potency of phthiocol and of 2-methyl-1,4-naphthoquinone. Data also are given to indicate the approximate maintenance requirement of this animal. Evidence at hand indicates that considerable amounts of vitamin K can be stored, provided the deficient animal is given large doses of vitamin K, or perhaps with smaller doses over a long period of time. The clinical implications of this are pointed out.

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Active and Passive Immunization Against the Virus of Malignant Panleucopenia of Cats.

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A common, acute infectious disease of cats characterized by intranuclear inclusion bodies, profound depression of the number of lymphocytes and granulocytes of the blood and an extensive aplasia of lymphoid tissue and of the bone marrow including erythropoietic elements has recently been described by Hammon and Enders[1, 2, 3] who showed that the etiologic agent was filterable.* The disease is probably identical with that previously reported by Lawrence and Syverton.[4] The experiments recorded here in detail† had as their objectives the determination of whether or not active immunization against subsequent inoculation of the virus could be effected in the natural host by means of formalinized suspensions of organs from infected cats and whether or not the immunity which had been found to result from an attack of panleucopenia could be passively transferred to susceptible animals by means of the blood serum.

Active Immunization. The data obtained in 2 experiments are pre-

1 Hammon, W. D., and Enders, J. F., *J. Exp. Med.*, 1939, **69**, 327.

2 Hammon, W. D., and Enders, J. F., *Ibid.*, 1939, **70**, 563.

3 Enders, J. F., Third International Congress for Microbiology, New York, 1939, Abstracts of Communications.

* Communications which we have received from a number of investigators indicate that the disease is present in Canada, Germany, Russia, and possibly South America.

4 Lawrence, J. S., and Syverton, J. T., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 914.

† Certain of the data included here were briefly described at the meeting of the Fourth International Congress for Microbiology held at New York, September 9-14, 1939.

TABLE I.
Immunization-Experiments with Formalinized Vaccine.

	Vaccinated						Controls				
Exp.	Kitten No.	Dose of vaccine	Mode of test-infection	Leuco-penia	Wt loss	Out-come	Kitten No.	Mode of test-infection	Leuco-penia	Wt loss	Out-come
1	206	2 doses, 2 cc (1)	Intraäbdominal	0	0	S	210	Intraäbdominal	8,300 (5) +	+	S
	207	,,	,,	0	0	S	211	,,	0 (3)	++	S
	208	,,	,,	0	0	S	212	,,	+	+	D
	209	,,	,,	0	0	S	213	,,	5,000 (5) +	+	S
2	236	2 doses, 2 cc (2)	Exposed and intraäbdominal	0	0	S	238	Exposed (4)	+	+	D
	237	,,	,, ,, ,,	0	0	S	239	Exposed and intraäbdominal	450 (5) +	+	S
						0 D					2 D
Total	6			0	0	6 S	6		5	6	4 S

(1) 0.3% formalin added to a 10% suspension of spleen from an infected animal, stored 4 days at room-temperature, then 7 days at 5°C until first used. Given subcutaneously.
(2) 0.3% formalin added to a 10% suspension of spleen and liver from an infected animal, stored 5 days at room-temperature, then 5½ months at 5°C. Given subcutaneously, 1 cc at 2 different sites.
(3) Developed progressive anemia with marked increase in erythrocytic fragility.
(4) This animal became infected through some unknown exposure and became ill, exposing in turn the vaccinated kittens and the other control before their inoculation with virus.
(5) Lowest leucocyte count obtained—counts made daily following inoculation.

sented in Table I. The kittens employed were secured from distant rural areas to avoid encountering a high percentage of naturally immune animals as is found normally in those collected in and near an endemic center such as Boston. The vaccine prepared according to the procedure included in the notations of the table was given in 2 doses at an interval of 7 days. The test dose of virus which consisted of 1 cc of a 10% suspension in infusion-broth, of spleen or lymph-node from animals dying or dead as a result of the specific infection, was administered 7 days following the last injection of vaccine. Normal kittens, which were litter-mates of the vaccinated animals, served as controls. These received an equal quantity of virus-containing suspension. In evaluating these results it should be considered that both vaccinated animals of the second experiment not only were inoculated with the virus but were exposed to kittens actually suffering from the infection.

The results in the vaccinated animals, none of which showed any indication of panleucopenia, while not conclusively demonstrating the value of the immunizing procedure, because of the relatively small number of animals involved—some of which, as we know from previous experience, might have possessed natural resistance—nevertheless strongly suggest that the vaccination did serve to prevent the appearance of any recognized signs of illness. In contrast, 2 of the 6 unvaccinated kittens died, and all exhibited definite indication of disease, although in one a significant leucopenia failed to appear.

