A BIOCHEMICAL STUDY OF PROTEUS VULGARIS HAUSER.¹

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In this study we deal with the cultural properties, the products of growth, the pathogenicity, and the toxicity, of two strains of Proteus vulgaris, Hauser. This is an exceedingly interesting organism on account of its wide distribution, the ease with which it is modified, and because it seems to stand between the true saprophytic and the purely pathogenic bacteria. It has been found under a great variety of abnormal conditions in the human body, but most frequently in the digestive tract, where it produces the greatest harm. Here it has been present in large numbers, in a variety of food poisonings (1, 2), in the summer diarrheas of children (3, 4), the diarrheas of calves (5), in severe cases of typhoid fever (6), and in other more obscure abnormal intestinal conditions.

Its association with other pathogenic organisms is of great importance and probably has not received the attention it deserves. Theobald Smith (7) found that when the hog cholera bacillus and Proteus were grown together, in what he called a mixed pure culture, the virulence of the former was lowered, while that of the latter was apparently raised. Kühnau (8) found virulent strains of Proteus in severe cases of diphtheria. Levy and Thomas (9) find that the virulence of the cholera spirillum was raised by injecting cultures of Proteus precipitated by alcohol. Its presence in severe cases of typhoid has already been mentioned (6) and in the same article Vincent notes that mixed cultures of Proteus and the typhoid bacillus are very virulent for rabbits.

¹ Aided by a grant from the Rockefeller Institute for Medical Research.
² Figures in parenthesis refer to bibliography at the end of this paper.
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DESCRIPTION OF CULTURES.

Both the cultures used in this study were obtained from Dr. Theobald Smith. Culture A had been in his possession for some time, while Culture B was isolated from some putrid material in January, 1910. Culturally these two strains do not agree in all points, but they will be described together and their differences noted.

Morphology. Both organisms are short rods which vary considerably in length. They are motile, do not produce spores, and are negative to Gram’s stain. In Culture A the organisms occur in long chains while in Culture B they are isolated.

Cultural characteristics. On an agar slant there is a moderate, spreading, glistening growth covering the entire surface of the media. The condensation water is turbid. In bouillon there is an abundant growth with a small amount of flocculent white sediment. Culture A shows a tendency to form a surface membrane.

When it was received, Culture A liquefied gelatin slowly and when plated it was found that the majority of the colonies were non-liquefiers. The liquefying colonies were of the swarming type so well described by Hauser (10) and Smith (11). Subcultures were made from the latter and from time to time the culture was plated and subcultures always made from the liquefying colonies. In May, 1910, it was found that the culture did not digest casein, but its action on gelatin was not tested until the following July when it was found that there was no sign of liquefaction in a stab culture in three weeks’ time. In August another culture of this strain was received from Dr. Smith and it was found that the liquefying properties were the same as when the culture was first studied by us.

Culture B liquefied gelatin rapidly throughout the period in which it was studied, although it was treated in exactly the same way as Culture A. In July, 1910, a stab culture was liquefied to a depth of 5 cm. in twelve days. Swarming colonies were observed in one instance only, in which case the culture had been passed rapidly through a series of milk tubes and then plated on glucose gelatin. However, the culture was not plated on 5 per cent gelatin and it is probable that on this medium swarming colonies would have been found.
Culture A was unfortunately not studied in milk at the time (April) it was received, but in July it caused no apparent change in twelve days. The reaction at the end of this period was about +1.5 per cent.¹

Culture B in milk shows no apparent change in two days but on the third day there is a soft curd and signs of digestion. In five days the milk is about five-eighths digested and the reaction is about +2.5 per cent. In a milk fermentation tube the bulb is very slowly acted upon, being half digested in twelve days.

Dr. Theobald Smith has repeatedly pointed out the fundamental character of the fermentative power of Proteus and of other organisms. He says (12),

In view of this confused state of affairs (the various forms of Proteus) I would suggest that in the study of doubtful forms we should apply the fermentation test and class those species which fail to act on lactose, but which ferment dextrose and saccharose under Proteus. I found the fermentation test such a valuable group reaction, that I can safely recommend it in the study of all saprophytic forms in supplying a broader view of the relationship of physiological species.

If one follows his method, taking into consideration the amount of acid formed in the bulb and branch, the information one gains is truly remarkable. In different organisms of the same species one may get variations in the reaction of the bulb due probably to different rates of growth. In the branch, however, the amount of acid produced is very typical of the species. It is to be hoped that before long we will have a classification of bacteria based on the growth in the fermentation tube, as this seems to be the surest and easiest means of differentiating a great many species.

As Dr. Smith has shown, Proteus forms from 20 to 30 per cent of gas in dextrose bouillon and the acid produced is between 2 and 3 per cent of a normal solution.

