inhibitor in solution are important for the phenomenon and that it is possible to modify some of these properties without appreciably affecting biological activity. Solutions of purified inhibitor have a high viscosity, suggesting the presence of a highly asymmetric component.[1,3] Although it has not been possible to observe birefringence during electrophoresis of these solutions, it is possible that predisposition to aggregation results from orientation of asymmetric particles in the electric field, as in the Kerr effect.[4] The application of the electrical precipitation phenomenon to the final purification of the EW inhibitor is being explored.

Summary. Precipitation occurs in solutions of egg-white or purified egg-white inhibitor of virus hemagglutination submitted to an electric field. The precipitate includes a great part of the inhibitor of the preparations in a state substantially purer than that of the starting material. This result suggests application of the phenomenon to the final purification of the inhibitor.

[3] Eckert, E. A., Lanni, F., Beard, D., and Beard, J. W., *Science*, 1949, **109**, 463.

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17150. Propagation of Canine Distemper Virus on the Chorio-Allantoic Membrane of Embryonated Hen Eggs.

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Several attempts have been made by various investigators to cultivate canine distemper virus in the tissues of developing chick embryos. Most of these attempts were not too successful. Thus, Mitscherlich[1] found the virus to survive for 6 days after inoculation onto the chorio-allantoic membrane, but not to be active after subinoculation to other eggs. Plummer[2] in two attempts maintained the virus for 5 and 6 passages respectively on the chorio-allantois but failed to carry it further. Beveridge and Burnet[3] obtained no evidence that canine distemper virus multiplied in chick embryo tissues although various routes of inoculation were tried.

However, Haig[4] reported the successful cultivation of Green's distemperoid virus through 30 serial passages on the chorio-allantoic membrane of chick embryos, and later reported[5] that the virus had been maintained on the chorio-allantoic membrane for more than 90 serial passages over a period of 15 months. During this time the egg-adapted virus was found to have become partially attenuated for ferrets and to have lost some of its highly contagious characteristics for this species. A temperature of 37°C seemed to be the most suitable for incubation of the inoculated eggs since both higher virus titers and more pronounced macroscopic lesions on the chorio-allantoic membranes were attained. Attempts to maintain the virus by serial passage by the yolk-sac, allantoic cavity or amniotic cavity routes of inoculation ended in failure.

For some time attempts have been made in this laboratory to adapt canine distemper virus to some host other than dogs or ferrets. In

[1] Mitscherlich, E., *Dtsch. tierarztl. Wschr.*, 1938, **46**, 497.

[2] Plummer, P. J. G., *Canad. J. Comp. Med.*, 1939, **3**, 96.

[3] Beveridge, W. I. B., and Burnet, F. M., Med. Res. Council, Sp. Report Series, No. 256, 1946, p. 76.

[4] Haig, D. A., *Onderstepoort J. Vet. Sci. and Animal Industry*, 1948, **23**, 149.

[5] Haig, D. A., *J. South Afr. Vet. Med. Assn.*, 1948, **19**, 73.

accordance with the experience of most previous investigators, most of our attempts ended in failure. However, in studies initiated before the work of Haig[4,5] appeared in print, we too obtained results indicating that canine distemper virus may be successfully passed for a number of generations on the chorio-allantoic membrane of developing chick embryos. The object of this paper is to present our experimental data.

Material and methods. The distemper strain was the stock virus used by the Lederle Laboratories* for the production of canine distemper vaccine and immune serum. This strain of virus, hereafter referred to as the *Lederle* strain, was isolated from a sick dog in these laboratories in 1941 and has been maintained since then by irregular alternate passages through ferrets and dogs. When inoculated into ferrets, this strain produces the usual symptoms and lesions characteristic of distemper: pyrexia, general erythema with rash around the mouth, which usually becomes pustular; erythema and edema of the anus; inflammation of the nasal passages and mucous membranes of the eyes, which is shown first by a watery discharge that later becomes purulent, causing edema and glueing of the eyelids. All inoculated ferrets show these symptoms 6 to 10 days following inoculation and usually die 2 to 3 days later.

The ferrets used in the experiments were usually between 3 and 10 months old. They were bred on an upstate New York farm and when brought to the laboratories were kept in strict isolation at an adequate distance from the infected area. They were cared for by attendants who never came in contact with dogs or the infected ferrets.

