EXPERIMENTAL EVIDENCE OF A HEAT-RESISTANT GASTRO-INTESTINAL IRRITANT PRODUCED BY BACILLI OF THE SALMONELLA GROUP.

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The Salmonella group contains the organisms which are mainly responsible for outbreaks of food poisoning and the possibility of the production by these strains of bodies which are toxic, or which act as gastro-intestinal irritants, when administered by the oral route, is of practical as well as theoretical interest. This is especially the case if it can be shown that these irritant substances are heat resistant.

The importance of the subject has led to extensive investigation the results of which are very discrepant. It has not even been settled if members of this group are capable of producing true exotoxins, although existing evidence is rather against this view. It is not proposed to give a detailed summary of the experimental data but a number of important investigations will be referred to under different sections.

I. Experiments with mice.

Three series of investigations with mice may be mentioned before discussing my own results.

Bahr and Dyssegaard (1927) worked with 28 strains from various sources, including *B. paratyphosus* B, *B. voldagsen*, *B. aertrycke*, *B. enteritidis* and a number of Ratin types. Only a few were associated with food poisoning cases and many had been isolated many years previously. The only culture medium used was a meat extract peptone broth with 4 per cent. glucose. The cultures were incubated for 12–15 days at 30° C. Under these conditions the authors found that heated cultures were regularly toxic to mice by intra-peritoneal injection (0·2–0·5 c.c.), but that when fed in 5 c.c. doses, *i.e.* as much as ten times the intra-peritoneal fatal dose, no harmful effects were observable. Rats, rabbits and monkeys yielded similar results.

Geiger and Meyer (1928) working with *B. aertrycke* in suitable media (not indicated in the paper) incubated at 37° C. for 3–4 days, report that boiled cultures were rapidly fatal when fed to mice in doses as small as 0·5 c.c. Definite symptoms were exhibited in from 1 to 3 hours and death resulted in from 6 to 24 hours. They describe typical post mortem appearances.

Branham, Robey and Day (1928) used 17 Salmonella strains, but all isolated 6 years or longer. 100 per cent. of mice fed with living cultures died. At least 7 different culture media were tested and they found great variations, some being quite non-toxic. Combining all their results they found

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that 40 per cent. of mice fed with heat-killed broth cultures died and 41 per cent. of mice died when fed with similar cultures, but filtered through a Berkefeld filter. None exhibited diarrhoea, and death when it occurred was between the 5th and 14th day. With filtrates of different ages the younger were the most toxic, *i.e.* 24 hours' filtrates gave 40 per cent. fatality, 3 days' 20 per cent., 7 days' 10 per cent., 14 days' 0 per cent. With a beef heart medium culture, boiled for 10 minutes and fed to mice, a mortality as high as 70 per cent. in one series and 95 per cent. in another was obtained. Agar cultures 24 hours old emulsified in normal saline and boiled were non-toxic when fed to mice.

The discrepancies between these three investigations are very apparent and in an endeavour to clear them up a long series of experiments was carried out.

Work on the Salmonella group extending over 27 years has proved to me how important it is to work with virulent strains and with recently isolated strains. Salmonella strains lose, I believe, their ability to produce heat-resistant toxins when cultivated for a long time upon laboratory media. The work was done with fairly recently isolated strains, unless for control purposes comparison with long isolated strains was desired, and all were passed through guinea-pigs before being used for toxin production.

The four strains *B. aertrycke*, *B. enteritidis*, Newcastle and Newport were kindly sent me by Dr W. M. Scott of the Ministry of Health. The aertrycke strain was isolated in July 1930 from a human food poisoning outbreak. The Newcastle strain was isolated in August 1930 from beef causing a non-fatal outbreak of food poisoning. Dr Scott has described this type and shown it to be closely related to *B. enteritidis*. The *B. suipestifer* strain is of suipestifer (American) type and was kindly sent to me by Dr Menton, who isolated it from a food poisoning outbreak in Staffordshire in 1930. The Newport strain was isolated by Dr Scott from a fatal case in a food poisoning outbreak at High Wycombe in September 1932. The sources of the other strains are indicated in the text.

