THE TOXIN OF THE BACILLUS ENTERITIDIS OF GÄRTNER.

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Cases of illness arising from the ingestion of unsound food may have their origin in food that is really putrid, *i.e.* the material has all the objective appearance of contaminated food, it smells bad, has an unhealthy colour and usually a decidedly peculiar flavour. There are, however, two other forms of unsound food which are much more likely to be dangerous on account of the absence of obvious signs of putridity; in one there is only a very faint odour and the other is quite without smell. The former arises from infection with the *B. botulinus* of van Ermengem, while the latter is caused by the *B. enteritidis* of Gärtner or other allied organisms.

Due attention has been paid to the first two forms of food poisoning, but the same care has not been given to the third—the most dangerous of the three, as cooking does not destroy the toxicity. It was considered, therefore, that an investigation into the toxin of the *B. enteritidis* might be of some value, more especially as most of the previous work done on this bacillus dealt with the organism itself.

Fischer (1902) gives a very excellent résumé to date of the literature of meat poisoning, particularly of the Gärtner or Gärtner-like cases. He states that most of the infected meat comes from animals which have been ill, especially after calving, and which have been killed in order to save them from dying. Some space in this communication is devoted to the consideration of the toxin: the figures given, although somewhat incomplete, yield the main result that one can separate the toxin from a culture of the organism and that the toxin withstands boiling. He also gives a very accurate description of the clinical and post-mortem appearances of the experimental animal.

Bonhoff (1904), working with another member of the same group of organisms, the *B. typhi murium* of Loeffler, likewise obtained a toxic fluid free from bacilli which retained its toxicity after boiling.

Schottmüller (1904) found that he obtained fairly active toxic fluids after boiling cultures of *B. paratyphi*, another closely allied bacillus.

Trautmann (1903), working also with a member of this same group which he had isolated from a case of food poisoning, found that boiled cultures were toxic and that the filtrates from cultures possessed a slight degree of toxicity. According to this author there is some doubt about the heat-resisting properties of the toxin of the *B. morbificans bovis* described by Basenau (1893), and of the Moorseele bacillus of van Ermengem (1903), but the toxin of the bacillus isolated by Kaensche (1896) at Breslau resists boiling.

Van Ermengem (1903) stated that the toxin of the bacillus he found in the Moorseele outbreak did not lose its potency when heated to 100° C. or even to 120° C.

Morgan (1905) obtained a toxin from bacilli of the Gärtner type which he isolated from normal intestines. It was very toxic for guinea-pigs.

Throughout the following experiments mice were used as the test animals as they were found to be particularly susceptible to the toxin of the *B. enteritidis*, and were therefore peculiarly suited for the demonstration of the action of small doses. All injections were intraperitoneal.

The strain of the *B. enteritidis* which was used was, except where otherwise stated, a virulent strain that had been recently repeatedly passed through guinea-pigs. All filtrates were carefully tested as regards their sterility before injection.

1. In the first place an attempt was made to obtain toxic material for the experiments by growing the bacillus in ordinary broth, and, after varying periods of growth, filtering through a Chamberland filter. It was found that cultures up to three days old yielded practically no toxin, although there had been abundant growth in the flasks. The filtrates obtained from the older cultures however showed varying degrees of toxicity. The fact that filtrates from the older cultures were more toxic than those from early growths indicates that the toxin of the Gärtner bacillus is of the class of endotoxins, for it seems probable that it was only after the death of the bacilli and the

subsequent disintegration of the cell bodies that one obtained the toxin in a free state.

TABLE I. *Intraperitoneal injection of Filtrates of broth cultures.*

Age of culture	Amount injected				
	2 c.c.	1 c.c.	0·5 c.c.	0·1 c.c.	0·05 c.c.
3 days	Not dead in 40 days	Dead in 24 hrs.	Not dead in 40 days	Not dead in 40 days	Not dead in 40 days
5 days	Dead in 24 hrs.	Dead in 24 hrs.	Not dead in 40 days	Not dead in 40 days	Not dead in 40 days
7 days	Dead in 5 days	Dead in 10 days	Not dead in 40 days	Not dead in 40 days	Not dead in 40 days
9 days	Dead in 24 hrs.	Not dead in 40 days	Dead in 24 hrs.	Dead in 14 days	Not dead in 40 days

As cultivation in ordinary broth did not yield positive results, other fluid media were tried but without success. Cultures in milk were likewise tried, but these, too, gave quite unsatisfactory results.

