thyroid tissue, some quantitative dimensions may be assigned to the free inositol in this species. Since average dry weight of sheep thyroid gland constitutes 20.3 ± 3% of wet weight(7), the average inositol level of 355 mg/100 g observed in the present study would account for 1.7% of dry weight of ovine thyroid. In terms of ultimate disposition, only a small fraction of this free inositol need be necessary for incorporation into phosphoinositide. For example, lipid phosphorus in the sheep thyroid averages 191.5 μg P/g wet weight and 9% of this is present in the phosphoinositide(2). Thus, assuming that thyroid phosphoinositide is a monophosphoinositide, its inositol content maximally would equal only about 0.3% of free inositol.

For the moment, the physiological significance of free myo-inositol in thyroid tissue is unclear, nor has it been determined whether the thyroid can concentrate or synthesize inositol. Eagle has demonstrated the role of myo-inositol as an essential growth factor in tissue culture(8). Tissue analyses in rats, guinea pigs and rabbits (to be published elsewhere), have indicated that free myo-inositol is significantly more abundant in thyroids of these species than in salivary gland, heart, liver, spleen, kidney cortex, adrenal, pancreas, testicle, ovary, lung, mammary gland and diaphragm. Screening studies of multiple tissues have indicated some correlation between levels of free inositol and protein elaboration. Thus, conceivably, the abundant myo-inositol within the thyroid could be linked to the active intrathyroidal accumulation of amino acids (or other anions such as iodide), protein synthesis, or secretion of thyronines. Alternatively, in view of known conversion of inositol to glucuronic acid(9,10) some of the free myo-inositol within the thyroid might be implicated in active formation of intrathyroidal mucopolysaccharides(11). Experimental efforts to assess these and other possibilities and to correlate inositol levels with thyroid activity are in progress.

Summary. Bioassay with Kloeckera Brevis yeast has been employed to measure content of free myo-inositol in human, rat, rabbit, sheep and guinea pig thyroid glands. In all species, thyroid/plasma concentration differentials for free myo-inositol were greater than 100. Chromatographic documentation has also been secured, and the possible significance of the abundant intrathyroidal myo-inositol has been discussed.

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Propagation of Canine Distemper (CD) Virus in Tissue Culture. (24694)

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Repeated attempts by the authors to grow CD virus (canine distemper virus) of dog or ferret origin in tissues of those 2 species yielded essentially negative results. Presumably experience of others has been similar; no report of a successful attempt has come to our attention, among numerous publications in the past 5 years on tissue-culture propagation of many different animal viruses. This communication describes the successful propaga-

tion in chick-embryo tissue culture of chick-embryo-adapted CD virus, with no evidence of cytopathogenic effect.*

Materials and methods. Virus strains. American Cyanamid (Lederle) strain of chick-embryo-adapted CD virus(1) was used. Experimental ferrets were challenged with American Cyanamid (Lederle) stock strain of virulent CD virus(2). *Tissue culture.* The virus was serially passed in stationary tube cultures of trypsinized total chick-embryo tissues (9 - day - old embryos) prepared essentially by the method of Dulbecco and Vogt(3). Povitsky bottle cultures of same tissue were used for virus pools for dried experimental vaccine. Growth medium for both cultures was Eagle's solution (4) containing 10% horse serum, in amounts of 1 ml/tube and 100 ml/Povitsky bottle. After 24-hour growth at 37°C, cultures were renewed with mixture 199(5), and thereafter no changes were made. *Virus titrations.* Live 7- or 8-day-old chick embryos were inoculated on the chorioallantoic membrane (CAM) by the technic described by Gorham (6). Six embryos were inoculated with 0.2 ml doses of each serial 10-fold dilution of infected tissue-culture fluid. After 7 days incubation at 35°-36°C, results were recorded by removing the top of each CAM and examining it under a strong light for lesions. Infected membranes were thickened and showed the opaque foci associated with American Cyanamid (Lederle) strain of CD virus. Fifty-%-chick-embryo-infective end points (CED_{50}) were calculated by the Reed and Muench formula (7). *CD dog immune serum.* To prepare serum, a susceptible puppy was injected subcutaneously with 2 ml of a 30% CD-infected CAM suspension. Repeated titrations of this serum by the neutralization method gave values between $10^{-3.0}$ and $10^{-4.0}$ against virus CED_{50}'s of 20 to 200. Before use in the neutralization test, both immune and normal sera were inactivated at 56°C for 30 minutes. *Neutralization test.* Triple sets of 10-fold dilutions of infected tissue-culture fluids were prepared in hormone broth. Dilutions of the first set were mixed with equal volume of hormone broth, the second set with equal volume of 1:8 dilution of CD immune dog serum, the third set with equal volume of 1:8 dilution of normal dog serum. After standing at 4°C for 4 hours, each mixture was inoculated in doses of 0.2 ml on the CAM of 6 chick embryos, as described above. After 7 days incubation at 35°-36°C, results were recorded and 50% endpoints calculated, as also described above. *Susceptible ferrets.* These were procured and maintained under the same conditions of strict isolation reported previously(2).