Seven-day-old developing chick embryos were used throughout. Inoculations were made either onto the chorio-allantoic (C-A) membrane or into the yolk sac. The inoculated eggs were incubated either at 35° or 38°C for 4 to 5 days at which time the chorio-allantoic membranes and/or yolk sacs, embryos and allantoic fluids were harvested under aseptic conditions. All suspensions were made in beef heart infusion broth free of dextrose.

Experimental. Of the various routes and series of inoculations made, the series that was followed with best success is described in detail: A 20% suspension of infected ferret spleen was centrifuged at 2,000 r.p.m. for 10 minutes. To the supernate penicillin and streptomycin was added to give 500 units and 100 μg of each respectively per ml and after standing for one hour at room temperature, 12 eggs were inoculated with 0.2 ml each on the C-A membranes and then incubated at 35°C. One embryo was dead on the first day and was discarded. The remaining 11 embryos were still alive at the end of the 5th day. The C-A membranes appeared normal. They were harvested and a 20% suspension of their pooled tissue, after treatment with penicillin and streptomycin as described above, was inoculated on the C-A membranes of a second series of 15 eggs. A total of 4 serial passages in eggs was thus made. C-A membranes of the 4th passage were pooled and 0.5 ml of a 20% suspension of the pool was injected subcutaneously into each of 2 ferrets. Both ferrets developed symptoms typical of distemper 9 and 10 days later, respectively, and were found dead on the 12th day.

A 20% spleen suspension of one of the ferrets was inoculated into 10 developing chick embryos by the yolk-sac route. Four days later the yolk sacs of 8 surviving embryos were harvested and a portion of the pooled tissue suspension subinoculated onto the C-A membranes of 10 eggs. Two more serial passages were made on the C-A membranes and then pooled membranes of the last passage were made up to a 20% suspension and injected subcutaneously in 0.5 ml amounts into each of 2 ferrets. Both ferrets showed symptoms indicative of distemper on the 7th day and were found dead on the 9th day. A suspension of their pooled spleens was prepared and a portion inoculated onto the C-A membranes of 14 eggs. These eggs constituted the first passage of our current strain in eggs which now has been carried for 74 successive serial transfers on the C-A membranes.

* The authors are indebted to Mr. H. M. Kroll, Animal Industry Section, Lederle Laboratories, for his cooperation in the initial stages of the work.

Our experience with the *Lederle* strain propagated on the C-A membranes has been very similar in many ways to that reported by Haig[4,5] for the *Onderstepoort* strain. Up through the 9th to 10th passage of the *Lederle* strain very little, if any, changes were noticed in the appearance of the C-A membranes. However, starting at that passage level some of the membranes were noted to be thickened and moist, showing fairly numerous small areas whitish-gray in appearance. These changes became more marked with increasing number of egg passages so that to date, at the 74th passage, the membranes are markedly thickened and the accompanying lesions quite numerous, heavy and opaque (see Fig. 1).

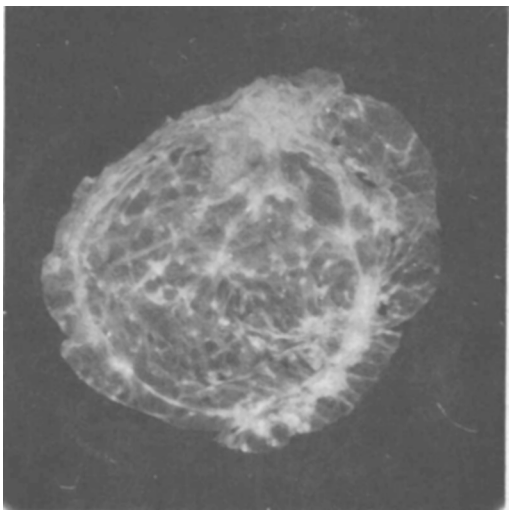

FIG. 1.
Distemper infected chorio-allantoic membrane.

Appropriate bacteriological tests carried out routinely with both aerobic and anaerobic media have consistently shown the infected tissues to be free of any detectable bacterial contaminants.

Subinoculation of the egg-adapted virus into ferrets. At intervals membranes of a certain passage level were pooled, homogenized to a uniform suspension and inoculated into ferrets for testing of virulence. The data pertaining to the maintenance of the virus in eggs and subinoculation into ferrets are summarized in Fig. 2.