As most of this work was carried out in 1930 the strains when used were quite recently isolated.

Evidence of the presence of a substance toxic to mice by the mouth in sterile boiled Salmonella cultures.

It will be simplest to consider first the positive experimental evidence and subsequently to discuss the various factors influencing the production of such a substance. After a considerable amount of negative work a medium was obtained which gave approximately 90 per cent. of positive results. This medium was a comparatively simple one consisting of fresh beef, chopped up, mixed with tap water and after boiling, brought to a reaction of *p*H 6·0 to 6·1.

In many batches this was the natural reaction. An essential point appeared to be the *p*H and a little water more or less made no difference. This medium is referred to as *standard medium*. Most batches so made up were found satisfactory, but from time to time a batch apparently identical seemed almost non-toxic. I have been unable as yet to explain these anomalies.

Table I.

All mice feeding experiments with cultures on standard medium were grown for 2 days at 37° C. (unless otherwise stated). All cultures were heated for 30 minutes at 100° C. "Meat" means that the meat itself was fed; "juice" that 2 c.c. of juice was fed on bread.

Strain	Material fed	No. of mice	Results
Aertrycke	Meat	7	Typ. s.* Death within 36 hrs.
,,	Juice	9	6. Typ. s. Death in 2–4 days 1. Illness only on 6th day and death on 7th day 2. No ill effects noticeable
,,	Meat (24 hrs. culture)	1	Typ. s. Death within 2 days
,,	Meat (7 days)	1	Typ. s. Death within 26 hrs.
Enteritidis	Meat	2	Typ. s. Death within 48 hrs.
,,	Juice	2	Typ. s. Death within 2–3 days
Newcastle	Meat	2	Typ. s. Death within 2 days
Suipestifer	Meat	2	Typ. s. Death within 2–3 days
,,	Juice	2	One, no observable effect; one death within 3 days
Newport	Juice	2	Typ. s. and death 2–3 days
Aertrycke (Meirelbeek)	Meat	1	Delayed onset of symptoms; death on 7th day
,,	Juice	3	One died on 4th day; two unaffected
B. paratyphosus B (virulent strain)	Meat	1	Typ. s. Death in 26 hrs.
B. paratyphosus B (old lab. strain)	Juice	2	No observable effect

* Typical symptoms.

The results obtained with this standard medium are summarised in Table I. Including all the 37 experiments, positive results were obtained in 30 (81·1 per cent.). With meat alone the positive results were 100 per cent.: with juice alone they were 65 per cent. The table includes mice fed with strains of definitely low virulence. If these six experiments are excluded the percentage of positive results is 90·3 and with juice tests only, 80 per cent. The meat dose is of course enormous (about 4 g.), but the significance of this is lessened by the results obtained with other media, which are described in Table II.

When virulent strains were used the symptoms nearly always occurred in the following sequence. The mice (unfed during the previous 18 hours) were usually fed between 10 and 11 a.m. As a rule no symptoms were noted during the working day, but often the animals were quiet and occasionally the hair was ruffled; in some cases there was considerable bowel action. Seen at 9 a.m. next day the mice were generally alive but there had been marked diarrhoea during the night, the hair was ruffled, the animals hunched and quiet, usually the hind legs were splayed with definite loss of power almost amounting to paralysis. These conditions persisted, death in typical cases resulting within 48 hours. A few autopsies were made, but apart from a few petechial haemorrhages in the stomach nothing very definite was observable with the naked

eye. Salmonella bacilli were invariably absent from the intestines or internal organs (heart blood and liver).

The high proportion of positive results with this medium makes it possible to consider some of the factors involved.

The influence of the nutrient medium employed.

It is remarkable that this definite and fatal irritant or toxic action is entirely dependent upon the use of a suitable culture medium. My first experiments with two other media were negative. Using the same strains, all other conditions being precisely similar, negative results were obtained with a number of other media. These findings with a brief note on the media used are set out in Table II.

