TABLE 2.—TOXICITY OF THE GERM SUBSTANCE OF *BACILLUS PYOCYANEUS* INJECTED INTRAPERITONEALLY INTO GUINEA-PIGS AFTER BEING HEATED.

30 Number.	240 Weight.	4.40 jected, mg.	+ Results.	12 Hours.	Proportion to body weight.
29	180	4.50	+	12	1 to 40,000
30	240	4.40	+	12	1 to 45,000
31	140	2.80	+	12	1 to 50,000
(30 minutes at 75 C.) (3 + in 12 hours.)					
32	250	5.00	+	12	1 to 50,000
33	230	4.60	+	12	
34	225	5.00	+	12	30 minutes at 100 C.
35	245	6.10	+	12	
(4 + in 12 hours.)					
36	220	4.40	—		1 to 50,000
41	190	3.80	+	12	
37	190	4.20	+	12	1 to 45,000
40	230	5.11	+	12	
38	240	6.00	+	18 days.	1 to 40,000
39	205	5.12	+	12	
(30 minutes at 110 C.) (5 + in 12 hours to 18 days, 1 —.)					
44	245	4.9	+	18	1 to 50,000
45	250	5.00	—		
47	235	5.22	+	18	1 to 45,000
43	225	5.00	+	18	
42	200	5.00	+	16 days	1 to 40,000
46	220	5.50	+	12	
(30 minutes at 120 C.) (5 + in 12 hours to 16 days, 1 —.)					

The intracellular toxin can not be extracted, at least not in appreciable quantity, by physiologic salt solution; 100 mg. of the germ substance was suspended in 50 c.c. of physiologic salt solution, placed in a sealed tube, and vigorously agitated in a mechanical shaker for twelve hours, at the end of which time the emulsion was filtered through heavy Swedish filter paper, and the filtrate found to be without toxic effect. This is found to be true, whether the germ substance was freshly taken from agar cultures or had been previously extracted with alcohol and ether.

When the germ substance was extracted with 1 per cent. of sulphuric acid and this extract treated with absolute alcohol, a precipitate was obtained. This precipitate, after having been washed until the washings were free from acid, dried and injected into animals, proved to be toxic, but much less so than the original germ substance.

While no appreciable amount of toxin could be obtained from the cell substance, a hemolytic body was extracted with physiologic salt solution; 100 mg. of the germ substance which had been hardened by the action of alcohol and ether, was suspended in 50 c.c. of physiologic salt solution and agitated for twelve hours, after which it was filtered through heavy Swedish paper. To three tubes containing each 10 c.c. of a 5 per cent. solution of defibrinated blood, the following quantities of physiologic salt solution extract of the germ substance were added:

Tube 1 received	10 c.c. of the extract.
Tube 2 received	1 c.c. of the extract.
Tube 3 received	0.1 c.c. of the extract.

These tubes were placed in the incubator for twenty-four hours, at the end of which time complete hemolysis had occurred in Tube 1. In Tube 2, about one-third of the blood had been dissolved, while in Tube 3 the hemolytic action was found to be very slight.

The hemolytic substance was also extracted from the unhardened fresh germ. Some of this germ substance was shaken with physiologic salt solution for twelve hours and then passed through a Berkefeld filter; 13 c.c. of this filtrate was found to completely dissolve all the blood corpuscles in 10 c.c. of a 5 per cent. blood solution in twenty-four hours at incubator temperature.

My conclusions may be stated as follows:

1. *Bacillus pyocyaneus* does contain an intracellular toxin.

2. This toxin can not be extracted from the cell by physiologic salt solution. It is in part extracted by dilute sulphuric acid.

3. Heat to 120 C. in the autoclave continued for thirty minutes only slightly diminishes the toxicity of the cell substance.

4. The cell substance of *Bacillus pyocyaneus* contains a hemolysin, which may be extracted from the cell, either in the fresh state or after treatment with alcohol and ether by physiologic salt solution.

