might be overlooked if reliance were placed only on such standard liver-function tests as per cent esterified cholesterol, bilirubin levels or BSP clearance.

Tests on the dog when treatment was stopped after 5 months, indicate ready reversibility of fatty infiltration of the liver. It is therefore possible that intermittent use of the drug might lower serum cholesterol without causing excessive fatty livers. The prompt return of plasma cholesterol to pretreatment levels, observed in Exp. I and II upon cessation of treatment, indicates lack of permanent alteration of cholesterol-synthesizing mechanisms.

Additional work in this laboratory with species other than the dog showed that SKF No. 525-A produces hypocholesteremic effects in rats(13), mice and monkeys. In both rats and mice treated with SKF No. 525-A, we observed fatty infiltration of liver; monkeys were not autopsied for hepatic examination.

Summary. β-Diethylaminoethyl diphenylpropylacetate hydrochloride (SKF No. 525-A) reduced significantly plasma total cholesterol and aortic cholesterol in dogs. Chronic daily administration of the SKF No. 525-A to dogs for 5 months resulted in marked fatty infiltration of the liver, which was rapidly reversible upon withdrawal of the compound.

The authors wish to thank Dr. C. A. Leonard for drug studies in monkeys and Dr. L. Z. Saunders for histopathological studies.

1. Cowgill, G. R., Drabkin, D. L., *Am. J. Physiol.,* 1927, vol. 36.

2. Anderson, J. T., Keys, A., *Clin. Chem.,* 1956, v2, 145.

3. Zilversmit, D. B., Davis, A. K., *J. Lab. Clin. Med.,* 1950, v35, 155.

4. Kunkel, H. G., Ahrens, E. H., Jr., Eisenmenger, W. J., *Gastroenterology,* 1948, v11, 499.

5. Brown, H., Reingold, A. M., Samson, M., *J. Clin. Endocr. Metab.,* 1953, v13, 444.

6. Hawk, P. B., Oser, B. L., Summerson, W. H., *Practical Physiological Chemistry,* 13th ed., Blakiston Co., New York, 1954.

7. Popper, H., Schaffner, F., *Liver: Structure and Function,* McGraw-Hill, New York, 1957, pp48-49.

8. Cooper, J. R., Axelrod, J., Brodie, B. B., *J. Pharm. and Exp. Therap.,* 1954, v112, 55.

9. Kuriaki, K., Marumo, H., *Jap. J. Pharmacol.,* 1959, v8, 96.

10. Blohm, T. R., Mackenzie, R. D., *Arch. Biochem. and Biophysics,* 1959, v85, 245.

11. Huff, J. W., Gilfillan, J. L., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 41.

12. Lena, E. T., *Boll. chim. farm.,* 1959, v98, 201.

13. Rice, E., Greenberg, S. M., *Fed. Proc.,* 1960, v19, 15.

Received November 18, 1959. P.S.E.B.M., 1960, v104.

Further Evidence of Immunologic Dissimilarity of Distemper (CD) and Measles (M) Viruses.* (25897)

V. J. CABASSO, J. E. AVAMPATO, K. H. KISER AND M. R. STEBBINS
(Introduced by H. R. Cox)
Viral and Rickettsial Research Section, Lederle Labs., Amer. Cyanamid Co., Pearl River, N. Y.

Although a possible relationship between canine distemper (CDV) and measles (MV) viruses has been proposed by various investigators(1-5), others find experimental evidence against such a theory(6,7). Inasmuch as modified canine distemper virus has been suggested for use in immunizing children against measles(8), further investigation of the relationship of these 2 viruses is required. Additional experimental evidence of their dissimilarity is presented here.

Materials and methods. Virus strains used: Edmonston strain† of MV adapted to chick embryo tissue culture(9); HeLa-grown MV with titers of $10^{4.5-5.5}$ TCD_{50} for inoculation of monkeys and ferrets and for neutralization

* Presented in part at SAB meeting in Philadelphia, May 1, 1960.

