I am thoroughly convinced that in Case 1 the function of the bowel was so impaired by adhesions, not only of the coils of the ileum to each other, but the tension caused by adhesion of the appendix to the lateral parietal peritoneum, which favored solution in the continuity of the bowel at the point of ulceration at the site of Peyer's patches, was entirely responsible for the tremendous rent that took place. This was demonstrable at the postmortem, which showed the great amount of tension on the superficial sutures.

In Case 2, the only interpretation that could be placed on the extensive adhesions, so like those found in Case 1, is that a like result should have been expected. The inflammatory exudate, which takes place after every attack of appendicitis, it matters not how mild the type, is certain to impair to a degree the function of the bowel, not only immediately about the ileocecal region, but often extending over a considerable extent of the ileum disarranging the circulation, and in this way bringing about structural change in the bowel, interfering as well with the fecal current, and together serving as a most potent factor in the production of perforation.

Where the appendix has once been the seat of inflammatory change, a disease like typhoid fever is sure to light up a recurrence, and as at best its powers of resistance are poor, it could hardly be expected to escape rupture within the organ itself, or what would seem more often to take place, rupture of a Peyer's patch, which, if not actually caused by impairment of the function due to pre-existing disease in the appendix, may be regarded as at least favoring it. Not only do the two cases here reported bear out this belief, but many of those recorded, notably two of Osler's cases; one on admission had an inflammatory mass the size of a walnut in the appendix region, subsequently dying from perforation in the ileum, while the other at the autopsy showed perforation of the ileocecal region and the appendix embedded in an inflammatory mass. One of Hare's two cases also showed pre-existing disease in the appendix. Indeed, proof of pre-existing disease in the appendix in cases of perforation in typhoid fever is not wanting if careful analysis be made of the literature on the subject. Toft says that from personal investigation of 300 cases of illness of all sorts, every third person between the ages of 20 and 70, showed evidence of present or past traces of inflammation of the appendix, and that actual structural change existed in 5 per cent. of all bodies examined. Kraussald declares that this percentage is rather too low than too high.

If these percentages can be considered as accurate, it is within the power of the surgeon to reduce the mortality in perforative peritonitis in typhoid fever from 80 per cent. to 73, first by eliminating the appendix as a factor *per se*; second, by freeing the agglutinated intestines from adhesions caused by previous attacks of appendicitis, which afford possibility of still further serving as prophylaxis against occurrence of perforation in the ileum. Most observers agree that acute appendicitis, occurring during the course of typhoid fever, is merely a coincidence, and is probably in no instance the result of the bacillus of Eberth, nor to the general infective process resulting from the fever.

Hopfenhausen⁴ shows in a study of thirty appendices removed from typhoid subjects dying from either asthenia or toxemia, that no special change existed in the appendix further than slight cellular infiltration, in

no way sufficient to interfere with the function of the organ, and only that change that would be found anywhere in the intestinal tract that is supplied with lymphoid tissue. The type of appendicitis, therefore, that may be looked for during the course of typhoid fever, will usually be the recurrent.

Clinicians are generally agreed that the opinion formerly held of doubting the existence of typhoid fever where bowel symptoms were absent, is entirely erroneous, and as a matter of fact, bowel lesions are, if the bowel be previously healthy, rather atypical than typical symptoms at the onset of the disease.

With this knowledge in mind, when a case of typhoid fever presents among its earliest symptoms pain to the right of the hypogastric region with tenderness, nausea or vomiting, aching or discomfort in the groin, oftentimes extending down the leg, anorexia, and last but not least, rigidity of the right rectus muscle, a relighting up of a pre-existing disease should be suspected, knowing, as we now do, that the right iliac fossa is not tender early in typhoid fever; at least not until after the seventh or eighth day, when the bacillus of Eberth is deposited in the glands of the intestines (Peyer's patches). Immediate laparotomy would seem the only wise course to pursue, removing not only the appendix, but thoroughly freeing the agglutinated coils of the ileum from its tied-down position, which must of necessity impair nutrition of the parts, and consequently diminish its structural resistance.

