reported here appears identical in its properties to "product 2" of Bergsagel and Hougie (2). Our studies and those of Streuli(3) indicate that the only necessary component provided by platelets is their phosphatide. Since the thromboplastic material is probably a phosphatide-protein complex, and because of its sedimentation properties, it has characteristics resembling those of tissue thromboplastin prepared by Chargaff(9). It differs from the latter in its ability to convert prothrombin to thrombin in the absence of factor V or Stuart factor, and in its dissociation in the absence of calcium ion.

Our studies support the concept of blood thromboplastin formation as a chain reaction, since there did not appear to be haphazard complexing of clotting components to the phosphatide emulsion. This was demonstrated by failure of new activity to form when the cephalin reagent was incubated with either plasma or serum reagents separately. The data do not contribute to the question as to which clotting factors are complexed or whether any act in enzymatic manner. However, the present technics may provide a means by which such problems may be more effectively handled. As purified clotting factors become available, it would be of considerable interest to determine which are sedimented with appropriate clotting phosphatide.

Summary. A reagent with activity resembling that of blood thromboplastin was sedimented on crude cephalin. Activity was lost if washing of sediment was undertaken in absence of calcium ion. The thromboplastic reagent normally clotted plasmas deficient in Stuart factor or factor V; activity was irreversibly destroyed following incubation with serum. Certain properties similar to those of tissue thromboplastin are indicated.

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Action of Rabies Vaccine Derived from Embryonated Duck Eggs Against Street Virus. (25101)

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Two alternatives appeared possible in attempts toward decreasing the chances of encephalomyelitis following use of rabies vaccine derived from rabbit brain or other species of brain. These are: (1) chemical or physical treatment of the vaccine to remove as far as possible factors contributing to this type of side reaction, and (2) preparation of vaccine from virus infected tissue initially devoid of such factors. We experimented mainly along the latter line since some early results indicated fixed rabies virus could be grown reasonably well in embryonated duck eggs(1). Such duck embryo (DE) killed rabies vaccine suffices as a useful agent for treatment of persons bitten by animals or otherwise exposed to rabies street virus, and degree of antibody response and minimal extent of side reactions in such human patients have been reported(2). Although DE rabies vaccine fulfills requirements of potency and safety for human usage, we have thus far reported only

on its action against fixed virus. We have unpublished evidence concerning 90 dogs immunized with the vaccine and challenged intramuscularly at different times with street virus in experiments designed for veterinary use, and the results are good for the purpose intended. We here present results of further successful immunization experiments in which we used street virus challenge by intracerebral route according to Semple's(3) original monograph now nearly forgotten. His chapter V is particularly informative to this day. We followed very closely his original technic in tests of phenolyzed or "Semple vaccine."

Materials and methods. Five to 6 lb. normal rabbits were used. DE rabies vaccine of a lot exactly one year of age (in ice box storage) was used. This lot had passed all pertinent tests including potency maintenance after 30 days heating at 37°C. Street virus, as a first mouse passage from a fatal human case, was supplied by Dr. Edwin H. Lennette of Calif. State Dept. of Health Laboratories. Following their de-coding several left-over sera obtained from persons treated with DE and Semple rabies vaccines respectively were on hand following an antibody study of coded serum samples supplied by Dr. Morris Greenberg of N. Y. City Dept. of Health. These sera were used in serum + virus neutralization tests in standard white mice obtained from local breeders. The CVS strain of fixed virus was obtained from our laboratories, while Phillips and Park strains of fixed virus were obtained from our biological production laboratories. *Immunization with vaccine and challenge with street virus.* In following Semple's original procedure, we gave 8 rabbits each a subcutaneous dose of 1.5 ml of diluted 2% vaccine daily for 24 days. DE vaccine is 10% virus tissue when normally rehydrated, therefore it was diluted 1:5 further before use in rabbits. A week after last dose of vaccine, a small bleeding was taken from the rabbits and the resultant sera subjected to standard serum + virus neutralization test in mice. Then the rabbits, divided into 4 pairs, were injected intracerebrally with 0.25 ml street virus diluted 1:200, 1:400, 1:800, and 1:1600 respectively. A pair of normal control rabbits received each of these 4 dilutions of virus, also 2 additional pairs of normal controls received 2 higher dilutions of street virus, namely 1:3200 and 1:6400. *Serum + virus neutralization tests.* Sera of immunized rabbits and human patients' sera were tested for virus neutralizing antibody by standard methods using doubling dilutions of inactivated serum and virus so diluted that mice injected with the mixtures received 100 LD_{50} of virus/mouse. Groups of 6 mice were used/serum dilution, and 4 additional groups of 10 mice each were given amounts of virus computed to be 100; 10; 1.0; and 0.1 LD_{50} of live virus as controls. Rabbit sera were tested against CVS virus alone, while human sera were tested against CVS, Phillips, and Park fixed viruses, and also against street virus. Titers were computed by the method of Reed and Muench(4).