† Courtesy of Dr. J. F. Enders, Children's Hosp., Boston.

tests; Lederle chick-embryo adapted CDV (10) for chick-embryo tissue culture and neutralization tests and for vaccination of ferrets. Virus stock was prepared as described earlier (6). Ferrets were challenged with Lederle virulent CDV(11). *Tissue culture.* Growth medium consisted of Eagle's solution(12) containing lactalbumin hydrolysate, to which was added: 2% calf serum for monkey kidney cultures, 10% horse serum for chick embryo and 30% calf serum for HeLa cells, all prepared by the method of Dulbecco and Vogt (13). Renewal fluids were medium 199(14) plus 2% hydrolyzed gelatin and 1% liver digest for monkey kidney cultures; medium 199 alone for chick embryo; and Eagle's solution plus 20% horse serum for HeLa cultures. *Human sera.* Thirteen paired acute and convalescent measles sera and 3 convalescent sera collected during a 1947 epidemic in Cleveland‡ were stored in a dry ice cabinet until tested. *Measles immune monkey serum.* Serial bleedings from cynomolgus monkeys immunized with MV§ were tested for MV and CD neutralizing antibodies. *Neutralization tests.* CD antibodies were titrated in chick embryos(6). MV neutralization tests were carried out as follows: 2-fold dilutions of inactivated serum (30 minutes at 56°C) and approximately 100 TCD_{50} of virus were mixed and incubated one hour at room temperature. Each of 2 HeLa culture tubes was inoculated with 0.1 ml of each serum-virus mixture, incubated for 14 days at 37°C and examined daily for multinucleated cells characteristic of MV. Renewal fluid was changed every 4th day. Paired serum specimens were always tested at the same time, and normal and immune serum controls as well as a virus titration, were included in each test. Serum neutralizing titers (SN_{50}) were calculated by Reed and Muench formula(15). *Control immune sera* were the same as those used in a previous study(6).

Results. Immunization of Ferrets with MV. Blood samples from 4 groups of 3 ferrets were pooled by group. Two groups were then inoculated with a HeLa-grown MV suspension according to following schedule:

Day	Route	Dose
0	IM in 2 sites	2 ml MV-adjuvant mixture‖
	IP	1 " ¶
7	IP	1 "
14	IM in 2 sites	2 " -adjuvant mixture
	IP	1 "
21	IP	1 " (Test bleeding done on this day.)

‖ MV-adjuvant mixture: 5 parts virus suspension, 4 parts Bayol F, and 1 part Arlacel A.

¶ MV: virus suspension—$10^{4.5}$ to $10^{5.5}$ TCD_{50}/ml.

The other 2 groups were inoculated according to the same schedule with a normal HeLa cell preparation. Sera from 2 ferrets were pooled and the ferrets then vaccinated with modified CDV. On day 21, all ferrets were re-bled and their sera pooled by group. CDV and MV neutralizing antibody titers on all pools had been determined by day 31, and third blood specimens were obtained on that day before the animals were challenged with virulent CDV. One MV, one HeLa control and the CD vaccine groups of ferrets received 10,000 ferret LD_{50}, and the other MV and HeLa groups received 1,000 LD_{50} of CDV. All serum pools were again tested for CD and MV neutralizing antibodies (Table I). With use of adjuvant, demonstrable MV antibodies were elicited in MV inoculated ferrets, yet no CD antibodies were formed and all animals succumbed to CDV challenge. CD vaccination induced CD antibodies and immunity to challenge, but did not induce MV antibodies. *MV and CDV SN_{50} titers from 16 measles patients.* Time of bleeding and virus titers are given in Table II. Variable amounts of available serum account for the different starting dilutions. Appearance of MV and CD antibodies in the same individual are clearly not correlated. The highest CDV levels (between 1:10 and 1:30) were found in sera from 2 patients, of whom one had no history of clinical measles, and from MV-immune monkeys. No significance is attributed to CD titers of less than 1:20, as dogs with even slightly higher titers have been found fully susceptible to distemper challenge(16). Ac-

‡ Courtesy of Dr. A. B. Sabin, Children's Hosp., Cincinnati.

