TABLE I.
Infectivity of *H. pertussis* via the Yolk Sac in the Chick Embryo.

Inoculum				
Source	Dilution	No. of viable *H. pertussis*	Age of eggs when infected (days)	Results*
B-G culture	10^{-7}	144	7	D6, D7, D8
			11	D7, D9, 0
	10^{-8}	14	7	D6, D8, 0
			11	D6, D7, 0
	10^{-9}	1.4	7	D8, 0, 0
			11	D8, 0, 0
Infected yolk	10^{-8}	15	8	D5, D8, D9, D9
	$1/3x10^{-8}$	5	8	D6, D7, D8, D8, D9
	$1/9x10^{-8}$	1.7	8	D5, D6, D6, S10, 0
Infected allantoic fluid	10^{-9}	2	8	D8, D9, D9
	$1/3x10^{-9}$	0.7	8	0
			12	0, 0

* Results:
D_6 signifies death after 6 days' infection with many *H. pertussis* in yolk.
0 signifies no evidence of infection.
S_{10} signifies survival for 10 days, with positive culture from yolk.

in cultures from the heart's blood and amniotic fluid; none were found in the allantoic fluid. The heavily-infected yolk of embryos succumbing to infection was diluted in broth and titrated by the same route in other embryos. As few as 5 organisms deriving from the yolk were found to be capable of initiating a fatal infection. Table I summarizes experiments which exemplify these results.

H. pertussis from cultures on Bordet-Gengou medium or from heavily-infected yolk were also introduced via the allantoic cavity of 10- or 11-day embryos. A considerably larger number of organisms, usually at least several thousand, was necessary for the initiation of a fatal infection and deaths were infrequent. In those embryos succumbing, however, many organisms were seen in smears of the allantoic fluid and cultures revealed them to be present also in the heart's blood and tracheal fluid. *H. pertussis* in the allantoic fluids proved to be of high virulence when tested by the yolk-sac route (Table I).

Conclusion. The chick embryo is highly susceptible to *H. pertussis* when infected via the yolk-sac. The results of inoculation via the allantoic cavity are much less satisfactory. The chick embryo would seem to promise considerable utility in the quantitative experimental evaluation of antibody preparations and chemotherapeutic compounds versus *H. pertussis*.

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Prothrombin Level and Effect of Vitamin K Substitutes in Thrombocytopenic Purpura in Rats.*

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The administration of antiplatelet serum to animals produces purpura, a prolonged bleeding time and a low capillary resistance. These abnormalities are generally attributed

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to the low level of blood platelets and a vascular defect.[1] An additional etiological factor has been suggested by the recent results of Fleck and Lille,[2] who reported that the blood prothrombin level is reduced in rats and guinea pigs which had been given antiplatelet serum. According to these workers, the "symptoms" of experimental purpura can be almost completely inhibited by the prophylactic administration of the vitamin K substitute 2-me-1,4-naphthohydroquinone acetate. Their abstract does not indicate whether or not the vitamin prevents the fall in the prothrombin level or platelet count. Others in their laboratory have reported[3] that in human essential thrombocytopenia (Werlhof's disease), in which the prothrombin time is unchanged, the vitamin K substitute "stops the hemorrhagic symptoms and restores the bleeding time to normal" without raising the platelet count. Since these results, if correct, would necessitate a radical revision of our concepts of the action of vitamin K and of the pathological physiology of thrombocytopenic purpura, it seemed important to test their validity. Consequently, the experiments of Fleck and Lille on experimental purpura were repeated.

Methods. Purpura was produced in rats by the subcutaneous injection of antiplatelet serum. The serum was prepared by the intravenous injection of a rabbit with a saline suspension of the washed blood platelets of 10 or 11 heparinized rats. Four injections were given at approximately weekly intervals. Five days after the last injection, the rabbit was bled and the serum was preserved in a frozen state until within 10 days of its use.

