CANINE DISTEMPER IN THE RHESUS MONKEY (MACACA MULATTA)

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PLATE 12

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Canine distemper has been described in various animals but never, to the best of our knowledge, in monkeys. Nicolle (1) inoculated one monkey with blood from a dog sick with distemper but the monkey remained well.[1] This appears to be the only effort to transmit the disease to monkeys, a surprising fact in view of the lively speculation that has at times centered about the nature of canine distemper.

Materials and Methods

Distemper virus was supplied to us in frozen dog spleen[2] and represents the strain or strains used in the manufacture of distemper vaccine. In most cases the virus was first passed to ferrets, using 0.5 cc. of the supernatant fluid of a 20 per cent emulsion injected subcutaneously into one or two animals and passed from ferret spleen to monkeys after the characteristic signs of distemper had appeared (reddening and swelling of the footpads, conjunctivitis, thick, mucoid secretion in the nostrils and coma). Ferret spleen was prepared in the same manner as the dog spleen.

20 per cent emulsions have regularly been used. The supernatant fluid has been given in doses of from 0.2 to 1 cc. For blood passage 4 to 7 cc. of freshly drawn blood has been given as a single dose. Inoculation has been successful by

[1] Baló, Josef, (Die unsichtbaren Krankheitserreger. Filtrierbare Vira, Berlin, S. Karger, 1935) quotes Veith as authority for the statement that the monkey is susceptible to distemper, but does not give the reference. Veith is not elsewhere quoted on the subject. The reference appears to refer to Joh. Elias Veith, author of Handbuch der Veterinärkunde issued last century. However no reference to the matter appears in either the edition of 1817 or 1842.

[2] Courtesy of Dr. Norman Pyle, Lederle Laboratories, Pearl River, N. Y.

TABLE I

Source of Virus and Route of Inoculation in 63 Successful Transmissions of Canine Distemper to Rhesus Monkeys

Source of virus	Route of inoculation					
	Intracutaneously	Subcutaneously	Intracerebrally	Intranasally	Subcutaneously and intracerebrally	Intraperitoneally
Ferret spleen		2	7	6	3	1
Dog spleen		6		4		
Monkey spleen		1		17		
Monkey blood		5				
Dried virus	2	4				2
Monkey brain			2			

Dried virus was desiccated dog spleen as prepared for the immunization of dogs against distemper.

TABLE II

Canine Distemper

Serial Transmission in Monkeys and Transmission from Monkey to Ferret

Pooled distemper dog spleen

↓

Monkey 1, inoculated subcutaneously with 0.5 cc. of the supernatant fluid of a 20 per cent emulsion of dog spleen. Developed fever after 5 days. Sacrificed on 6th day. Inclusion bodies present in tissues

↓

Ferret 1, inoculated with similar emulsion but prepared from spleen of monkey 1. Developed typical signs and febrile reaction of canine distemper. Sacrificed on 13th day. Inclusion bodies present in tissues

↓

Monkey 2, inoculated with supernatant fluid of a 10 per cent emulsion of ferret spleen 1 given intranasally by means of a ureteral catheter on each of 3 successive days. Developed typical signs and febrile reaction of distemper. Died on 15th day with massive tuberculous pneumonia. Inclusion bodies in tissues

Pooled distemper dog spleen

↓

Monkey 3, given 0.5 cc. of the supernatant fluid of a 20 per cent emulsion of dog spleen subcutaneously. Typical response Sacrificed on 12th day

↓

Monkey 4, inoculated similarly with an emulsion prepared from the spleen of monkey 3. This and two other animals similarly inoculated all developed usual fever and signs and recovered

↓

Monkey 5, inoculated subcutaneously with 4 cc. of venous blood taken from monkey 4, 7 days after inoculation. Distemper manifest in usual way. Sacrificed on 11th day. Inclusion bodies in various organs

↓

Monkey 6, one of eight animals in which three successive nasal instillations of a 10 per cent emulsion of monkey spleen No. 5 were given. All developed signs of distemper. No. 6 died on 20th day of the disease. Inclusion bodies numerous

all the routes tried. These, together with the sources of virus, are shown in Table I. Monkey tissues have frequently been tested for virus by inoculating ferrets. A typical example of transmission appears in Table II. Two failures to transmit the disease to ferrets have occurred, once when monkey spleen was used which was taken 12 days after inoculation and once 17 days after inoculation. All earlier transfers have been successful.

