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THE FERMENT-ACTION OF BACTERIA.

BY

T. LAUDER BRUNTON, M.D., F.R.S., AND
A. MACFADYEN, M.D., B.Sc.

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The Ferment-action of Bacteria."

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In the course of the research the following micro-organisms were sed:—

1. Koch's comma spirillum (Flügge, 'Die Mikro-organismen,' Leipzig, 1886, p. 334).
2. Finkler's comma spirillum (Flügge, 'Die Mikro-organismen,' Leipzig, 1886, p. 382).
3. A putrefactive micrococcus.
4. Scurf bacillus (Klein).
5. A bacillus isolated from milk by Dr. Klein, which for convenience we may call the "Welford Bacillus."

All of these liquefy gelatine, the two last most energetically. nthrax was not used, on account of the resistance of its spores and ıe consequent difficulty of completely sterilising the culture media. he experiments were made in each case with pure cultures.

The first question which we tried to solve was, What is the nature f the substance by which bacteria liquefy gelatine? Is it an enzyme? here are two ways in which they might do this. They might ecrete some fluid which would dissolve the gelatine mechanically, ithout altering it chemically, as saliva dissolves sugar in the mouth; or ıey might do it by secreting a specific enzyme, which would dissolve ıe gelatine by altering it chemically, as the ptyalin of the saliva ffects the solution of starch. If the solution were effected in the rst way by the secretion of a mere solvent, we should expect that hen the microbes were removed or destroyed, either by heat or ıemical means, the portion of the medium already dissolved would ot have any extensive action on fresh media. But if it had any ıch solvent action, it would probably continue after the solution had een heated to a temperature sufficient to destroy the action of an nzyme. If, on the other hand, the microbes liquefied the media by ecreting an enzyme, we should expect that the liquefied portion ould probably dissolve a considerable amount of new medium when dded to it, but that its solvent action would be arrested by exposure) a temperature sufficient to inhibit enzyme action.

The culture medium was made by adding to meat broth: gelatine,) per cent.; peptone, 1 per cent.; and sodic chloride, 0·5 per cent. he reaction was rendered faintly alkaline with carbonate of soda. ı all the experiments Koch's methods to ensure sterile media and ıre cultures were followed out.

Tubes of 10 per cent. gelatine were inoculated with the microbes, and placed in the incubator at 37° C., with the exceptio the putrefactive micrococcus, which was kept at 22° C.

When liquefaction was complete the fluid was filtered into st tubes, the bacterial deposit being washed with a small quantit sterile distilled water.

Of the filtrate, one, three, and five drops were added respecti to fresh gelatine, and the tubes placed in the incubators as bef The gelatine liquefied, and in all cases bacteria were present.

This liquefied gelatine was in its turn taken and subjected temperature of 50° C. for one hour. Then one, three, and five d were added to fresh gelatine. After incubating, some of the cho comma tubes did not liquefy, but in all cases where liquefaction t place it was due to the active bacteria, as proved by their growth control plates. The control plates were made by adding a drops of the liquefied gelatine to fresh gelatine, and pouring it ou a sterile glass dish. After incubating at 22° C., the gelatine examined microscopically, and the presence or absence of bacte colonies noted.

The liquefied gelatine was next subjected to a temperature 100° C. for fifteen minutes. The same number of drops were ad to gelatine. This fresh gelatine did not liquefy. Finally, 5 c.c. w added to fresh gelatine, but still it did not liquefy.

The control plates showed no colonies.

We therefore conclude that exposure to a temperature of 100° C. destroys—

(*a.*) The bacteria.
(*b.*) The liquefying power of the fluid.

(II) 50° C. does neither. It was not deemed advisable to conti the sterilisation too long, having regard to the injurious action of h on soluble ferments.

It was next necessary to determine the temperatures between 50 and 100° C., which would be sufficient to kill the bacteria with rendering any ferment which might exist inactive. A series experiments led to the following results:—

60° C. for half an hour killed Koch's and Finkler's spirillum.

75° C. for fifteen minutes, on two successive days, killed the sc and "Welford" bacilli.

70° C. for fifteen minutes, on two successive days, destroyed putrefactive micrococcus.

