hormone of the pituitary (LH) when injected into hypophysectomized males will produce pronounced enlargement of the seminal vesicles and will stimulate, probably indirectly, gametogenesis; but the follicle stimulating hormone (FSH) will not induce any enlargement of the seminal vesicles. In the female Foster and Hisaw[5] have shown that FSH will stimulate the development of follicles only after they have developed antra. Epithelioid cells atrophy after hypophysectomy and enlarge again after injections of LH.[6] Thus, if the above mentioned conclusions be accepted, the enlarged seminal vesicles and the correction of priapism with anterior lobe injections in the male dwarf mouse may be taken to indicate the presence of LH and a partial or complete deficiency of FSH; and in the female, likewise, the presence of large numbers of epithelioid cells and the development of follicles only to medium size again indicate the presence of LH and a deficiency of FSH. It seems probable, therefore, that the dwarf mouse not only lacks growth hormone, but has a partial or complete deficiency of at least one of the gonadotropic hormones.

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Method for Separation of Micro-organisms from Large Quantities of Broth Culture.

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This paper contains a report of a rapid and simple method for removing bacteria from broth cultures. Large volumes can be handled conveniently. Preliminary tests indicate that the antigenic properties of the bacteria remain unaffected.

The method consists in the formation of a precipitate of calcium phosphate in the broth. This adsorbs the bacteria, settles quickly, and can be removed by siphoning away the supernatant liquid and lightly centrifuging the remaining sediment. The calcium phosphate may then be redissolved by suspending the precipitate in water and acidifying to about pH 5.0. The bacteria can be recovered by centrifugation and then washed.

[5] Foster, M. A., Foster, R. C., and Hisaw, F. L., *Endocrinol.*, 1937, **21**, 249.

[6] Hisaw, F. L., personal communication.

The amounts of bacterial culture employed are limited only by the available apparatus. A preliminary test is made of the amount of calcium phosphate necessary to effect practically complete removal of the microörganisms. This may be done by preparing a series of tubes containing 10 cc. of broth to which is added 0.1-1.0 cc. of the calcium reagent, followed by N NaOH, to pH 7.0. After light centrifugation, the supernatant liquid will be crystal-clear in the tubes containing enough calcium. In the routine treatment of any one type of culture, a single test should suffice.

We conduct the precipitation of 5-liter quantities of culture by adding half the required amount of 0.5 M acid calcium phosphate,* and half the amount of N NaOH that will be required to bring the pH to 6.5-7.0 (Nitrazine paper). The rest of the calcium phosphate and NaOH are then added. This is probably more efficient than adding the whole amount at one time, and helps to prevent large clumps of precipitate from forming. For the same reason, the mixture should be agitated vigorously during the precipitation. There is little danger of excessive local hydroxyl ion concentration because of the buffer action of the protein and peptone present in the broth.

The precipitate is allowed to settle for 10 minutes or more and the supernatant liquid, if not turbid, siphoned off and discarded. The amount of bacteria lost in this way is negligible. The precipitate is then separated by light centrifugation for 2-3 minutes or suction filtration. Should the supernatant liquid be turbid, it is well to determine whether this is due to the calcium phosphate or to bacteria. If due to the former, it will clear readily with a little acid, and flocculate with a little alkali. If the supernatant liquid does not become crystal clear after acidification, insufficient calcium phosphate was used and the treatment should be repeated.

The precipitate of calcium phosphate and bacteria should be mixed with water (so that the final volume is half the volume of the calcium reagent used) to form a homogeneous suspension. One or 2 N HCl is added, with constant stirring, to a pH between 5 and 4. The calcium phosphate precipitate dissolves, and the bacteria may be collected with the aid of centrifugation. If it is desired to restrict the acidity of the bacterial suspension as much as possible, the calcium phosphate may be redissolved at about pH 5 if sufficient water is used, or the acidity may be controlled more safely by adding HCl to pH 5.5, followed by enough normal acid sodium phosphate to complete the solution.

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* To prepare a $Ca(H_2PO_4)_2$ solution in 0.5 M concentration, acidification with HCl to pH 3.5-3.0 is necessary to dissolve the salt completely.

In the case of a culture containing a phosphate buffer, it is only necessary to add an adequate amount of calcium chloride, followed by neutralization with NaOH, in the original precipitation.

We found that a culture of *Staphylococcus aureus* and one of *Bacillus typhosus* grown in beef-heart infusion-broth† required approximately 0.014 Mols of acid calcium phosphate per liter, for practically complete precipitation. A glucose phosphate broth culture‡ of indifferent streptococcus (Strain Q155) required 0.04 Mols of $CaCl_2$ per liter.

We have tested some of the antigenic properties of the Q155 Strain of indifferent streptococcus and found that the sera of rabbits, immunized with a vaccine prepared from bacteria precipitated by this method, when compared with the sera of rabbits immunized with a vaccine prepared from centrifuged organisms, contained antibodies (type-specific agglutinins and precipitins) detectable in equally high dilutions.

Furthermore, crude extracts containing the type-specific carbohydrate (S)[1] were prepared, both from precipitated and centrifuged bacteria, and they were found to be active in the same dilutions. The extracts were prepared according to Lancefield's method.[2]

Quantitative tests of viability have not been made, but we can say that the majority of bacteria is viable after separation by this method.

We find that less time is required to separate bacteria from 20 liters of broth, by this method, than was formerly necessary to handle 3 liters; and larger quantities require very little additional time. Furthermore, the yield of bacteria is greater than after the usual centrifugation procedure.

We are investigating the possibility of using other salts as precipitating agents, and the effect of these procedures on labile antigenic fractions of some bacteria.

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† Beef heart infusion broth: beef heart infusion; 1% peptone; 0.5% NaCl.

‡ Glucose phosphate broth: beef heart infusion; 1% peptone; 0.2% Na_2HPO_4; 0.5% glucose; adjusted to pH 7.6-7.8 (N NaOH).

1 Hitchcock, C. H., *J. Exp. Med.*, 1928, **48**, 393.

2 Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.