rabbits will be reported elsewhere.

Summary. Thin sections of rabbit papillomas reveal pools of dense virus particles in only highly keratinized cells. Viral bodies were readily distinguishable only in papillomas which had been soaked in glycerine-saline for some time. Viral bodies in fresh warts were masked by keratin, melanin and other granules. The bodies are spherical with a non-homogeneous density. They appeared to vary from 25 to 35 mμ in diameter, the most frequent size being 33 mμ. Particles in tissues soaked 1 year or 6 years were identical and equally active in test animals. Sectioned pellets of virus purified by a fluorocarbon process revealed the same particles as those seen in the tissue.

Since submitting this paper we have found the virus in fresh papillomas from cottontail rabbits. It is first seen in the nucleolus, but later fills the entire nucleus and may form crystalline-like arrays.

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Received May 19, 1959. P.S.E.B.M., 1959, v101.

A New Virus of Ducks Interfering with Development of Malaria Parasite (*Plasmodium lophurae*). (25023)

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The avian malaria parasite *Plasmodium lophurae* has been maintained for 14 years in my laboratory by weekly transfer in young ducklings of infected blood. In Feb. 1958 my technician, Mary Chichester, and I noted that in one passage the infections lost their synchronicity(1) and failed to develop with usual severity, and the parasites appeared abnormal. On a subsequent passage parasitemias remained very low but the ducklings died. Since these deaths could not have been a result of malarial infections it appeared likely that some other infective agent had been present in a duckling used for passage of malaria and that this agent had been enhanced in virulence by rapid passage. The following experiments were therefore undertaken to separate this agent from the malaria parasites, to characterize the agent, and to study the effect of introducing the agent into ducks simultaneously infected with *P. lophurae*.

Experimental. 1. *Recovery of P. lophurae free from contaminating agent.* Blood was taken from a duckling showing 17 parasites, many of them abnormal, per 100 red blood cells, and mixed with one-tenth its volume of physiological saline containing heparin at 27 mg/100 ml. When the heparinized blood was inoculated to other ducklings these died in 5 days with parasitemia of 1/100 red cells. When blood cells were separated from plasma by centrifugation, washed with centrifugation 3 times in 0.85% sodium chloride solution, and finally resuspended in saline to original vol-

ume of the blood, inoculation of resulting suspension to a duckling produced 5 days later a parasitemia of 28/100 red cells, with only some of the parasites abnormal. Erythrocytes of this duck were washed in a similar way and inoculated to another which, 4 days later, had 17 parasites/100 red cells, most of them quite normal. Two more transfers in this fashion at intervals of 4 and 3 days respectively completed the process of diluting away the contaminating agent. Thereafter *P. lophurae* was again passed in series by weekly inoculation of infected whole blood appropriately diluted in saline. The parasites appeared normal and developed synchronously during the first 5 days of infection, just as they had done before contamination. The success of this procedure for freeing the parasites of contamination shows that contaminant was not present in either erythrocytes or parasites.

2. *Characteristics of infective agent.* The agent was easily obtained free from malaria parasites by subinoculation of plasma of a duckling infected with both entities. It was soon found that the infection could be transmitted readily by intravenous inoculation of plasma from an infected bird. White Pekin ducks, used throughout the work, were susceptible at all ages tested (3 days to 3½ months). Death generally occurred suddenly within 3 to 5 days after inoculation of as little as 0.1 ml of a 1:10 dilution of infective plasma into a 200 g duckling. With smaller doses which did not kill so quickly, the only signs of illness were weakness, loss of appetite and brown discoloration of bill. A few birds inoculated with very small doses survived the infection and were later immune to a dose of infective plasma which killed previously uninfected controls. For example, one duckling was inoculated with a 1:100 dilution of infective plasma. Eight days later this duckling was thin and had a brown beak, but after another 2 weeks it had recovered. This bird then showed no effect when it was challenged a month after initial inoculation with a dose of infective plasma which killed controls in 5 days. The infection in ducks was accompanied by severe anemia. Thus, two 3-month-old ducks which appeared weak 5 days after inoculation had a red cell volume of only 20% instead of the normal 35%. In younger ducklings anemia was generally even greater. Plasma of high infectivity had a greenish tinge and a characteristic, almost milky, cloudiness when viewed by reflected light. No consistent changes in leukocytes were noted.

