pear simultaneously. They emerge one or two at a time as in the human, their period of eruption extends over 2 to 7 months.

The dental records of a series of 38 normal and 16 experimental males born in the colony have not been analyzed in full, as yet, but on the basis of preliminary surveys of the material, certain striking differences are apparent between the ages at tooth emergence in normal animals and those receiving injections of testosterone propionate.

The dentition records of 3 experimental animals in which early bone union of the skeleton and sexual precocity were induced(2) has been compared with similar data relating to the dentition of 10 normal males. The treatment with androgen began in Mm 22 at 6 months and 21 days of age; Mm 18 at 7 months, 6 days and Mm 43 at 1 year, 10 months and 2 days. Mm 22 and Mm 18 received 7.5 mg testosterone propionate‡ per kilo body-weight per week and Mm 43 received 22.5 mg per kilo. In these 3 monkeys an acceleration of growth and differentiation resulted in skeletal maturation at 2 years, 10 months instead of around 7 years, the age at which bone union is completed in the normal male.

Conclusions. A study of Table I reveals that in no normal male were the 4 permanent canines recorded as having emerged more or less simultaneously. The average age of the injected animals at time of canine emergence is seen to be about a year earlier than that of the controls. In fact, the earliest permanent canines in the latter group did not appear as early as the latest ones in the experimental group. It is thus evident that when androgen is employed as a growth-stimulating agent, the development with emergence of the canine teeth is also accelerated.

TABLE I.
Data on Permanent Canine Tooth Emergence for 10 Normal Male Monkeys and 3 Males Injected with Testosterone Propionate.

Animal No.	Age at emergence of earliest permanent canine yr.	mo.	day	Age at emergence of the fourth permanent canine yr.	mo.	day
A. Control Monkeys (Males):						
25	3	11	0	4	3	1
82	3	6	29	3	10	28
86	4	1	20	4	4	28
40	4	1	24	4	8	22
49	4	2	15	4	8	7
77	3	10	11	4	2	9
83	4	0	20	4	3	19
84	3	9	18	4	1	15
53	4	3	10	4	4	14
56	4	2	22	4	7	23
Age avg	4 yr.	0 mo.	7 days	4 yr.	4 mo.	11 days
B. Monkeys injected with testosterone propionate (Males):						
18	3	3	28	3	3	28
22	3	2	1	3	2	1
43	3	2	3	(2 lower canines present; autopsied)		
Avg age	3 yr.	2 mo.	21 days			

Received January 13, 1950. P.S.E.B.M., 1950, v73.

2. van Wagenen, G., *Fed. Proc.*, 1947, v6, 219.

‡ Oreton was supplied by the Schering Corporation through the interest of Dr. Edward Henderson.

A Test for the Antigenicity of Rabies Vaccine. (17661)

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It is our purpose to report here a test for the immunizing ability of rabies vaccine that is more logical and less time-consuming than the official Habel test(1). It consists of one intraperitoneal injection of a vaccine into guinea pigs, followed 3 weeks later by the

1. Habel, K., *U. S. Pub. Health Rep.*, 1940, v55, 1473.

intramuscular inoculation of street virus. The minimum amount of vaccine that will protect against a given dose of street virus is determined.

Materials and methods. The guinea pigs come from a closed stock that has been maintained in this laboratory for 30 years and is practically free from disease. In an experiment using 50 or more guinea pigs no deaths from an intercurrent infection usually occur. These guinea pigs are fed a good quality of hay, oats, and cabbage during the winter, and oats and fresh alfalfa during the warmer months. Animals weighing from 250-350 g are used and the controls are kept with those receiving vaccine. In early experiments 5 guinea pigs were injected with each dilution of vaccine but at the present time 6 or more are used.

The vaccines to be tested are diluted in buffered salt solution and 1 cc is injected intraperitoneally into each animal. The dilutions commonly used are 1:50 and 1:250 of the 10% brain suspension.

The virus used for testing immunity was the brain of a dog developing rabies in Atlanta, Ga., received from Dr. Harald Johnson in 1943. A 20% suspension of this brain and also material from the first intracerebral passage through guinea pigs have been kept in sealed ampules frozen under CO_2 ice. The challenge virus is the 20% suspension 2nd guinea pig passage brain that has also been kept in sealed ampules under CO_2.

The time between the inoculation of vaccine and the challenge is approximately 3 weeks. An ampule of the 2nd guinea pig passage virus is removed from the CO_2 box, thawed, and diluted in cold sterile skim milk that has been adjusted to pH 7.3. Guinea pigs are immediately inoculated deep into the gastrocnemius muscle with 0.5 cc, using an automatic syringe* and a 2 inch, no. 22 gauge needle. The dilution used is approximately 10 times the MLD_{50} dose and is either 1:50 or 1:100. The inoculations with street virus are made in a special room, the animals are kept in an isolation unit, and persons who care for them wear heavy rubber gloves which are placed in diluted creolin after they are removed.