Table II. *Results with media yielding negative results with mice.*

Medium	No. of mice fed	No. of mice killed	Percentage fatal
A	6	0	0
B	8 (all fed with meat)	0	0
C	14 (10 meat; 4 juice)	4	28·5
D	3	0	0
E	3 All fed with meat	0	0
F	2	0	0
	36	4	11

Virulent Salmonella strains (*B. aertrycke* or *B. enteritidis*) employed in every instance.

In more than half the experiments the mice were maintained at a high temperature (usually 25–30° C.) to facilitate any gastro-intestinal effect.

Notes on the media:

Medium A. A liver, peptone broth made slightly alkaline.

„ B. A meat-water medium made up like the "standard" medium but quite non-effective for unknown reasons. *p*H not estimated.

„ C. A meat-water medium with 1 per cent. peptone and made slightly alkaline. Salmonella bacilli grow very abundantly in this medium.

„ D. Heart meat-water medium made up to a *p*H 7·0.

„ E. „ „ 6·8.

„ F. „ „ 7·0 and with 1 per cent. glucose added.

The successful standard medium is an acid medium and in it Salmonella strains do not multiply abundantly. As a growth-promoting medium it is far inferior to most of those used in Table II, Media A and C for example yielded very abundant growth. Climatic conditions also could be ruled out, as although the experiments were spread over a long period, parallel experiments with two different media carried out at the same time showed the same striking differences. With these media yielding negative results at least half of the mice were kept at about 30° C. for most of the time to see if this influenced the results, as the work of Arnold (1929–30) suggests that this is an important factor.

The following is a typical parallel experiment. *B. aertrycke*, after two recent consecutive guinea-pig passages, grown in standard medium and in two tubes of Medium C, both for 2 days and each boiled for 30 minutes. Of 4 mice fed, the mouse fed with the meat and that with 2 c.c. juice of the standard medium both died with typical symptoms within 48 hours, while

2 mice each fed with the meat and juice of Medium C showed no symptoms although observed daily for 12 days.

It will be noted that only 4 out of 36 mice died, all with Medium C.

The positive results were obtained with a medium which initially was definitely acid. In this medium *B. aertrycke* first produces more acid and only reaches neutrality after 4 days' growth at 37° C. The following are actual figures: at start, *p*H 6·1; 24 hours, 5·7; 48 hours, 5·8; 3 days, 6·8; 5 days, 7·3. From 5 days and onward the reaction is definitely alkaline. The same strain in the peptone meat medium (Medium C) gave the following *p*H figures: start, 7·4; 2 days, 7·1; 4 days, 8·0; 5 days, 8·2; 8 days, 8·4; 9 days, 8·4; 11 days, 8·6; 15 days, 8·6.

These results suggest that the irritant is only produced in an acid medium. In one experiment a standard medium tube was adjusted to *p*H 7·1 by alkali addition and inoculated with *B. aertrycke*. The boiled 2 days' growth was however fatal to a mouse with marked diarrhoea and death in about 24 hours. A fresh batch of medium was adjusted to *p*H 7·1 (*i.e.* approximately neutral) and this yielded positive results in one experiment in which a mouse was fed with the meat. The essential factors are obviously complicated and I have not found time to investigate them.

With another medium however positive results were obtained as uniformly as with the standard medium. This medium was litmus milk. These results are shown in Table III.

Table III.

All feeding experiments with cultures boiled for ½ hour. Dose 2 c.c.

Strain	Medium		No. of mice	Result
Aertrycke	Litmus milk	2 days	4	All died 24 hrs.—4 days with marked diarrhoea
Enteritidis	,,	2 ,,	2	Death within 48 hrs.
Meirelbeek	,,	2 ,,	1	Ill after 24 hrs. Died on 4th day
Aertrycke	,,	14 ,,	1	Typical symptoms. Death within 2 days
Aertrycke	Whey		1	Death in 4 days
			9	All died

The *p*H of the milk could not be obtained by colorimetric means, but whey was made up and tested and gave *p*H 6·7. This was equally active. The 14-day old milk was markedly alkaline and the experiment was to test if the irritant principle was destroyed by the alkali.