Dieulafoy states that if an appendicitis exists, the course of the fever will be higher early in the disease, and it may also be inferred that the morning drop in temperature will be less pronounced.

A study of the temperature and pulse in the cases recorded by Finney, Osler, Fitz, Cushing and others confirms this observation, especially in reference to the pulse, which is found to be continuously higher than is found in uncomplicated cases of typhoid fever.

THE ACTION OF MINERAL ACID ON THE CELLULAR SUBSTANCE OF BACILLUS TYPHOSUS.*

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This research was undertaken for the purpose of ascertaining whether or not the cellular substance of the typhoid bacillus shows any tendency to uniform and definite cleavage under the influence of the mineral acids, the ultimate object being to learn something of the chemical composition and structure of the molecule making up the substance of the cell. In this paper, which must be considered only a preliminary contribution, general results only are given, with the hope of being able to go more into detail subsequently.

METHODS.

The material used in these studies consisted of the cellular substance of a typical typhoid bacillus, which was obtained from the mesenteric glands after death from typhoid fever, and which gave in a typical manner all the characteristic cultural manifestations of this organism. The toxicity of the living culture was tested on rabbits, guinea-pigs and white rats, and then the

* This and the following paper were in a series of studies of bacterial cells, research in the Hygienic Laboratory of the University of Michigan, 1903, read at the Fifty-fourth Annual Session of the American Medical Association, in the Section on Pathology and Physiology.

4. Revue med. de la Suisse Romande, Feb. 20, 18.

organism was grown on the large tanks used in this laboratory, and the germ substance obtained in sufficient quantity to carry out the investigations to be detailed later. The germ substance was washed with alcohol, then placed in Soxhlet extractors and the fat thoroughly removed with ether. After this extraction the cellular substance was ground in agate mortars and separated from any traces of agar or other impurity by being passed through fine meshed sieves. As thus obtained, the cellular substance exists as a white powder that is fatal to the lower animals when injected in suspensions in water into the abdominal cavity in the proportion of 1 to 40,000 of body weight.

It has been suggested¹ that the intracellular toxins are not contained as such in the cells, but that they consist of atomic groups or side chains of a more complex molecule. At any rate, to extract them, more active agents than mere solvents have been found necessary. In this research the action of a hydrolytic agent, namely, dilute sulphuric acid, has been tried. It was proposed to find out whether or not this acid produced any definite cleavage—something as to the nature of the split product—whether it was a toxin, a protamin or something else, and also to study the distribution of the nitrogen in the germ substance by aid of the action of the acid.

STUDY OF THE DETAILS OF CLEAVAGE.

First, acids of two different strengths were employed to determine whether or not the split product was affected by a variation in the strength of the acid. Two 10-gram samples of the powdered germ substance were extracted, one with 0.1 per cent. sulphuric acid, and the other with 1 per cent.; extractions being made in the cold first, then of the residue on a boiling water bath, at 110 C., and at 120 C. Extractions of these residues at 134 C. split off nothing further precipitable with the reagent used. The extracts were precipitated with three volumes of 95 per cent. alcohol, the precipitate filtered out, washed with alcohol, dried and powdered.

Two points of interest were observed, namely, that a definite cleavage seemed to take place in both cases, either the weaker or the stronger acid making the separation, and that in both cases the total amount of split product that could be obtained by extraction in this way was practically 1 gram, or 10 per cent. It is interesting to note in passing that two years ago, with the colon bacillus Vaughan obtained in one experiment, from the 50 grams of germ substance used, 5 grams of the substance split off with dilute sulphuric acid, and precipitated by alcohol,² which is 10 per cent., as found with the typhoid. The fact that treatment with acids of two different strengths yielded practically the same amounts of split product indicated that a certain definite cleavage had taken place. The figures for the amounts of split product are given in Table 1.

