

TABLE I.
Cerebral Oxygen Uptake After Pyocyanin.

, Min. after injection of pyocyanin	A.-V. Difference, vol. %	Blood sugar, mg%		Remarks
		Arterial	Venous	
	9.15	90	79	Control
15	15.68	83	68	150 mg pyocyanin, clonic spasms, vomiting
30	11.70	81	65	Panting
43	9.52			"
58	5.77	74	66	
		54	48	150 mg pyocyanin
20	11.52	52	20	

behavior. In all instances marked muscular weakness was noted. Those animals that were able to walk at all sank to the floor after a few steps. The uncontrolled movements of the head resembled those of an animal with disturbance of vestibular apparatus. In 2 instances the animals gave evidence of a release of emotional mechanism. For example, the animals ran about in a frightened manner with tail hairs widely spread. Six of the 10 cats succumbed later, although they received no additional dosage of pyocyanin. In those animals that survived, the neurological changes persisted for 3 weeks and gradual signs of improvement were noted. Further studies in the biochemical and morphological changes of the brain are now in progress.

Conclusions. Pyocyanin administered *in vivo* produces an increased cerebral oxygen utilization which may be followed by a decrease. These changes are accompanied by symptoms of disturbed function of the central nervous system.

10934

The Filterability of Bacteria.

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If one filters a 1:30,000 solution of night blue, a colloidal basic dye, through a siliceous filter Berkefeld N, the filtrate is colorless.

If, now, we add a so-called "wetting agent" to the dye solution it is found that the dye passes the filter. A number of such agents have been tried, of which I give a partial list: Blood serum, bile, sodium taurocholate, glycocholic acid, turkey red oil, mucin, soap, castor oil soap, lecithin, sodium lauryl sulfate, etc.

Similarly, one can, by adding such agents before inoculation to nutrient broth cultures of bacteria which are non-filterable through siliceous filters when grown on broth without such agents, render the bacteria filterable. The following experiments will illustrate:

The experiments were made as follows: In each case the filter flask contained before sterilization 100 cc of the nutrient broth plus any addition which was used in the broth culture which was to be filtered, so that the filtrate passed directly into fresh, sterile material without further handling.

The ordinary laboratory meat broth is made by extracting ground lean beef in the cold room for 24 hours, then autoclaving at 120°C for 30 min, adding NaCl and peptone, and filtering and adjusting the pH to the required degree.

Berkefeld N filters were used throughout.

April 20, 1939. Ten different samples of an ordinary house meat broth culture of *Streptococcus hemolyticus* Group C were passed through 10 different Berkefeld N filters. All the filtrates showed growth of the streptococcus. The same organism grown in the Liebig meat extract broth were filtered through 10 different Berkefeld N filters. None of the filtrates showed growth.

June 6, 1939. A 4-day culture of *Streptococcus hemolyticus*, Group C, grown in meat extract broth containing 1:1000 taurocholate of sodium, was passed through 4 different filters.

June 8, 1939. Three of these filtrates were infected with streptococcus.

April 24, 1939. A 24-hour culture of typhoid bacillus (Rawlings) in meat extract broth was passed through 4 different Berkefeld filters.

April 25, 1939. All filtrates were sterile and there was then passed through the same filter an 11-day culture of typhoid bacillus in meat extract broth to which was added 1:5000 of sodium lauryl sulfate.

April 26, 1939. All filtrates were infected.

The above experiment was repeated with culture containing 1:2000 sodium taurocholate. All filtrates were infected.

June 4, 1939. A 48-hour culture of typhoid bacillus in meat extract broth was filtered through each of 4 different Berkefeld filters.

June 6, 1939. All filtrates were sterile. There was then passed through the same filters a culture made in another portion of the same broth which contained 1:1000 sodium taurocholate.

June 7, 1939. All filtrates were infected.

When filtrates were infected, the identity of the microorganism in the filtrates was established by stained smear culture, and also in the case of the typhoid bacillus, serologically.