Having established these facts, a series of cultures at 37° C. w made in small glass flasks, each containing about 100 c.c. of 10 cent. gelatine. The liquefied gelatine was filtered, and the dep washed with sterile distilled water.

These filtrates from the five series of cultures were sterilised as scribed above. Then 5—10 c.c. of each were added to 10 per cent. latine (20 c.c.) and kept at 37° C., as well as control tubes of sterile latine.

On the third day the tubes were removed from the incubator and aced in ice-cold water.

Results:—

Scurf bacillus, Welford bacillus: The gelatine does not stiffen, but remains liquid.

Koch's spirillum, Finkler's spirillum: The gelatine is semi-liquid, and does not completely re-gelatinise.

Putrefactive micrococcus, Control gelatine: The gelatine stiffens.

Control plates. No bacteria.

Kept at the ordinary room temperature, these phenomena persisted, e liquid gelatine remaining liquid, and the solid gelatine not lique-ing.

Here, then, we have complete liquefaction of the gelatine produced the first two cases, partial liquefaction in the next two, and no ect in the last.

That this liquefaction was brought about without the presence of tive bacteria is proved by the fact that control plates inoculated om the liquefied gelatine remained sterile. The complete lique-ction was produced by the sterile fluid from the microbes which ere more active liquefiers of gelatine than the others. In the case the two comma spirilla the enzyme action in gelatine was idently more feeble. The negative result with the putrefactive crococcus, and also the fact that tubes inoculated from it, and kept the optimum temperature of 22° C., also gave negative results, re probably due to the preliminary sterilisation having destroyed th the microbes and any enzyme which they might have formed.

These introductory experiments led to the following conclusions:—

1. 100° C. destroyed both the bacteria and the liquefying power.
2. 50° destroyed neither the bacteria nor the liquefying power.
3. Temperatures between 60° and 75° C. destroyed the bacteria, t not the liquefying power in four cases.
4. The liquefied gelatine treated as under 3, and added to fresh atine, liquefied it, although active bacteria were proved to be sent.
5. The liquefaction must, we think, be due to a soluble enzyme, asmuch as liquefaction still took place after the elimination of the crobes, while it was prevented by exposure to such a temperature would destroy the activity of an enzyme but would not be likely affect the action of a simple solvent.

II.

Having regard to the fact that the peptonising action in ge tine was slow, and in two cases partial, it was next sought to d termine whether more active liquefaction of the gelatine could obtained by growing the microbes in some other albumenoid soil.

Two culture fluids were made with meat broth as follows :—

A. Meat broth—	B. Meat broth—
Peptone, 1 per cent.	NaCl 0·5 per cent.
NaCl 0·5 ,,	

Both were rendered faintly alkaline with the carbonate of soda.

The bacteria grew well in both of these media, and so rapidly a abundantly in B. that further experiments were made with it onl *i.e.*, without peptones. For each culture, 100 c.c. meat broth we used. After inoculation and four days' incubation at 37° C., th broth was filtered, and the bacterial deposit washed with steri distilled water. It was then sterilised as already described, an 10 c.c. added to tubes of 10 per cent. gelatine. These tubes we placed in the incubator, as well as control tubes of sterile gelatin When taken out, and placed in ice-cold water, the following resul were obtained :—

(1.) After 24 hours :

Scurf bacillus Welford bacillus	Liquid.
Koch's spirillum Finkler's spirillum	Semi-liquid.
Putrefactive micrococcus Control gelatine	No liquefaction.
Control plates. No colonies.	

(2.) After 48 hours :

Koch's spirillum Finkler's spirillum	Liquid.
Putrefactive micrococcus Control gelatine	No liquefaction.
Control plates. No growth.	

From these experiments it will be seen that the enzyme developed in meat broth is more active than that formed in gelatine. In twenty-four hours the gelatine was liquefied by the scurf and Welford bacilli ; in forty-eight hours by Koch's and Finkler's comma spirilla Again the putrefactive micrococcus gave negative results.

Conclusions :—

1. An enzyme is formed in meat broth which liquefies gelatine,

ıd does so more surely and quickly than the enzyme formed in elatine itself.

2. The liquefaction is produced by a soluble ferment, since its ction can be demonstrated apart from the microbes which pro- ıce it.