TABLE 1.—RESULTS WITH VARIED STRENGTH OF ACID.

	0.1 per cent. H ₂ SO ₄	1 per cent. H ₂ SO ₄
Cold	0.2073	0.2015
Water bath	0.5427	0.6555
110° C.	0.1045	0.2215
120° (1 per cent. instead of 1/10 per cent.) ..	0.2290	0.0905
	1.0835	1.1690

In both cases no more was obtained by further extraction at a higher temperature, and while in the case of the series with 0.1 per cent. acid, a 1 per cent. acid

was used for the extraction at 120; it is probable that several extractions with the 0.1 per cent. acid at that temperature would have accomplished the same result.

All these split products, as well as the final residue, were toxic for guinea-pigs, though not so toxic as the germ powder itself. It may be that the split products are not a single substance, but that they consist largely of some inert body with which the real toxin is mixed. The extracts all give the xanthoproteic test, also a fair Millon's, among the proteid color reactions, and those above 100, the biuret test.

From the 20 grams of germ powder used, there remained, after all extractions, a residue of 1.505 grams.

THE NATURE OF THE SPLIT PRODUCTS.

In order to investigate further the split products, a larger sample of germ substance was used, so that the yield might be greater, and in order to avoid as far as possible any decomposition of the split product by the acid, the weaker acid (0.1 per cent.) was chosen, since it seemed that by repeated extraction as much could be obtained as with the stronger acid. Accordingly, 200 grams of germ substance was extracted with 0.1 per cent. sulphuric acid in the cold, then the residue at 100 C. (flowing steam in the autoclave), and at 120 C. in the autoclave. The exact temperatures used for the smaller samples were found to be impracticable here, because with the larger amount, extraction on the water bath was not successful. Four liters of acid were used for each operation, and the extractions at a given temperature continued until no more was split off at that temperature, when the residue was again treated with acid, and extracted at the next higher temperature used. This required three extractions in the cold, four at 100 C. and two at 120 C. The amount of split product obtained by precipitating the extracts at each temperature with alcohol was the same relative proportion of the weight of the whole sample as the products obtained from extractions of the smaller samples, and the whole was approximately 20 grams, or 10 per cent., as before, the residue remaining this time amounting to 26.96 grams. The extracts each time were precipitated with three volumes of 95 per cent. alcohol, the precipitate after settling being filtered out, washed with alcohol, and then purified by dissolving, so far as possible, in water and reprecipitating with alcohol. In this way the split products were divided into a soluble and insoluble portion. The different soluble portions from each temperature were put together and repurified. The insoluble portions were saved except that at 100 C. This was very small in amount and so slimy and mucilaginous that it was not possible to remove it from the filter, and it was consequently lost. Determinations of ash, phosphorus and nitrogen were made in both the soluble and insoluble portions, and likewise in the final residue and original germ powder. First, the ash was determined, then the phosphorus in the ash. The nitrogen in each case was determined by the Kjeldahl method. The figures are given in Table 2.

It is evident that the extraction in the cold brought down first of all a large part of the inorganic constituents of the germ, among which iron and sodium were found, and it is interesting that the split product which was most toxic, the soluble part at 120 C., was the one containing the largest percentage of nitrogen.

An ultimate analysis was made of the soluble part obtained at 100 C., this being the only one of which there was a sufficient quantity for the purpose. The

1. Vaughan: A study of bacterial cells, Trans. Assn. Amer. Phys., 1902.

2. Marshall and Gelston: Toxicity of the cellular substance of the colon bacillus, Trans. Assn. Amer. Phys., 1902.

powder was dried to constant weight, and its constituents determined with the results given below. Carbon and hydrogen were determined by combustion, nitrogen by the Kjeldahl method, phosphorus in the ash by precipitation first with ammonium molybdate, then with

On the whole, the indications are that the split product obtained by the action of dilute mineral acid is of the nature of a phosphorized glycoprotein.