5. The minimum lethal dose of the cellular toxin is for guinea-pigs when given intraperitoneally about 1 part of the cell substance to 50,000 parts of body weight.

6. When injected subcutaneously in guinea-pigs in the proportion of 1 part of the cell substance to 10,000 parts of body weight or less no permanent ill effect results.

7. Subcutaneous injections do not give immunity to subsequent intraperitoneal injections.

THE EFFECT OF PUTREFACTIVE BODIES ON THE CHEMICAL TESTS FOR MORPHIN.*

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In at least three important trials for murder, the possibility of putrefactive products being mistaken for morphin has arisen. The first of these was the Sonzogna trial at Cremona, Italy, in which it is probable that the experts confounded a putrefactive body with morphin. This substance was extracted from either alkaline or acid solutions with ether, but could not be removed with amyl alcohol. It reduced iodic acid, but in its other reactions, also in its physiologic properties, it bore no close resemblance to morphin. In the Buchanan case in New York every test for morphin was duplicated with putrefactive products, so that the distinguished chemists present were not able to tell which of the two portions contained the alkaloid. In the case of Dr. Urbino de Freitas of Oporto, Portugal, who was accused of attempting the wholesale murder of his wife's family by the administration of morphin, the toxicologists who investigated the case relied for the detection of this alkaloid on the color reactions applied to residues obtained by following Dragendorff's method. The chemists reported not only morphin, but narcein and probably delphinin. The defense questioned the reliability of the tests used, and many of the most prominent toxicologists of Europe became more or less involved in the controversy.

These cases are enough to illustrate the desirability of further investigation along this line. Many methods have been suggested for the extraction of morphin in medicolegal cases, without confounding it with any putrefactive body. The best of these methods is that advanced by Kippenberger, who holds that the alkaloidal tannates are soluble in glycerin, while the putrefactive bodies likely to interfere with the tests for morphin are insoluble in this agent. A careful reading of Kippenberger's paper shows that this statement does not rest on any extensive experiments. The method of work in the investigation herewith reported may be described as follows: Six liter flasks were cleaned, rinsed with distilled water, and in each of these there was placed 500 gm. of chopped beef liver and 1,000 c.c. of distilled water. These flasks were then placed in the autoclave and sterilized at 120 C. for forty-five minutes. After having been heated, the flasks were allowed to stand at room temperature for twenty-four

* See note to previous paper.

hours, when their sterility was proven by inoculating tubes of beef tea with material from each flask. After this these flasks were treated as follows:

No. 1 was inoculated with *Bacillus pyocyaneus*; No. 2 was treated with 100 mg. of morphin and then inoculated with *Bacillus pyocyaneus*; No. 3 was treated with 200 mg. of morphin and then inoculated with *Bacillus pyocyaneus*; No. 4 was inoculated with *Bacillus coli communis*; No. 5 was inoculated with *Bacillus coli communis* and treated with 100 mg. of morphin; No. 6 was treated with 200 mg. of morphin and inoculated with *Bacillus coli communis*.

All of these flasks were then placed in the incubator at 37 C., where they were kept for two weeks. At the expiration of this time they were all autoclaved at 120 C. for forty-five minutes. A 2 per cent. solution of tannic acid in glycerin was poured into each flask in an amount equal to the mass therein contained. The flasks were then placed in an incubator and kept for forty-eight hours at 40 C. Then the flasks were removed from the incubator, the contents strained through muslin, and the filtrate heated on a water bath at 60 C. for one-half hour in order to bring down the coagulable proteids. After filtration through paper the filtrates were shaken with twice their volumes of petroleum ether. After separation of the ether the material was heated in order to drive off traces of the solvent, and then rendered slightly alkaline with sodium hydrate and shaken with chloroform. The chloroform extract from each flask promptly reduced iodic acid and potassium permanganate. Like results were obtained with amylic alcohol extracts from an alkaline mixture. None of these residues gave either the ferric chlorid or the Froehde test for morphin in a satisfactory manner. Great difficulty was experienced in obtaining clean pure residues. This was true with both the chloroform and amylic alcohol extracts, and it was found to be necessary to repeatedly take up the residues with water slightly acidulated with acetic acid, and again render alkaline and shake with the solvent. Crystalline residues were finally obtained from only two flasks, one of which contained morphin, while the other did not. The crystals in both of these cases seemed identical microscopically with those obtained from an alkaline solution of morphin shaken with chloroform, and the chloroform extract evaporated. The crystalline residue was so small in amount that the possibility of determining the melting point was excluded.