III.

Instead of using heat sterilisation some experiments were made ith menthol and thymol.

It was found that when these substances were added in amounts ıfficient to prevent the growth of the bacteria—the ferment action as likewise inhibited.

IV.

The presence of a soluble ferment being demonstrated, can we olate it?

(1.) From gelatine.

(2.) From meat broth.

(1.) *From Gelatine Cultures.*

Flasks containing 250 c.c. of 10 per cent. gelatine were inoculated ith the five microbes. They were left in the incubator at 47° C., putrefactive micrococcus, 32° C.), till liquefaction was complete. he liquefied gelatine was treated with absolute alcohol and filtered. he precipitate was extracted with glycerine, and finally repre- pitated with alcohol. The precipitate, after drying in a sterilised ask, was taken up in a small quantity of sterile distilled water, and llowed to stand over night. About 5 c.c. were then added to 10 per ent. gelatine, and incubated at 37° C.

Results.—Negative. No liquefaction was produced.

(2.) *Meat Broth Cultures.*

In each case 250 c.c. were treated in a similar manner—with cohol and glycerine, and the precipitate and sterile distilled water dded to 10 per cent. gelatine.

Results :—

Koch's spirillum Finkler's spirillum Putrefactive micrococcus	No liquefaction.
Scurf bacillus Welford bacillus	In a few tubes the gelatine was viscid. The rest resolidified.
Control plates.	No colonies.

Concluding that the prolonged method of extraction had weakened

the action of the enzyme, a modification of the process was n made in the following manner:—500 c.c. of meat broth were ino lated with the microbes, and left in the incubator for seven da The precipitate, with an excess of alcohol, was allowed to sta overnight, and, after drying, was dissolved in sterile distilled wat and then reprecipitated by alcohol. This precipitate was dried a taken up in distilled water. The next day about 20 c.c. were add to 100 c.c. of a 5 per cent. gelatine, and placed in the incubator 37° C.

Results after four days:—

The only positive results were obtained with the scurf bacillus a the Welford bacillus. In these cases the gelatine remained liqu while the control gelatine resolidified. The control plates gave colonies.

Conclusion.—The bacteria do form a soluble enzyme which can isolated, and its action demonstrated on albumenoid gelatine.

V.

Are the microbes which liquefy gelatine capable of exerting a li action on other proteid bodies?

To test this, experiments were made with—

(*a.*) Egg-albumen.
(*b.*) Fibrin.

In the first place, it was necessary to find out what resulted fro the direct action of the microbes.

Faintly alkaline meat broth, as developing the most active enzym was used.

(a.) *Egg Albumen.*

To flasks containing 100 c.c. of meat broth were added sma pieces of coagulated egg albumen. The flasks were then sterilise and inoculated with Koch's spirillum, Finkler's spirillum, the scu and Welford bacilli. They were then placed in the incubator a 37° C.

Results:—

(1.) Scurf bacillus.
Welford bacillus:—
1st day. No marked change.
2nd day. Albumen broken up into small fine flocculent fragments.
3rd day. Disintegration almost complete.
4th day. Disintegration complete.

(2.) Koch's spirillum.
Finkler's spirillum :—
1st day. No marked change.
2nd day. Translucent.
3rd day. Thinned and transparent.
5th day. Disintegration.

The bacteria are therefore able, by means of their peptonising ction, to disintegrate egg albumen.

(b.) *Fibrin.*

To 100 c.c. of the meat broth small pieces of boiled fibrin were dded, and after sterilisation the flasks were inoculated with the same icrobes, then placed in the incubator at 37° C.
Results :—

(1.) Scurf bacillus.
Welford bacillus.
1st day. No marked change.
2nd day. Fibrin eroded.
3rd day. Breaking up.
4th day. Disintegration complete.
5th day. Fluid has become turbid.

(2.) Koch's spirillum.
Finkler's spirillum :—
1st day. No change.
2nd day. Slight erosion.
3rd day. Frayed appearance.
4th day. Commencing to break up.
5th day. Disintegrated.
6th day. Turbidity.

Here again we have a marked disintegrating action on fibrin.
Conclusion.—The bacteria exert a disintegrating action on egg bumen and fibrin, as well as on gelatine.