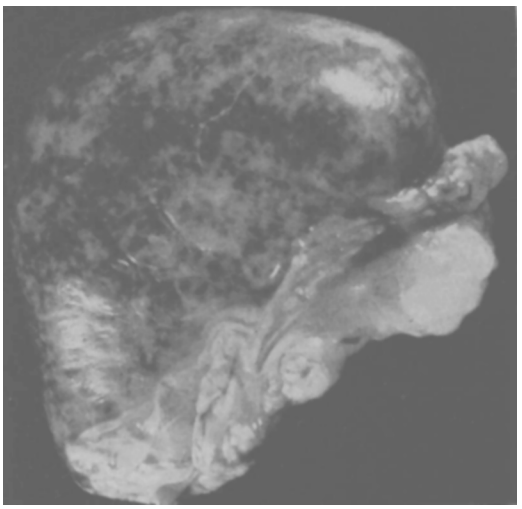

FIG. 1. Spleen from 3-mo-old duck killed 6 days after inoculation with spleen necrosis virus.

At autopsy a constant finding was enlarged spleen more or less full of pale whitish areas (Fig. 1). The liver was often enlarged and reddish, and sometimes showed hemorrhagic areas. Some deaths, but by no means all, were associated with obvious hemorrhage into the peritoneal cavity.

The disease was not contagious. No instance of infection of ducklings by contact occurred although there was ample opportunity for this.

Of four 2-day-old chicks inoculated with duck plasma which killed ducklings in 4 days, one died on 10th day. It had an enlarged liver and a white tumor-like lesion at one side of spleen. Another died on 19th day with no characteristic pathology. The other 2 survived and showed no lesions when killed a month after inoculation. The agent does not seem to infect chicks readily.

Plasma from infected ducks, stored in deep freeze several weeks or in dry-ice box for nearly a year, was still highly infective. Filtration of infective plasma through Selas 03 porcelain filter, known to retain bacteria, did not reduce its infectivity. In view of this fact

TABLE I. Neutralization Tests of Spleen Necrosis Virus.

Infective plasma diluted 1:5 in nutrient broth and incubated ½ hr at 37° with equal vol of:	Days to death* Exp. A	Exp. B
Nutrient broth	13, 11	4, 4
Normal duck serum	12, 11	3, 4
Serum from recovered, immune duck	18, 26	9, 14
Anti-duck hepatitis serum	16, 14	4, 4

* In each experiment, each mixture was inoculated intrav. to two 5-day-old ducklings, 0.1 ml per duckling.

and of failure to obtain any cultivable organism from infective plasma, or to see any micro-organism by light microscopy, the infective agent is almost certainly a filterable virus.

3. *Neutralization of virus by serum from immune duck.* Two experiments (Table I) showed clearly that the virus was neutralized by serum from a duck which had recovered from initial inoculation and had proved immune to challenge with a dose fatal to control birds. Plasma of exceptionally low activity was used for Exp. A and of usual high activity for Exp. B. The virus was not neutralized by normal duck serum or by an anti-duck hepatitis serum obtained through the courtesy of Dr. E. Dougherty, 3rd, of Duck Disease Research Laboratory, Eastport, L. I., N. Y.

4. *Microscopic pathology.* Only the spleen and liver of ducklings near death from the viral infection have been studied. Tissues were fixed in Zenker with 5% formaldehyde and sections stained with hematoxylin-eosin. In the spleen there were obvious large necrotic areas corresponding to the whitish lesions visible grossly. One spleen had a region of still normal basophilic cells with numerous mitotic figures. The spleens were hyperemic as were also livers of diseased birds. In liver as in spleen there was necrosis. In addition, liver sections showed conspicuous cuffing of blood vessels with fairly numerous mitotic figures among these cells.