The nature of the disease in ferrets has been established by their clinical response and the presence of inclusion bodies in their tissues. These have regularly been found.

The only failures to transmit the disease by monkey tissue preparations occurred after brain and spleen had been stored for 167 days in 50 per cent glycerin. This experience is comparable to that of DeMonbreun (2) who found dog tissues lost their infectivity after 85 days storage under similar conditions.

An emulsion prepared from normal ferret spleen was given subcutaneously and intracerebrally into five monkeys. None developed a febrile response or symptoms of any kind.

Experimental Distemper in Monkeys

By these methods distemper has been transmitted to the 63 monkeys which form the basis of the present report. No appreciable difference in the response of the animals was associated with source of virus or method of inoculation. The group may therefore be described as a whole.

Clinical Course of the Disease

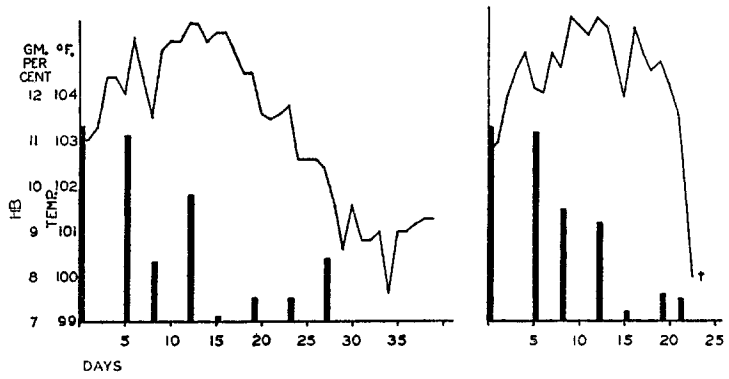
Based on our observations distemper in *rhesus* monkeys could be characterized as an acute, febrile disease having an incubation period of from 2 to 10 (average 5) days followed by 2 weeks of fever which in half the cases is distinctly biphasic and which is accompanied by extreme weakness, diarrhea, emaciation, hemoglobinemia and sometimes, in the early days of the febrile period rhinitis, conjunctivitis and irritability. The febrile period is usually followed by 1 to 3 weeks of subnormal temperatures. The mortality rate has been 9 per cent and the fatal outcome due to either encephalitis (three animals) or pneumonia (one animal).

The febrile response serves as an excellent guide to the course of the disease. Typical curves are shown in Text-fig. 1. The biphasic character of the first of those cases occurred in precisely half of our animals. The descent to normal or nearly normal may be of short duration, however, and our practice has been to measure the body temperature twice each day. This is all the more important

since the maximum temperature for any given day may occur in either the morning or afternoon.

Weakness appeared in one instance on the 5th day. Half the cases were first appreciably weak from the 9th to 11th days and the rest developed this sign during the following week. In most animals weakness became so marked that the monkeys hung limply from the walls of the runway when uncaged and often collapsed on the floor. In their cages they sat hunched and apathetic. Recovery, after the period of subnormal temperatures, usually arrived suddenly, and animals transferred to the open air pen quickly recovered their natural appearance and activity.

Loss of appetite and diarrhea occur during the period of weakness and even a robust animal rapidly wastes away until it resembles a terminal case of tubercu-



TEXT-FIG. 1. Changes in body temperature and blood hemoglobin in two monkeys suffering from canine distemper. The normal temperatures for these particular animals were 102.6° and 103°F.

losis. Loss of appetite is not as frequent as weakness. Often animals refuse to eat everything but fruit. The diarrhea is characterized by thin, yellow stools which the animal passes whenever disturbed.

Before these symptoms occur but by no means as commonly a degree of conjunctivitis may be present. Bright red streaks at the canthi of the eyes are often associated but these have frequently been seen in animals sick of other diseases. In one-fifth of our cases a few small vesicles were seen on the bridge of the nose or the lips and a thin watery secretion from the nares was about as common. Other symptoms which occurred were tremors, ataxia, cyanosis and irritability. Alopecia, described in distemper in dogs, also occurred but did not seem more severe than the confinement alone produced in control animals. The days on which these signs first appeared in a representative group of monkeys are shown in Table III.

