local sources and tested in the same way as the European series, give results generally speaking confirmatory of the above, except that one culture from a food poisoning epidemic slowly affected saccharose. Salicin was attacked slowly by this, and by another culture from a food poison-

ing epidemic. Arabinose was attacked by *all of this series except the swine disease cultures*, thus confirming the fact noted before, that such strains do not affect this sugar, and that this may be used as a means of differentiation. The action on dulcit on certain of the Bureau strains, however, showed wider variations, for in two swine fever cultures and in one food poisoning culture, no action occurred up to three weeks.

The result of the above tests is in agreement with previous observers with two noteworthy exceptions. Whereas I find arabinose is unaffected by old cultures from swine, Bainbridge states that acid and gas are formed on this sugar. I do not think there is any possibility of error of observation on my part as the results have been repeated more than once. The only alternatives are firstly that my arabinose is not the same as Bainbridge's (mine is from Merck), secondly that he has in the preparation of his arabinose media somehow facilitated the breaking down of it by the strains used. In the preparation of my sugar media, the ordinary steam sterilisation (twice for half hour) is used, and the media is neutral, so breaking up is out of the question. Again, none of my Gaertner types affect dextrin, while three observers above quoted, found that acid and gas is produced. The above remarks *re* arabinose apply equally well to dextrin.

Notes on certain recently discovered Pseudo-Gaertner type organisms recovered from various sources.

(See Table III.)

Case I. Purcell.—This organism was recovered from the blood of a septicaemic case, the history of which is as follows:—The patient was operated upon for epithelioma of lip and neck glands; these removed, he was progressing to recovery when suddenly the temperature shot up and the pulse became very quick. An erysipelatous condition

Table III.

No.	Morph.	Gram.	LITMUS MILK.			All positive results shown occurred before the fourth day unless otherwise marked. All negative results shown were observed up to three weeks.																	Labelling of Culture.
			1 day.	3 days.	7 days.	Glucose.	Mannit.	Dulcit.	Lactose.	Saccharose.	Maltose.	Dextrin.	Galactose.	Inulin.	Amygdalin.	Salicin.	Arabinose.	Raffinose.	Sorbit.	Erythrit.	Adonit.	Indol. 7 days.‡	
8789	b	–	a	alk	alk	AG	AG	–G	–	–	AG	–	AG	–	–	AG	AG	–	AG	–	–	+	Fæces.
Caton	b	–	a	ALK	ALK	AG	AG	–	–	–	AG	–	AG	–	–	AG	AG	–	AG	–	–	+	Fæces (normal).
Pennington	b	–	a	–	ALK	AG	AG	–	a[18]	–	AG	–	AG	–	–	–	–	–	–	–	–	+	Fæces (normal).
3511	b	–	a	–	ALK	AG	AG	–	a[18]	A[18]	AG	–	AG	–	–	–	–	–	–	–	–	+	Ice cream.
Purcell	b	–	a	alk	ALK	AG	AG	AG[7]	–	–	AG	–	AG	–	–	–	AG	–	AG	–	–	–	Blood case of Streptococcic septicaemia.
*Levenberg	b	–	a	AC	AC	AG	AG	AG	A[6]	–	AG	–	AG	–	–	AG	AG	–	AG	–	–	+	Fæces (normal).
*McMillan	b	–	a	A	AC	AG	AG	AG[5]	AG[5]	AG[5]	AG	–	AG	–	–	–	AG	–	AG	–	–	+	Fæces (normal).
*Iggulden	b	–	a	A	AC	AG	AG	AG	AG[3]	–	AG	–	AG	AG	–	a	AG	AG	AG	–	–	+	Fæces (normal).
*3202 (11)	b	–	a	A	AC	AG	AG	AG	A[3]	–	AG	–	AG	–	–	AG	–	–	–	–	AG	+	Fæces (intestinal infection).
†9658	b	–	a	–	alk	AG	AG	–	–	–	AG	–	AG	–	–	–	AG	–	AG	–	–	+	Fæces (suspect typhoid carrier).

* These four are quite obviously not Gaertner type, but are shown to demonstrate the fallacy of reading the sugar reactions too early.

† This culture had only been tested one week when this table was made up. ‡ Indol tested by benzaldehyde method.

developed in head, neck, and spread to trunk (not on arms). About forty-eight hours after the onset, streptococci were recovered from blood. Three days later, from a blood culture taken in the same way as before, no streptococci were found but staphylococci and the bacillus now under discussion. Two days later the blood was sterile. The patient was treated with an autogenous vaccine of streptococci from which he seemed to derive much benefit. I am inclined to think the Gaertner type bacillus may have been a secondary invader in the lowered condition, which was quickly killed off as the patient progressed to improvement. It gives all the reactions of a typical Gaertner type organism, including a negative indol reaction tested by the benzaldehyde method on the eighth day.

Case II. (8789).—In faeces from a patient in hospital, condition unknown, but as far as I can find out not typhoid, paratyphoid or food poisoning, a Gaertner type organism was found giving the typical cameleonage on litmus milk, and not affecting lactose or cane sugar, and giving acid and gas on glucose, mannit and dulcit. The fermentation of salicin on the third day should be noted. The indol reaction (benzaldehyde method) was strongly positive in seven days.

Case III. (Caton).—This organism was recovered from the faeces of a healthy man on a ship on which there had been several cases of true enteric fever. It is distinguishable from the Gaertner type in that on salicin acid and gas are produced, also dulcit and arabinose are not attacked up to twenty-one days. The indol reaction was strongly positive after a week's incubation (benzalehyde method).

Case IV. (Pennington).—This organism was recovered from the faeces of a healthy man on the same ship. At a week's incubation it is indistinguishable from a true Gaertner type organism. By twenty-one days dulcit and

arabinose were not affected, and on lactose a trace of acid was produced. The indol reaction (benzaldehyde method) was strongly positive in seven days.