Results of street virus challenge. Table I shows results of successful immunization of rabbits with DE rabies vaccine in terms of resistance to intracerebral challenge with street virus. Semple found subdural inoculation of living (street) rabies virus a very severe test of immunity. He used 4 monkeys, 2 dogs, and 2 rabbits immunized with freshly made phenolyzed vaccine. All 4 monkeys, one of 2 dogs, and one of 2 rabbits survived subdural challenge. Rabbits immunized with DE vaccine resist several fatal doses, (up to about 32) of street virus, by more acute intracerebral challenge (Table I). It is of added interest that this vaccine was 1 year old when used. This action parallels that of freshly prepared Semple vaccine, and total dose of vaccine used/rabbit was the same as that used by Semple. It may be mentioned that DE vaccine is first DE passage fixed virus.

Results of neutralization tests. Table II shows results of virus neutralization tests of human sera following treatment with DE and Semple vaccines respectively. In addition to customary use of CVS fixed virus, we used 2 other strains of fixed virus, and also street virus. It appears there is little difference in the heterologous virus neutralizing action of serum following use of DE or Semple vaccine. The only differences noted are the strain to strain differences which both sera show simultaneously.

TABLE I. Rabbits Immunized with DE Rabies Vaccine and Challenged Intracerebrally with Street Virus.

Intracer. challenge dose of 0.25 ml of street virus diluted:	Rabbits immunized with DE rabies vaccine: Antibody titer	Result	Normal rabbits: Result	
1:200	85	Rabies, day 17	Rabies, day	15
	48	S	*Idem*	15
1:400	25	S	"	17
	35	S	"	15
1:800	14	Rabies, day 19	"	15
	26	S	"	15
1:1600	6	S	"	12
	27	S	"	13
1:3200	Not done		"	15
			"	15
1:6400	*Idem*		"	15
			S	

Street virus used was mouse brain passage from a fatal human case. Antibody titers are reciprocals of dilutions of serum computed to save half the mice from 100 LD_{50} of CVS virus/mouse.
S = surviving and normal at 30 days.

Discussion. Our DE vaccine had a "potency" (times better than N.I.H. standard vaccine No. 159A in mouse tests) of 1.27 when first made. Potency of a sample after heating 30 days at 37°C was 1.54, which indicates no decrease had taken place, and these figures are representative of the product (2). Degree of activity of this vaccine in rabbits is sufficient to cause these animals to resist from one to several fatal doses of street virus injected intracerebrally. This showing in light of Semple's results and those of many subsequent investigators using intracerebral street virus challenge is proof of direct capacity as an antirabies prophylactic. Human sera following use of DE and Semple vaccines respectively neutralize street virus as well as other fixed viruses in mouse tests. Titers of both kinds of sera, especially against street virus, are lower than when CVS fixed virus is used, however this would be anticipated by nearly all workers with these viruses. Such differences appear to be due to differences in sensitivity of virus to antibody.

TABLE II. Antibody Titers of Human Sera Measured against Various Rabies Viruses.

Serum following use of DE and Semple vaccine		Fixed virus strains: CVS	Phillips	Park	Street virus
(DE)	1-31-59	1010			128
"	1-14	1350	730	560	
"	1-29	1600	790	670	
(Semple)	3-26	1650		512	142
"	1-26	2450	850		

The above sera were of sufficient volume to allow only the number of tests shown. Antibody titers are expressed as in Table I.

Summary. Treatment of rabbits with DE rabies vaccine causes them to withstand otherwise fatal doses of street virus administered intracerebrally. These results with 1 year old DE vaccine parallel Semple's original experience with freshy prepared phenolyzed vaccine. Furthermore, human serum following treatment with these vaccines neutralizes different fixed viruses and also street virus as tested in white mice.

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Metabolism of Folic Acid in Folic Acid and Biotin Deficient Rat. (25102)

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In the course of a vitamin balance study with germ-free rats fed purified diets, Luckey *et al.*(1) observed that biotin administration to a rat reared on diet free of biotin and folic acid (pteroylglutamic acid, PGA) caused a 50-fold increase in PGA excretion over that