2. As it appeared, therefore, impossible to obtain the toxin, at least in reasonable amount, by the above methods, the bacilli themselves were next dealt with. Suspensions of the bacilli in broth heated to 60° C. for 30 minutes proved to be fairly active. Positive results were also obtained with suspensions of bacilli killed by exposure to the vapour of chloroform; these experiments will be referred to in more detail later. It was not, however, until the bacilli were dealt with in mass and material obtained either by autolysis or by grinding in the cold by Macfayden and Rowland's method that one had good and reasonably constant results. The method employed in the present research was the autolytic one.

The manner in which the mass of bacteria for autolysis was obtained and the autolysis carried out was finally as follows: Two to four large (Roux) bottles containing agar were inoculated with an emulsion of the bacilli and after from 18 to 20 hours at 37° C. the resultant growth was brushed off the agar surface with the aid of a sterile brush and a small quantity of salt solution. The emulsion so obtained was then centrifugalised and the bacterial sediment mixed with a little sterile normal saline or distilled water, distributed into sterile bottles in approximately equal amounts, and, after the addition of a drop or two of toluol, placed in an incubator at 37° C. for varying periods. At the end of the period allowed for autolysis, the mass was diluted with an equal volume of distilled water or normal saline and filtered through a Chamberland filter.

As regards the time requisite for autolysis, it was found that about eight or nine days was on the whole the best. Before the expiration of that time one frequently, it is true, obtained toxic filtrates but they were not quite reliable. On the other hand if the autolysis was allowed to go on too long one obtained filtrates which were quite innocuous.

TABLE II. *Autolysis without toluol.*

Duration of autolysis	Filtrate. Amount injected					Unfiltered emulsion. Amount injected			
	2 c.c.	1 c.c.	0·5 c.c.	0·2 c.c.	0·1 c.c.	1 c.c.	0·5 c.c.	0·2 c.c.	0·1 c.c.
5 days	Dead in 24 hrs.	Dead in 24 hrs.	Dead in 24 hrs.	—	Not dead in 40 days	—	—	—	—
9 days	—	Dead in 2 days	Dead in 35 days	Dead in 11 days	Dead in 35 days	Dead in 24 hrs.	Dead in 24 hrs.	Dead in 2 days	Dead in 30 days
19 days	—	All alive 40 days after injection				Dead in 24 hrs.	Dead in 24 hrs.	Dead in 2 days	Not dead in 40 days

It is to be noted, however, that toluol, which was added in the first instance to prevent growth of any foreign bacteria which had possibly obtained entrance to the mass, apparently rendered the autolysis more rapid. In this series, as in that without toluol, too prolonged digestion caused the disappearance of the toxin from the filtrate.

TABLE III. *Autolysis with toluol.*

Duration of autolysis	Filtrate. Amount injected				Unfiltered emulsion. Amount injected			
	2 c.c.	1 c.c.	0·5 c.c.	0·2 c.c.	1 c.c.	0·5 c.c.	0·2 c.c.	0·05 c.c.
6 days	Dead in 24 hrs.	Dead in 24 hrs.	Dead in 24 hrs.	Dead in 24 hrs.	Dead in 24 hrs.	Dead in 24 hrs.	Dead in 24 hrs.	Not dead in 40 days
	1 c.c.	0·5 c.c.	0·2 c.c.	0·1 c.c.	1 c.c.	0·5 c.c.	0·2 c.c.	0·1 c.c.
10 days	Dead in 24 hrs.	Dead in 24 hrs.	Dead in 5 days	Not dead in 40 days	Dead in 24 hrs.	Dead in 24 hrs.	Dead in 24 hrs.	Dead in 2 days
19 days	Not dead in 40 days	Not dead in 40 days	Dead in 31 days	Not dead in 40 days	Dead in 24 hrs.	Dead in 24 hrs.	Dead in 24 hrs.	Dead in 2 days

In both sets of experiments the toxicity of the autolysed mass was tested as well as the toxicity of the filtrates, the fluid or emulsion being rendered absolutely sterile by heating for 30 minutes at 60° C. In every case this preparation was much more toxic than the filtrate. It will be noted that although the filtrates after prolonged autolysis were atoxic, such was not the case with the bacterial mass. The explanation of this fact may be that it is only the free toxin which is destroyed, whereas that which still remains within the bodies of the bacilli and which would naturally be effective on injection is untouched. Some

support is lent to this view by the fact that cell-juice obtained by cold grinding very rapidly deteriorates even when kept in the cold.