A small number of susceptible animals show a furious type of rabies for a short period of time and care is taken that the workers are not bitten. The great majority of the animals develop a paralysis 9 to 15 days after inoculation and death occurs from 1 to 4 days later. With a few exceptions all deaths occur within 20 days after the injection, and it has been our practice to terminate the experiments a month after the challenge injection. If this time is extended to 2 months some of the few animals that survive in the control group may develop rabies but it is probable that this is an infection from their cage mates. No new cases occur among the vaccinated animals.

An occasional animal, either vaccinated or control, will show paralysis at the usual time but will live for a much longer period and occasionally one will recover. These animals have been called positive because the symptoms have developed at the proper time. Tests for virus in their brains and salivary glands have been negative but their sera show a much higher content of neutralizing antibodies than do sera from vaccinated guinea pigs.

Results. Street virus has been used in the past for challenge but on the whole the tests have not been satisfactory because only a portion of the control animals developed rabies. In our experiments 90% of the control animals develop rabies; for example, with one 2nd passage brain that has been in use for 17 months, 145 out of 152 normal animals have been infected.

There are several factors which have to be considered that may influence the development of the disease. The inoculum is large, 0.5 cc, and it is injected with considerable force, injuring the tissues. If the inoculum is reduced to 0.1 cc and the concentration increased so that the animals receive the same amount of virus, or if the inoculum is kept at the same level but is introduced slowly, the number of takes is reduced. The effect of the milk used as a diluent has not been determined, but it is not essential, for 10% horse serum can be used in its place. The

* Cornwall syringe No. 1250, Becton, Dickinson and Co., Rutherford, N.J.

TABLE I. A Typical Protocol.

Vaccine	10/8. Intraperitoneal injection, 1 ml		Guinea pig			10/31. Intramuscular injection 0.5 ml, 2nd guinea pig passage street virus		
						Date of		
	Dilution	% brain	No.	Wt 10/8	Wt 10/30	Symptoms	Death	Dead/inoculated
A. 10% rabbit brain inactivated	1:1250	0.008	1	325	440			
			2	350	415	11/11	11/15	
			3	340	440		11/11	2/5
			4	280	370			
			5	350	470			
	1:250	0.04	6	290	410			
			7	335	445			
			8	300	430			0/5
			9	340	430			
			10	300	475			
	1:50	0.2	11	295	440			
			12	315	440			
			13	270	420			0/5
			14	330	430			
			15	310	430			
B. 10% guinea pig brain inactivated	1:1250	0.008	16	355	510	11/14	11/17	
			17	345	460	11/11	11/16	
			18	295	425	11/11	11/13	4/5
			19	270	395			
			20	350	460	11/ 9	11/13	
	1:250	0.04	21	325	480	11/10	11/12	
			22	345	465			
			23	310	410			3/5
			24	335	455	11/15	11/19	
			25	310	425	11/11	11/15	
	1:50	0.2	26	290	395			
			27	340	475			
			28	330	425			0/5
			29	300	425			
			30	275	405			
Controls			31	335	490	11/11	11/14	
			32	315	470	11/10	11/14	
			33	325	480	11/11	11/18	5/5
			34	265	390	11/11	11/13	
			35	270	415	11/13	11/15	

Experiment terminated 12/1.

TABLE II.
Summary of Tests on Phenol Vaccines for Canine Immunization.
Intraperitoneal injection of vaccine 8/15. Intramuscular injection of street virus 9/13.

Vaccine	Days to expiration	% brain suspension and results		
		0.5	0.1	0.02
1	294	0/7	4/5	4/5
2	54	1/7	2/5	4/5
3	8	4/7	4/5	5/5
4	215	0/7	2/5	4/5
5	49	2/7	3/5	5/5
Rabbit brain, 3 mo. after preparation		0/6	1/5	1/5
Controls		7/7		

Exp. terminated 10/14.

strain of guinea pig may be important. In one test in another laboratory on a group of guinea pigs that were in poor condition and were dying from a respiratory disease, the controls did not show a high number of takes. However, groups of our guinea pigs on a poor diet or in an environment above (80-83°F) or below (60°F) the usual room temperature (70°F) have shown the same susceptibility as control animals.

After a great number of tests of various kinds we have come to consider a vaccine satisfactory when 1 cc of a 0.2% brain suspension protects all animals and the same amount of a 0.04% protects at least half of the animals. In some vaccines we consider especially good, 1 cc of an 0.008% suspension protected. In Table I are given in detail the results of the test of a good and an excellent vaccine. In Table II are results of a test made on 5 samples, purchased on the open market, of phenol-inactivated rabies virus for the immunization of dogs, together with the vaccine prepared in this laboratory as a control. Further results on the comparison of vaccines will be given in another paper.