VI.

Can we demonstrate the action of the enzyme on proteid bodies ch as egg albumen and fibrin, in the same way that its action was monstrated on gelatine ?
The alcoholic precipitate from 500 c.c. of the meat broth culture as dried at 35° C., and then dissolved in sterile distilled water. It as then reprecipitated by alcohol and filtered. This precipitate was ied in sterile plugged flasks, and to it were added 50 c.c. of sterile

distilled water, and 5 c.c. of a $\frac{1}{2}$ per cent. chloroform water. Ca bonate of soda was finally added to render the fluid faintly alkaline.

In each flask was placed a small piece of boiled fibrin. After fo days in the incubator they were taken out and examined :—

A. From each, gelatine plate cultures were made.
B. The appearance of the fibrin was noted.
C. After filtration the filtrate was tested for digestive products.

A. Some of the plates showed bacteria. The flasks from whi these had been made were rejected; only those were used whi had remained sterile.

B. In none did the fibrin break up and disappear. But it becar thinned and frayed at the edges. This was most marked with t scurf and Welford bacilli.

C. The filtrate was examined for soluble products :—

On neutralising with dilute hydrochloric acid a precipitate a peared. This was filtered off and the filtrate tested for peptone A solution of caustic soda was added, and then a highly dilute soluti of cupric sulphate was filtered down the side of the test tube. At t line of demarcation the rose-coloured peptone reaction was strong marked.

The simple boiled solution of the ferment only gave the fainte peptone reaction.

These results were obtained with the scurf and Welford bacil and Koch's and Finkler's spirillum. To sum up :—

1. The fibrin was visibly affected.
2. Neutralisation produced a precipitate.
3. The peptone reaction was very distinct.

The enzyme therefore, apart from the bacteria, *can* form solub products from fibrin, and amongst these peptones.

VII.

Are the microbes capable of forming a diastatic, as well as a pe tonising ferment ?

A. Scurf bacillus.
Welford bacillus :—

Starch was heated with water so as to form a thin paste. To th was added sodic chloride (0·5 per cent.). About 100 c.c. were plac in each flask, which was then plugged with cotton wool a sterilised.

After inoculation they were placed in the incubator (37° C.) alo with flasks of sterile starch paste.

Flasks were opened on successive days and examined :—

2nd day. Starch has lost its opalescence. Iodine gives a blue colour.
3rd day. Iodine gives a red colour.
5th day. No reaction with iodine.
6th day. Was tested for a reducing sugar. The reactions were as follows :—

(1.) Iodine.—No reaction.
(2.) Caustic soda.—On gently boiling fluid becomes yellow.
(3.) Cupric sulphate and caustic soda.—A yellow precipitate on boiling.
(4.) Fehling's reagent.—A red precipitate.
(5.) Barfoed's reagent.—No reaction on gently heating.

(*Barfoed's Solution* :—One part of neutral acetate of copper dissolved n 15 parts of water, and then to 200 c.c., 5 c.c. of acetic acid 38 per cent.) added.)

The control starch gave blue colour with iodine, but none of the bove reactions.

B. Putrefactive micrococcus—
Results were negative.
C. Koch's spirillum.
Finkler's spirillum :—

The same starch solution was used, but a few drops of meat broth ere added in each case. The usual control experiments were ade :—

3rd day. Starch has lost its opalescence. Iodine strikes a blue colour.
4th day. Iodine gives a violet colour.
5th day. Iodine gives red reaction.
7th day. Iodine.—Red.
Caustic soda.—Yellow on boiling.
Cupric sulphate and caustic soda.—No reduction.
Fehling's solution.—No reduction. On previous addition of H_2SO_4 a slight reduction.
Barfoed's reagent.—No reduction.
Control starch.—Iodine strikes blue.

From these experiments the following conclusions may be rawn :—

1. The putrefactive micrococcus did not grow on the carbohydrate il, and so we are left in doubt as to its diastatic action.

2. The scurf bacillus and Welford bacillus were both capable of ltivation, and evinced a marked diastatic action, in addition to eir peptonising power. The failure of the iodine test, and the

precipitates obtained with Fehling, &c., indicate the presence of reducing sugar. The failure with Barfoed's reagent suggests th the sugar is in great part, at any rate, maltose.