A few experiments were carried out to see whether one could not obtain a still more toxic filtrate in the autolytic experiments, if, instead of leaving the bottles containing the autolysing bacteria at rest, they were repeatedly shaken. The result seems rather to point to the fact that the shaking on the whole gives more toxic filtrates.

TABLE IV. *Filtrate from* 8 *days' autolysis of Gärtner's bacillus.*

	Shaken	Unshaken
Dose in c.c.	Effect	Effect
1·0	Dead in 2 days	Dead in 5 days
0·5	Dead in 2 days	Not dead in 30 days
0·2	Dead in 2 days	Not dead in 30 days
0·1	Not dead in 30 days	Not dead in 30 days
	Filtrate from 10 *days' autolysis of Paratyphoid B.*	
1·0	Dead in 24 hrs.	Dead in 24 hrs.
0·5	Dead in 24 hrs.	Dead in 2 days
0·2	Dead in 2 days	Dead in 2 days
0·1	Dead in 24 hrs.	Dead in 12 days

3. The question as to what part the medium in which the autolysis was carried out played was next considered and several experiments under this head were done. For instance the distilled water or normal saline was replaced by a 1 % sodium carbonate solution or by a 2 % cane sugar solution, but it was found that they either hindered autolysis or destroyed the toxin as soon as formed. With sugar the autolysis on inspection appeared to be far from complete, whereas with the sodium carbonate it seemed to have progressed further than usual.

TABLE V. *Autolysis of Gärtner bacilli in varying media.* (9 *days autolysed.*)

	Dose of Filtrate	
Medium	0·2 c.c.	0·1 c.c.
Aq. dist.	Died in 6 days	Died in 6 days
Sod. Carb. sol. 1 %	Not dead in 40 days	Not dead in 40 days
Cane Sugar sol. 2 %	Ditto	Ditto

The influence of the use of chloroform vapour in the preparation of the *toxic* material was considerable. Ordinary test-tube cultures 18 to 20 hours old had the wool plugs removed and corks substituted so

as to hold small pieces of cotton wool saturated with chloroform in position. The tubes were then placed in the incubator for two to three hours on their sides with the agar slightly elevated to prevent any of the chloroform running down on to the growth. In every case the bacteria on testing were found to be dead. Suspensions made from bacilli so prepared were found to be much less toxic than suspensions made with the same quantity of organisms suspended in the same amount of broth and made from cultures of exactly the same age, but sterilised by heating to 60° C. for 30 minutes.

TABLE VI. *Suspension of 4 mgm. Gärtner culture in 2 c.c. broth. Cultures killed by $CHCl_3$ vapour and by heat.*

Dose in c.c.	$CHCl_3$	Heat (30 mins., 60°)
1·0	Dead in 5 days	Dead in 4 days
0·5	Not dead in 30 days	Dead in 2 days
0·2	Not dead in 30 days	Dead in 2 days
0·1	Not dead in 30 days	Not dead in 30 days

4. As regards the effect of heat it was proved conclusively that the statement that this toxin can stand boiling without being destroyed is correct. Very many experiments were made on this point and all gave similar results.

TABLE VII. *Autolysed Gärtner bacilli heated for 30 mins. to different temperatures. Dose injected 0·5 c.c.*

Temp.	Result
60°	Dead in 2 days
80°	Ditto
90°	Ditto
100°	Ditto

TABLE VIII. *Autolysed Gärtner bacilli heated to 60° for 30 mins.*

Dose in c.c.	Autolysed 9 days	Autolysed 8 days
1·0	Dead in 24 hrs.	Dead in 24 hrs.
0·5	Ditto	Dead in 48 hrs.
0·2	Ditto	Dead in 24 hrs.
0·1	Ditto	Dead in 48 hrs.