Case V. (Levinberg).—This organism was recovered from the faeces of a healthy man on the same ship. At two days' incubation it was indistinguishable from a true Gaertner type organism, but by a week on lactose acid was produced, and on salicin acid and gas. The litmus milk by this time had clotted. The indol reaction (benzaldehyde method) was strongly positive in seven days.

Case VI. (McMillan).—This organism was recovered from a healthy man on the same ship. At three days incubation it was only distinguishable from a true Gaertner type organism by the strong acidity on milk. At a week, however, the lactose and cane sugar tubes showed small amounts of acid and gas. The indol reaction (benzaldehyde method) was strongly positive in seven days.

Case VII. (3202).—In the faeces of a case of intestinal infection firstly diagnosed as typhoid, but subsequently clearly showing itself a quite different condition, was isolated amongst other organisms a gram negative colon-like bacillus. This tested, gave reactions closely akin to b. paratyphosus A. Milk was acidified slightly at first, later becoming strongly acid. At forty-eight hours the only difference from b. paratyphosus A. was that on adonit acid and gas were produced, while sorbit was not affected. Later the lactose tube became acid, and gas was not produced up to twenty-one days. The indol reaction (benzaldehyde method) was strongly positive in seven days.

Case VIII. (3511).—From ice cream, an organism agreeing with the Gaertner type in many particulars. Dulcit and arabinose were not fermented. The organism between the eighteenth and twenty-first day gave slight acid on lactose and cane-sugar. Litmus milk shows cameleonage.

The indol reaction (benzaldehyde method) was strongly positive in seven days.

It will be noted that only one of these organisms is quite typical in its reactions as compared with the previous tabulated results of standard types or cultures isolated from sources where infection with some of the recognised types might be presumed.

All of these "pseudo-gaertner" except Case I. gave a strong positive indol reaction. Four of them though at first non-lactose fermenters, attack this sugar within a week and clot milk. Two others attack lactose very slowly though giving typical cameleonage on litmus milk. Two others ferment salicin energetically and early.

Notes on Certain Anaerogenes.

The last Table No. IV. shows the biochemical reactions of certain anaerogene organisms labelled suipestifer and fowl cholera. They are of interest as being closely similar to the strain found by Bainbridge which he assumed was a variant Gaertner type.

The principal resemblance to this type is the cameleonage of milk and the non-fermentation of lactose.

Table IV.—*Showing bio-chemical reactions up to one week of anaerogene strains of Suipestifer-Fowl Cholera, etc.*

No.	Gelatine.	Litmus Milk. 1 day.	Litmus Milk. 7 days.	Litmus Milk. 3 days.	Glucose.	Mannit.	Dulcit.	Lactose.	Cane sugar.	Maltose.	Dextrin.	Galactose.	Inulin.	Amygdalin.	Salicin.	Arabinose.	Raffinose.	Sorbit.	Erythrit.	Inosit.	Adonit.	Labelling of Culture.
A41	–	a	–	alk	A	A	A	–	–	A	–	A	–	–	–	A	–	.	–	.	–	Suipestifer, Kral.
A30	...	a	–	alk	A	A	A	–	–	A	–	A	–	–	–	–	–	.	–	.	–	Suipestifer, Botany.
A36	..	a	alk	alk	A	A	A	–	–	A	–	A	–	–	–	A	–	.	–	.	–	Cholera suum, Kral.
A49	...	a	–	–	A	A	A	–	–	A	–	A	–	–	–	.	–	.	–	.	–	Fowl cholera, Sydney
A48	–	a	–	–	A	A	A	–	–	A	–	A	–	–	–	A	–	.	–	.	–	Fowl cholera, French
A47	–	a	–	–	A	A	A	–	–	A	–	A	–	–	–	A	–	.	–	.	–	Fowl cholera, Klein.

Final Conclusions.

I. No definite bio-chemical distinction can be drawn between b. paratyphosus (B), b. enteritidis Gaertner (or the other food poisoning strains), the rat virus bacilli, b. typhi murium, b. psittacosis or b. morbif bovis.

II. The bio-chemical distinction between b. paratyphosus A, and b. paratyphosus B, is one of degree only.

III. *The Gaertner type organisms from swine are separable from the other Gaertner strains by their inability to attack arabinose.*

IV. The action on dulcit of a number of the Gaertner type cultures shows considerable variations, and dulcit therefore cannot be considered of much value in differentiation.

V. Salicin is only exceptionally attacked by any of the above strains. A trace of action occurred in one rat virus strain and in two locally isolated food poisoning strains, but occurring late may possibly be due to accidental contamination.

VI. From a number of sources where infection with Gaertner type organisms was not likely, *i.e.* normal faeces and in one ice-cream, Gaertner-like organisms were found, but in every case could be differentiated bio-chemically from the true type. These pseudo-gaertners may or may not ferment salicin, which is therefore not an absolutely reliable differential test. Lactose and cane-sugar are sometimes affected late, but in some cases the indol (benzaldehyde) reaction, which is always positive in strong contrast to the invariable negative result with the true Gaertner types, was the sole distinguishing feature.

VII. In the blood of a case of erysipelatous septicaemia after wound infection, an organism bio-chemically indistinguishable from the true Gaertner type was detected.

VIII. Two cultures labelled suipestifer, one culture labelled cholera, and three cultures labelled fowl cholera, were found to be non-gas formers, approximating closely to Bainbridge's variant form. The most simple conclusion is that as these stains are quite easily mistaken for Gaertner type, unless gas formation is noted, that this error has been made by several observers. They are evidently a definite enough type and widespread in distribution in pigs and fowls. Whether of any pathological significance cannot be said.

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