April 11, 1939. A 24-hour culture of *Bacillus subtilis* was passed through each of 5 Berkefeld filters.

April 12, 1939. All filtrates were sterile. The filters were then sterilized in the autoclave and after cooling, there was passed through 3 of the filters a 2-day culture of *Bacillus subtilis* in broth containing 2:1000 sodium taurocholate. All these filtrates were infected the next day. Through the other 2 sterilized filters there was passed a broth culture of *subtilis* without any "wetting agent", and both of these filtrates remained sterile.

In the course of this work it was found that when control experiments were made with broth cultures grown on the ordinary laboratory meat broth without any added "wetting agent" a considerable percentage of the controls showed that the organisms had passed the filter. It was found that if the dye is added to this ordinary laboratory meat broth before heating it did not pass the filter. When added to the broth which had been autoclaved, the dye passed the filter.

Further, it was found that when the broth was made with Liebig meat extract ($\frac{1}{2}\%$) the dye did not pass the filter. I finally reached the conclusion that in the case of broth made with ground beef there was always a considerable amount of fat present and the heating converted this into soap, and this small amount of soap present was the "wetting agent" which aided the dye and bacteria to permeate the filter.

When meat extract, in the preparation of which the temperature never goes above the boiling point of water, was used to make the culture medium, neither the dye nor bacteria passed the filter.

This was supported by experiments which showed that a solution of castor oil soap of 1:50,000 passed the dye through the Berkefeld filter.

Analysis of the 2 broth culture media by the Division of Chemistry, National Institute of Health, showed in the case of the one made from ground beef, 45 parts per million of fatty acid, and in the one made from meat extract 24 parts per million. It is not unlikely that Kendall¹ in his experiments with typhoid bacilli grown on his "K" medium, which he found to be filterable, had by accident one of these "wetting agents" present. His medium was prepared from the intestines of swine or sheep and autoclaved and it is entirely possible that he had present either soap or bile salts, or both.

As a working hypothesis to explain the action of the various wetting agents on filters, I believe the wetting agent "insulated" the surface of the filter pores.

¹ Kendall, A. I., *Trans. Assn. Am. Physicians*, 1932, **57**, 100.

Siliceous filters have a negative charge and hold the basic dye particles which have a positive charge. When the filtering surface is covered with the "wetting agent" this prevents the siliceous surface from holding the positively charged dye particles.

This hypothesis is borne out by the following experiment. A clean glass rod was immersed in the dye solution in distilled water and allowed to stand over night. The rod will be found stained blue and the dye can not be washed off with water. Another glass rod is immersed in a dye solution containing 1:2000 castor oil soap, or a similar solution of sodium taurocholate, or a solution of sodium lauryl sulfate of similar concentration. The rods are allowed to stand in these solutions over night, yet when they are removed and washed off with water, they are unstained.

The "wetting agent" has insulated the surface of the glass rod, which has a negative charge, so that it can not hold the positively charged dye particles.

It is a pleasing duty to acknowledge my thanks to the staff of the National Institute of Health for the facilities placed at my disposal.

10935

Effect of Certain Endocrines on the Excretion of an Anti-diuretic Substance.

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The activity of the posterior pituitary has been investigated with renewed interest during the past few years. It has been shown² recently and confirmed^{1, 4, 5} that an antidiuretic substance is excreted in the urine following dehydration of animals. Gilman and Goodman believe this substance is of pituitary origin since it is absent in the urine of animals dehydrated after hypophysectomy.

The relationship of the antidiuretic activity of the posterior pituitary to the other members of the endocrine system is still not clear. It has been reported that it is related to adrenal cortical function only

¹ Boylston, G. A., and Ivy, A. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 644.

² Gilman, A., and Goodman, L. S., *J. Physiol.*, 1937, **90**, 113.

⁴ Ingram, W. R., Ladd, L., and Benbow, J. T., *Proc. Am. Physiol. Soc.*, 1938, 107.

⁵ Martin, S. J., Herrlich, H. C., and Fazekas, J. F., *Am. J. Physiol.*, 1939, **127**, 51.