Note. About a year later 3 mice feeding experiments, (*a*) with the above aertrycke strain, (*b*) with the Newport strain and (*c*) with a fresh *B. aertrycke* (isolated but a month previously in the laboratory from a fatal case of food poisoning) were all negative; 2-day old, ½-hour boiled cultures being used. Evidently therefore litmus milk is not invariably positive and may show irregular results like the meat medium.

The influence of heat treatment upon the toxic products of Salmonella strains.

It is well authenticated that heat treatment at 70–80° C. or even boiling at 100° C. for 20–30 minutes does not diminish the toxicity of these products.

Certain facts in relation to food poisoning outbreaks suggest that the action upon the gastro-intestinal tract may be enhanced through boiling.

As regards mice the following parallel feeding experiments with two strains of Salmonella were conducted as set out in Table IV. In every case the standard meat-water medium was used with growth for 2 days at 37° C. and the meat itself was fed.

Table IV.

	Strain	Parenteral virulence	Unheated meat	Heated 1 hr. at 65° C.	Heated 30 min. at 100° C.
1.	*B. aertrycke*	Fairly high	No symptoms. B. not recovered when killed after 8 days	Typ. s. Death 2 days	Typ. s.* Death 2 days
2.	,,	,,	Ill, died 2 days. B. recovered from liver not heart blood	,,	,,
3.	,,	After another guinea-pig passage	Death 2 days. Only recoverable from intestine	Not affected	Typ. s., but recovered
4.	,,	,,	Death 24 hrs. Only recoverable from intestine	Death 48 hrs.	Death 48 hrs.
5.	,,	After further animal passage	Death on 3rd day with general infection	,,	,,
6.	*B. newcastle*	Low virulence	No ill effects	No ill effects	Typ. s. Death 24 hrs.
7.	,,	,,	—	,,	Typ. s. Death 30 hrs.
8.	,,	,,	No ill effects	,,	No ill effects
9.	,,	After guinea-pig passage	—	Very ill but gradually recovered	Death 24 hrs.
10.	,,	After another guinea-pig passage	Death in 24 hrs.	No symptoms. Found dead after 10 days	Typ. s. Death 24 hrs.
11.	,,	After a further guinea-pig passage	—	Death 24 hrs.	Death 24 hrs.

* Typical symptoms.

With the highly virulent *B. aertrycke* strain no differences between 65° and 100° C. were observable. With the low virulent Newcastle strain the boiled cultures were definitely more toxic to mice than those only just killed at 65° C.

The same problem was studied by feeding experiments with the same culture medium, grown for 2 or 3 days at 37° C. and then filtered through a Berkefeld filter (all filtrates tested for sterility). Table V summarises the results. The dose of filtrate was 2 c.c.

These experiments on mice show that the sterile filtrates possessed considerable toxicity when fed in doses of 2 c.c. on bread. Out of 14 mice 6 died within 3 days while 3 others showed gastro-intestinal symptoms.

The results show clearly that boiling the filtrates for 20 minutes did not diminish their toxic action. The results do not prove definitely that heating the filtrate to 100° C. enhanced their toxic action.

The influence of the strain employed.

With strains of marked virulence to guinea-pigs by parenteral introduction no definite differences were noticeable with different types of Salmonella. The data recorded in the different tables suggest that more constant results were obtained with more virulent strains. This was specially tested with an aertrycke type (Meirelbeek) which had been cultivated in the laboratory for over 20 years and not recently passed through animals. Of four mice fed on different occasions with this strain grown on the standard medium and regularly giving positive results with other Salmonella strains, one mouse fed with the *meat* showed delayed onset of symptoms with death only on the 7th day while of 3 mice fed with 2 c.c. of *meat juice* 1 mouse died after 4 days while the other two were unaffected. Two *B. paratyphosus* B strains were tested. One strain sent me by Prof. Wilson was somewhat abnormal since it was pathogenic to mice on feeding and this behaved like a virulent *B. aertrycke* when the boiled *meat* was fed. The other was the stock laboratory strain and

Table V.

