were brought about in this way. Following operation, however, we were not able to raise the calcium above the normal level. It should be added that after large doses of irradiated ergosterol has been given for a period of some weeks, removal of the parathyroid glands does not result in the precipitous fall in the calcium which we are accustomed to observe following extirpation. This distinction is due probably to a storing of the calcium in the tissues. The results of this series of experiments may be summarized by the statement that our preliminary observation has been substantiated, in that hypercalcemia could not be induced or the calcium level elevated markedly, after the parathyroids had been removed.

Irradiated ergosterol was found to increase not only the calcium concentration but that of the inorganic phosphorus in the serum. The phosphorus rose frequently to a level of 12 to 14 mg. per 100 cc. of serum several days after irradiated ergosterol had been given. In this respect the action of irradiated ergosterol differs from that of parathyroid extract, which does not bring about an increase of inorganic phosphorus until hypercalcemia is marked and the animal is in a highly weakened condition. This effect may not be associated with the activity of the parathyroid glands. It should be mentioned also that the hypercalcemia brought about by irradiated ergosterol is far more prolonged, both in man and in animals, than that which follows injections of parathyroid extract.

The mechanism of the action of irradiated ergosterol is not entirely clear, but these experiments seem to be of interest as demonstrating that a vitamin may function, at least to a certain extent, through the medium of one of the endocrine glands.

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Attempts to Cultivate Vaccine Virus in the Growing Chick Embryo.

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The propagation of vaccine virus in the skin and cornea of susceptible animals has been supplemented by evidence of mass growth in the testicle (Noguchi[1]) and perhaps the brain (Levaditi[2]). At-

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[1] Noguchi, *J. Exp. Med.*, 1915, xxi, 565; xxvii, 423.

[2] Levaditi, Harvier and Nicolau, *Compt. Rend. Soc. Biol.*, 1921, lxxxv, 345.

tempts to cultivate vaccine virus elsewhere than in the skin of living animals have as their objectives purification of the virus, the production of larger amounts at less cost as well as to throw light on the nature of the virus itself.

These objectives would all be reached and extended if it were possible to grow vaccine virus outside the animal body in ordinary or in tissue culture. It is the generally accepted opinion that viruses in general will increase only in the presence of living cells (Rivers, p. 13[3]) for which they have specific affinity.

The claims of Fornet[4] and of Volpino[5] of having grown vaccine virus in serum in the presence of sugar or glycerine have not been confirmed. The addition of animal tissue to such media with positive results in the hands of Pröscher[6] and of Plotz[7] has not only been unconfirmed but is denied by Gins.[8] The remarkable results recently claimed by Maitland and Maitland,[9] who apparently obtained marked increase of the virus in a series of cultures comprising minced hen kidney, hen serum and Tyrode's solution, and grown aerobically certainly inspire repetition.

Distinct evidence of the growth of vaccine virus in tissue cultures of adult corneal epithelium (Steinhardt, Israel and Lambert[10]; Gins[11]) and in testicular extract (Flores Cordova[12]) or testicular cells (Parker[13]; Parker and Nye[14]; Minervin and Schmerling[15]; Haagen[16]) has been generally accepted. Even more interesting positive results in tissue culture with embryonic rabbit and guinea pig cornea were obtained by Cracium and Oppenheimer.[17] Carrel and Rivers[18] in tissue cultures from the skin, cornea and brain of em-

[3] Rivers, "Filterable Viruses," 1928, Williams & Wilkins.

[4] Fornet, *Berlin. Klin. Wochenschr.*, 1913, 1864. *Centralbl. Bakt.*, 1922, lxxxvii, 36.

[5] Volpino, *Pathologica*, 1921, xiii.

[6] Pröscher, *Centralbl. Bakt.*, 1906, xl, 337. *Berlin. Klin. Wochenschr.*, 1915, 886.

[7] Plotz, *Compt. Rend. hebdom. des seances de l'Acad. des Sciences*, 1922, clxxiv, 1265.

[8] Gins, *Berlin. Klin. Wochenschr.*, 1913, 2349; 1914, 391. *Z. f. Hyk.*, 1916 lxxxii, 98.

[9] Maitland and Maitland, *Lancet*, 1928, ii, 596.

[10] Steinhardt, Israel and Lambert, *J. Inf. Dis.*, 1913, xiii, 294.

[11] Gins, *Z. f. Hyg.*, 1916, lxxxii, 101.

[12] Flores Cordova, *Cronica Medica*, 1926, xliii, 377.

[13] Parker, *J. Med. Res.*, 1924, xliv, 645.

[14] Parker and Nye, *Am. J. Path.*, 1925, i, 325.

[15] Minervin and Schmerling, *Centralbl. Bakt.*, 1926, xcix, 558.

[16] Haagen, *Centralbl. Bakt.*, 1928, cix, 31.