§ Data to be published.

TABLE I. Immunization of Ferrets with MV and Subsequent CDV Challenge.

Ferret group No.	Inoc.	Neutralizing antibodies MV/CD			Mortality ratio after CD challenge
		Pre-vac.	21 days	31 days	
1	HeLa control	<4/<5*	<4/<5	<4/<5	3/3†
2		"	"	"	" ‡
3	HeLa MV	"	<60/<5	20/<5	" †
4		"	60/<5	10/<5	" ‡
5	CD vaccine	"	<4/1800	<4/1300	0/2

* Reciprocal of dilution. † Challenge dose 10,000 LD_{50} CDV. ‡ Challenge dose 1,000 LD_{50} CDV.

tively immune dogs often possess titers of 1:1000 or more, as illustrated by the control CD-immune dog serum, which was devoid of measles antibodies. Judging from convalescent phase titers, all but 2 patients had had clinical measles, perhaps even the 3 from whom no acute phase sera were available. Yet distemper antibodies are absent or insignificant in all. *Effect of homologous and heterologous antisera on multiplication of MV and CDV.* MV was grown in 3 groups of HeLa culture tubes. In one group the medium contained 5% normal horse serum; in the second group, 5% control MV-immune monkey serum; in the third, 5% control CD immune dog serum. After 14 days incubation at 37°C, pooled fluids from each group were titrated in HeLa cultures. Groups of chick-embryo culture tubes were renewed with medium 199, alone or plus one of the following: 2.5% normal dog serum, 2.5% control CD-immune dog serum, or 2.5% control MV-immune monkey serum. Tubes were inoculated with tissue culture adapted CDV(17) and incubated for 4 days at 37°C. Tissues and fluids from each group were harvested, ground together and titrated on the CAM of 7-day-old chick embryos (Table III). MV growth was significantly inhibited (3 logs) by the homologous, but not at all by the heterologous sera. MV-antiserum in the medium did not interfere with CDV growth, but CD-immune serum did. *MV and CD antibody titers of measles-immune monkeys.* One of 6 susceptible monkeys developed MV antibodies one week after vaccination, but died of pneumonia on the 13th day. The others developed MV titers by the second week post-vaccination and maintained them at high levels for 6 weeks of observation. Except for animals Nos. 4 and 5, which lacked significant CD titers, CD antibodies were absent despite the

TABLE II. Distemper and Measles Antibody Titers of 16 Measles Patients.

Clinical signs at first bleeding	Serum neutralization titers* Acute/Convalescent MV†	CD‡
Koplik spots	<8/44	<5/ 10
	<8/64	<25/ 5
	<8/90	<5/ 5
	<4/20	<5/<5
	<8/64	<25/<5
	<8/90	<5/<5
	<4/128	<25/<5
	6/80	"
Rash—2nd day	10/64	<5/ 5
3rd "	8/8	"
Idem	10/64	"
"	8/32	"
Not obtained	—/40	—/<25
Idem	—/128	—/<5
"	—/128	"
No clinical measles	64/90	10/ 30
Control measles immune monkey serum	140	20
Control distemper immune dog serum	<4	4,000

* Reciprocal of dilution.
† Tested against 100 TCD_{50} of virus.
‡ " " 100-500 CED_{50} of virus.

TABLE III. Multiplication of MV and CD Viruses in Presence of Homologous and Heterologous Antisera.

Type serum added to medium	MV titers in HeLa cells	CD titers in chick embryos (EID_{50}/ml)
None		4.2
Normal horse	5.5*	
" dog		4.2
MV-immune monkey	2.5	4.3
CD- " dog	5.5	Neg. und.

* Log to power of 10.

TABLE IV. Measles and Distemper Antibody Titers of Cynomolgus Monkeys Immunized with Measles Virus.