In lactose one sometimes gets a small bubble of gas but as this is so rare, it may be due to some other sugar in the media. The reaction of the media is near the neutral point.

The production of gas in saccharose is slow, often not beginning before the third or fourth day, but it amounts to 25 or 30 per cent

¹ In this paper + represents acidity and - represents alkalinity, the reaction being determined by titrating 5 cc. of the diluted and boiled media with 1/50 NaOH.
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in ten days. The amount of acid produced is smaller than in the case of dextrose, being from +1.5 to +2.0 per cent. In some cases the reaction in the bulbs is low but in the branch the reaction is very constant.

It will be seen that the only constant distinctive cultural characteristics shown by these two strains are the fermentative powers. Yet it was found that they formed practically the same chemical products and that their pathogenic powers were the same. Culture A, after being passed through a mouse, liquefied gelatin and resembled typical Proteus vulgaris.

An interesting point brought out was the cause of the coagulation of milk by these organisms. Smith (12) says that

The contemporaneous power to liquefy gelatin and precipitate casein in milk indicates that the two processes are due to a ferment, and that the changes produced by Proteus vulgaris in milk are not the result of an acid fermentation of the milk sugar, as is the case with the colon group.

Roger (13), on the other hand, admitting that milk sugar is not fermented, notes that when the casein is precipitated the media is acid and thinks the acid is responsible for the curdling.

We noticed that there was a greater amount of acid produced by the peptonizing strain of proteus in milk and it was thought that this might be the cause of the coagulation. However, on the addition of 0.5 per cent of calcium carbonate to the media the coagulation was more prompt and the digestion more rapid than in the control tube, while the reaction in the calcium carbonate tube when coagulation took place was +1.5 as against a reaction of +2.8 in the control. When 1 per cent magnesium carbonate was added to the milk, the acid formed was all neutralized and here also the coagulation was more prompt and firmer than in the plain milk. Coagulation, then, must be due to an enzyme which acts best in a neutral or slightly acid medium.

PRODUCTS OF GROWTH.

A bouillon containing 0.4 per cent of Liebig's beef extract, 1 per cent of Witte's peptone and 0.5 per cent of sodium chloride was used in studying the products of growth of these organisms. No differences could be found between the two strains, so that the following notes apply to both cultures.
Lewandowsky (14), using plain bouillon, reports Proteus vulgaris as an organism that forms phenol. We were never able to detect this substance in the distillate from acidified cultures, although a number of cultures were examined, including a fifteen-day culture in 5 per cent peptone bouillon and a sixteen-day culture in bouillon containing 0.02 per cent of tyrosin (15). Skatol has also been reported as one of the products of growth of Proteus vulgaris, but using the delicate paradiethylamidobenzaldehyde (Ehrlich's aldehyde) test, we were never able to find it in any of our cultures, including those mentioned under phenol.

Mercaptan was absent, even when cystine had been added to the media. Alcohols, aldehydes and ketones could not be found. Indol was present in moderate amounts but seldom in concentrations sufficient to give a precipitate with β-naphthaquinonesodium-monosulphonate. Indol-acetic acid was present in cultures four days old, it being identified by its color reaction with concentrated hydrochloric acid and a few drops of 0.01 per cent sodium nitrite, its spectrum, and its color reaction with hydrochloric acid and ferric chloride (16). Aromatic oxyacids were present in moder-
Hydrogen sulphide was present even in twenty-four-hour cultures.

Volatile alkali is produced in large amounts as is shown in the curve on the preceding page. This is probably for the most part ammonia, but primary amines are present as shown by the carbamidine test. Putrescine and cadaverine could not be demonstrated in cultures three weeks old.

In estimating the volatile alkali and acids a series of flasks containing 60 cc. of bouillon and with paraffined plugs, was inoculated and incubated at 37° C. From time to time a flask was opened and 25 cc. taken out for each determination. A mark placed on the outside of the flask at the surface of the media showed that the loss by evaporation of the media was so slight that it could not be detected. In order to liberate the alkali, 25 cc. of the culture was diluted and about 1 gram of magnesium carbonate added and the volatile alkali determined as in the Kjeldahl method. The volatile acids were liberated by the addition of about 1 cc. of phosphoric acid to another 25 cc. of the culture, and after distilling, the distillate was titrated with $\frac{1}{17}$ sodium hydrate.

The volatile acids are small in amount but their molecular weight is remarkably high as will be seen in Table II.