Because of the frequency of spontaneous infections during the isolation-period of 2 weeks required for vaccination, we are able at this time to present only these 2 experiments. But something has been learned from many others of the same type which have been attempted. Thus they clearly showed that vaccination following exposure is ineffective, and that exposure soon after the beginning of vaccination results in a typical attack.

We have no knowledge of the duration of the immunity presumably induced by vaccination. Observations, however, on the persistence of the resistant state in animals which have recovered from infection suggest that a permanent immunity could be obtained by the inoculation of active virus following vaccination with formalinized material. A procedure of this sort apparently gives rise to humoral immune factors sufficient to protect kittens passively against a subsequent inoculation of active virus (see 4th experiment in Table II) and would be analogous to that which Laidlaw and Dunkin[5, 6]

[5] Laidlaw, P. P., and Dunkin, G. W., *J. Comp. Path. and Therap.*, 1927, **41**, 1.
[6] Laidlaw, P. P., and Dunkin, G. W., *Ibid.*, 1928, **41**, 209.

found successful in the immunization of dogs and ferrets against distemper.

Passive Immunization. Four separate experiments were carried out with the object of testing the prophylactic properties of convalescent and "hyperimmune" serum. Table II furnishes a summary of the significant facts. Kittens from 2 to 5 months of age were employed, and, in most instances, these were obtained in litters. The littermates were divided as equally as possible between the "serum-protected" and the control groups. This was done to equilibrate in so far as possible the immunologic status of the 2 groups. As in the experiments on active immunization, all kittens were obtained from country districts. Serum was given in one instance 33 days prior to the test-inoculation of virus and in the cases of 4 animals was not given until 3 days after adequate exposure. In addition to exposure this group of 8 kittens was also given virus by mouth at the time the serum was injected. Other kittens exposed at the same time as this group became infected without the additional administration of virus. In other experiments the serum was administered either at the same time as the virus or on the previous day.

The single death due to panleucopenia which occurred among the total 15 "serum-protected" animals was in one of 5 kittens which was not given serum until 3 days after exposure, or about the middle of the incubationary period, which in naturally acquired infections is from 6 to 9 days. One other death occurred in the serum-protected group in an animal suffering at the time of inoculation from a severe upper respiratory infection. At necropsy, including examination of tissue-sections, no evidence of viral infection could be found.

Only 2 of the 15 animals receiving serum developed a leucopenia: the fatal case mentioned above, and one other which had a count on one day of 8,150 leucocytes per cmm. This count falls just outside the lower limit of normal variation expected in this particular animal on the basis of statistical criteria established by Hammon[7] in a study of the physiological fluctuation of counts in a group of 66 kittens. All counts here recorded as leucopenic have been tested by these criteria.

The control kittens, which were equal in number to the serum-protected animals in each experiment, were given the same test-inoculation of virus at the same time, and kept in the same room and not infrequently in the same cage with the serum-protected animals. Eight of the 15 controls succumbed but only in 7 can death be attributed to infection with the virus, for one kitten died following

[7] Hammon, W. D., *Anat. Rec.*, March, 1939.

TABLE II.
Immunization-Experiments with Convalescent and Hyperimmune Serum.

	Serum inoculated							Controls without serum			
Exp.	Kitten No.	Type of serum	Dose, cc	When given	Mode of test infection	Leuco-penia	Out-come	Kitten No.	Mode of test-infection	Leuco-penia	Out-come
1	129	Convalescent (1)	2	33 days before test inoculation	Intraäbdominal	0	S	128	Intraäbdominal	3,900 (8) +	S
		" (1)	"								
	133	Hyperimmune (2)	"	15 " " " "	"	0	S	131	"	+	D
		Convalescent (1)	"								
	134	Hyperimmune (2)	"	15 " " " "	"	0	D (6)	135	"	+	D
2	169	Convalescent (3)	3	3 days after exposure and at same time as virus was given *per os*.	Repeated exposure and *per os*	0	S	171	Repeated exposure and *per os*	6,750 (8) +	S
	170	"	"		"	0	S	173	"	+	D
	172	"	"		"	0	S	174	"	+	D (7)
	175	"	"		"	0	S	176	"	+	D
	177	"	"		"	+	D	178	"	400 (8) +	S
3	200	" (4)	4	1 day before test-inoculation	Intraäbdominal	0	S	203	Intraäbdominal	1,200 (8) +	S
	201	"	"	" " " " "	"	8,150 (8) +	S	204	"	+	D
	202	"	"	" " " " "	"	0	S	205	"	+	D
4	244	Hyperimmune (5)	4	Same time as " "	"	0	S	240	"	6,900 (8) +	S
	245	"	"	" " " " "	"	0	S	241	"	+	D
	246	"	"	" " " " "	"	0	S	242	"	4,550 (8) +	S
	247	"	"	" " " " "	"	0	S	243	"	6,050 (8) +	S
							2 D				8 D
Total	15					2	13 S	15		15	7 S