TABLE III

The Days on Which Certain Symptoms Were Observed in a Group of 24 Monkeys Inoculated with the Virus of Canine Distemper

	Days																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Day fever occurred		5	3	5	1	5	2	1	2											
Duration of fever							1	1		1	1	3		5	1	3		3		5
Conjunctivitis				1	3		2	4		1			1	2						
Weakness									4	7		2	1	1		2				
Diarrhea								1	2	3		3	3		1					
Loss of appetite					3				4				1							
Nasal discharge			1		2	1	2		1											
Cyanosis					1	1										2	1			
Ataxia (?)									1	2						1				
Tremor									2											
Vesicles										1								1		
Irritability					1			1												
Paraocular swelling					1									1						

TABLE IV

Clinical-Pathological Observations in Three Cases of Canine Distemper in Monkeys

		Day of the disease									
		0	1	3	5	8	12	15	19	23	27
Blood sugar, *mg. per cent*	Case 1	83		84	89	74	83	77	78	94	94
	Case 2	83		74	85	65	100	91	89	84	105
	Case 3	83		89	91	63	105	88	95		
Hemoglobin, *gm. per cent*	Case 1	11.3		11.1	8.3	9.8	7.1	7.5	7.2	7.5	8.4
	Case 2	12.8			12.4	11.1	12.3	9.2	10.6	8.0	10.5
	Case 3	11.3			11.3	9.5	9.2	7.2	7.6	7.5	
Erythrocytes, *millions*	Case 1	5.0				4.7	4.4	4.8	5.0	2.1	3.7
	Case 2	4.4			4.8	4.6	4.9	4.9	5.8	5.2	5.3
	Case 3	4.9			4.0	3.9	4.3	5.1	5.2	3.7	
Leucocytes, *thousands*	Case 1	16.8		36	15.4	22.8	11.4	11.0	11.6	11.8	12.8
	Case 2	12.8	17	9.7	7.0	4.8	9.6	9.2	15.2	11	11.6
	Case 3	9.8	16.8	14.8	12.4	8.6	10.0	11.2	9.6	11.6	
Polynuclears, *per cent*	Case 1	55			45	21	21	29	30	40	29
	Case 2	51		58	12	47	43	43	41	42	37
	Case 3	56			57	40	51	60	56	47	
Lymphocytes, *per cent*	Case 1	41			58	72	77	71	66	55	66
	Case 2	44			42	83	51	55	55	56	55
	Case 3	39			37	49	48	40	37	44	

In view of the observations of Wharton and Wharton (3) that a constant manifestation of canine distemper in dogs is a reduction in erythrocytes, hemoglobin and blood sugar clinical-pathological studies were made in a number of animals. One group of three monkeys was periodically examined and the results of this study are incorporated in Table IV. In a group of twelve normal monkeys we found the average hemoglobin value to be 9.7 gm. per cent and the blood sugar 104 mg. per cent, values with which those in Table IV may be compared. The change in hemoglobin concentration in the peripheral blood has also been included in Text-fig. 1 to show the correlation between the anemia and the fever.

Cerebrospinal fluid pleocytosis has been observed in three monkeys in which repeated cisternal punctures were performed (Table V).

TABLE V

Increase in Number of Leucocytes in Cerebrospinal Fluid, in Distemper in Monkeys Following the Subcutaneous Inoculation of Ferret Spleen Emulsion

Animal	Normal	Number of days following inoculation		
		6	14	16
1	2	Bloody	30	36
2	4	6	10	56
3	7	22	1600	2110

Complications

Encephalitis was the cause of death in three of our animals, death occurring on the 18th, 23rd and 52nd days following inoculation. These animals were first stuporous and weak. They could be aroused only with difficulty, ceased to eat and slowly died after a period of falling temperature. Pneumonia occurred but once as a cause of death. The lungs were massively involved and showed characteristic lesions histologically. The animal had been dyspneic and cyanotic for several days. The same clinical features appeared in a few other animals which ultimately recovered.