Four pairs of white rats (Sherman strain, 210 to 330 g) were used in each of 3 experiments. Each pair consisted of a male and a female. Pair 1 received no injections, pair 2 received vitamin K, pair 3 was given vitamin K and antiplatelet serum, and pair 4 received only antiplatelet serum. The platelet count, prothrombin time and capillary resistance were determined. Blood for the platelet count was obtained by cutting the tail, and was diluted with 3.8% sodium citrate. Two methods were used to measure the prothrombin time. It was usually determined by Quick's micromethod,[4] in which about 10 mm^3 of whole blood was taken from the tail and added to an equal amount of thromboplastin solution.† At the end of each experiment, the prothrombin time was determined on oxalated plasma[5] which was obtained from cardiac blood samples (nembutal anesthesia). The capillary resistance was measured on the abdominal skin by Dalldorf's method.[6]

The 3 experiments differed in the dosages of antiplatelet serum and vitamin K, and in the vitamin preparation employed. Control determinations were made on all rats on the first day. In the first experiment, pairs 2 and 3 were given 10 mg/kg of 2-me-1,4-naphthoquinone bisulfite‡ intramuscularly on the first day. Antiplatelet serum (0.75 cc/kg) was administered subcutaneously to pairs 3 and 4. Determinations were repeated on the second and third days. On the fourth day, the rats were autopsied after taking cardiac blood samples. In the second experiment, 5 mg/kg of 2-me-1,4-naphthohydroquinone diphosphoric ester tetra sodium salt§ were injected into pairs 2 and 3 on the first day. The injections were repeated twice daily on the second and third days. Pairs 3 and 4 received 0.96 cc/kg of antiplatelet serum on the second day. Measurements were repeated on the third and fourth days. In the third experiment, 40 mg/kg of 2-me-1,4-naphthoquinone bisulfite‖ was administered to pairs 2 and 3 on the first, second and third days. Since the volume of fluid injected was large, the remaining rats received 10 cc/kg of isotonic saline as a control measure. Anti-

[1] Elliott, R. H. E., Jr., and Whipple, M. A., *J. Lab. and Clin. Med.*, 1940, **26**, 489.

[2] Fleck, L., and Lille, F., *Am. Rev. Soviet Med.*, 1945, **3**, 174.

[3] Groër, F., Baranowski, T., and Rosenbusch, J., *Am. Rev. Soviet Med.*, 1945, **3**, 173.

[4] Quick, A. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 788.

† Bacto thromboplastin from rabbit brain, Difco Laboratories.

[5] Quick, A. J., *Am. J. Clin. Path.*, 1940, **10**, 222.

[6] Dalldorf, G., *J. Exp. Med.*, 1931, **53**, 289.

‡ 2.5 cc/kg Hykinone, Abbott Laboratories.

§ 1 cc/kg Synkayvite, Hoffmann-La Roche, Inc.

‖ 10 cc/kg Hykinone.

TABLE I.
Effect of the Administration of Antiplatelet Serum to Rats 3 and 4, and of Vitamin K to Rats 2 and 3.

		1st day			2nd day			3rd day			4th day
Exp.	No., sex	Platelet count, per mm³ × 1000	Prothr. time, sec.	Cap. res.,* cm Hg.	Platelet count, per mm³ × 1000	Prothr. time, sec.	Cap. res.,* cm Hg.	Platelet count per mm³ × 1000	Prothr. time, sec	Cap. res., cm Hg	Plasma prothr. time, sec
1	1 ♂	600	40	30	866	49	20	940	45	19	20.5
	1 ♀	914	41	26	898	44	30	722	46.5	>25	19.4
	2 ♂	808	45	30	1218	51	20	766	40	25	19.9
	2 ♀	682	46	20	958	49	25	1042	39	24	23.4
	3 ♂	976	44	30	294	49	10	74	42	5	23.6
	3 ♀	777	36	28	176	37	15	16	40	10	22.2
	4 ♂	856	40	>30	350	36	20	20	34.5	15	21.4
	4 ♀	584	43	24	194	48	10	46	34.5	10	19.5
					3rd day			4th day			
2	1 ♂	640	30	17	874	36	26	818	39	>25	21.4
	1 ♀	642	30	>25	606	36	>26	820	33	20	20.8
	2 ♂	616	35	15	868	36	>26	834	39	>25	20.3
	2 ♀	812	37	18	818	37	27	770	38	>25	20.1
	3 ♂	928	30	18	254	35	20*	150	40	10	19.4
	3 ♀	681	31	>23	62	35	>27*	—	—	—	—†
	4 ♂	910	30	22	220	32.5	>26*	78	30	20	20.2
	4 ♀	724	35	>24	72	34	>24*	214	35	15	19.8
3	1 ♂	1346	42	35	662	39	25	480	—	10	24.6
	1 ♀	1202	35	15	1130	40	25	1190	—	15	21.6
	2 ♂	1156	40	30	224	40	25	700	—	>26	21.0
	2 ♀	934	36	15	1136	42	35	1012	—	27	21.0
	3 ♂	612	32	35	210	—	30	64	—	20	22.0
	3 ♀	802	40	35	136	32	20	168	—	10	21.0
	4 ♂	880	32	30	64	44	10	164	—	>25	20.4
	4 ♀	942	40	25	56	38	20	140	—	15	19.5

* These high values can probably be attributed to cutaneous vasoconstriction following excessive bleeding from the tail. The following determinations of capillary resistance, except those done on the first day of experiment 3, were therefore made before the animals were bled.