It must be evident from these results that a satisfactory method of extracting morphin in medicolegal examinations is not furnished by Kippenberger.

THE EXTRACTION OF A TOXIN FROM LIVER CELLS.

A PRELIMINARY REPORT.*

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The liver of an ox, just after the animal had been slaughtered, was obtained and passed through a sausage machine, finely dividing the structure and removing a considerable portion of the connective tissue. This finely divided material was stirred up with five times its volume of 1 per cent. sulphuric acid, and the mixture was heated for three hours and thirty minutes in the

water bath at a temperature of 81 C. Then it was heated for three hours longer over the direct flame at 100 C. and filtered. The filtrate proved to be a clear, light amber-colored fluid, which was added drop by drop to three volumes of 95 per cent. alcohol, with constant stirring. The precipitate which formed was collected on a hard filter, washed with alcohol, and then dissolved in a minimum amount of water. This solution was again precipitated by adding it drop by drop to three times its volume of 95 per cent. alcohol. This precipitate was washed with alcohol and ether, then redissolved in distilled water. This process was repeated until the aqueous solution failed to give a test for sulphuric acid with dimethylamidoazobenzol. The substance thus obtained was dried in vacuo and ground, first in a porcelain and then in an agate mortar.

The cleavage product thus obtained, when dissolved in water and injected intraperitoneally into guinea-pigs and rabbits, kills the animal when the proportion is 1 part of the extract to 500 parts of body weight. When used in smaller quantities there is more or less marked emaciation, depending on the quantity injected, from which the animal recovers very slowly. So far we have not been able to secure any marked degree of immunity by beginning with small doses and gradually increasing the quantity.

After acute poisoning with the quantity above mentioned, postmortem examination shows the liver of the dead animal to be deeply congested. The spleen is soft and mottled, with dark and pale red spots. The kidneys show no gross changes, but the adrenals are markedly congested. The gastric and mesenteric vessels are greatly dilated, and there are frequently found small hemorrhagic areas under the peritoneum. We are now engaged in studying the effects of very small doses frequently repeated on the structure of the liver and other organs.

A METHOD OF MICROSCOPIC OBSERVATION BY MEANS OF LATERAL ILLUMINATION.

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INTRODUCTION.

Last year two German physicists, Siedentopf and Zsigmondy, published an article¹ in which they described an apparatus for the observation of ultramicroscopic particles. The object which they selected for examination consisted of a piece of glass through which was diffused uniformly particles of metallic gold in a very fine state of division. By means of a powerful light—either the sun or an electric arc—passed through a complicated system of condensers, a very small but exceedingly intense focus of illumination was obtained. The piece of glass containing the gold was placed in this focus so that the light entered the glass from the side. It was then observed with an ordinary microscope from above. By this arrangement none of the light entered the microscope directly. To the naked eye, or with the ordinary microscope, the glass appeared perfectly homogeneous. But by this special illumination the individual particles of gold so reflected and refracted the light that they could be observed as distinct luminous points. This being the only light that entered the microscope, the particles appeared as bright points against a dark background.

* See note to paper of Dr. MacIntyre.

1. *Annalen der Physik*, 1903, x, 1.