[17] Cracium and Oppenheimer, *J. Exp. Med.*, 1926, xliii, 815.

[18] Carrel and Rivers, *Compt. Rend. Soc. Biol.*, 1927, xcvi, 848.

bryo chicks succeeded in increasing 25 vaccine units to 10,000 in 10 days. These last results have been confirmed by Levaditi[19] with his neuro-vaccine, and by Eagles and McLean.[20]

It occurred to us that since tissues of the embryonic chick in culture appear to furnish the most suitable medium for the growth of vaccine virus, a technically simpler and possibly better result might be obtained by direct inoculation of fertile and incubating hens' eggs. It was first ascertained that egg white is distinctly destructive for vaccine virus when incubated with proper control at 37° C. for 24 hours, whereas egg yolk is apparently harmless. The virus inoculated in the yolk of an infertile egg survives in the incubator for 4 but not for 8 days.

Fertile hens' eggs in a number of different lots were incubated at 40° C. for from 5 to 6 days and were then inoculated aseptically in the yolk with the Noguchi strain of rabbit testicle vaccine-virus. The incubation was continued for from 4 to 10 days, the eggs were opened after treating with alcohol and the free flame, or else the yolk withdrawn through a freshly sterilized area of the shell. The embryos examined at successive stages after introduction of the virus in the yolk showed first a marked congestion of the vessels followed by a shrinking of the embryo which was then found to be dead and fragile. In some instances, of course, bacterial contamination occurred. In nearly every sterile although usually dead embryo up to 10 days after inoculation (total incubation 15 to 16 days; length of embryo up to 30 mm.) living virus was found as tested intradermally in the rabbit skin. There was more virus present in the embryo than in yolk per unit of volume, and more virus than in fresh vaccine diluted with equal parts of ground embryo of the same age. In other words, there was distinct evidence of multiplication of the virus and of its concentration in the embryo from the yolk. A second series of incubated eggs was inoculated in the yolk from the first incubated yolk, and the virus was still present several days later. A third transfer was negative. In such series experiments, transfers from the embryonic tissue which shows greater amounts and increase of virus in the first series, although technically more difficult, might well have yielded more favorable results.

Direct inoculation of vaccine virus into the yolk of fertile, incubated hens' eggs gives evidence, then, of some increase in the virus but leads to the death of the embryo in a few days. It is still pos-

[19] Levaditi, *Compt. Rend. Soc. Biol.*, 1927, xcvi, 967.

[20] Eagles and McClean, *Brit. J. Exp. Path.*, 1929, xvi, 35.

sible that by modifying the conditions of these experiments a new method of cultivating vaccine virus may be obtained.

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Attempted Production of Vaccinal Encephalitis in Rabbits with a Testicular Virus.

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The production of vaccinal encephalitis in rabbits is of interest at this time chiefly in view of the prevalence of post vaccinal encephalitis in some European countries. The possible use of the rabbit's brain as a medium for mass production of sterile vaccine (advocated by Levaditi) also adds importance to the question.

A considerable number of European workers have succeeded in producing what they regard as a vaccinal encephalitis in rabbits. Levaditi[1] and his co-workers since 1921 have developed a strain of vaccine virus, known as neurovaccine, which produces regularly on intracerebral injection into rabbits a typical encephalitis with paralytic symptoms and death in 4-7 days. They regard it as a virus adapted to the central nervous system by passage and consider that it has acquired neurotropic properties. They first adapted it to the brain by alternate brain and testicular passage—later omitting the testicular passages. Blanc and Caminopetros[2] found that ordinary calf vaccine passed through the rabbit's cornea and then to the brain was sufficiently adapted to cause a fatal encephalitis. Herzberg[3] produced a vaccinal encephalitis by the intracerebral injection of a virus adapted to the rabbit's testicle.

The great majority of workers, however, have found no adaptation necessary and consider the encephalitogenic property as one inherent in ordinary vaccine virus. Marie,[4] Krumbach,[5] Condrea,[6]

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1 Levaditi, Harvier and Nicolau, *Compt. Rend. Soc. Biol.*, 1921, lxxxv, 345; Levaditi and Nicolau, *Compt. Rend. Soc. Biol.*, 1922, lxxxvi, 77; Levaditi, *J. State Med.*, 1924, xxxii, 151.

2 Blanc and Caminopetros, *Compt. Rend. Soc. Biol.*, 1923, lxxxviii, 1020.

3 Herzberg, *Cent. f. Bakt.*, 1925, xcv, 211.

4 Marie, *Compt. Rend. Soc. Biol.*, 1920, lxxxiii, 476.

5 Krumback, *Z. f. Immuntatsforsch.*, 1922, xxxviii, 1.

6 Condrea, *Compt. Rend. Soc. Biol.*, 1922, lxxxvi, 897.