

such shedding is due to the absence of thyroid activity is demonstrated by the fact that the feeding of 0.5 mg. of desiccated thyroid tissue for each gram of body weight to both hypophysectomized and thyroidectomized snakes prevented shedding. About 30 snakes were put under hibernating conditions for several months, and none of them shed its skin during that time. This indicates that the shedding is not induced by lowered metabolism alone, but probably is due to the absence of thyroid activity.

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Purification of Phage by Adsorption and Elution.

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A precise study of the nature of viruses is rendered difficult by various extraneous substances. Although these substances do not appear to affect the action of the virus on its specific host, they modify the behavior of the virus in tests designed to ascertain its nature. Recently two methods have been reported for purifying viruses. One is based on ultrafiltration,¹ the other on adsorption and elution.²

The latter procedure is more easily carried out in the laboratory. However, it is difficult to standardize the technique and hence some authors have reported positive, others negative results. This paper deals with the standardization of the technique and the further purification of *B. coli* bacteriophage.

Media and Reaction: The character of the medium influences the adsorption, elution and amount of phage produced. In a synthetic medium the concentration of phage at the outset is low and elution is unsatisfactory. Adsorption is best in an acid and elution in an alkaline medium. The following protocol is typical:

Parallel tubes of broth and synthetic media were seeded at the same time with the same amounts of phage and culture, incubated 24 hours at 37°C., and filtered through a Seitz filter.

The phage titre in the broth was 10⁹, in the synthetic media 10⁵.

Method of Eluting. Adsorption is more complete at pH 5.5 than

¹ Elfold, W. J., *J. Path. and Bact.*, 1931, **34**, 505.

² Kligler, I. J., and Olitzki, L., *Brit. J. Exp. Path.*, 1931, **12**, 172.

TABLE I.

	Phage Concentration*		Eluate**
	Supernatant fluid after adsorption	Resuspended kaoline sediment	
<i>Broth</i>			
Adsorbed with kaoline at:			
pH 5.5	10 ²	10 ⁶	10 ⁸
pH 7.6	10 ³	10 ⁹	10 ⁷
<i>Synthetic medium</i>			
Adsorbed with kaoline at:			
pH 5.5	0	10 ³	10 ²
pH 7.6	10 ¹	10 ⁴	10 ¹

*The numbers equal phage plaques.

**See reference.

at pH 7.6 and the former adsorbate yields the best eluate. In the protein containing broth the phage is not so completely removed as in the synthetic medium. On the other hand it is apparently less firmly bound to the adsorbent and more readily removed by the eluting fluid.

The kaoline is also a variable factor. Each lot must be tested for optimal concentration. A 50% suspension in saline adjusted with phosphate solution to pH 5.5 and autoclaved served as the stock. Adsorption was carried out by adding equal volumes of kaoline suspension and phage, the mixture shaken thoroughly and left overnight in the ice box.

Elution was most effective with a N/100 solution of N/100 NH₄OH. Twice the original volume was added to the kaoline sediment after thorough centrifugation and removal of the supernatant fluid.

Purification of the Eluate. The eluate thus obtained had a phage content of 10⁸, gave negative tests with Esbach and sulfosalicylic acid and a positive ninhydrin reaction. The total non-ammonia N was 9 to 11 mg. per 100 cc.

Further purification was effected by re-adsorbing 4 cc. eluate with 2 gm. kaoline (pH 5.5), and eluting again with 8 cc., N/100 NH₄OH. The second eluate had a phage content of 10⁷, and the ninhydrin test was slightly positive.

This eluate was dialyzed under sterile conditions for 6 days. The phage content then was 10⁶, protein and ninhydrin tests were negative and the N content was 1.4 mg. per 100 cc. These results indicate a phage suspension of a high degree of purity. Experiments are now under way to determine the behavior and antigenic character of this purified phage.

The above experiments indicate that it is possible to obtain a potent phage practically free of extraneous proteins or amino acids.

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Cause of Onset of Labor. An Hormonal Investigation.

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With the increase in our knowledge of the rôle played by the pituitary and ovarian hormones, attention is being directed toward the possibility of a hormonal source as a fundamental cause which induces labor. Animal experiments suggest that there is a synergistic action between oestrin and pituitrin. If the isolated uterus is first bathed with oestrin, it becomes more sensitive to pituitrin as manifested by increase of contraction.¹⁻⁵ On this hypothesis, a hormonal theory as to the cause of the onset of labor may be summarized as follows: During early pregnancy oestrin is held in abeyance by the presence of the luteinizing factor in the ovary, placenta, and anterior hypophysis, and as maturity of gestation approaches release of this inhibiting action upon oestrin is observed. The uterine sensitivity to pituitrin, stimulated by the released oestrin, becomes more marked and continues until the threshold is reached, when labor is precipitated.

To test this theory clinically, a series of hormonal injections was undertaken on 45 pregnant negro women at term. The ovarian follicular hormone, in the form of theelin, was administered in various dosages, singularly and daily, to 10 pregnant women who were at term. Labor occurred within 36 hours in only 2 cases. Ovarian follicular fluid, aspirated at operation, was given to 2 women in a similar state; neither delivered in the allotted time. This same hormone in the form of amniotin or progynon was administered alone, or in combination with pituitrin or pitocin, the active uterine stimulating principle of pituitrin, in various dosages and at different

¹ Bourne, A. W., and Burn, J. H., *Lancet*, 1928, **2**, 1020.

² Brouha, L., and Simmonnet, H., *Compt. Rend. Soc. de Biol.*, 1928, **94**, 759.

³ Dixon, W. E., and Marshall, F. N. A., *J. Phys.*, 1924, **59**, 276.

⁴ Jeffcoate, T. N. A., *J. Obs. and Gyn. Brit. Empire*, 1932, **39**, 67.

⁵ Witherspoon, J. T., *Proc. Soc. Exp. Biol. and Med.*, 1932, **29**, 1063.