Results. Serial propagation of CD virus in tube cultures. The 48th passage of CD virus in live embryos was inoculated into 10 chick-embryo-culture tubes, 0.1 ml of a 40%-infected CAM suspension/tube. Tubes were incubated at 37°C and examined microscopically for 7 days. No gross cytopathogenic effect was observed. Monolayers then were detached into the fluid, pooled, and ground in Tenbroeck grinder. The undiluted suspension was inoculated on the CAM of chick embryos, 0.2 ml/egg. A second tissue-culture passage then was made in 10 new tubes, 0.2 ml of suspension/tube. Again virus was detected in the inoculated embryos, but no cytopathic changes occurred in tissue-culture passage. Serial transfers were continued in the same manner every 7 days for 14 passages. None showed cytopathogenicity, but in undiluted form all produced on the CAM lesions characteristic of CD virus.

From 15th passage on, in total of 64 passages, all without cytopathology, tissue-culture harvests were not inoculated as undiluted

TABLE I. Optimum Time of Harvest of CD Virus Propagated in Chick-Embryo-Tissue Culture.

Day of harvest	CED_{50}/ml Passage level 19th	20th
4	2.9*	4.2
5	1.9	3.4
6	2.7	2.4
7	2.4	3.2

* Power of 10.

* The following article, on cultivation of a "street" strain of CD virus in dog kidney tissue culture, came to the attention of the authors of this manuscript after it had been submitted for publication. Rockborn, G. Canine Distemper Virus in Tissue Culture, *Archiv Virusforsch.*, 1958, v8, 485.

TABLE II. Serum Neutralization Results of Tissue-Culture-Grown CD Virus, and 1st Chick-Embryo Passage Thereof.

Virus suspension plus:	Passage level: CED_{50}/ml					
	Tissue culture			Tissue culture plus 1 chick-embryo passage		
	21st	34th	55th	21st	34th	55th
Sterile broth	3.4*	3	3	4	4	4
Normal dog serum	3	3.5	3	4	3.5	3.7
CD-immune dog serum	<1	<1	<1	<1	<1	<1

* Power of 10.

suspensions but, instead, were titrated in the chick embryo. Titers ranged from $10^{2.1}$ to $10^{5.2}$/ml, the majority falling between $10^{3.0}$ and $10^{4.5}$. These values are of the same order as those obtained with CAM suspensions of infected live embryos.

To determine optimum time of harvest, tissue cultures of 19th and 20th tissue-culture passages were titrated after 4, 5, 6 and 7 days of incubation. Since results (Table I) indicated that maximum virus yields are reached in 4 days, subsequent passages were harvested routinely on 4th day.

The tissue-cultured virus was identified by the neutralization method and by ferret immunization.

Serum neutralization. Results of this test (Table II), performed at 3 different transfer levels of the virus, in tissue culture and on CAM suspensions derived from their first back-passages in live chick embryos, prove unequivocally the immunological identity of the tissue-culture-propagated agent with the original chick-embryo-adapted CD virus.

Ferret immunization. Two normal ferrets were each inoculated intraperitoneally with 1 ml of undiluted suspensions of 22nd and 34th tissue-culture passages. These animals remained normal for 3 weeks and then they and 2 normal control ferrets were challenged, also intraperitoneally, with virulent distemper virus.

Later, the 42nd tissue-culture passage was diluted in 10-fold steps and 1 ml of each dilution, from undiluted through 10^{-5}, was inoculated intraperitoneally into 3 normal ferrets. During the next 3 weeks 2 ferrets (10^{-2} and 10^{-4} groups) died of pneumonia. The others remained normal, and, together with 2 normal controls, were challenged with virulent CD virus. Results of both experiments (Table III), recorded 26 days after challenge, also established the identity of the tissue-culture agent with CD virus. Control animals died of typical distemper, but all others survived, whether they had been injected with 150,000 or as few as 1.5 CED_{50}'s.

