plement-deviation reactions with rabbit-melanin, has suggested the possibility of modifying hereditary chromogenic functions by heterophile antimelanin immunization.

To test this possibility, a series of 12 pedigreed female albino rabbits were given 6 subcutaneous, 6 intraperitoneal, followed by 6 intravenous injections with native beef-melanin, at 5-day time intervals, the technic identical with that used by Adant. About 4 days after the final immunizing dose, the presumably melanin-immune albino females were mated with a pedigreed heterozygous black. Subcutaneous melanin injections were resumed 3 to 6 days later and continued at weekly intervals throughout pregnancy.

Eight litters, born to these presumably melanin-immune albino mothers, and 4 control litters born to non-immune albino females, are summarized in Table I.

TABLE I.

Litter number	Pigment Distribution			Non-immune Controls		
	Black	Gray	White	Black	Gray	White
1	4	0	4	3	0	3
2	4	0	3	4	0	4
3	2	0	1	4	0	3
4	1	1	1	4	0	4
5	3	0	6	—	—	—
6	6	0	4	—	—	—
7	4	0	2	—	—	—
8	2	0	9	—	—	—
Total	45%	2%	53%	52%	0%	48%

Within the limits of the experimental error, no modification of hereditary chromogenic functions is demonstrable by this method of heterophile immunization.

7800 C

A Test for Filtrable Forms of M. Tuberculosis Using Ultrafilters.

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A vast amount of work has been done which apparently supports the theory that a filtrable stage exists in a life cycle of *M. tuberculosis*. It should be noted that the outstanding criticism of all such work lies in the fact that in each case use has been made of Cham-

berland or Berkefeld filter candles which are difficult to standardize inasmuch as they involve factors such as electrical charge of the particle, medium in which the particle is suspended, as well as pore size. Until a filtration process can be devised which would permit exact duplication at any time and which could be standardized and controlled to retain a given size of particle, proof would be inconclusive that a filtrable form of *M. tuberculosis* exists.

For this reason it was felt that more significant data could be obtained with the ultrafilters of Krueger and Ritter[1] in which such factors as filter charge and adsorption of filtrate play practically no part, the membranes actually operating on the principle of a sieve. By carefully controlled experiments they not only graded the collodion membranes of varying pore sizes on the basis of permeability to colloidal particles of known size but also determined the degree of negative pressure which could be used in each case without causing distortion of the membrane. It was found that by using a negative pressure of 20 cm. a 4.5% collodion membrane (with pore diameter 40$\mu\mu$) retained all known bacteria.

The following filtration experiments, therefore, were carried out on 4.5% and 5.0% collodion membranes. The strains of tubercle bacilli used had been freshly isolated from sputum and cultivated both on Sweaney's egg-glycerol medium and on glycerine veal broth. Microscopic examination of the culture on Sweaney's medium after 6 weeks' incubation revealed many smoothly staining acid-fast rods with few granular forms while in the latter case the acid-fast granules predominated after 3 months' incubation.

The criterion for complete retention by this method as tested upon filtrates of the centrifugalized suspensions of the organisms was considered to be: 1. Lack of infection of guinea pigs as determined by tuberculin tests and absence of lesions and organisms, acid-fast or otherwise, in the spleen, liver, lungs and lymph glands of the test animals following subcutaneous inoculation of the filtrate in the inguinal region.

2. The absence of any microscopic forms, acid-fast or otherwise.

3. The absence of growth on Sweaney's egg-glycerol medium.

The controls for these experiments consisted of the following tests on the original saline suspension before filtration:

1. The failure of *B. prodigiosus* to pass through the collodion membrane when filtered simultaneously with the saline suspension of *M. tuberculosis*.

2. The experimental infection of tuberculosis-free guinea pigs;

[1] Krueger, A. P., and Ritter, R. C., *J. Gen. Physiol.*, 1929, **13**, 409.

the test animals were found to give positive tuberculin tests after intervals of 4 and 8 weeks. Recovery of acid-fast organisms was made from the liver, spleen and lymph nodes.

3. The cultivation of the organisms on Sweaney's egg-glycerol medium.

Three separate series of tests were made on each type of membrane. Two guinea pigs were used for the controls and 2 for each test. Each animal was previously found to be tuberculosis-free as determined by the tuberculin test. An even emulsion of the organism was obtained by thoroughly grinding the growth as washed from the solid media in physiological saline.

Our results show that the ultrafiltrates did not contain infectious particles capable of producing: (1) a positive tuberculin reaction; (2) anatomical evidences of the infection in experimental animals or growth of tubercle bacilli on appropriate media for their cultivation. It may be concluded, therefore, that the cultures tested did not develop "filterable forms" smaller than 40 μμ in diameter.

7801

Colloidal Calcium Phosphate of Blood and Calcium Partition in Serum.

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It has now been thoroughly shown that under certain conditions the inorganic phosphate of the serum becomes incompletely diffusible.[1] A logical explanation of this phenomenon is that the nondiffusible phosphate which is formed is combined with some of the serum calcium in a compound which is in a colloidal state. As yet, no direct evidence is available as to the exact composition of this colloidal calcium phosphate.

On the basis of the view which has recently received strong support from the work of McLean and Hastings,[2] namely, that there is an equilibrium between the ionized and the protein bound calcium in

[1] See Schmidt, C. L. A., and Greenberg, D. M., *Physiol. Rev.*, in press, for literature.

[2] McLean, F. C., and Hastings, A. B., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 529; *J. Biol. Chem.*, 1934, **107**, 337.