Distribution of Virus

No deliberate effort has been made to study the distribution of distemper virus in the monkey. However a number of observations

have been made which indicate the periods in which blood, brain and spleen have been infectious, demonstrated either by passage to ferrets or to other monkeys in which the nature of the disease was later established by the presence of inclusion bodies. This information is summarized in Table VI.

Morbid Anatomy

Histological study of the tissues of animals sacrificed or dying on the 5th, 7th, 9th, 11th, 18th, 19th and 23rd days suggests a sequence of changes in the reticulo-endothelial tissues of considerable intensity. These may well be followed in the spleen. By the 5th day an intense congestion of the splenic sinuses is present which persists to the 19th day. The organ is engorged with blood cells and the sinus endothelium slowly thickens by proliferation about these masses of cells.

TABLE VI

Distribution of Distemper Virus in Infected Monkeys

	Days after inoculation tissues were demonstrated to contain virus										
	6	7	8	9	10	11	12	13	14	23	52
Blood	x	x			x	x					
Spleen						x	x				
Brain										x	x

By the 3rd week the endothelial reaction subsides and the pulp is marked by heavy deposits of hemosiderin.

The germinal centers of the follicles also respond by the 5th day. The earliest change seems to be necrosis of the germinal cells, slowly followed by an active regeneration and growth of the center. The total size of the entire follicle is not conspicuously enlarged, however, the increase in splenic size being due chiefly to the congestion.

Throughout the lymphatic tissue both in germinal centers of follicles and in the sinus endothelium, amorphous, neutral staining material is common which we believe represents necrotic endothelial cells.

The suprarenal cortex also shows changes related to the course of the disease. These consist of an early degeneration of the cortical epithelium, associated with the presence of inclusion bodies and soon

followed by intense subcapsular regeneration. The cells containing inclusions have commonly been in process of disintegration and careful examination is often necessary to determine whether an inclusion body is in the cytoplasm or the faded remains of a nucleus.

The changes in the central nervous system have been observed in so few animals it seems unwarranted to characterize them. The most conspicuous change we have seen has been enlargement and evidently multiplication of the larger glial elements. Perivascular cuffing and round cells in the substance of the brain have not been seen. The changes in the neurons have been limited to early stages of cell deterioration.

The most striking evidence of the disease is the occurrence of inclusion bodies. These have been identified in tissues from all animals in which death occurred before the 20th day following inoculation but not in cases dying later. They have also been present in tissues from ferrets, both animals infected with dog spleen and monkey tissues. In all cases they have been identical in appearance.

The inclusions are usually round or oval, occasionally shaped like an Indian club. They range in size from less than 1 micron to occupy the entire nucleus of an epithelial cell of kidney or suprarenal cortex. Usually homogeneous they sometimes have a darker center and are often surrounded by a halo. They stain brilliantly with acid dyes. They have been most numerous in the kidney and suprarenal and the germinal cells of the splenic follicles but have also been found in the lung, testis, brain and liver. They were numerous in the conjunctival margin of a ferret which had conjunctivitis. Most of the inclusions are intranuclear but in every case some have been seen in the cytoplasm and twice masses of them have been seen within kidney tubules. They are readily stained by Giemsa's mixture in Zenker fixed tissue but show to better advantage when stained by Laidlaw's method (4). In the central nervous system they are most numerous in Ammon's horn. A number of inclusion bodies are shown in Figs. 1 and 2.

The fatal case of pneumonia showed, in addition to the infiltration of the lungs, a serofibrinous pleurisy and pericarditis. The pneumonitis was distinctly interstitial in nature, a feature in harmony with reported observations in dogs.

Lack of Spontaneous Cases

A surprising feature of the present work has been the absence of cases of spontaneous infection. While simple precautions were taken

to prevent this, normal and infected animals were several times confined to the same room. Since all animals have had their temperatures measured twice daily and all were carefully watched throughout the experiments, it is very doubtful that cross infection occurred despite the intranasal administration of virus. This is in striking contrast to the behavior of ferrets and dogs.

DISCUSSION

The disease in monkeys seems distinctly more benign than in other reported hosts. The reported mortality in dogs is higher and in the ferret a fatal outcome is the rule. We have seen no seasonal fluctuation in virulence although the experiments have extended throughout a calendar year. At present we have no evidence that repeated passage in monkeys will increase the virulence of the infection, although the disease is being propagated with this in mind.