<table>
<thead>
<tr>
<th>AGE OF CULTURE</th>
<th>MOLECULAR WEIGHT OF VOLATILE ACIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proteus A</td>
</tr>
<tr>
<td>7 days</td>
<td>84</td>
</tr>
<tr>
<td>14 days</td>
<td>97.5</td>
</tr>
<tr>
<td>25 days</td>
<td>100.3</td>
</tr>
</tbody>
</table>

When the organisms were grown in flasks containing 1 per cent casein and 0.06 per cent sodium carbonate, the culture precipitated with Hedin's tannic acid mixture (17), and the soluble nitrogen determined in the filtrate, it was found that Proteus A had failed to act on the casein, while Proteus B destroyed 64.2 per cent of the protein in three weeks' time. The failure of Proteus A to act on the protein corresponds with its loss of power to digest gelatin. When to the casein solution was added 1 per cent of dextrose or lac-
tose, the protein was not attacked by Proteus B. This is rather peculiar, as in a fifteen-day milk culture of this organism there was 18.1 per cent more soluble nitrogen than in the control milk. We failed to get any action on a 1 per cent solution of dried egg albumin but there was apparently little growth and it seems probable that with the addition of some salts a more suitable medium could be made in which the protein would be acted upon.

The study of the products of the growth of Proteus shows that, to some extent at least, it is a putrefactive organism. It destroys a native albumin (casein) and it produces ammonia, primary amines, hydrogen sulphide, fatty acids of a high molecular weight, aromatic oxyacids, indol and indolacetic acids, all of which are associated with putrefaction. Given the proper conditions in the digestive tract, it would probably prove harmful to the host through the formation of these substances (18).

PATHOGENIC PROPERTIES OF PROTEUS.

Injected under the skin of guinea-pigs, bouillon cultures of these organisms caused large ulcers which healed slowly. When injected into the peritoneal cavity, the weight of a guinea-pig fell from 575 to 301 grams in sixteen days, and the animal died apparently from starvation and a diffuse peritonitis.

In mice the lethal dose of a twenty-four-hour bouillon culture was about 1 cc., but by passing the cultures through seven mice, the virulence was raised so that the lethal dose was 0.1 cc. for Proteus A and 0.25 cc. for Proteus B.

Young guinea-pigs weighing 200 grams were fed, for a week, on a cake made of bread and 7 cc. of a twenty-four-hour bouillon culture of the organisms. They lost a few grams in weight but this was probably due to their somewhat limited diet rather than to the cultures.

Nursing kittens three days old were fed bouillon cultures as follows, the cultures being injected into their stomachs through a catheter:

- August 1 each kitten received 0.5 cc. of a 24-hour culture.
- August 3 each kitten received 1.0 cc. of a 24-hour culture.
- August 4 each kitten received 1.0 cc. of a 24-hour culture.
- August 5 each kitten received 1.0 cc. of a 24-hour culture.
- August 6 each kitten received 5.0 cc. of a 24-hour culture.
Kitten I received cultures of Proteus A, the virulence of which was 0.1 cc. for mice. Kitten II received cultures of Proteus B, the virulence of which was 0.25 cc. for mice, and Kitten III, the control, received plain bouillon. The kittens showed no apparent effects from the cultures throughout the experiment nor during the following month.

Two monkeys (Macacus rhesus) on a diet of eggs and corn-meal were fed daily for a month, 50 cc. of twenty-four-hour cultures of Proteus A and B, the virulence of which for mice was 0.1 cc. and 0.25 cc. respectively. No ill effects were noticed in either monkey during or following the feeding, although the organisms fed were found in the feces of both monkeys.

A monkey (Macacus rhesus) on a diet of meat and eggs was fed 50 cc. daily of a twenty-four-hour bouillon culture of Proteus B. This caused a marked diarrhoeal stool of a greenish color. The organisms were found in the feces in great numbers for the first two weeks but after that about two in ten colonies were Proteus. After a month and a half, the monkey became very weak and was chloroformed. The walls of the cæcum, ascending and transverse colon were thickened and congested. Small ulcers, about 4 mm. in diameter, were scattered over this area. The remaining parts of the digestive tract were apparently normal. Cultures from the spleen and blood were sterile. The blood showed a slight agglutinative reaction toward the organism fed, there being a microscopic agglutination in dilutions of 1:50. Spectroscopic examinations of the blood made from time to time showed it to be normal.

The results of these feeding experiments are rather indefinite and correspond to those recorded in the literature. Vincent (19) and Schumburg (20) state that cultures of Proteus isolated by them from the vomited material of patients suffering from meat-poisoning caused diarrhoea and death when fed to mice. Myerhof (21), on the other hand, says that his cultures of Proteus, when fed to mice, caused neither sickness nor immunity.

Metschnikoff (22), working with proteus, isolated from cases of summer diarrhoea in children, failed to get any effect when the cultures were fed to young macacus and cynocephalus monkeys. When fed to nursing rabbits and young chimpanzees, a characteristic diarrhoea resulted which caused the death of the former.
TOXIN.

