

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.\*

V. C. VAUGHAN, M.D., LL.D., J. F. MUNSON, A.B.,

AND

FRANK R. SPENCER, M.D.

ANN ARBOR, MICH.

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.

D. J. DAVIS, M.D.

CHICAGO.

(From the Pathological Laboratory of Rush Medical College.)

### INTRODUCTION.

Last year two German physicists, Siedentopf and Zsigmondy, published an article<sup>1</sup> 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.