In brief, it was found that canine distemper virus may be maintained by serial passage on the C-A membranes of developing chick embryos and that the virus still retains its

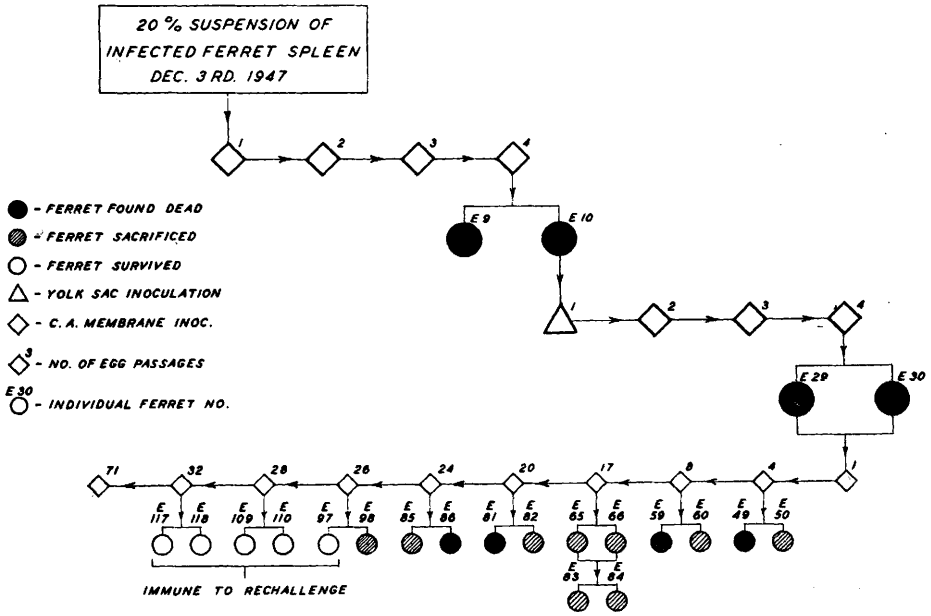

FIG. 2.
Ferret and egg passage series of distemper virus.

lethal effect for ferrets through at least the 20th egg passage. Starting at the 26th passage level, however, the C-A membrane material was definitely of lessened virulence since 1 of 2 ferrets survived infection following a very mild illness and was found to be completely resistant on rechallenge with approximately 100 MLD of virulent ferret spleen virus 30 days later. Likewise ferrets inoculated with C-A membranes of the 28th and 32nd egg passages showed no signs of infection although they too were found completely resistant to rechallenge with approximately 100 MLD of virulent ferret spleen virus 30 days later.

Further proof for identity of the virus. In order to eliminate the possibility of having viruses other than that of canine distemper present, the following animals were inoculated with a 20% suspension of C-A membranes of the 48th egg passage: 6 Swiss albino mice with 0.03 ml each intracerebrally; 3 guinea pigs with 0.15 ml each intracerebrally; 3 guinea pigs with 1.0 ml each subcutaneously; 2 rabbits on the scarified corneas with 0.1 ml each, and 2 ferrets with 1.0 ml each intraperitoneally. None of the inoculated animals showed any signs of illness, therefore infection with other viruses such as lymphocytic choriomeningitis or herpes simplex was improbable. The 2 ferrets were challenged at the end of 30 days and were shown to be immune to at least 100 MLD of virulent ferret spleen distemper virus.

Neutralization tests in eggs. Dogs, ferrets and guinea pigs were hyperimmunized with repeated injections of ferret spleen virus. Blood samples were drawn and the sera from each species pooled. Pools of normal ferret serum and normal guinea pig serum were also prepared. The 5 serum pools were filtered separately through single Seitz EK pads and inactivated at 56°C for 30 minutes. Each serum pool was then mixed with an equal volume of a 20% suspension of C-A membranes of the 55th egg passage. As a further control a mixture of equal volumes of sterile broth and C-A membrane suspension was similarly prepared. All 6 mixtures were incubated at 37°C for 2 hours before inoculation into 6 eggs each on the C-A membranes. All eggs were opened after 5 days' incubation at 35°C and the embryonic membranes and tissues carefully examined macroscopically. The results may be summarized by stating that those eggs inoculated with the mixtures containing normal ferret serum, normal guinea pig serum or sterile broth all showed the markedly thickened membranes and numerous opaque foci that are usually observed with the *Lederle* strain of egg-adapted canine distemper virus. In contrast those eggs inoculated with the mixtures containing immune guinea pig, ferret or dog sera showed only the typically thin, sparkling transparent appearance of normal membranes with no visible lesions whatsoever.