5. *Effects of virus on Plasmodium lophurae.* In ducklings in which a contaminating infective agent was first suspected, development of malaria parasites was sharply inhibited and many of the parasites were clearly abnormal. Abnormal parasites in blood films stained with Giemsa showed any or all of the following characters: diffuse or fragmented nuclear material; excessive, large, colorless vacuoles; generally pale or diffuse purplish stain; fragmentation of cytoplasm. Sometimes the parasite was completely broken down with only some pigment grains left within the erythrocyte. Some difficulty was encountered in trying to reproduce these effects by simultaneous inoculation of ducklings with the 2 separated agents—*P. lophurae* and the virus. With too large a dose of virus the ducklings would die in 3 days before any effects on malaria parasites were apparent. With too small a dose the malarial infection would reach a peak before striking effects of the virus could be seen. In several experiments, however, appropriate dosage was achieved, resulting in some reduction in numbers of malaria parasites, in appearance of abnormal parasites and in a disturbance of synchronous development. For example, in Exp. C (Table II) the control ducklings on 4th day had the usual highly synchronous infection with nearly all parasites uninucleate trophozoites. In presence of virus the parasitemias were somewhat lower and numerous abnormal parasites were present, but more important, moderate to large numbers of parasites were in the multinucleate stage, which had been passed by controls during the previous night. Similar retardation is shown in Exp. D, in which there was no effect on total parasite count. On 5th day, most parasites in control ducks were large multinucleate forms whereas in ducks which had received the larger dose of virus (No. 15-18) over half of the parasites were still young forms much as they had been on the previous day. A small retarding effect and fewer abnormal parasites were seen in the ducks which received the smaller dose of virus. In both experiments treatment with a mixture of folic acid, pantothenate and coenzyme A appeared partially to overcome the inhibitory effects of the virus, suggesting that the virus was in one way or another interfering with the nutrition of malaria parasites.

Discussion. The new transmissible disease of ducks here described may be designated spleen necrosis of ducks. It is characterized especially by its effect on the spleen, which becomes greatly enlarged and more or less

TABLE II. Retardation in Development of *P. lophurae* in 22 Ducks Simultaneously Infected with Spleen Necrosis Virus.

Exp.*	Virus, ml†	Parasites per 100 red cells	% parasites Uni-nucleate	% parasites Abnormal (all stages)
C	0	240	95	0
	0	540	90	0
	.05	87	61	23
	.05	140	19	35
	‡ .05	290	67	2
	.05	90	14	21
D	0	770	25	0
	0	110	7	0
	0	730	28	0
	0	425	26	0
	.025	610	26	4
	.025	540	31	6
	.025	660	30	2
	.025	480	28	10
	.05	700	50	4
	.05	570	69	7
	.05	540	85	13
	.05	650	54	11
	‡ .05	450	45	6
	.05	540	44	7
	.05	640	49	7
	.05	710	45	3

* Parasite counts are given, in Exp. C for 4th day after inoculation, and in Exp. D for 5th day.

† Virus inoculum was a 1:10 dilution in broth of infective duck plasma, given at indicated dosage/100 g body wt.

‡ Ducks 5 and 6 and 19-22 each received intrav. on each of days 1-4 after inoculation 1 ml of a solution containing, in mg/100 ml, folic acid 1.7, calcium pantothenate 70, liver coenzyme A concentrate 30 (15 units CoA/mg).

replaced by pale necrotic tissue. No disease of ducks with similar pathology has been previously reported. Duck viral hepatitis(2) has no marked effect on the spleen, although the enlarged liver with hemorrhagic areas characteristic of hepatitis is also often seen in spleen necrosis. The 2 entities may be further distinguished by failure of duck anti-hepatitis serum to neutralize the spleen necrosis virus. Infectious serositis of ducks, possibly caused by psittacosis-like organism(3), produces an enlarged, pale and mottled spleen, but there is also marked fibrinous inflammation of serous membranes and yellowish exudate over the liver, changes not seen in the new disease. Moreover, infectious serositis, like fowl cholera and duck plague, is highly contagious, whereas the spleen necrosis appeared not at all contagious.

The origin of the infection and its mode of transmission in nature are unknown. One might suppose that it is present in some ducks as a latent, egg-transmitted virus and that pathogenicity appeared as a result of rapid transfer at weekly intervals in connection with maintenance of strain of *P. lophurae*.