(1) Details of source uncertain.
(2) 10 months following recovery the cat from which the serum was obtained was reinoculated with virus, found resistant, and bled 17 days later.
(3) Bled 13 days after recovery.
(4) Bled 12 days after recovery.
(5) Bled 12 days after test-inoculation of virus following vaccination with formalinized tissue-suspension (cat 236, Table I).
(6) Had respiratory infection with temperature 103.4°F. when inoculated and died 7 days later with pneumonia. Necropsy showed no evidence of panleucopenia. Leucocyte-count at death was 68,800.
(7) During recovery (11 days after inoculation) pneumonia developed and was cause of death. Leucocyte-count had returned to normal.
(8) Lowest leucocyte-count obtained—counts made daily.

pneumonia several days after the leucocyte-count had become normal. All the unprotected kittens, however, developed a significant leucopenia (6,900 cells per cmm or less).

If the animals dying with pneumonia are eliminated from both protected and control groups, the results, on the basis of death or recovery when evaluated by statistical tests, are significantly different (χ^2 with the Yates correction for small numbers[8] $= 4.37$. P lies between .05 and .02). When tested for significance on the basis of infection as indicated by leucopenia the results become highly significant.

That convalescent serum given shortly before the appearance of symptoms fails to modify the disease is apparent from the following observation. Three kittens were given 4 cc of hyperimmune serum intraäbdominally 1 or 2 days before noticeable illness appeared. The ensuing disease ran a course similar to that occurring among the 3 control animals included in the experiment, although 2 cats died in the latter group and only one among those treated with serum.

Natural Immunity. An observation has been made which strongly suggests that the resistance of a naturally immune female cat is transferred to the offspring. During the early phase of our work in the winter of 1937-1938 we assembled a number of pregnant animals and mothers with suckling kittens, all of which were obtained in the vicinity of Boston, where the disease is endemic. Precautions were then taken to eliminate, in so far as possible, the introduction of infection among this group of about 30 cats. These proved eventually ineffective for after all the pregnant animals had given birth to their litters and at a time when the young ranged in age from 2 weeks to 2 months, one adult female and her 2 kittens died of panleucopenia. Heavy exposure of all other animals to the virus seems to have been assured, since they were loose in a common enclosure, the kittens appeared to suckle indiscriminately at times, and all attempts at further isolation were abandoned. Nevertheless, no other apparent infections occurred during the next few months, nor did the inoculation of any of these animals with organ-suspensions from infected cats lead to overt illness. This immunity of the kittens appears to be most easily accounted for *a priori* by a passive transfer of protective substance across the placenta. The duration of this type of immunity under effective isolation would at best be probably only a few months, but it would seem likely that when frequent exposure to the virus occurs, active immunity might fortify a waning passive

[8] Hill, Bradford A., *Principles of Medical Statistics*, The Lancet, Ltd., London, 1937, p. 93.

immunity. A chain of events of this sort could account for the fact, which we have noted, that relatively few manifest cases of this disease are seen in a community where it is endemic.

Comment. The evidence for the prophylactic action of convalescent or "hyperimmune" serum, provided this is given before or within 3 days after exposure, is, we believe, sufficient to justify its use to confer temporary protection upon cats before or after known contact with the disease. This information should be of value to those who employ cats for experimental purposes.

Should our observations on the immunizing properties of formalinized virus be supported by further experimental trials, the method would appear to offer a practical mode of inducing active immunity which, it is suggested, might be fortified and rendered more permanent by a subsequent inoculation of active virus. The use of the vaccine, however, would seem to be limited to animals which have not been exposed or which do not come in contact with the virus at least during the earlier period of time required for the procedure of vaccination.

Throughout our work we have employed a number of "strains" of virus obtained from animals derived from areas of the northeastern United States. These all produced the same clinical picture and the same pathological changes. Nevertheless, we have borne in mind the possibility that antigenic differences might distinguish strains of virus of different origin. The experiments reported here afford some evidence, however, which points to an antigenic homogeneity. Thus in the second experiment on active immunization and the fourth on passive protection, the viruses used in the production of vaccine or antiserum were obtained from sources other than those from which the viruses used for testing the immunity were secured. Apparently complete cross-protection occurred in both instances.

Summary. Evidence is presented which suggests that the injection of formalinized suspensions of organs from cats infected with the virus of malignant panleucopenia induces resistance in susceptible animals against a subsequent injection of active virus. The serum of cats convalescent from infection or that of animals vaccinated with formalinized material and then inoculated wth living virus, will protect susceptible cats from a subsequent or recent exposure to an animal suffering from the disease or from a subsequent injection of active virus.