Discussion and summary. This is a test of the ability of a vaccine to produce immunity to street virus injected peripherally. It seems more logical than the official Habel(1) test which tests the ability of a vaccine to produce a resistant state, which may be interference or immunity, to a given strain of fixed virus injected intracerebrally. In the Habel test a given strain of fixed virus must be used, as the resistant states cannot be demonstrated to all strains of fixed virus(2,3). While most of our tests have been with a single strain of street virus, we have used enough other strains to know that they will give good results. Our work has been done with a given strain of guinea pigs, but it is probable that animals from any healthy stock can be used; whereas in the Habel test special strains of mice and animals of a given weight must be used.

The time-saving factor may not be important when a single vaccine is tested, but when many are under study it is of considerable importance. For instance, if the Habel test were used to compare the vaccines given in Table II, 300 intraperitoneal injections would be required on each of 6 days, plus 330 intracerebral injections. The time taken for inoculation of the guinea pigs in the test was not more than 2 hours. It is very desirable that comparative tests be made using the one described and the Habel test on a variety of vaccines. We have not done this because of the work involved and because the test as we have used it gave satisfactory results in comparing vaccines. In our work we considered each experiment as an entity and were not interested in securing a highly standardized test. Repeated tests made at different times on the same vaccine have given essentially the same results.

Working with street virus involves a certain

2. Habel, K., and Wright, J. T., *U. S. Pub. Health Rep.*, 1948, v63, 44.

3. Wright, J. T., and Habel, K., *J. Immunol.*, 1948, v60, 503.

risk and this has to be considered. Carefully trained and dependable help must be used, and with such help the risk does not appear to be very great. We have not felt that vaccination of the personnel was necessary.

Some strains of fixed virus injected intramuscularly will cause death in guinea pigs in as high dilutions as the street virus and it is possible that they could be substituted for the latter with satisfactory results.

Maximum immunity develops at 3 weeks, but if more vaccine is injected the interval between the injection of vaccine and of virus can be shortened.

Injury to the tissues by injecting a large volume of virus with considerable force helps in establishing the infection and is responsible for the almost 100 per cent takes in the control animals. This procedure might well be used when testing dogs for immunity.

Conclusion. A simple test for evaluating the antigenicity of rabies vaccine is described and its advantages and disadvantages are discussed.

Received January 27, 1950. P.S.E.B.M., 1950, v73.

Complementary Correction of the Defective Coagulation Mechanism of Hemophilic and Thrombocytopenic Blood.* (17662)

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Evidence has been presented that at least two factors are necessary for the activation of thromboplastin: one contained in plasma and the other supplied by the platelets. According to Quick(1), active thromboplastin is formed through the action of an enzymatic agent liberated by the platelets (thromboplastinogenase) on a plasmatic precursor (thromboplastinogen). A deficiency of thromboplastin capable of severe impairment of the clotting mechanism may be caused either by lack of thromboplastinogen or of thromboplastinogenase, or by the presence in the circulation of an agent capable of inhibiting the platelet factor (antithromboplastinogenase)(2). All 3 conditions have been found in definite clinical states: the first in hemophilia(1), the second in thrombocytopenic purpura(3,4) and the third in some rare types of acquired hemophilia-like disease(2). Thus in hemophilia there is adequate thromboplastinogenase (platelet factor), but a lack of thromboplastinogen(2). In thrombocytopenic blood there is adequate thromboplastinogen, but a lack of thromboplastinogenase. If these assumptions are correct, the addition of hemophilic to thrombocytopenic blood should produce a mixture with a normal clotting mechanism.

We have recently been afforded the unusual opportunity of studying simultaneously a case of classical hemophilia and a 7-year-old girl with a severe thrombocytopenia (9,700 platelets per cm) of unknown cause presenting, as occasionally found in severe purpura(3), a somewhat prolonged clotting time (23 minutes in glass tubes). Blood from the two patients was mixed in various volumetric proportions. Clotting time, percentage of prothrombin activity left in serum, clot retraction and "accelerator effect" of serum were determined in each sample and in the two original bloods.

Methods. Blood was collected from both

* Aided by grants from the Charlton Fund and the American Cancer Society, Massachusetts Division

† This work was completed during the tenure of a Damon Runyon Clinical Research Fellowship, administered by the American Cancer Society.

1. Quick, A. J., *Am. J. Med. Sc.*, 1947, v214, 272.

2. Quick, A. J., and Stefanini, M., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 111.

3. Quick, A. J., Shanberge, J. N., and Stefanini, M., *J. Lab. and Clin. Med.*, 1949, v34, 761.

4. Soulier, J. P., *Rév. d'Hématol.*, 1948, v3, 302.