Strain	Unboiled filtrate	Filtrate boiled for 20 minutes
B. aertrycke	Death 3rd day. Diarrhoea etc.	Diarrhoea, typ. s.* but recovered
,,	No symptoms. Found dead on 10th day	Diarrhoea etc. Death within 2 days
,,	A little diarrhoea, otherwise no action	Typ. s. but recovered
B. enteritidis	No ill effects	Death within 24 hrs.
B. aertrycke	,,	,,
,, †	,,	No ill effects
,,	Death within 24 hrs.	Death within 24 hrs.

* Typical symptoms.

† This experiment was made with a new batch of medium (pH = 6·2) which for some unknown reason was not very effective.

was without effect when 2 c.c. of the boiled *meat juice* was fed to mice (3 experiments). Only two experiments were carried out with *B. typhosus*. When the boiled *meat* was fed it killed the mouse within 48 hours with typical symptoms, but 2 c.c. of boiled *meat juice* only caused some diarrhoea in another mouse and it soon recovered.

It is interesting that this toxic effect on mice was not confined to Salmonella strains. With several Staphylococci negative results were obtained. Five different *B. coli* strains from water, human purulent material etc., were employed. Of 5 mice fed with the 2-day meat cultures after 30 minutes at 100° C. two showed no effects while three died 24–48 hours afterwards with typical symptoms. Of 3 mice fed with 2 c.c. of boiled meat juice one showed no effects while but 2 died after 4 and 5 days respectively without definite symptoms. The coli strains responded therefore rather like Salmonella strains.

Experiments bearing upon the nature of the substance toxic to mice.

Parenteral experiments favour the view that the toxic action of Salmonella strains is due to toxins within the bacillary bodies or to these bodies released by autolysis. It is generally assumed that any positive action obtained by

feeding has the same explanation. G. Elkeles (1930) discusses other possibilities, but only to reject them; he gives a good summary of current views.

(*a*) *Is the toxic action due to Salmonella endotoxins?* The fact that young cultures give as good, or better, results in feeding experiments as old cultures, and the fact that positive results were obtained with a medium which was not a good growth-promoting medium, suggests a different explanation. It was possible however to carry out a series of experiments which excluded endotoxins as an explanation of the toxic action exhibited with my mice.

The following experiments were carried out with three Salmonella strains of high virulence which at the time (when grown in the standard medium and boiled) regularly caused death when fed to mice.

(1) The whole of the thick growth of *B. aertrycke* on two agar plates was emulsified in nutrient broth and boiled for half an hour. A half of the foregoing was fed to a mouse. No symptoms were observable, but the animal was found dead at end of 9 days.

(2) A similar strain of *B. aertrycke* tested in the same way caused no symptoms for 12 days, then the mouse became obviously ill, but it recovered completely in a day or two.

(3) *B. aertrycke* was grown on four agar plates and so far as possible the whole growth was emulsified in 8 c.c. of broth and boiled for 1 hour. Two mice were fed with the equivalent of one agar plate of growth each. One mouse was kept at room temperature, the other at 30° C. They showed no ill effects, but one died after 23 days.

(4) *B. aertrycke* was grown on two agar plates and emulsified in the juice of a standard medium tube, the juice and bacilli being mixed again with the meat. The mixture was boiled for 1 hour and the whole of it fed to a mouse kept for the first day at 30° C. to enhance any action. After about 24 hours the mouse was quiet with ruffled hair, but quickly recovered; it showed no further symptoms and survived. In this experiment the possible irritant action of the meat was superadded.