Blandine⁹ reports the isolation of a nuclein and nuclealbumin from cultures of the typhoid bacillus,

TABLE 2.—SOLUBLE AND INSOLUBLE PRODUCTS.

Cold.						100° C.						120° C.						Residue.			Germ.		
Soluble.			Insoluble.			Soluble.			Insoluble.			Soluble.			Insoluble.								
Ash.	P.	N.	Ash.	P.	N.	Ash.	P.	N.	Ash.	P.	N.	Ash.	P.	N.	Ash.	P.	N.	Ash.	P.	N.	Ash.	P.	N.
72.6	.4058	3.75	55.65	8.43	5.89	5.28	.385	10.34	. . .	. . .	. . .	3.02	.87	14.5	4.78	1.50	12.26	2.60	.21	13.04	5.7	1.85	11.55
Ash free . . . 13.72 13.28						10.918						15.01 12.89						13.424			12.258		

diluted acidified phosphotungstic acid, dried, weighed and powdered, and the nitrogen determined by the Kjeldahl method. The nitrogen was also determined in the filtrates from these precipitates and in the dried residue remaining from the acid extraction. The results are given in Table 5. All the acid extracts gave the xanthoproteic and Millon reactions for proteid, and all, save the 33 1/3 per cent., the biuret test.

TABLE 5.—NITROGEN AS AMMONIA.

	1 %	5 %	33 1/3 %
Amount of extract.....	200 c.c.	200 c.c.	200 c.c.
Wt. N as NH ₃ in 200 c.c....	0.0112 gms.	0.0112 gms.	0.0112 gms.
TOTAL NITROGEN OF EXTRACTS (Including N as NH ₃).			
	1 %	5 %	33 1/3 %
Amount of extract.....	200 c.c.	200 c.c.	200 c.c.
Wt. N in extract.....	0.4284 g.	0.7644 g.	1.0164 g.
PER CENT. TOTAL NITROGEN OF EXTRACT COMING OFF AS NH ₃ .			
	1 %	5 %	33 1/3 %
	2.6143	1.4652	1.1019

The amount of amido nitrogen split off was the same in each case, which pointed to a definite cleavage regardless of the strength of the acid. The percentages varied, of course, since the total nitrogen of the extracts was different. The largest amount of total nitrogen was found in the 33 1/3 per cent. extract, and hence the smallest per cent. of amid nitrogen, while the smallest total nitrogen was in the 1 per cent. extract. The amid nitrogen varied from 1.1 per cent. to 2.6 per cent. Hausmann¹¹ found approximately 1.2 per cent. amid nitrogen in true proteids, which was in accordance with the results of other investigators.

TABLE 6.—CALCULATED FOR THE GERM SUBSTANCE (10 gm. sample used).

	Amid nitrogen.	Per cent. of total N of germ as amid nitrogen
Total nitrogen.	11.55 %	0.97 %
	0.112 %	

The total nitrogen of the germ was determined by kjeldahling samples of the germ powder. The per cent. of amid nitrogen was found from the fact that 0.0112 grams nitrogen was obtained from each of the three extracts from 10-gram samples.

TABLE 7.—NITROGEN OF THE PHOSPHOTUNGSTIC PRECIPITATES.

	1 %	5 %	33 1/3 %
Wt. ppt.....	7.8220	12.8602	16.9792
Wt. N in ppt.....	0.2014	0.3429	0.2029
Per cent. N in ppt.....	2.575 %	2.667 %	1.197 %

The largest amount of diamido nitrogen was found in the precipitate from the 5 per cent. extract. It is probable that the 33 1/3 per cent. acid was strong enough to further decompose the diamido compounds and so lessen their quantity. In working for hexon bases a 5 per cent. acid extract might be expected to produce the largest yield. The per cent. of nitrogen in the precipitate from the 1 per cent. and 5 per cent. extracts were notably close together, and it is of interest that the 2.66 per cent. nitrogen found in the precipitate from the 5 per cent. extract was quite close to the 2.68 per cent. nitrogen calculated by Gulewitsch¹⁴ in the phosphotungstic precipitate of arginin, but the carbon and hydrogen found were about twice what he calculated. His figures were C, 3.44 per cent.; H, 0.74 per cent., while we found C 8.34 per cent. and H 1.68 per cent.

