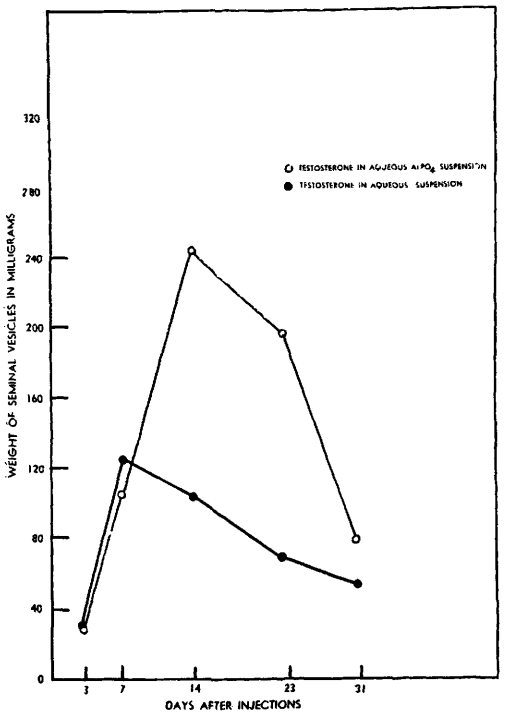

FIG. 3.

Potentiation of a commercial aqueous testosterone suspension by aluminum phosphate. Seminal vesicle response of immature rats to a single intramuscular injection.

slower release from the depot site, it follows that a depot of micro-crystalline testosterone, subcutaneously or intramuscularly, should be ideal for slow absorption. As yet, there is no experimental demonstration of the mechanism by which aqueous $AlPO_4$ suspension of testosterone elicits a greater androgenic response than the aqueous suspensions without $AlPO_4$.

Summary. The experimental data confirm the depot effect described by Carlinfanti and his co-workers following subcutaneous injection of ATAP suspensions in the guinea pig. The effect also has been demonstrated in immature castrate rats after intramuscular injection. In both instances marked increase in response and duration of biological activity were obtained. Potency ratios and indices of duration of action indicate that each milligram of testosterone in aqueous aluminum phosphate suspension is (1) two or more times as active, and (2) acts approximately two to three times as long as the same quantity of testosterone in aqueous suspension alone.

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Inhibition of the Phagocytic Action of Leucocytes by Mumps and Influenza Viruses. (18005)

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In previous experiments(1-3), it has been shown that mumps virus has a destructive effect on the erythrocytes from a number of species which results in hemolysis. It was therefore of interest to determine if this virus would damage other cells. Polymorphonuclear leucocytes of the guinea pig were selected for study since their ability to engulf killed bacteria offered a ready test of their intact functional activity for which a quantitative technic has been developed for direct observation with the microscope(4). The erythrocytes of this species are very susceptible to the hemolytic action of mumps virus (3).

The effect of influenza virus on the phagocytosis of bacteria was also studied since reports(5) indicated that this virus caused

1. Morgan, H. R., Enders, J. F., and Wagley, P. F., *J. Exp. Med.*, 1948, v88, 503.

2. Chu, L. W., and Morgan, H. R., *J. Exp. Med.*, 1950, v91, 393.

3. Chu, L. W., and Morgan, H. R., *J. Exp. Med.*, 1950, v91, 403.

4. Merchant, D. J., *J. Inf. Dis.*, in press.

agglutination of guinea pig leucocytes. However, influenza virus did not possess the hemolytic property demonstrated by mumps virus(1).

Materials and methods. The egg-adapted strain of mumps virus described in previous studies was employed(1,2). The virus was cultivated in the amniotic sac of 7-8-day-old chick embryos harvesting the amniotic and allantoic fluids after incubation at 35°C for 5 days. The PR8 strain of influenza A virus was inoculated into the allantoic cavity of 10-day-old embryos and the allantoic fluid was collected after 48 hours incubation at 35°C. All of these virus-containing egg fluids were dialyzed against saline buffered with phosphate at 4°C for 48 hours before use in these experiments. The mumps antiserum employed was prepared by immunizing guinea pigs.

Estimations of the phagocytic activity of the polymorphonuclear leucocytes of the guinea pig were carried out by the technic recently described in detail using killed suspensions of *Bacillus anthracis* as the test organism(4). The phagocytosis of these bacteria was observed directly in a slide preparation on a warm stage using darkfield illumination. The mixture placed on the slide for observation consisted of 0.7 ml of homologous serum, 0.1 ml of a leucocyte suspension containing 45,000 to 50,000 cells per cmm and 0.1 ml of a suspension of anthrax bacilli. The suspension of anthrax bacilli was added after 15 minutes of incubation at 37°C. In the test experiments, 0.1 ml of the dialyzed virus preparation was added, and, in the inhibition experiments, 0.1 ml of the immune serum was added to 0.6 ml of the normal guinea pig serum. Phagocytosis was evaluated after 15 minutes at 37°C by determining the percentage of cells that had engulfed the anthrax organisms.