Following the method used by Levy (23) and Vaughan (24) we precipitated cultures of Proteus by adding alcohol to the strength of about 70 per cent, centrifuging and drying the precipitate in a vacuum over sulphuric acid. When dry the substance is of a brownish color and has a slightly putrid odor. The yield is about as follows:

500 cc. bouillon 8 day culture at 37° = 0.48 grams.
500 cc. gelatin 7 day culture at 37° = 1.41 grams.
Growth from agar slants in 3-quart bottles incubated 7 days at 37° = 0.5 grams.

The material used for most of the work was obtained from seven-day growths on agar slants, as it was found that this was the easiest and quickest way of working with the cultures.

The precipitate obtained is sparingly soluble in salt solution, 1 part dissolving in about 200 parts of physiological salt solution. It is soluble in 1 per cent sodium carbonate; precipitated by alcohol, \( \frac{1}{20} \) hydrochloric acid, magnesium sulphate, ammonium sulphate; is not coagulated by heat, gives the xanthoproteic, Millon's and biuret reactions.

It is completely digested by trypsin but only partially by pepsin. The substance obtained from gelatin cultures gave 15.4 per cent nitrogen, while that from agar cultures gave 9.96 per cent nitrogen. Prepared in this way, the precipitate probably consists largely of nucleoprotein but other substances are undoubtedly present.

When dissolved in sterile salt solution and injected into the peritoneal cavity of guinea-pigs, this substance caused the death of the animal in from six to twenty hours, the lethal dose per 100 grams of body weight being as follows:

Precipitate from gelatin culture, 11.5 mg.
Precipitate from bouillon culture 9 mg.
Precipitate from agar culture, 8.2 mg.

After a fatal dose is injected, the symptoms are as follows: The pig quickly appears to be very sick, has a staring coat, and remains quiet in the corner of its cage. Soon it lies on its side, as though in an exhausted condition and its temperature falls often as low as 32° C. No evidence of pain or convulsions were
observed when the injection amounted to 8 or 10 mgs. per 100 grams of body weight. When larger amounts were given, the animal cried out and there were spasmotic movements of the abdominal muscles, as though the animal would vomit, although this act never took place in a guinea-pig.

At autopsy it was found that the walls of the peritoneal cavity are congested and small punctiform haemorrhagic spots are often present. In the cavity there is a decided increase in the amount of straw-colored fluid, often tinged with blood. The liver, spleen, stomach and intestines are covered by a fibrin exudate, rich in polymorphonuclear leucocytes. The liver is swollen and somewhat darkened. The spleen is swollen, darkened and often shows on the surface haemorrhagic spots, the size of a pin-head. The adrenals show a surface congestion, while the kidneys are apparently normal. The vessels of the digestive tract are often enlarged and the stomach and caecum contain varying amounts of gas depending upon the length of time the animal has lived after the inoculation. In the pleural cavity the lungs appear to be normal, while the vessels of the heart are engorged with blood and under the visceral pericardium small haemorrhagic spots are often to be seen.

Microscopical examination of the tissues shows a slight granular degeneration of the liver, accompanied, at times, by a perilobular lymphocyte infiltration. In some animals there was a marked degeneration of the liver which was probably dropsical, as the cells showed large vacuoles. The kidney shows a slight granular degeneration. The spleen shows marked congestion with a distension of the sinuses, while the lungs and adrenals are apparently normal. In the heart the capillaries are greatly distended with blood and at times it seems as though they had burst, causing haemorrhage between the muscle fibers.

What would be a fatal dose if injected into the peritoneal cavity, when given under the skin does not produce these results. There is a slight rise in temperature and the loss of ten grams or so in weight, this loss, however, being soon regained. An induration is felt in the subcutis and in about three days the hair comes out and an ulcer is formed. The ulcer is at first small but after several days the opening is about 1.5 cm. in diameter and extends down to the muscular layers, with a sharply circumscribed edge. There
is a slight discharge, and a thick, yellowish pus can be seen in the depths of the ulcer. The healing process is very slow, extending over a period of about three weeks. The same type of lesion is produced by 1 cc. of a twenty-four-hour culture of Proteus and by the bodies of the bacteria killed by heat.

In white mice the lethal dose lies between 2.5 and 5 mgs, the mouse dying in from five to twelve hours. The macroscopical changes noted are a mottling of the liver, a congestion of the spleen, adrenals and at times, of the kidneys. The contents of the intestine are fluid, and gas is often present. The heart is very much engorged and its vessels are dilated.

In cats the picture is as follows:

August 4, 1910. 10:15 a.m. Cat weighing 1740 grams anaesthetized with chloroform and 30 mg. toxin No. 15 in 10 cc. sterile salt solution injected into a superficial vein of the leg.
10:50 a.m. Cat crying out and vomits a considerable amount of partly digested food.
12:30 p.m. Cat seems brighter but from time to time makes violent attempts to vomit and is unsteady on its feet.
5 p.m. Cat rouses up when disturbed but if left alone it sits in a corner with its eyes closed and seems to feel very badly.