Time of bleeding (wk)	Monkey No. and serum neutralizing antibodies											
	1		2		3*		4		5		6	
	CD	MV	CD	MV	CD	MV	CD	MV	CD	MV	CD	MV
0	<5†	<4	<5	<4	<5	<4	<5	<4	<5	<4	<5	<4
1	"	"	"	"	"	44	"	"	"	"	"	"
2	"	40	"	20			"	30	"	30	"	44
3	"	90	"	180			"	360	10	260	"	180
4	"	"	"	260			"	"	"	510	"	"
5	"	60	"	"			"	510	<5	260	"	90
6	"	90	"	180			10	"	"	180	"	180

* Died of pneumonia on 13th day. † Reciprocal of dilution.

presence of elevated levels of MV antibodies (Table IV).

Discussion. Tests with the strains used in this study indicate little antigenic similarity between MV and CDV, and any relationship between them seems remote. Warren *et al.* (5) have suggested that strain and passage histories of CDV might bear on its degree of similarity to MV. Since only one serologic type of CDV has been recognized, such variations in the antigenic mosaic of the virus are difficult to assume. Our tests on sera from patients convalescing from measles showed no evidence of simultaneous development of CD and MV antibodies. Possibly CD antibodies develop later. Absorption experiments with human sera containing antibodies to both viruses are in progress and results should furnish further clues to their antigenic relationship.

Summary. Additional experimental evidence of dissimilarity of canine distemper (CDV) and measles (MV) viruses are presented: 1. Ferrets inoculated with repeated doses of MV-adjuvant mixture and which developed M antibodies proved as susceptible as control animals to challenge with CDV. 2. Paired convalescent sera from measles patients showed significant measles antibody rises, while CD antibodies remained essentially absent. 3. Growth of MV in HeLa cells was not inhibited by presence of CD antiserum or normal serum, while MV antiserum apparently reduced virus multiplication. Presence of measles antiserum did not interfere with propagation of CDV. 4. By 14th day after MV infection, serologically negative monkeys developed high antibody titers for MV but none for CDV. It remains to be determined whether results obtained are attributable to particular virus strains used, or apply also to other isolates of MV and CDV.

Authors thank Marie Murrin and Hubert Klaver for technical assistance and E. Manoogian for help in preparation of manuscript.

1. Adams, J. M., Imagawa, D. T., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 240.
2. Carlström, G., *Lancet,* 1957, v273, 344.
3. ———, *Arch. ges. Virusforsch.,* 1959, v8, 527.
4. ———, *ibid.,* 1959, v8, 539.
5. Warren, J., Nadel, M. K., Slater, E., Millian, S. J., *Am. J. Vet. Res.,* 1960, v21, 111.
6. Cabasso, V. J., Kiser, K. H., Stebbins, M. R., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 227.
7. Enders, J. F., McCarthy, K., Mitus, A., Cheatham, W. J., *New England J. Med.,* 1959, v261, 875.
8. Adams, J. M., Imagawa, D. T., Wright, S. W., Tartan, G., *Virology,* 1959, v7, 351.
9. Katz, S. L., Milovanic, M. V., Enders, J. F., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 23.
10. Cabasso, V. J., Cox, H. R., *Cornell Vet.,* 1952, v42, 96.
11. Cabasso, V. J., Stebbins, M. R., Johnson, D. W., *Vet. Med.,* 1956, v51, 119.
12. Eagle, H., *J. Exp. Med.,* 1955, v102, 595.
13. Dulbecco, R., Vogt, M., *ibid.,* 1954, v99, 167.
14. Morgan, J. F., Morton, H. J., Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 1.
15. Reed, L. J., Muench, H., *Am. J. Hyg.,* 1938, v23, 493.
16. Gillespie, J. H., Baker, J. A., Burgher, J., Robson, D., Gilman, B., *Cornell Vet.,* 1958, v48, 103.
17. Cabasso, V. J., Kiser, K., Stebbins, M. R., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 551.

Received April 1, 1960. P.S.E.B.M., 1960, v104.