Effects of viral infections on the course of experimental malaria have been noted occasionally before. Jacobs(4) found that ducklings infected with ornithosis virus and inoculated 10-12 days later with *P. lophurae* mostly survived the latter infection. He attributed the effect to hyperplasia of the reticulo-endothelial system brought about by previous viral infection. It will be noted that the time relations here were quite different from those obtaining in the present study where both virus and parasites were inoculated simultaneously. Huff (personal communication) observed that malaria parasites in canaries infected with fowl pox became vacuolated and did not develop normally. In a more extensive series of observations, Yoeli *et al.*(5) found that West Nile virus interfered with development of *P. berghei* in mice. The parasites showed abnormalities like those of *P. lophurae* in ducks with spleen necrosis.

In no case has the mechanism of these effects been worked out. It may be that the virus prevents the parasite from getting some essential nutrient. Ability of a folic acid-coenzyme A supplement to counteract somewhat the effect of spleen necrosis virus on *P. lophurae* would be in line with such a hypothesis.

Summary. In the course of passage of *P. lophurae* in ducklings by weekly transfer of infected blood, a contaminating infective agent appeared which inhibited malarial infection and caused rapid death of ducks. The agent was present in plasma of infected birds and was readily separated from malaria parasites. It was a filterable virus, producing, in ducks inoculated with appropriate doses, a rapidly fatal disease characterized especially by enlargement and necrosis of the spleen. This spleen necrosis virus was neutralized by serum from a recovered immune duck but not by antiserum to duck hepatitis virus. In ducklings simultaneously inoculated with *P. lophurae* and a suitable dose of spleen necrosis

virus, development of malaria parasites was retarded and many of them appeared abnormal.

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Received May 19, 1959. P.S.E.B.M., 1959, v101.

Effect of Gastric Emptying Upon Propulsive Motility of Small Intestine of Rats. (25024)

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The influence of antral peristaltic activity upon motility of small intestine has been considered by Alvarez(1) and others before him. Existence of a feeding response alerting the entire intestine has been observed repeatedly in dogs with exteriorized loops(2), but this does not necessarily involve gastric motility for it has been observed in the sham fed(3). Obviously gastric motility is necessary for introduction of material before the intestine can propel it. However, we wished to determine whether the continued propulsion of the material is influenced by the activity of the stomach in emptying.

Material and methods. We further modified Macht's technic so that amount of intubated mixture in both stomach and intestine might be quantitated at end of experimental period. The mixture consisted of 1 g methylcellulose, 200 mg trypan blue, 20 g powdered tetrafluorethylene resin (Teflon) and 200 ml H_2O. The trypan blue was used as a marker, while Teflon was chosen because it is practically indestructible. Up to 3 ml of this mixture was given by gastric intubation in 38 unanesthetized male adult albino (Wistar) rats weighing 210 to 410 g and fasted overnight. After killing the rats at end of 20 min and determining the distance traversed by the blue mixture, the stomach and small intestine were digested separately in nitrosyl sulfuric acid upon a steam bath. The Teflon remaining was collected upon sintered gooch crucibles, and after washing with chloroform to remove traces of fat, was dried to constant weight. In 12 similar rats killed immediately after intubation, it was found that 10% of the Teflon had left the stomach at the moment of intubation and traversed 6% of the small intestine.

Results. From the amount of Teflon recovered from the intestine and total amount intubated (Teflon recovered from stomach plus that from intestine) the per cent gastric emptying was calculated. When this value for each rat was plotted against per cent of small intestine traversed the scatter diagram of Fig. 1 resulted. (For conservation of space, 6 extreme points to the left and above have been omitted from the Figure but not from the calculations.) Also plotted on the

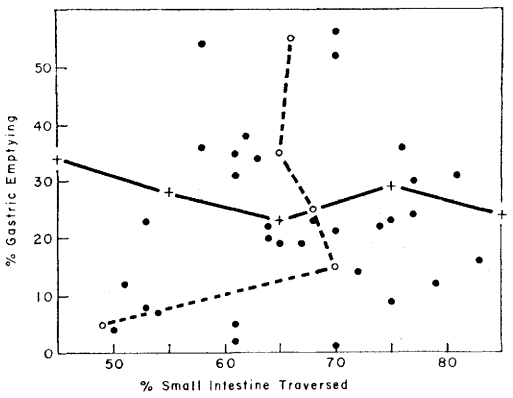

FIG. 1. Simultaneous gastric emptying and proportion of intestine traversed; each filled circle represents a rat. Also shown: empirical regression lines, % emptying on % traversed (solid); % traversed on % emptying (dashed).