Technical Papers

Relation of Prothrombin to the Prolongation of Clotting Time in Aestivating Ground Squirrels

Arthur Svihla, Howard Bowman, and Ruth Ritenour[1]

Department of Zoology, University of Washington, Seattle

A change in the blood clotting time was found to take place in two species of ground squirrels (*Citellus columbianus* and *C. parryi ablusis*) when these mammals are in a dormant state during either aestivation or hibernation. A complete clot did not form even when the blood sample was exposed to the air for several days (*1*). Although blood clotting has been studied intensively for a long time, this phenomenon is still imperfectly known. The most generally accepted theory of blood coagulation, as formulated by Morawitz (*2*), includes these steps:

1. Prothrombin (plasma factor) + calcium ions + thrombokinase (thromboplastin, tissue and platelet factor) = thrombin.
2. Thrombin + fibrinogen = fibrin.

The first equation has recently been modified by Owren (*3*), Ferguson (*4*), and Quick (*5*), but essentially the above-mentioned components of the blood are necessary for the formation of a clot. The decrease of any of these substances may prolong clotting time or completely inhibit clot formation. This has been shown in the classic work of Campbell, Link, *et al.* (*6*) in their investigation of the hemorrhagic diseases of such domestic animals as sheep and cattle, which became hemophilic when fed moldy sweet clover hay and died of internal hemorrhages. Link (*7*) found that this hemophilic condition was brought about by the destruction of the prothrombin in the blood by the substance coumarin, which is present in large amounts in moldy sweet clover hay. Previously, Quick (*8*) found that laboratory rabbits that were fed moldy sweet clover hay became hemophilic and bled to death when their hearts were punctured with a hypodermic needle.

Similarly, our ground squirrels developed a pericarditis when the heart was punctured while they were dormant. This did not occur when they were normal and active. This seasonal hemophilic condition in ground squirrels might be considered as caused by decreased amounts of various blood substances such as (*a*) prothrombin, (*b*) thromboplastin, (*c*) calcium, (*d*) fibrinogen, as well as (*e*) the possible presence of an anticoagulant. It was therefore determined to test for the amounts of each of these substances in the blood of both active and dormant squirrels. This report concerns the relationship of prothrombin to the prolongation of clotting time in these animals.

Quick (*9*) has developed a technique to determine the amount of prothrombin in the blood by diluting the blood and supplying ample amounts of all other components such as thromboplastin, calcium, etc., and then recording the coagulation time. Any decrease in the amount of prothrombin in the blood sample is indicated by the increase in clotting time. In our experiments we followed Kato's modification (*10*) of Quick's technique, since less blood is required, and used commercial thromboplastin manufactured by Difco Laboratories. Table 1 and Fig. 1 show our results.

TABLE 1

CLOTTING TIMES AT DIFFERENT PROTHROMBIN CONCENTRATIONS

	Active animals						Dormant animals				
	10	20	30	50	100		10	20	30	50	100
	65.5	34.4	25.0	20.9	19.7		104.0	68.4	61.3	44.4	30.0
	60.7	27.3	23.2	22.2	21.7		541.0	78.4	55.1	26.5	25.3
	134.6	65.0	45.2	27.8	21.1		61.2	39.9	33.1	25.3	25.3
	61.2	31.3	26.4	19.6	18.5		256.0	58.2	74.9	27.2	23.5
	81.8	48.5	26.8	20.1	16.0		95.0	63.5	35.5	27.6	25.0
	69.0	38.4	32.7	22.1	20.2		70.0	42.7	27.2	25.3	20.1
	64.8	40.5	28.8	19.7			243.0	41.4	30.6	25.6	20.2
Av	76.5	40.7	29.7	21.7	19.5 sec		105.0	49.2	40.0	29.9	24.5
							92.0	44.2	34.2	19.6	18.7
								56.2	43.0	33.1	20.1
						Av	174.1	54.2	43.5	28.5	23.3

A reduction in the amount of prothrombin in the blood of dormant squirrels is apparent. At all concentrations of prothrombin used, the clotting times were much longer for the dormant animals than for the active ones. For the latter, the normal blood or that with 100% concentration of prothrombin shows an average clotting time of 19.5 sec, whereas the blood of the dormant squirrels at the same concentration clots on an average of 23.3 sec. At lesser concentra-