August 5. 8 a.m. Cat found dead, stiff. Autopsy at once.
Kidneys. The vessels on the surface of the organ and those going to it are distended with blood. On section there seems to be a congestion of the medullary rays.
Adrenals. Small and apparently normal.
Stomach. Very much distended with gas. Large scattering hemorrhagic areas lay in the mucous membrane near the pylorus.
Intestines. Moderately distended with gas. No hemorrhagic areas found.
Pleural Cavity. Lungs apparently normal. Heart congested and shows small hemorrhagic areas under the visceral pericardium.
No bacteria could be found in smears from the blood or spleen.

Cat No. 2.

August 22, 1910. Cat weighing 3525 grams anaesthetized and 15 mg. toxin No. 17 digested twenty-four hours with trypsin injected into the femoral vein.
10:20 a.m. Cat feeling badly. Strings of saliva hanging from mouth. No vomiting.
12. Noon. Cat has had nothing to eat since yesterday morning. Vomits a greenish substance containing bubbles of gas. Looks very sick. During the afternoon the cat vomited twice and seemed very sick.

*August 23.* 8 a.m. Large amount of bloody diarrhoeal feces in cage. Cat slept all morning and refused to eat.

*August 24.* No feces. Cat still sleeping most of the time and refuses to eat.

*August 25.* Cat feeling better but eats very little. Weight 3180 grams. From this time on the cat returned to normal.

When sublethal doses are injected into the peritoneal cavity of a guinea-pig it often is found at the end of twenty-four hours that part of the rectum is protruding from the anus. This might indicate that the substance causes an increased peristalsis and it was found that in one of these animals which was autopsied immediately after chloroforming that the peristaltic movements were very marked.

The properties of this toxin were studied to a certain extent, although we have not been able to get it in a pure form.

*Effect of heating.* Heating to 100° C. for thirty minutes does not destroy the toxin, as the following protocols show.

Guinea-pig 58. Weight, 252 grams. Intraperitoneal inoculation of 30 mg. toxin No. 11 heated 30 minutes in solution, in a sealed tube in boiling water. Guinea-pig found dead in fifteen hours. Autopsy showed typical picture of toxin action.

Guinea-pig 63. Weight, 297 grams. Intraperitoneal inoculation of 30 mg. toxin No. 11 heated 30 minutes at 70°C. in a sealed tube. Found dead in fifteen hours. Autopsy showed typical picture of toxin action.

Guinea-pig 62. Control. Weight, 298 grams. Intraperitoneal inoculation of 30 mg. toxin No. 11. Dead in four and a-half hours.

Guinea-pig 66. Weight, 300 grams. Intraperitoneal inoculation of 28 mg. toxin No. 16 digested twenty-four hours with trypsin and heated 30 minutes in boiling water in a sealed tube. Found dead in twenty hours and autopsy showed typical picture of toxin action.

Guinea-pig 75. Control. Weight, 317 grams. Intraperitoneal inoculation of 28 mg. toxin No. 16 digested twenty-four hours with trypsin. Found dead in twenty hours and autopsy showed typical picture of toxin action.
DIGESTION OF PROTEUS TOXIN WITH TRYPSIN.

Digestion in a 0.25 per cent trypsin solution containing 0.15 per cent sodium carbonate does not destroy the toxic action as is shown in Table III. After digestion the solution was heated to the boiling point to destroy the enzyme.

TABLE III.

<table>
<thead>
<tr>
<th>ANIMAL</th>
<th>WEIGHT</th>
<th>TOXIN NO.</th>
<th>AMOUNT INJECTED INTO PERITONEAL CAVITY</th>
<th>HOURS OF DIGESTION</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>grams</td>
<td>13</td>
<td>milligrams</td>
<td>No trypsin (Control) 3.75</td>
<td>Found dead in 23 hrs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>2.5</td>
<td></td>
<td>Found dead in 20 hrs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>2.5</td>
<td>9</td>
<td>Found dead in 14 hrs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>2.5</td>
<td>24</td>
<td>Found dead in 23 hrs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>5</td>
<td>Trypsin+ NaCO₃ 24</td>
<td>Lived Dead in 48 hrs.</td>
</tr>
<tr>
<td>Guinea-Pig. No.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>69</td>
<td>360</td>
<td>15</td>
<td>24</td>
<td>(Control) 0</td>
<td>Dead in 42 hrs.</td>
</tr>
<tr>
<td>71</td>
<td>312</td>
<td>15</td>
<td>24</td>
<td></td>
<td>Dead in 6 hrs.</td>
</tr>
<tr>
<td>67</td>
<td>380</td>
<td>16</td>
<td>28</td>
<td>(Control) 0</td>
<td>Dead in 20 hrs.</td>
</tr>
<tr>
<td>75</td>
<td>317</td>
<td>16</td>
<td>28</td>
<td></td>
<td>Dead in 20 hrs.</td>
</tr>
</tbody>
</table>
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DIGESTION WITH PEPsin.

