were used as controls. Practically all the animals showed the calcification phenomena now well described in the literature. The animals were killed and the organs dried in high vacuum, powdered, extracted with ether (dry), treated with saturated alcoholic barium hydroxide to remove soaps, extracted with petrol ether, the petrol ether evaporated and the residue taken up in dichloroethylene (1 cc.) and treated with one-half cc. of saturated aqueous trichloroacetic acid.

The results show a most striking accumulation of ergosterol in the adrenals and brain with small amounts in the liver and kidneys. Muscular organs appear to be almost free from this substance.

Judging from the color reaction the bone marrow especially seems to contain fairly large amount of a $\Delta^{1,2}$ sterin, the nature of which we are now investigating.

The results are all consistent but the amount of ergosterol found by the color test was not in proportion to the amount of ergosterol (Vigantol) fed, *e. g.*, the highest dosed animal did not have a great deal more ergosterol in his body. *A priori* one would of course expect this result.

Human arteriosclerotic aortas and adrenals from elderly patients showing arteriosclerosis contain a substance which strongly suggests by its color reactions and spectrogram the presence of ergosterol and its irradiation products whereas aortas from young individuals dying from varied causes seem to contain none or but traces of these materials.

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The Relation of Washed Granules From Commercial Vaccine to the Virus.

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The work of MacCallum and Oppenheimer[1] and of Craciun and Oppenheimer[2] offered at least the hope that the "washed granules" prepared from commercial calf vaccine might represent purified vaccinia virus. We have accordingly investigated further the nature

[1] MacCallum, W. G., and Oppenheimer, E. H., *J. Am. Med. Assn.*, 1922, lxxviii, 410.

[2] Craciun, E. C., and Oppenheimer, E. H., *J. Exp. Med.*, 1926, xliii, 815.

of the washed granular material obtained by the method of Craciun and Oppenheimer.[2]

In confirmation of the earlier work we have found that the granular centrifugalization sediments after repeated washings in Ringer's solution were infectious even in high dilution; characteristic lesions of vaccinia resulted from intradermal injection into rabbits; the supernatant fluids were infectious only in low dilution. When

TABLE I.

Inactivation of Washed Vaccinia Granules at Reactions More Acid Than pH = 5.2–5.3.

Time of exposure	McI. 2.8	McI. 3.3	W. 3.5–3.6	W. 4.0–4.1	W. 4.4–4.5	W. 4.8	W. 5.2	W. 5.3	S. 5.3	S. 5.6	S. 5.9	Granules control
3 hours	—		—	—	—	—	—		+	+	+	++
20 hours in ice box	—		—		—		+		+	+		++
2½ hours at room temp.		—	—	—	—				+	+	+	++
2½ hours at room temp.		—		—		tr.		+		+	+	

One volume of each suspension of granules in Locke solution was diluted with 3 volumes of buffer; after the interval indicated, 0.1 cc. of the mixtures were injected into a rabbit. Intensities of "takes" indicated by plus signs. The letters above the columns indicate McIlvaine's citrate, Walpole's acetate and Sorensen's phosphate buffer mixtures, respectively, of the indicated pH values. (See Clark, W. M., "The Determination of Hydrogen Ions," 1922, 2nd edit., Baltimore, ch. vi.)

mixed with the sera of rabbits recently recovered from vaccinia the granules were rendered non-infectious; the infectivity of the granules was not neutralized by normal rabbit sera. Consideration of the results of previous work[1, 2, 3] and of this work leaves no doubt in our minds that the property of infectivity is borne by some of the particles in the washed granular material.

The washed granules have been found to become non-infectious at hydrogen ion concentrations more acid than pH = 5.2 or 5.3 (See Table I). This is in sufficient agreement with the results of Douglas and Smith,[4] who found that "diffusates" of vaccine virus from infected rabbits' testes were rapidly inactivated at reactions more acid than pH = 5.5.

We have found that infectivity could be restored to non-infectious mixtures of granules and immune serum by washing the serum-treated granules in saline or Ringer's solution.[5, 6, 7] Yet recovery of infectivity on washing was not complete; the infecting power of the rewashed granules treated with immune sera was considerably inferior to that of granules similarly treated with normal sera. Two of our experiments which show these effects most clearly are given in Tables II and III. It would seem that immune sera do produce some specific effect on the virus *in vitro,* even though it has proven difficult to demonstrate this without the aid of animal inoculation.[6, 8]

We have attempted to produce agglutination and reduction of cataphoretic velocity[9] of the four-times "washed granules" by several types of sera, as follows (a) The sera of rabbits vaccinated and revaccinated with crude commercial calf vaccine. Such sera gave pronounced precipitin reactions against bovine serum, and protected against vaccinia. These sera caused pronounced although incomplete agglutination of the washed granular material and markedly reduced cataphoretic velocity.

(b) The sera of rabbits inoculated with infectious products of

[3] Ward, H. K., *J. Exp. Med.*, 1929, l, 31.

[4] Douglas, S. R., and Smith, W., *Brit. J. Exp. Path.*, 1928, ix, 213.

[5] Todd, C., *Brit. J. Path.*, 1928, ix, 244.

[6] Andrewes, C. H., *J. Path. and Bact.*, 1928, xxxi, 671.

[7] Long, P. H., and Olitsky, P. K., *Science*, 1929, lxix, 72.

[8] Schultz, E. W., Bullock, L. T., and Lawrence, F., *J. Immunol.*, 1928, xv, 243. See, however, Bedson, S. P., *Brit. J. Exp. Path.*, 1929, x, 364; Bedson, S. P., and Bland, J. O. W., *ibid.*, 393; Rivers, T. M., Haagen, E., and Muckenfuss, R. S., *J. Exp. Med.*, 1929, l, 673.