(5) Newcastle strain grown on four trypt-agar plates for 2 days and the very abundant growth emulsified in the juice of a standard medium tube; boiled for half an hour and then added to the meat portion and the mixture divided and fed to two mice, the one kept at room temperature, the other at 30° C. There was no evidence of any irritant action and the mice remained unaffected.

(6) Newcastle strain grown on two trypt-agar plates for 24 hours and the abundant growth emulsified in about 4 c.c. of ordinary nutrient broth and boiled for 1 hour. A mouse inoculated intraperitoneally with 0·2 c.c. of the emulsion was dead next morning. The rest, *i.e.* practically 2 plates of growth, was mixed with the meat of a standard medium tube and fed to a mouse, kept at 30° C. for the first 24 hours. It showed no symptoms or ill effects.

(7) *B. aertrycke* grown thickly upon two agar plates and the growth emulsified in meat juice and this added to the meat and the whole boiled for

30 minutes. The whole mixture was fed to a mouse. The animal showed no symptoms but was found dead on the 11th day.

These experiments show definitely that enormous doses of heated bacilli of high virulence are devoid of any irritant action when fed to mice and are devoid of toxicity apart from the occasional exhibition of what may be a late toxic effect, comparable to that found by Branham, Robey and Day (1928). The cause of death in my experiments was definitely not due to endotoxins of the Salmonella group.

(*b*) *Is the toxic action attributable to autolytic products from Salmonella bacilli?* In my experiments the growth period for cultures was short, *i.e.* 2 days as a standard. The media found to give positive results were conspicuously inferior in promoting the growth of organisms when compared to several media which gave copious growth, but gave negative results in respect to toxic effects on mice. A number of parallel experiments were carried out with cultures 2, 6, 9 and 14 days old. While the 2-day cultures were always positive the older cultures were less constant and occasionally negative.

In one experiment a virulent aertrycke strain was grown on two agar plates, the abundant growth was emulsified in a tube of nutrient broth and the mixture was incubated for 9 days (to obtain abundant autolytic products). This was boiled for 30 minutes and 2 c.c. were fed to a mouse. No symptoms were noticeable until the 6th day when the animal was ill, dragged its hind legs and had a roughened coat, but no diarrhoea. The mouse was found dead on 7th day. Even with this massive dose there was no irritant action, but a late toxic effect.

It is, I think, evident that the cause of death of the mice under my experimental conditions was not due to autolytic products derived from the breakdown of Salmonella bacilli.

(*c*) *Is there evidence that the irritant or toxic effect is specific?* The fact that strains other than Salmonella, *e.g. B. coli* have yielded similar reactions is against a definite specific action confined to the Salmonella group. Parallel experimental feeding of normal mice and mice immunised against the Salmonella strains used would throw light on the problem. Unfortunately, although many efforts to immunise mice were made, most of them failed, due to the death of the mice. The few mice that were left available gave variable results. When the massive dose of the *meat* was given no differences were observable as all the mice died. With doses of meat juice the results were inconstant.

II. EXPERIMENTS WITH KITTENS.

Experiments conducted many years previously, which yielded positive results, suggested that kittens might be of service in this investigation.

Eleven young kittens fed with boiled cultures of various Salmonella strains grown in the standard meat medium (dose about 15 g. meat and 25 c.c. juice) exhibited no symptoms and remained well. A further experiment, using litmus

milk as the culture medium, was also negative. Very massive doses of bacilli grown on agar plates and emulsified in broth were without ill effects upon two kittens. In five experiments, three being with boiled cultures in the standard meat medium and two growths upon agar plates, the kittens were killed 5 to 6 hours after feeding. No haemorrhages or other naked eye abnormalities were detectable in the stomach or in the duodenum. Feeding with living Salmonella strains was equally ineffective in four experiments and caused no infection in the animals.

These experiments indicate a complete insensitiveness on the part of the kittens to the five different Salmonella strains used, all of which were highly virulent to guinea-pigs and mice by injection.

Discussion.