The toxin was made up with a 0.5 per cent pepsin solution in \( \frac{3}{10} \) HCl and incubated for twenty-four hours before it was injected. Digestion was not complete, there being a residue left in the tube. In table IV the results of this experiment show that pepsin has as little effect as trypsin.

<p>| TABLE IV. |
|-----|-----|-----|-----|-----|</p>
<table>
<thead>
<tr>
<th>ANIMAL</th>
<th>TOXIN NO.</th>
<th>AMOUNT INJECTED INTO PERITONEAL CAVITY</th>
<th>HOURS OF DIGESTION</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>15</td>
<td>milligrams 5</td>
<td>Toxin + ( \frac{3}{10} ) HCl (Control)</td>
<td>Found dead in 21 hrs.</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>5</td>
<td>24</td>
<td>Dead in 3 days</td>
</tr>
<tr>
<td>Guinea Pig No. 73</td>
<td>15</td>
<td>20</td>
<td>Toxin + ( \frac{3}{10} ) HCl (Control)</td>
<td>Dead in 21 hrs.</td>
</tr>
<tr>
<td>Weight 310 g.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>20</td>
<td>24</td>
<td>Dead in 21 hrs.</td>
</tr>
</tbody>
</table>

We evidently are dealing with a very stable substance as neither tryptic nor peptic digestion; \( \frac{3}{10} \) HCl nor 0.15 per cent Na$_2$CO$_3$, acting for short periods of time, affect its toxic action. Dissolved in 1 per cent Na$_2$CO$_3$ the toxic properties were diminished but not lost.

An attempt was made to separate the toxin from the protein by adding enough HCl to make a \( \frac{3}{10} \) solution, filtering off the precipitated protein and suspending it in salt solution. Injection of the precipitate and filtrate showed that the toxic substance had been carried down with the precipitate as is shown in Table V.

<p>| TABLE V. |
|-----|-----|-----|-----|-----|</p>
<table>
<thead>
<tr>
<th>ANIMAL</th>
<th>TOXIN NO.</th>
<th>AMOUNT OF TOXIN REPRESENTED IN INJECTION INTO PERITONEAL CAVITY</th>
<th>PORTION INJECTED</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>15</td>
<td>milligrams 5</td>
<td>Control</td>
<td>Dead in 20 hrs.</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>5</td>
<td>Precipitate</td>
<td>Dead in 21 hrs.</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>5</td>
<td>Precipitate</td>
<td>Dead in 3 days</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>5</td>
<td>Filtrate</td>
<td>Alive</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>5</td>
<td>Filtrate</td>
<td>Alive</td>
</tr>
</tbody>
</table>
EFFECT OF DIALYSIS.

In order, if possible, to separate the toxin from the protein, the solution was dialyzed in running water in a tube, the end of which was covered by parchment paper. The tube was shaken from time to time and before injection the fluid inside was brought back to its original level.

TABLE VI.

<table>
<thead>
<tr>
<th>ANIMAL</th>
<th>TOXIN NO.</th>
<th>AMOUNT INJECTED INTO PERITONEAL CAVITY</th>
<th>HOURS OF DIALYSIS</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse........</td>
<td>13</td>
<td>2.5</td>
<td>12</td>
<td>Dead in 5.5 hrs.</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>5.0</td>
<td>48</td>
<td>Dead in 15 hrs.</td>
</tr>
<tr>
<td>Guinea-pig</td>
<td>16</td>
<td>28</td>
<td>60</td>
<td>Dead in 21 hrs.</td>
</tr>
</tbody>
</table>

Since the substance is non-dialyzable we must conclude that it is of a complicated chemical structure or that it is firmly bound to or is a part of the protein.

In order to separate, if possible, the toxin from the protein, we next tried the effect of dialysis after digestion with trypsin and pepsin, but the results were not as definite as in the preceding experiments. In the majority of cases after dialysis for two or three days the toxin was present inside the parchment paper in undiminished strength. In one case after digestion for twenty-four hours with trypsin and dialyzing seventy-two hours, 20 mgs. killed a guinea-pig, weighing 290 grams, in sixteen hours and autopsy showed a typical picture of toxin action.

EFFECT OF OXIDATION ON THE TOXIN.

The effect of oxidation on the toxic substance was tried by adding Parke, Davis and Company's hydrogen dioxide solution as follows:
Proteus Vulgaris

*Tube A.* Control. 2 cc. of a 0.5 per cent solution of toxin 17 (digested twenty-four hours with trypsin) + 0.5 cc. salt solution.