[9] For technic, see Mudd, S., Lucké, B., McCutcheon, M., and Strumia, M., *J. Exp. Med.*, 1929, xlix, 779.

commercial calf vaccine; these sera gave negative precipitin tests with bovine serum; also the sera of rabbits vaccinated with rabbit testicular or neurovirus. These sera protected against vaccinia. With the

TABLE II.
Protection from Infective Granules by Immune Sera, with Partial Recovery of Infectivity of the Same Granules After Washing.

Granules in presence of serum from:	Dilutions of Serum				
	1:4	1:16	1:64	1:256	1:1024
Vaccinated rabbit No. 1890	0	0	+	+	++++
Vaccinated rabbit No. 1892	0(?)	++ to +	++++ to +++	++++ to +++	
Revaccinated rabbit No. 2883	0	++	+++	+++	
Revaccinated rabbit No. 3024	0	trace	+++	++	
Normal rabbit No. 3179	++++ to +++	++++	+++		
Normal rabbit No. 3187	+++	++++	++		
The above granules washed after treatment with serum from:					
Vaccinated rabbit No. 1890	+ to tr	++ to +	++ to +		++++
Vaccinated rabbit No. 1892	+++	+++ to ++			
Revaccinated rabbit No. 2883	+++	+++ to ++	+++		
Revaccinated rabbit No. 3024	tr	++	+++ to +		
Normal rabbit No. 3179	+++ to +++	+++	+++		
Normal rabbit No. 3187	+++ to ++	+++ to ++	++ to +		

Plus marks indicate intensities of lesions resulting from intradermal injections of 0.1 cc. each. Lesions in rabbit inoculated with mixtures of granules and sera read on fifth day; lesions in rabbit inoculated with serum-treated granules after washing read on fourth day.

"washed granules" they gave only very weak agglutination and reduction of cataphoretic velocity. We could not be sure whether or not the agglutination and cataphoretic results obtained with these

sera were to be regarded as very weak positive specific antivirus reactions or as negative reactions.

(c) The sera of rabbits injected with the skin scrapings of a normal calf. These sera gave positive precipitin reactions with bovine serum, but did not protect against vaccinia (See Table III). They

TABLE III.
Protection from Infective Granules by Immune Sera, with Partial Recovery of Infectivity of the Same Granules After Washing.

Granules in presence of serum from:	Dilution of Serum			
	1:4	1:16	1:64	1:256
Revaccinated rabbit No. 1890	0	0	0	tr
Revaccinated rabbit No. 1892	0	0	0	0
Normal rabbit No. 3151	++	++	++	
Normal rabbit No. 3152	+++	+++	+++	
Anticalf rabbit No. 3132	+++	+++ + to ++	+++ to +++	
Anticalf rabbit No. 3134	++	++ to +	++ to ++	
The above granules washed after treatment with serum from:				
Revaccinated rabbit No. 1890	+ to tr	+	++	++
Revaccinated rabbit No. 1892	++ to +	++	++	++
Normal rabbit No. 3151	+++ to +++	+++ to +++	+++	
Normal rabbit No. 3152	+++	+++ to +++	+++ to +++	
Anticalf rabbit No. 3132	+++	+++ to +++	+++ to +++	
Anticalf rabbit No. 3134	+++	+++	+++ to +++	

Lesions in rabbit inoculated with mixed granules and serum read on third day; lesions in rabbit inoculated with treated washed granules read on sixth day. "Anticalf" rabbits immunized with skin scrapings from a normal unvaccinated calf.

caused partial agglutination of "washed granules" to a slightly greater degree than the sera of group b.

(d) Normal rabbit sera. These caused a trace of agglutination and reduction of velocity of "washed granules," in these reactions being slightly less positive than the sera of groups b and c but much less positive than those of group a.

The only conclusions we feel justified drawing from these *in vitro* serological tests is that the washed granular material contains calf protein in addition to the virus. This conclusion seems the more probable when one remembers that the "washed granules" are obtained by centrifugation of ground-up material which includes calf epidermis and lymph as well as vaccinia virus.

Therefore we regard the "top layer" or "washed granules" obtained from commercial calf vaccine by the method of Craciun and Oppenheimer[2] as advantageous material with which to start, but by no means as the final product, in the identification and purification of the virus.

The vaccines used were furnished us through the kindness of Dr. W. F. Elgin, of the H. K. Mulford Laboratories, and of Dr. T. M. Rivers.

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B. Pyocyaneus as a "Carrier" of B. Coli Bacteriophage.

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The phenomenon here reported was first observed in the course of an unsuccessful attempt to discover among a number of specimens of *B. coli* bacteriophage one that would be active upon *B. pyocyaneus*. Of 25 filtrates showing marked lysis of one or more strains of *B. coli* none could be demonstrated to have any lytic power for any of the 7 strains of *B. pyocyaneus* tested. The bacteriophage specimens were obtained from the following sources: sewage 4; urine 7; feces 15; pus 2; unknown source 1. The phages were tested on the pyocyaneus cultures both as fresh filtrates and after their lytic power for *B. coli* had been materially enhanced by repeated passage. Neither clearing of young broth cultures nor plaque formation could be demonstrated with any of the strains of *B. pyocyaneus*.

An attempt was then made with one bacteriophage specimen to bring about pyocyaneus lysis by serial passages upon cultures of the latter organism. A filtrate having marked lytic activity on several strains of *B. coli* was chosen for this test and its activity was brought to a maximum by a number of passages upon a strain of *B. coli*, S 1. This coli strain was isolated from a case of pyelitis, belonged to the slow lactose-fermenting group, gave gas on saccha-