Neutralization tests in ferrets. Two ferrets were hyperimmunized by injecting them subcutaneously at 5-day intervals with 1 ml of a 20% C-A membrane suspension representing the 59th, 60th, 61st, 62nd, 63rd and 64th egg passages. The ferrets were bled by cardiac puncture one week after the last injection and their sera pooled. Three guinea pigs which were hyperimmunized in a similar manner were likewise bled and their sera pooled. In addition 2 other ferrets were submitted to the same schedule of immunization with the exception that they received an additional intraperitoneal injection of 2.0 ml of a 20% suspension of the 65th C-A membrane passage. They were also bled one week after the last injection and their sera pooled. Pools of normal ferret and of normal guinea pig sera were also prepared. The 5 serum pools were filtered separately through single Seitz EK pads and inactiviated at 56°C for 30 minutes. Mixtures, containing 8 ml of the respective serum plus 2 ml of infected ferret spleen suspension (containing at least 100 MLD of virulent virus per ml) were incubated at 37°C for 2 hours and then injected intraperitoneally into 2 ferrets, each ferret receiving 5 ml of the serum-virus mixture. The results may be summarized by stating that those ferrets inoculated with the mixtures containing normal ferret or guinea pig serum showed typical symptoms of distemper starting either on the 7th or 8th day and were dead by the 11th to 13th day. In contrast the 6 ferrets inoculated with the

mixtures containing immune ferret or guinea pig sera remained symptom-free and survived in a healthy state for more than 35 days following inoculation.

From the two neutralization tests described above it is apparent that both the egg-adapted virus strain and the parent strain of ferret spleen virus are antigenically (immunologically) related.

Comment. During the course of the work described above, Haig[4,5] reported his studies in which he successfully maintained the *Onderstepoort* strain of virus for more than 90 serial passages on the C-A membranes of chick embryos over a period of 15 months. Through the courtesy of Dr. D. A. Haig and Dr. R. Alexander,[6] Director of the Division of Veterinary Services, The Veterinary Research Institute, Pretoria, Onderstepoort, Union of South Africa, the *Onderstepoort* strain of virus was sent to us in December 1948 and was readily established and maintained in our laboratory on the C-A membranes of chick embryos. Studies concerning the comparative properties of the *Onderstepoort* and *Lederle* strains of chick embryo adapted distemper virus will be published at a later date.

Summary. Studies are reported in which the *Lederle* stock strain of canine distemper virus was adapted and maintained through 74 serial passages on the chorio-allantoic membranes of developing chick embryos. The chick embryo adapted strain retained its capacity to produce lethal infection in ferrets through the 24th egg passage level but apparently lost its ability to do so between the 24th and 28th egg passages. Ferrets inoculated with chick embryo adapted virus of passage levels higher than the 28th transfer failed to show any signs of disease, but were found immune on rechallenge with fully virulent ferret spleen virus. Cross neutralization tests carried out in chick embryos and in ferrets indicate that the chick embryo adapted strain and the parent strain of ferret spleen virus are immunologically related.

The authors wish to express their appreciation to Mr. Frank E. Smith for his able technical assistance during the course of these studies.

[6] Alexander, R., personal communication to H. R. Cox.

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17151. Chymotrypsin in Cancer.

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A trial of chymotrypsin as a therapeutic agent in cancer was initiated at the Laboratory of Experimental Oncology in August 1948. The isolation of this enzyme in the crystalline state was described by Kunitz and Northrop.[1] The basis of interest in chymotrypsin in cancer was not the highly tenuous theoretical considerations that apparently led to its use,[2] but the purported clinical results in one case that has appeared in the literature[3] and additional similar cases that were rumored in this area and elsewhere.[4] These reports claimed prompt relief of pain after 3 or 4 parenteral injections of chymotrypsin, and subsequent decrease or disappearance of lesions stated to have been malignant neoplasms.

Ten patients (Table I) with inoperable neo-

* Aided by Grant 396 from the National Advisory Cancer Council.

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