*Tube B.* 2 cc. toxin as in A. + 0.5 cc. \( \text{H}_2\text{O}_2 \) solution.

*Tube C.* 2 cc. toxin as in A. + 0.5 cc. \( \text{H}_2\text{O}_2 \) solution + small crystal of \( \text{FeSO}_4 \).

The tubes were incubated at 37° C. for two hours and at the end of this time the oxidation in tube C was very marked. Mice were inoculated, each mouse receiving what corresponded to 5 mg. of the dried toxin into its peritoneal cavity.

<table>
<thead>
<tr>
<th>INJECTION</th>
<th>MOUSE 1</th>
<th>MOUSE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tube A.</strong> Toxin + salt solution (Control).......</td>
<td>Found dead in 21.5 hrs.</td>
<td>Found dead in 21.5 hrs.</td>
</tr>
<tr>
<td><strong>Tube B.</strong> Toxin + ( \text{H}_2\text{O}_2 )</td>
<td>Found dead in 45 hrs.</td>
<td>Found dead in 45 hrs.</td>
</tr>
<tr>
<td><strong>Tube C.</strong> Toxin + ( \text{H}_2\text{O}_2 ) + ( \text{FeSO}_4 ) ..........</td>
<td>Found dead in 50.5 hrs.</td>
<td>Lived.</td>
</tr>
</tbody>
</table>

This experiment shows that the toxin is partially destroyed by powerful oxidation.

**IMMUNITY TOWARDS THE TOXIN.**

Several attempts were made to establish an immunity in guinea-pigs toward the toxin by means of subcutaneous and sublethal intraperitoneal inoculations but these all failed. In some instances, where repeated, subcutaneous inoculations were made, the animal apparently became more sensitive towards the substance as the lesion appeared quicker and was more severe than in the first inoculation.

In rabbits the effect of sublethal intravenous inoculations was very interesting, as the following protocols show.

*Rabbit No. 5.*

*August 5.* Weight, 2160 grams. Intravenous injection of 10 mg. toxin No. 13. No apparent effect.

*August 8.* Weight, 1860 grams. Temperature, 38.5°. Intravenous injection of 10 mg. toxin No. 15.
August 10. Temperature, 38.4°, weight, 1765 grams.
August 11. Temperature, 39.8°. Intravenous injection of 10 mg. toxin No. 15.
August 13. Weight, 1710 grams.
August 17. Weight, 1717 grams. Intravenous injection of 20 mg. toxin No. 16 digested twenty hours with trypsin.

Immediately after the inoculation the rabbit lay on its side and kicked rather violently. This kicking was not of a spasmodic character, however. Soon it became quiet and the breathing became gradually slower until it stopped altogether. The heart was beating a short time after the breathing stopped but the animal was dead in five minutes from the time it was inoculated.

Autopsy at once. Pupils slightly dilated.

Peritoneal Cavity. Liver swollen and slightly congested; surface very rough. Spleen slightly enlarged and bluish in color. Kidneys apparently normal. Adrenals apparently normal. The walls of the stomach appeared to be slightly thickened but this was not marked. Intestines normal.

Pleural Cavity. Heart empty and walls flabby. Left ventricle apparently normal. Lungs collapsed and very pale. No thrombi could be found in the pulmonary vessels.

Rabbit No. 4.

August 6. Weight, 1780 grams.
August 7. Intravenous injection of 10 mg. toxin No. 15. Immediately after the inoculation the rabbit appeared to be dizzy, falling on its side when it attempted to move. This effect passed off in a short time.

August 8. Rabbit appears to be quite sick and breathes with difficulty. Temperature, 38.3°, weight 1607 grams.
August 9. Temperature, 39.0°. Intravenous injection of 5 mg. toxin No. 15. After the inoculation the rabbit lay for about two hours on its side as though exhausted.
August 10. Temperature, 39.6°, weight 1525 grams.
August 11. Temperature, 40.4°. Intravenous injection of 5 mg. toxin No. 15.
August 13. Weight, 1515 grams.
August 17. Weight, 1575 grams.
August 20. Weight, 1607 grams.
August 22. Weight, 1560 grams. Rabbit had not been fed. Intravenous injection of 10 mg. toxin No. 17, digested 24 hours with trypsin. Immediately after the inoculation the rabbit fell from side to side and then lay and kicked rather violently, the kick becoming more feeble until it was quiet. The breathing was at first rapid and then gradually slowed until it had stopped altogether for quite an interval. Then the rabbit gave several deep gasps and died. During this time and for a short time after the last
Proteus Vulgaris

breath was taken the heart beat at first rapidly and then slower and slower until it stopped. The rabbit died five minutes after being inoculated.

Autopsy at once. Pupils normal.