Experimental vaccines. Three experimental lots of vaccine were prepared, 2 from 33rd and one from 34th serial passage of the virus. Povitsky-bottle cultures were inoculated with 10 ml of infected tissue-culture suspensions representing these passages, and harvested 4 days later. Harvests were ground in Waring blendor for 2 minutes, distributed in 2 ml amounts in glass vials, and dried from the frozen state. Virus titrations made on fresh harvests and after lyophilization indicated an average loss of about 1 to 1.5 logs as a result of drying (Table IV). Nevertheless, all 3 preparations were shown in ferret immunization tests to be effective vaccines.

TABLE III. Immunization of Ferrets with Tissue Culture CD Virus.

Exp. No.	Passage level	Inoculum dilution (1 ml)	CED_{50} inj.	Challenge results: No. dead from challenge/total No. challenged	
				Immunized ferrets	Control ferrets
1	22	Undil.	2.9*	0/2	2/2
	34	"	3.4	0/2	
2	42	"	5.2	1/2†‡	
		10^{-1}	4.2	0/3	
		10^{-2}	3.2	0/2‡	"
		10^{-3}	2.2	0/3	
		10^{-4}	1.2	0/2‡	
		10^{-5}	.2	0/3	

* Power of 10.

† One ferret died of pneumonia 10 days after challenge; no distemper symptoms.

‡ Immunized ferrets which died of inter-current infection before challenge and showed no distemper symptoms were omitted from Table.

TABLE IV. Titers of Fresh and Post-Drying Experimental Vaccines Prepared in Chick-Embryo Tissue Culture.

Vaccine lot No.	Passage level	CED_{50}/ml	
		Before drying	After drying
13A	33	3.7*	2.7
14A	33	3.7	2.2
15A	34	4.2	3.2

* Power of 10.

Lots 13A and 14A were reconstituted to volume with sterile distilled water and, without further dilution, each was injected intraperitoneally into 3 normal ferrets in 1 ml doses containing, respectively, 500 or 150 CED_{50}'s. Lot 15A, in addition to similar administration to 6 ferrets in undiluted form, also was injected into 3 ferrets each as a 10^{-1}, 10^{-2} and 10^{-3} dilution, in 1 ml doses containing from 1,500 to 1.5 virus CED_{50}'s. Table V shows that, while normal challenge control animals died of the experimental disease, all immunized ferrets survived, regardless of number of CED_{50}'s of tissue-culture-propagated virus injected.

Discussion. Despite continued failure to grow CD virus of dog or ferret origin in cultures of tissues from these species, a chick-embryo-adapted CD virus was propagated successfully in chick-embryo-tissue culture for 64 serial transfers. The observation that gross cytopathology did not accompany virus multiplication is not unique. Possibly histological studies, including use of the fluorescent antibody technic, would reveal changes indicative of cellular infection. Such studies are under consideration.

TABLE V. Immunization of Ferrets with Experimental Tissue Culture CD Vaccines.

Vaccine lot No.	Passage level	Inoculum dilution (1 ml)	CED_{50} inj.	Challenge results: No. dead from challenge/total No. challenged	
				Immunized ferrets	Control ferrets
13A	33	Undil.	2.7*	0/3	2/2
14A	33	"	2.2	"	"
15A	34	"	3.2	1/6†	4/4
		10^{-1}	2.2	0/3	
		10^{-2}	1.2	"	
		10^{-3}	.2	"	

* Power of 10.

† One ferret died of inter-current infection; no distemper symptoms.

The tissue-culture-grown virus remained antigenic for the ferret through at least 42 passages, at remarkably low virus CED_{50} (0.15 - Table III). This observation suggests that while the chick-embryo CAM is of great practical value, it is less sensitive than the ferret for detection of minute amounts of CD virus, and that, as presently used, the CAM fails to reveal the entire virus content of infected preparations.

Summary. A chick-embryo-adapted canine distemper virus was propagated successfully for 64 passages in cultures of total chick-embryo tissues. As measured on the chorioallantoic membrane of live chick embryos, average virus titers of these passages ranged between $10^{3.0}$ and $10^{4.5}$/ml infected culture suspension. These titers are of the same order as those obtained with live-chick-embryo-grown virus. Identity of tissue-culture-grown agent with original CD virus employed was established by virus neutralization method and by ferret immunization. Effective freeze-dried vaccines were produced with different passage levels of tissue-culture-grown virus.

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