3. With regard to Koch's spirillum and Finkler's, though th evinced a diastatic action, it was feebler than in the former ca only traces of a reducing sugar being detected after the addition sulphuric acid. The red and violet coloration with iodine points the formation of dextrin (erythro- and achroo-dextrin).

At any rate, in the scurf and Welford bacilli we have two microb which evince a marked diastatic action; and a demonstration of t fact that the same germ can produce both a diastatic and peptonising ferment.

VIII.

Can we demonstrate the action of the diastatic enzyme apart fro the bacteria?

Starch cultures of the scurf bacillus and the Welford bacillus (t days' growth) were treated with chloroform water (1 per cent.) t they became sterile.

The fluid was then added to fresh starch, and incubated at 37° C

In eight to ten days the iodine reaction had disappeared. (boiling with caustic soda the fluid became yellow. Fehling's soluti was reduced. The fluid lost its opalescence. Control plates— growth.

These experiments point strongly to the existence of a diasta enzyme capable of isolation, and of acting apart from the bacteria.

IX.

That the peptonising enzyme bears the closest analogy to t pancreatic ferment will be seen from the following experimen Sterile meat broth, in which Finkler's spirillum and the Welfo bacillus had been cultivated, was added to 10 per cent. gelatine tub of differing reaction:—

	Gelatine.	*Results.*
A.	Acidified with dilute hydrochloric acid..	No liquefaction.
B.	Alkaline by adding sodic carbonate	Liquefied.
C.	Neutral	Liquefied.
D.	Boiled after adding the ferment.....	No liquefaction.

X.

The digestive action of the microbes was tested on several oth bodies.

1. *Fats.*—Alkaline meat broth and olive oil, 2 per cent.

The results were negative.

Experiments which were made by Manfredi* tend to show that fat-ontaining media impair the vegetative energy of bacteria.

2. *Dextrose.*—The culture fluid was prepared as follows:—

Dextrose	2 per cent.
Peptone.....	1 „
Sodic chloride	0·5 „
Reaction	Neutral.

After sterilisation, the flasks were inoculated with the scurf acillus and Welford bacillus. Incubated at 37° C. They were xamined on the fourth day.

Fehling's solution was no longer reduced. The fluid gave a arked acid reaction.

The control solution reduced Fehling's solution. Reaction was nchanged.

3. *Cane-sugar.*—Cane-sugar, 2 per cent.
Peptone, 1 per cent.
NaCl, 0·5 per cent.
Reaction, neutral.

Inoculated with scurf bacillus and Welford bacillus, and incubated 37° C.

The results were negative. No reducing sugar detected.

Muscle.—Alkaline meat broth cultures were used. Inoculated ith Finkler's spirillum and Welford bacillus.

With the Welford bacillus a marked effect—the muscular tissue ecomes disintegrated, and the striæ indistinct.

These experiments, though incomplete in themselves, are sufficient show that the bacteria which liquefy gelatine and diastase starch, an also exert a digestive influence on dextrose and muscle. The xact determination of the products of this action in the case of ese and some other organic bodies must be reserved for further vestigation.

To sum up briefly the results of this inquiry:—

1. The bacteria which liquefy gelatine do so by means of a soluble nzyme.

2. This enzyme can be isolated, and its peptonising action demon-rated apart from the microbes which produce it.

3. The most active enzyme is that formed in meat broth.

4. Acidity hinders, alkalinity favours its action.

5. The bacteria which form a peptonising enzyme on proteid soil an also produce a diastatic enzyme on carbohydrate soil.

* 'Accademia dei Lincei, Rendiconti,' vol. 3, sem. 1, 1887, p. 535.

6. The diastatic enzyme is not so readily separated from the microbes which produce it, but where that has been accomplished its action on starch can still be demonstrated.

7. The diastatic enzyme has no effect on gelatine, and *vice versâ*.

8. The bacteria are capable of evincing an adaptiveness to the soil in which they grow.

9. The microbes are capable of digesting other similar bodies such as dextrose and muscle.

10. Fatty matter was not affected.

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