Pleural Cavity. Lungs collapsed and apparently normal. No thrombi could be found. Heart showed white areas in the muscle wall of the right ventricle.

The fact that rabbits apparently became hypersensitive might indicate that an immunizing process is taking place, but we were never able to establish an immunity of such a degree that it would protect the animal against a fatal dose of the toxin.

In the guinea-pig the picture, in an animal dead from an intraperitoneal inoculation, is strikingly like that of the tuberculin reaction, and it may be that the two act in a similar manner.

On the other hand the effect produced in cats is very similar to that produced by Schmiedeberg's sepsin (25), as was pointed out by Levy (26). Faust (27) has repeated the work of the former and succeeded in isolating a crystalline substance to which he gives the formula,

$$\text{CH}_2\text{NH}_2\cdot\text{CHOH}\cdot\text{CHOH}\cdot\text{CH}_2\cdot\text{CH}_2\text{NH}_2.$$  

His yield was very small being only 0.03 gram of the sepsin sulphate, for 5 kilos of putrefying yeast. Twenty milligrams of this substance caused in dogs, vomiting, bloody diarrhœa, and finally death. Our relatively impure substance, in a much smaller amount, caused similar effects in cats, and the autopsy showed the intense congestion of the digestive tract similar to that produced by sepsin. Faust succeeded in converting his sepsin into cadaverin by repeated boiling but we were unable to do this with the proteus toxin showing that the two substances are probably not the same.

Vaughan and his pupils (28) have made an extensive study of the so-called endotoxins of a number of organisms and in looking over their work one is struck with the similarity of the action of all these substances.

Through the kindness of Dr. Theobald Smith we were able to compare the colon toxin with that of proteus. A freshly isolated culture of B. coli was grown on large agar slants and the seven
day growth precipitated with alcohol, the same as were our Proteus cultures. This substance, when dried, proved to be very toxic, 4.0 mg. per 100 gram of body weight killing a guinea-pig in less than twenty hours. It resisted heating to 100° for thirty minutes, tryptic and peptic digestion, the same as the proteus toxin. The picture in guinea-pigs differed in that there was less exudate into the peritoneal cavity and the venous congestion was not as marked with the colon as with the proteus toxin. Injected into the subcutis the necrosis was very slight. When injected intravenously into cats, the only effects we were able to observe were drowsiness, loss of appetite, and marked loss in weight. There was no vomiting nor diarrhoea. In rabbits an intravenous injection of 15 mg. of this colon toxin caused a drowsiness and a temporary loss in weight.

These precipitated cultures are probably made up of a great variety of substances, but it is conceivable that the cause of their toxic effects is a definite chemical group whose action is modified by a slight change in grouping or by the other substances present.

It should be noted that while this dried precipitate is in itself very toxic it comes from a relatively large amount of culture. One milligram from a bouillon culture represents 1 cc. of the culture and therefore a fatal dose for a 300 gram guinea-pig, 27 mg. represents 27 cc. of the bouillon culture. Myerhof (29) too noted the relative toxicity of living cultures of proteus to the dead bacteria, finding the living culture four times as toxic as the bodies of the bacteria and twenty times as toxic as the filtered cultures.

CONCLUSIONS.

1. Culturally, Proteus vulgaris varies greatly, its most constant property being the fermentation of dextrose and saccharose and its failure to ferment lactose.

2. In the absence of carbohydrates, proteus destroys some native albumins; and produces ammonia, primary amines, hydrogen sulphide, fatty acids of a high molecular weight, aromatic oxyacids, indol and indol-acetic acid. It does not produce phenol, skatol, mercaptan, alcohols, aldehydes, nor ketones.

3. Proteus vulgaris possesses both the properties of fermentative and putrefactive organisms.
Feeding experiments vary greatly and probably depend upon the virulence of the organisms fed and upon the diet.

The bodies of the bacteria precipitated by alcohol, contain a toxic substance which has the following properties:

- It is thermo-stable.
- It resists tryptic and peptic digestion.
- It is non-dialyzable and is either firmly bound to or is a part of the protein of the cells.
- It is partially destroyed by powerful oxidation.
- Injected into the peritoneal cavity of guinea-pigs it causes rapid death, but when the same amount is injected into the subcutis an extensive necrosis is the result.
- Intravenous injection in cats causes severe vomiting, bloody diarrhea and death. In this respect it resembles sepsin but in other ways it differs so that we cannot conclude that they are the same.
- Rabbits may become hypersensitive to repeated inoculations.
- We were not able to establish an immunity in guinea-pigs or rabbits.

We wish to express our indebtedness to Drs. Theobald Smith, H. D. Dakin and Alfred J. Wakeman for their advice and aid that they so freely gave throughout this work.

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A BIOCHEMICAL STUDY OF PROTEUS VULGARIS HAUSER
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