TABLE 8.—NITROGEN OF FILTRATES FROM PHOSPHOTUNGSTIC PPTS.

	1 %	5 %	33 1/3 %
Total amt. of filtrate.....	200 c.c.	260 c.c.	260 c.c.
Wt. N in whole filtrate.....	0.0315	0.07189	0.36218

The largest amount of monamido nitrogen was found in the filtrate from the 33 1/3 per cent. extract; the smallest in that from the 1 per cent.

TABLE 9.—NITROGEN OF RESIDUE LEFT FROM THE ACID.

	1 %	5 %	33 1/3 %
Wt. residue from 10 gms. germ substance.....	3.9630	1.7322	0.7308
Wt. N in residue.....	0.4633	0.1924	0.0457
Per cent. N in residue.....	11.6900	11.1064	6.2631

The per cent. of nitrogen in the residues from the 1 per cent. and 5 per cent. acids was not greatly changed from that of the original germ substance (11.55 per cent.), which would seem to indicate that the residue was still undecomposed.

CONCLUSIONS.

From this research it may be concluded that there is present in the cellular substance of the *Bacillus typhosus* one or more compound proteid bodies, one of which, at least, seems to be of the nature of a phosphorized glycoprotein. Further, that proteid bodies are split off by the use of dilute acid, cleavage taking place regardless of the strength of the acid, so long as it is quite dilute; and that the total amount of this cleavage product which can be split off by dilute acid composes about 10 per cent. of the weight of the dry germ substance. And, lastly, that the cleavage of amido nitrogen by acid is definite regardless of the strength of the acid; that the largest amount of diamido nitrogen is obtained by the use of 5 per cent. acid, as compared with that obtained by the use of 1 per cent. and 33 1/3 per cent., while the largest amount of monamido nitrogen is found in the 33 1/3 per cent. acid extract.

THE CHEMISTRY OF BACILLUS COLI COMMUNIS.*

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In a previous paper¹ I have given a résumé of the literature on the chemical composition of bacteria, and a report of some preliminary work on the colon bacillus. This work demonstrated that the dried germ substance after extraction with alcohol contains 8.6 per cent. of ash, one-third of which ash is phosphorus, while sodium and potassium are the most abundant metals. Extraction with dilute sulphuric acid and treatment of the residue with a weak solution of sodium hydrate sufficed to bring practically the whole of the germ substance into solution. In the acid extract indications were found of xanthin and hexon bases, carbohydrate, much phosphorus and a toxic substance insoluble in alcohol. From the alkaline extract a nuclein body containing 2.93 per cent. of phosphorus was obtained, and evidence of carbohydrate was found. In these preliminary experiments the amounts were too small to admit of the purification and identification of the products, but they served to show several lines of work that could be profitably pursued. Along two of these I can report progress, although neither is completed.

Pure colon cultures obtained from feces were grown on 2 per cent. agar in the large incubating tanks used in this laboratory. After from seven to fourteen days the growth was removed, freed from agar, extracted first with alcohol and then with ether, dried and pulverized. This material was heated on the waterbath for an hour, with forty times its weight of 1 per cent. sulphuric acid, and filtered. The extract was poured into from three to five volumes of alcohol. The precipitate formed was washed with alcohol until the washings were

14. Gulewitsch: Ueber das Arginin. Zeit. f. physiol. Chem., 1899, xxvii, 178.

* See footnote to previous article.

1. Trans. Assn. of Amer. Phys., 1902.