Feeding experiments with the Salmonella group are notoriously difficult and uncertain. Caution is therefore necessary before making any deductions as to the meaning of experimental results.

The experiments show that it is possible to produce toxic effects upon mice with considerable regularity when they are fed with Salmonella strains grown in certain selected media. It is remarkable that to produce this effect a suitable medium is essential. With two batches of media of similar composition, but somewhat different reaction, one may be ineffective and the other uniformly toxic. It may be advanced that the dose given when meat was included was enormous, but since entirely negative results were obtained with equally enormous doses when using an unsuitable medium the practical importance of this argument is not considerable.

Moreover, it was possible to obtain the same action with 2 c.c. of juice or milk fairly regularly. It has long been known that most animals are comparatively insensitive to Salmonella infections per os, either with living cultures or with toxic products, therefore large doses must be used to induce infection by this path.

The results show conclusively that the toxic action is not due to Salmonella endotoxins, or to autolytic products resulting from the breakdown of the bodies of the bacilli. This is of importance as explaining the negative results obtained by many workers and shows that because their experiments were negative, the inference is not valid that Salmonella bacilli do not produce heat resisting irritant toxic substances.

It is evident, so far as mice are concerned, that Salmonella bacilli do produce some toxic irritant substance which in considerable doses by the mouth is capable of regularly causing death with a fairly typical set of symptoms.

This irritant substance is not destroyed by boiling and there is some evidence that heat treatment at 100° C. enhances its activity. This is of interest in relation to food poisoning outbreaks since clinical experience shows

that the outbreaks associated with toxins are more severe in type, although of shorter duration, than those associated with infection with living bacilli.

The above conclusions are, I suggest, justifiable from the data, but other deductions are hardly more than hypothetical. For example, as regards the nature of this irritant substance, it can hardly be what is called a true exotoxin like that of *B. botulinus*. It is still a matter of controversy whether members of the Salmonella group produce exotoxins, the balance of evidence being against the supposition. Such bodies are readily inactivated by heat, a fact which alone invalidates the conception, while they are not so peculiarly sensitive to a suitable medium as seems to be the case with this irritant. The toxic irritant can only be a product of the action of the bacterium upon the substrate. The present series of experiments is inadequate to enable a decision as to whether the virulence of the strain is a fundamental factor, although the findings suggest that the more virulent strains produce a more powerful poison.

As the same reaction is obtainable, though less readily, with *B. coli* it seems as if other organisms in suitable media may produce irritant substances toxic to mice when fed to them in large amount. The evidence does not support, but does not entirely negative, the possibility of these irritant substances being non-specific bodies which happen to be toxic when fed to mice and having no relationship to food poisoning.

In considering these mice experiments there seem to be two toxic elements in operation: (*a*) The one exerts a direct, fairly rapid irritant effect upon the alimentary tract, severe enough to kill mice when fed in large doses, this having nothing to do with the presence of Salmonella endotoxins but being a product of the action of the bacilli upon the substrate and only formed under a narrow range of chemical conditions. (*b*) The other is a toxic effect probably due to bacillary products, it is exhibited late (usually after the 5th day) and is particularly potent when parenteral introduction is used. The deaths by oral administration in the mice experiments of Branham, Robey and Day would seem to be of this nature. The toxic effects by Bahr and Dyssegaard by intraperitoneal inoculation also appear to be of a like nature. On the other hand the results of Geiger and Meyer would seem to belong to the first group and comparable with my results.

My results with kittens are so uniformly negative that they add but little information. Evidently the strains used were non-toxic to the kittens. Even massive doses of living bacilli exerted no observable irritant effect. They did not invade the alimentary canal tissues and cause a general infection. With this inability to invade the tissues it seems unlikely that any irritant effect would be demonstrable.

My investigation is mainly concerned with evidence of heat-resistant substances, toxic by the mouth, being formed in Salmonella cultures, using mice as test animals. The possibility of such substances being toxic to man per os is not dealt with.

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