† This rat died during the night, probably as a result of blood loss.

platelet serum (0.75 cc/kg) was given to pairs 3 and 4 on the second day, and determinations were made on the third and fourth days.

Results and discussion. All of the rats which received antiplatelet serum (pairs 3 and 4) developed low platelet counts, and most of them showed a diminished capillary resistance (Table I). These changes were as great in the rats which received vitamin K (pair 3) as in the untreated rats (pair 4). Autopsies failed to reveal any difference in the degree of purpura in the skin and internal organs of the 2 groups. The purpuric rats usually bled profusely from the tail incisions whether or not the animals had been given vitamin K. The prothrombin time was unchanged in all experiments. Large doses of vitamin K lower the prothrombin time when diluted plasma is used in this test.[7] Our failure to observe a similar reduction may be attributed to the use of undiluted plasma, a less sensitive indicator of changes in prothrombin level.

Since Fleck and Lille gave no specific data concerning their criteria of therapy or the dosage schedule of vitamin K, an exact duplication of their work could not be carried out.¶ In the experiments reported here, very large doses of 2 vitamin K substitutes were used, and these were administered both before and after the injection of antiplatelet serum. The

[7] Field, J. B., and Link, K. P., *J. Biol. Chem.*, 1944, **156**, 739.

¶ Abstracts, written in English, were sent to the *American Review of Soviet Medicine* by the authors in Lvov. A more detailed account of the work is not available.

vitamin K substitutes which we employed were not identical with that used by the Polish workers. Since their compound and the 2 used in this study possess marked vitamin K activity,[8,9] it is most unlikely that our failure to confirm their work can be attributed to this difference. We can offer no explanation for the divergent results.

Summary. Contrary to the results of Fleck and Lille, the prothrombin level is not reduced after the administration of antiplatelet serum to rats, nor are the manifestations of experimental thrombocytopenic purpura modified by large doses of vitamin K substitutes.

[8] Dam, H., *Advances in Enzymology*, 1941, **2**, 285.

[9] Smith, J. J., Ivy, A. C., and Foster, R. H. K., *J. Lab. and Clin. Med.*, 1943, **28**, 1667.

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Control of Salmonella Infections in Mice by Streptomycin.

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Preliminary observations on the use of streptomycin to control paratyphoid infections in mice indicated that this antibiotic given orally may be an effective means of eliminating Salmonella infections in colonies of mice and other laboratory animals for considerable periods of time. This paper reports the results of work* started in November 1945 when it was found that Salmonella organisms were responsible for both decreased production of mice needed for the Japanese B encephalitis vaccine program and also were the cause of contamination of the final vaccinal product.

A commercial mouse colony which was supplying mice for the Japanese B program was selected for streptomycin feeding since mouse brain vaccine prepared from these mice contained Salmonella organisms. Individual fecal samples were collected from the 1400 breeders in the colony, and streaked on brilliant green agar. Of this number, 30 adult mice in 6 breeding units yielded positive cultures (*Salmonella enteritidis*). Streptomycin† was incorporated in the drinking water of the entire colony for 7 consecutive days so that the daily intake per mouse was approximately 100 units. Two weeks later 100 young mice were used from the same 6 Salmonella positive units for vaccine production. No paratyphoid organisms were found in the vaccine produced from this group. The 30 positive adult mice and 24 of their offspring were tested 4 weeks after the streptomycin was fed and at monthly intervals thereafter. Only one adult mouse yielded a positive fecal culture in the first test, and all 4 subsequent tests on both young F_1 and old mice were negative for the presence of paratyphoid organisms.

As a further development of these findings experiments are now in progress to study the effect of 25, 50, 100, 200, 400 and 1000 units of streptomycin daily on the spread of *S. enteritidis* infection in groups of mice consisting of infected and non-infected individuals. The results to date with one strain of *S. enteritidis* are encouraging but incomplete. In addition, mice have been injected with *S. enteritidis* and simultaneously with *S. typhi-murium* cultures isolated from mice of 20 other commercial colonies. In mice receiving the combined injection it was found that *S. enteritidis* was more susceptible to streptomycin than *S. typhi-murium*.

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