latter had been determined before exercise. Our experiments on this point thus check with those of Barcroft and Stephens within reasonable limits.

Having measured the extreme contraction immediately after exercise, we then traced the course of recovery by continuing measurements until the spleen had attained all or most of its resting size. We found that recovery begins as soon as the exercise ceases because our measurements (made within 1 minute of the cessation of the electrical stimulation) invariably show a spleen which is increasing in size. This increase appears to consist of 2 phases, of which the first is very rapid, particularly within the first 5 minutes. The second is slower, continuing until the spleen has returned to its resting size. This usually takes from 15 to 30 minutes.

The speed of the first, or rapid, phase of recovery appears to be nearly constant under all conditions, and is independent of the intensity or duration of the preceding exercise. When the data are plotted as per cent of normal size, against time, the slope of the resulting curves for the first 3 to 5 minutes is constant within the error of the method. The speed of the second, slower, phase of recovery is variable and our data have not as yet enabled us to relate it definitely to any of the experimental conditions.

A few cats were not decerebrated but were anesthetized with amytal. The same 2 phase recovery was here likewise observed.

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Ultrafiltration Studies on the Bacteriophage.

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Studies on the magnitude of the bacteriophage corpuscle have been carried out by a number of investigators with widely different result. Thus Bechhold and Villa,[1] Prausnitz,[2] and Stassano and de Beaufort[3] assigned a diameter of about 20$\mu\mu$ to the lytic particle; Eliava and Suarez[4] conclude that the diameter is considerably less

[1] Bechhold, H., and Villa, L., *Z. Hyg. u. Infektionskrankh.*, 1925-26, cv, 601.

[2] Prausnitz, C., *Klin. Woch.*, 1922, i, 1639.

[3] Stassano, H., and de Beaufort, A. C., *Compt. rend Soc. de Biol.*, 1925, xciii, 1378.

[4] Eliava, G., and Suarez, E., *Compt. rend. de Soc. de Biol.*, 1927, xcvi, 462.

than 20μμ, while Zinsser and Tang[5] place it within the range from 20μμ to 100μμ.

The mechanism of ultrafiltration, a procedure usually employed in making such determinations, has recently been critically studied by Hitchcock,[6, 7, 8] Grollman[9] and others, who emphasize the necessity of conducting experiments of this kind under as simple and well controlled conditions as possible.

In ultrafiltration studies on the bacteriophage more ideal experimental conditions are undoubtedly realized by employing protein-free suspensions, a procedure made possible by the work of Arnold.[10] Arnold devised a method based upon the diffusion of the lytic principle into agar whereby one may prepare highly active bacteriophage suspensions which neither give any chemical reaction for proteins, nor, when injected into animals, produce antibodies directed against the proteins normally associated with the bacteriophage.

The question of the magnitude of protein-free bacteriophage is of particular interest because of Bronfenbrenner's[11] conclusion that the substance responsible for the lytic action of bacteriophage is adsorbed on colloidal particles of various sizes. We decided, therefore, to undertake ultrafiltration studies for the purpose of eliciting any difference in particle size between purified (protein-free) and non-purified bacteriophage.

A highly virulent race of anti-coli bacteriophage (Phage B) was used in our experiments and was propagated exclusively on one strain of *B. coli* (k-13). The non-purified lysate in Martin's broth was adjusted to the same lytic titre as that of the purified phage extracted from agar according to the method of Arnold. These latter extracts were consistently protein-free when tested with the ordinary chemical tests for proteins. The pH of purified and non-purified suspensions varied between pH 6.0-7.6 within which range we have satisfied ourselves, by cataphoresis experiments, that both types of preparation contained lytic particles bearing a negative charge.

The membranes employed were made by impregnating Whatman No. 1 filter paper with glacial acetic acid containing various percentages of dried Anthony's negative cotton and were standardized with colloidal suspensions of predetermined particle size as well as

[5] Zinsser, H., and Tang, F., *J. Exp. Med.*, 1927, xlvi, 357.
[6] Hitchcock, D. I., *J. Gen. Physiol.*, 1925-27, viii, 61.
[7] Hitchcock, D. I., *J. Gen. Physiol.*, 1926-27, x, 179.
[8] Hitchcock, D. I., *J. Gen. Physiol.*, 1925-26, ix, 755.
[9] Grollman, A., *J. Gen. Physiol.*, 1925-26, ix, 813.
[10] Arnold, L., *J. Lab. and Clin. Med.*, 1923, viii, 720.
[11] Bronfenbrenner, J., *J. Exp. Med.*, 1927, xlv, 873.

by their permeability to water (Poisseuille's Law). In the following work "percentage" of a membrane refers to the weight of dried negative cotton per 100 gm. of glacial acetic acid used in its preparation. Filtration pressures varied between 10 and 20 mm. of mercury.