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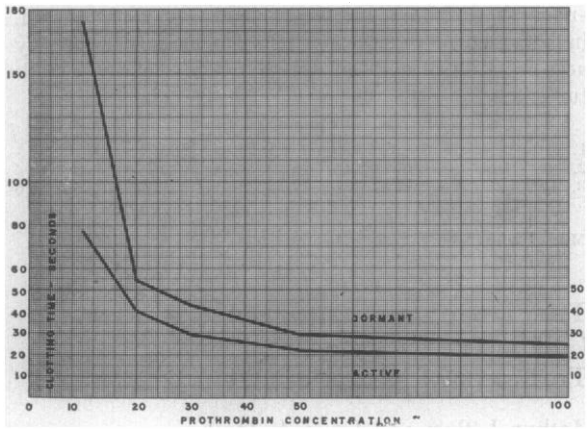

FIG. 1.

tions—for example, 10%—a much greater disparity in clotting times occurs, for now the blood of the active animals clots on an average of 76.5 sec, whereas that of the dormant squirrels requires an average of 174.1 sec.

Percentages of prothrombin dilutions may be compared by either of two methods that are in general use. The following formula of Ziffren *et al.* (*11*) is the one commonly used in clinics:

$$\text{Prothrombin percentage} = \frac{\text{average normal prothrombin time}}{\text{prothrombin time of patient}} \times 100.$$

Applying this formula to our data, we obtain a prothrombin percentage of 83.6 for dormant ground squirrels.

The second method for obtaining prothrombin dilutions is the use of a correlation chart, as employed by Nygaard (*12*). He has pointed out that more accurate results are obtained by this method. Quick (*13*) has also shown the fallacy of using Ziffren's formula, since the relation of clotting time to concentration of prothrombin is not linear but expressed by a hyperbolic curve. According to Nygaard's correlation chart, the prothrombin dilution for our dormant ground squirrels would be approximately 38%. This we take to be the more accurate percentage.

It is now well known that in the case of persons who are bedridden for any length of time, thromboses are apt to form, especially in lower extremities, as a result of lack of proper circulation. Post-operative cases are therefore encouraged to become ambulatory as soon as possible in order to stimulate circulation and thus prevent the formation of the thromboses. Seasonal changes in the blood picture of ground squirrels may be conceived as being an efficient adaptation to the dormant state. During this period the rate of blood flow is greatly reduced, thus increasing the danger of clots forming in the blood stream and causing death. A decrease in the amount of prothrombin during dormancy hence alleviates any danger of thrombus formation due to the lowered rate of blood circulation at this time. The same principle of decreasing the amount of prothrombin in the blood of humans is utilized in dicumarol therapy.

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Inactivation of Influenza Virus and of Viral Hemagglutinin by the Ciliate *Tetrahymena geleii*[1, 2]

Vincent Groupé and Leonora H. Pugh

Department of Microbiology, New Jersey Agricultural Experiment Station, New Brunswick

The experiments described in this report were undertaken to determine what effect, if any, the influenza virus and the free-living ciliate *Tetrahymena geleii* might have upon each other. Bacteria-free cultures of *T. geleii*[3] were propagated in 1.0% proteose peptone broth (Difco) at 28° C. Egg-adapted strains of influenza A (PR-8 strain) and influenza B (Lee strain) virus were cultivated in the allantoic sac, and allantoic fluid was collected 48 hr after infection from viable embryos. Hemagglutination tests (*1*) and infectivity titrations (*2*) were performed in the usual manner. Aseptic precautions were taken in all experiments.

In the experiments described below a constant amount of influenza B virus (10% by vol of infected allantoic fluid) was added to varying concentrations of viable and killed (frozen and thawed) cultures of *T. geleii,* respectively, as indicated in Table 1. The protozoal cultures used were previously incubated for 7–9 days at 28° C to ensure maximum growth. Samples were taken from each of the various cultures[4] at intervals during the incubation period, and hemagglutinin and infectivity titers were determined. Formalin, in amount sufficient to make a final concentration of 0.05%, was added to all samples used in hemagglutination tests to kill the protozoa. Samples used for infectivity titrations were serially diluted without treatment of any kind and inoculated into

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[3] Bacteria-free cultures of *T. geleii* were obtained through the courtesy of George W. Kidder.

[4] A total volume of 100 ml of culture medium containing protozoa and virus was placed in each of a series of Blake bottles and incubated in the horizontal position at 28° C.