A like result was obtained with guinea-pigs instead of mice and also when some pure cell-juice of the *B. enteritidis*, kindly given me by Dr Macfadyen, was used.

TABLE IX. *Gärtner bacilli autolysed* 10 *days, heated for* 30 *mins., dose* 1 *c.c. injected intraperitoneally into guinea-pigs of about* 150 *grms.*

Heated at 100°	Dead in 24 hrs.
,, 90°	Ditto
,, 80°	Lived
,, 70°	Dead in 24 hrs.
,, 60°	Ditto

TABLE X. *Gärtner cell-juice heated for* 30 *mins., dose* 0·1 *c.c. injected intraperitoneally into guinea-pigs of about* 350 *grms.*

Control (unheated juice)	Dead in 20 days
Heated at 100°	Dead in 21 days
,, 80°	Dead in 1 day
,, 70°	Dead in 21 days
,, 60°	Dead in 2 days

5. The question as to whether in the case of the *B. enteritidis* virulence and toxicity go hand in hand or are independent of one another came under review, but, as has proved to be the case with other organisms, these two properties were shown not to be in complete correspondence. The two strains of *B. enteritidis* used showed very well-marked differences in virulence; one had been isolated years ago and the other was the standard virulent culture used throughout these experiments and passed through guinea-pigs twice just before this experiment.

TABLE XI. *Virulence—tested on guinea-pigs.*

Old strain M.L.D. $= \frac{1}{10}$ of a loop.

Virulent strain M.L.D. $= \frac{1}{500}$ of a loop.

1 loop = 2 mg. moist culture.

The results obtained show a somewhat increased toxicity in the case of the virulent organism both when the toxic fluid from autolysis was used, and, under approximately quantitative conditions, when suspensions of equal amounts of bacilli killed by means of heat were taken.

TABLE XII. *Autolysed bacilli diluted with* 3 *volumes of dist. water heated to* 60° *for* 40 *mins.*

	Nature of culture. (Gärtner)	
Dose in c.c.	"Old"	"Virulent"
1·0	Dead in 24 hrs.	Dead in 24 hrs.
0·5	Dead in 24 hrs.	Dead in 24 hrs.
0·2	Not dead in 15 days	Dead in 24 hrs.
0·1	Not dead in 15 days	Dead in 24 hrs.

Suspensions of 2 mg. cult. in 2 c.c. broth, heated to 100° for 30 mins.

Nature of culture	Dose injected	
	0·5 c.c.	0·2 c.c.
Gärtner "Old"	Dead in 24 hrs.	Not dead in 6 days
,, "Virulent"	Dead in 24 hrs.	Dead in 2 days

6. A few experiments were carried out with the colon bacillus and the paratyphoid bacillus as regards their production of an endotoxin and in both cases results very similar to those with Gärtner were obtained. In the case of the paratyphoid B. bacillus the toxin was found to

TABLE XIII. *Paratyphoid B. bacilli autolysed for 9 days.*

Dose in c.c.	Filtrate	Unfiltered emulsion	
		Unheated	Heated to 60° for 30 mins.
1·0	Dead in 24 hrs.	Dead in 24 hrs.	Dead in 24 hrs.
0·5	Dead in 24 hrs.	Ditto	Ditto
0·2	Dead in 2 days	Ditto	Ditto
0·1	Dead in 24 hrs.	Ditto	Ditto

withstand heating to 100° as was noted by Schottmüller (*l.c.*), but 30 mins. at 100° rendered a suspension of fairly virulent colon bacilli innocuous. Schwarz (1906) has, however, recently noted that filtered

TABLE XIV. *Paratyphoid B. bacilli autolysed 11 days, heated for 30 mins., dose 0·5 c.c.*

Heated at 100° C.	Dead in 2 days
,, 90°	Dead in 1 day
,, 80°	Ditto
,, 70°	Ditto
,, 60°	Ditto

cultures of a variety of *B. coli* isolated from a spontaneous disease in a guinea-pig were very toxic to mice, guinea-pigs and rabbits after boiling. The clinical and post-mortem signs to which reference will presently be made were however somewhat different.