The following experimental results were obtained consistently in repeated tests to date: 1. Purified phage B. passes a 4.5% or 5% membrane without diminution in titre. The identical membrane, washed free of the lytic principle with 10-20 cc. portions of sterile distilled water, completely retains non-purified Phage B. 2. The sequence may be reversed. A 4.5% membrane completely retains non-purified Phage B. The identical membrane, without intermediate washing, passes purified Phage B quantitatively. This same result is obtained with 5% membranes. 3. Purified Phage B. added to water containing very small amounts of Martin's broth or bacterial autolysates, is completely retained by a 4.5% and 5% membranes. The identical membranes permit purified Phage B. not so treated to pass quantitatively. 4. Increased disperson up to one volume of non-purified Phage B. with 10 volumes of distilled water or Martin's broth does not render the non-purified phage more readily filterable. Thus, non-purified Phage B. is retained as usual by a 4% or 5% membrane. The same membrane retains Phage B. diluted with 10 volumes of distilled water or Martin's broth, but is permeable to purified Phage B.

Our data thus far indicate that protein-free bacteriophage is definitely smaller than ordinary, non-purified bacteriophage. The non-purified bacteriophage aggregate does not appear to be strongly adsorbed by the membranes, for merely washing with water removes it readily. Neither does non-purified bacteriophage alter detectably the effective pore size of retaining membranes, for the identical membranes which retain non-purified bacteriophage, pass purified bacteriophage without reduction of lytic titre, and further, give no evidence of reduced pore size when subjected to the usual tests (retention of colloidal particles of known magnitude, permeability to water, pressure required to force air through the moist membrane). Apparently the purified bacteriophage readily forms aggregates with protein or protein derivative contained in broth or bacterial autolysates, these aggregates possessing larger magnitudes than the purified protein-free bacteriophage itself. Increased dispersion in water or in Martin's broth does not appear to render the non-purified bacteriophage more filterable.

Preliminary experiments with denser membranes tend to place

the diameter of the purified bacteriophage in the neighborhood of 5.0 milli-microns. The detailed results of this work will be reported in another paper upon completion.

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Phosphocreatine in Insulinized Frogs.

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Fiske and Subbarow[1] have isolated phosphocreatine from protein-free muscle filtrates and find "that the compound is destroyed during contraction at a rate which rivals that of glycogenolysis and lactic acid production." Modern theories of muscle contraction have as their foundation the change of glycogen into lactic acid. But it has been shown that the muscles of insulinized frogs can contract in a normal manner even if no glycogen can be found in them.[2]

To determine whether there is a correlation between glycogen and phosphocreatine we have analyzed individual gastrocnemii of very large frogs for initial phosphorus, phosphocreatine, total phosphorus, lactacidogen, glycogen, and lactic acid.

One gastrocnemius was removed with as little stimulation as possible, frozen in liquid air, and weighed portions (0.5 gm. or more) were taken for each of the different estimations. The other gastrocnemius was removed at the same time and stimulated at second intervals nearly to exhaustion, then frozen, portions weighed, etc.

In normal frogs we found 6-20 mgm. of phosphocreatine per gm. of tissue in resting muscles, and very little or none in exercised muscles. Changes in lactacidogen (*i. e.*, total phosphorus minus initial phosphorus and phosphocreatine) were too irregular for any definite conclusions to be drawn from the data. The glycogen content of resting muscle was 6-8 mgm. per gm. of tissue, and the loss of glycogen in the exercised muscles, 1-2 mgm., was approximately balanced by the gain in lactic acid. Normal resting muscles are, therefor, high both in phosphocreatine and glycogen, and worked muscles lose all their phosphocreatine and much of their glycogen

In insulinized frogs which had had violent convulsions for several days we found the following conditions: The phosphocreatine con-

[1] Fiske, C. H., and Subbarow, Y., *Science*, 1928, lvii, 169.

[2] Olmsted, J. M. D., and Harvey, J. M., *Am. J. Physiol.*, 1927, lxxx, 643.