The infectiousness of the blood and the profound effects of the disease on the erythrocytes is interesting in light of the statement of Wharton and Wharton that distemper primarily affects these cells. The changes reported by these authors in the total leucocyte count and the lymphocytes closely correspond to our observations in monkeys.

It is not necessary to review at this time the similarity of the lesions and the appearance and distribution of the inclusion bodies to what occurs in dogs.

The evidence that the disease we have described is canine distemper may be summarized as follows:

1. It has regularly been produced by the inoculation of bacteriologically sterile suspensions of spleen taken from dogs sick with distemper.
2. It has likewise been regularly passed from ferrets suffering from distemper and showing the characteristic signs of that disease.
3. It has shown the characteristic biphasic febrile response of distemper in half the cases.
4. It has been passed to monkeys and back to ferrets with the development of distemper.
5. The lesions are undistinguishable from those of distemper.
6. The disease has regularly been associated, during the period of infectivity, with the presence of the inclusion bodies which occur in distemper.

7. The changes in the peripheral blood cells and blood sugar are identical with those described in canine distemper.

8. The cases with fatal issue have died of the fatal complications of canine distemper, encephalitis or pneumonia.

SUMMARY

Canine distemper has been transmitted to *rhesus* monkeys by a variety of methods. The disease is strikingly similar if not identical in its features with distemper in dogs.

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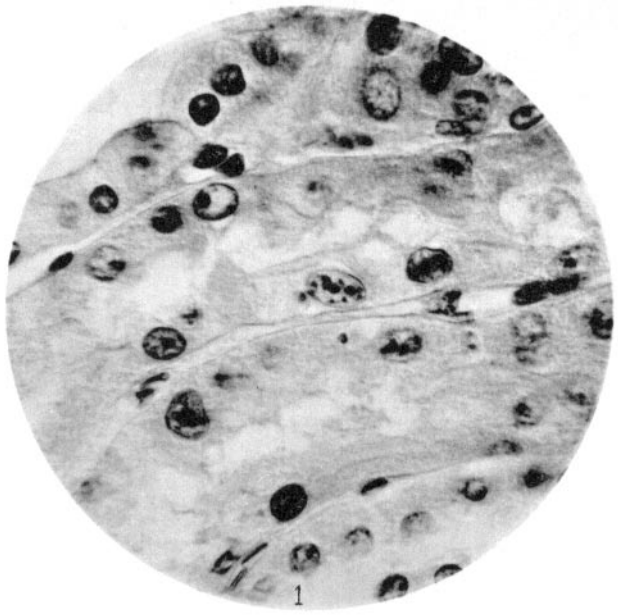
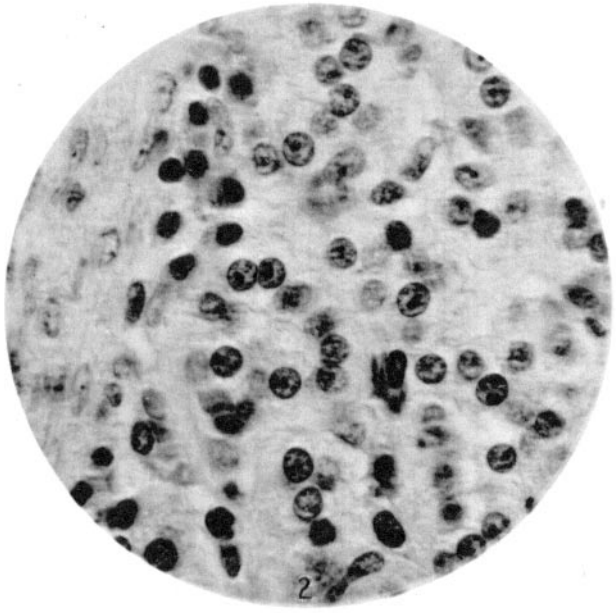
EXPLANATION OF PLATE 12

Typical inclusion bodies in monkeys inoculated with canine distemper.

FIG. 1. Inclusion bodies in kidney epithelium.

FIG. 2. Inclusion bodies in suprarenal cortex.





(Dalldorf *et al.*: Canine distemper in *rhesus* monkey)