Results. Table I presents a summary of the results of representative experiments. In the presence of mumps or influenza viruses, the ability of guinea pig polymorphonuclear leucocytes to engulf anthrax bacilli was reduced 20-30%. Amniotic or allantoic fluid containing mumps virus had identical effects. Heating of either virus at 50°C for 40 minutes destroyed this inhibitory action which was also specifically neutralized by the addition of homologous immune serum in the case of mumps virus. Amniotic or allantoic fluids harvested from normal embryonated eggs had no effect on the phagocytic activity of the leucocytes.

TABLE I.
Effects of Mumps and Influenza Viruses on Phagocytic Activity of the Polymorphonuclear Leucocytes of the Guinea Pig.

Materials added	Phagocytosis, %
Mumps virus	42
" " heated at 50°C, 40 min.	76
Mumps virus + mumps antiserum	70
Control	68
PR8 influenza virus	49
" " " heated at 50°C, 40 min.	81
PR8 influenza virus + mumps antiserum	48
Control	75

Another effect was observed after the addition of these viruses. Using an ocular micrometer, the diameter of the leucocytes was measured and found to be reduced 10-15%. This is a rough measure of their ability to spread out at a liquid-solid interface. This effect disappeared when the virus was heated or immune serum was added. No morphological changes were observed inside these cells.

Discussion. Evidence presented in these experiments indicates that mumps virus has an action on guinea pig leucocytes which decreases their phagocytosis of bacteria. The specific nature of this activity is indicated by its neutralization by homologous immune serum and the failure of normal egg fluids to exert any similar effect. This property of the virus is similar to its hemolytic activity in its susceptibility to inactivation by heat under conditions which leave the hemagglutinating capacity of the virus intact. It would not seem likely however that the action on leucocytes and erythrocytes are different manifestations of a single property of mumps virus since influenza virus, which has no

5. Nungester, W. J., Gordon, J., and Collins, K., *J. Inf. Dis.*, in press.

hemolytic activity, also inhibits phagocytosis. Unless, of course, the inhibition of phagocytosis produced by mumps virus is due to some property entirely different from that of influenza virus causing a similar inhibition. This question awaits further elucidation. One obvious implication of these studies is the possible relationship of inhibition of phagocytosis by these viruses to the development of secondary bacterial infections during the course of diseases caused by them. The significance of these findings must await parallel demonstration of such activity for those viruses in natural or experimental infections before any such conclusions can be drawn.

Summary. Mumps virus and influenza A virus (PR8) have been shown to reduce the phagocytosis of bacteria by the polymorphonuclear leucocytes of the guinea pig *in vitro*. This inhibitory effect is destroyed by mild heating of the viruses which leaves their hemagglutinating capacity intact. Mumps antiserum prevents the inhibition of phagocytosis by mumps virus but has no effect on the inhibitory action of influenza virus.

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A Note on Electrophoresis in Hypothyroidism.* (18006)

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A number of investigators have reported a moderate elevation of total protein in hypothyroidism and have attributed this to hemoconcentration(1-4). With adequate thyroid therapy the total protein concentrations returned to normal. Lewis, McCullagh and Clark(3) included six cases of hypothyroidism in their electrophoretic analyses of the plasma proteins in thyroid disease, and have described a pattern which they consider consistent. These authors worked with a phosphate buffer at pH 7.8 which was the condition under which sera had been studied at that time. However, at the present the general practice is to work in a veronal buffer of pH 8.6 and we have studied several cases under these conditions. Our results differed somewhat from those reported by Lewis *et al.*, and the differences probably are due to this variance of conditions.

Material and methods. The material was obtained from 3 females with spontaneous myxedema and 1 male with post-operative myxedema. In 2 of these patients samples were taken after treatment with desiccated thyroid. One of these latter cases was unable to tolerate adequate doses of thyroid (patient P) because of progressive heart disease. No attempt was made to differentiate pituitary from spontaneous hypothyroidism. The case histories of the patients follow: Case R, a 65-year-old white female housewife, noticed apathy, weakness and dryness of the skin one year prior to admission. Her physical appearance was typical of advanced myxedema *viz.* coarse dry skin, slow hoarse voice, puffy eyelids and absent axillary and pubic hair. The essential laboratory findings were BMR-21, EKG rate 60 with low voltage and flattened T waves, X-ray chest—diffuse

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1. Gildea, E. F., Man, E. B., and Peters, J. P., *J. Clin. Invest.*, 1939, v18, 739.

2. Shirer, J. W., *Trans. Am. Assn. Study Goiter*, 1932, p. 89.

3. Lewis, L. A., McCullagh, E. P., and Clark, J., *Am. J. Med. Sci.*, 1944, v208, 727

4. Moore, D. H., Levin, L., and Smelser, G. K., *J. Biol. Chem.*, 1945, v157, 723.