7. Feeding animals with infected meat was also tried, the meat being used both cooked and uncooked. As in the experiments of Fischer (*l.c.*), Trautmann (*l.c.*) and others, positive results were obtained. In the present instance meat for feeding purposes was obtained by killing a guinea-pig with a dose of living virulent culture. From the cadaver of the guinea-pig, muscle, spleen, liver, etc., were removed; in one case it was given to mice as it was, and in the other the material was mixed with a small quantity of water and then the whole was boiled for about half-an-hour. The meat, strained off from the broth, was given

to mice and the broth taken up with bread was used to feed still other animals. Death was delayed, in some cases, for quite a long period. As will be seen from the table, both animals fed with raw meat died, both with the cooked meat, and only one from the broth test. Mice from the same batch of animals, fed on ordinary diet, remained quite healthy throughout this period.

TABLE XV.

Nature of the food	Result
Raw	(*a*) Dead in 8 days (*b*) ,, 15 ,,
Cooked	(*a*) Dead in 30 days (*b*) ,, 38 ,,
Broth	(*a*) Dead in 9 days (*b*) Not dead in 50 days

P.M. signs were on the whole quite characteristic.
Gärtner bacilli recovered from heart's blood in raw feeding.

8. The clinical symptoms arising from poisoning with the Gärtner toxin are very marked and characteristic. At varying periods after the injection the experimental animal becomes comatose, and lies on its side with scarcely a movement. The eyelids are glued together. If, however, one pinches its tail or applies some other strong stimulus, the animal gives a few convulsive kicks with its four legs but quickly relapses into the comatose state. This state has been observed to last for as long as nine hours before death. There appears also to be some diarrhoea.

A post-mortem examination was always made, and the sterility of the fluids injected was always controlled by means of cultures made from the peritoneal fluid and often also from the heart's blood. In many cases organisms were obtained other than the Gärtner bacillus. A notable sign with both Gärtner and paratyphoid was the rapidity of the onset of putrefaction. The post-mortem appearances had much in common with those arising from other intestinal intoxications, *i.e.* hyperaemia of lung, spleen, suprarenals, etc. In Gärtner poisoning the filling of the small intestine with a clear yellow fluid was practically a constant phenomenon although the same was noted in some of the deaths from *coli* and paratyphoid B. infection. Another lesion which was noted fairly frequently in Gärtner animals and in one at least of the paratyphoid mice was small necrotic areas in the liver; these were also observed previously by Trautmann. Ballard (1890–1891) gives a very similar post-mortem picture for fatal cases in man.

The clinical appearances of paratyphoid B. intoxication so far as could be observed were very like those described for Gärtner poisoning,

but in the case of *coli* infection in the present experiments neither the narcosis nor the gluing together of the eyelids was noted.

In conclusion I wish to offer my best thanks to Dr Macfadyen for much kindly advice and criticism during the course of the above research.

SUMMARY OF RESULTS.

1. Cultures of Gärtner's bacillus grown on broth do not excrete any large amount of toxin into the fluid medium.

2. Suspension in broth made from ordinary agar cultures and killed by means of heat (30 mins. at 60° C.) are fairly toxic.

3. The most toxic preparations were obtained by autolysis of the bacilli, especially in the presence of toluol.

4. Cultures grown on agar and killed by means of chloroform vapour lose their toxicity to a considerable extent.

5. Autolysis takes place best in the presence of distilled water or of normal saline.

6. Autolysed material sterilised by heat is more toxic than the filtrate obtained from the same digest.

7. Shaking the bottles during the process of autolysis increases the yield of toxic substances.

8. Gärtner toxin withstands heating to 100° C. for 30 mins.

9. Paratyphoid B. bacillus cultures on autolysis yield a filtrate quite as toxic as those from the Gärtner bacillus itself.

10. Paratyphoid toxin is also heat resistant (100° for 30 mins.).

11. A colon bacillus gave a toxin which was fatal to mice. It was not heat resistant.

12. Feeding experiments with Gärtner-infected meat, both cooked and uncooked, proved successful.

13. So far as the present experiments go the connection between virulence and toxicity does not appear to be very definite.

14. The Gärtner bacillus contains a toxin of the endotoxin type as is shown in comparing results 1 and 3. This toxin gives rise to very definite clinical symptoms of which the gluing together of the eyelids and the prolonged narcosis before death are the most notable. The post-mortem signs have much in common with those arising from other intestinal intoxications.

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