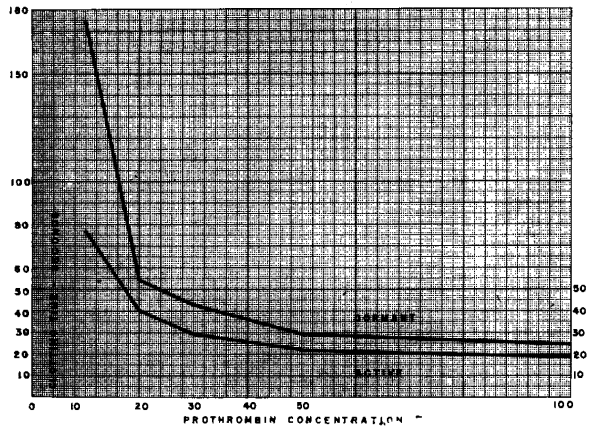

FIG. 1.

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]

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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.

TABLE 1

EFFECT OF *Tetrahymena geleii* ON INFLUENZA B VIRUS

Protozoal culture*	Viral hemagglutinin titer			Infective titer (ID_{50})
	Hr incubation at 28° C			
	0	24	48	48
None	64†	32	32	$10^{-6.5}$
Viable				
Undiluted	32	32	64	$10^{-2.7}$
½‡	32	32	16	
1/20	32	32	1	$10^{-4.3}$
Killed (frozen and thawed):				
Undiluted	32	32	32	$< 10^{-1.0}$
½	32	32	32	
1/20	32	32	32	$10^{-4.6}$

* Previously incubated 7–9 days at 28° C.
† Reciprocal of hemagglutinin titer of culture.
‡ Diluent used was 1% proteose peptone medium.

groups of 5 eggs each. Macroscopic and microscopic examination of the cultures at various intervals indicated that the presence of virus did not affect either the motility of the protozoa or their rate of multiplication. Little or no gross change in the concentration of protozoa was observed in mature undiluted cultures in the presence or absence of virus. However, the virus was affected in several ways by the protozoal culture.

The data from a typical experiment are summarized in Table 1. When the infective titers of the various cultures are compared after 48 hr of incubation it is evident that both viable and killed cultures of *T. geleii* contained a factor which inactivated influenza B virus. Protozoal cultures, killed by freezing and thawing, were found to be slightly more effective than viable cultures. Dilution of the protozoal cultures was accompanied by diminished viral inactivation. These data recall to mind the fact that antibacterial lipids have been obtained from similar cultures of *T. geleii* (*3*). Further inspection of the data indicates the existence of another phenomenon. It will be seen that viral hemagglutinin was markedly reduced by diluted, actively multiplying, viable protozoal cultures, whereas killed cultures or undiluted, mature, viable cultures had no effect on viral hemagglutinin. Identical end points (1/320) were obtained when viral hemagglutinin was titrated in the presence of culture medium from young (3-day) protozoal cultures, fresh broth, and saline, respectively. It is clear, therefore, that viral hemagglutinin, representing both infective and noninfective virus particles, was inactivated or destroyed only by actively multiplying (i.e., diluted) protozoal cultures. Similar results were obtained in comparable experiments with influenza A virus. It is well known that *T. geleii* and other ciliates feed upon certain bacteria and it would appear that a similar mechanism obtains in the case of the influenza viruses.

The influenza viruses, then, do not affect *T. geleii*, but the protozoal culture may affect the virus in at least 2 ways: first, viral inactivation by a factor present in both killed and viable cultures, and, second, inactivation or destruction of viral hemagglutinin by actively multiplying but not by mature protozoal cultures.

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The Importance of Protective Urinary Colloids in the Prevention and Treatment of Kidney Stones

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A calculus is generally defined as a concentration formed of crystalloids (*1*) held together by, and incorporated in, a colloidal matrix. The formation of urinary calculi is, however, far more complex and is still not fully understood (*2–6*).

Most investigations concerning the prevention and treatment of kidney stones have so far been directed only toward trying to ascertain how the concentration of crystalloids excreted in the urine could be diminished. With the exception of correcting certain metabolic disorders, all attempts to control calculous formations have so far not proved entirely successful. The influence that hydrophilic colloids exert in the etiology of kidney stone formation and prevention has never received sufficient attention. We therefore felt that a more systematic study of the action of hydrophilic colloids might offer valuable information on how the formation of urinary concretions could be completely avoided or at least stopped. Such knowledge might even lead to the development of a more efficient method by which some stones could be removed without surgery.

From a colloid-chemical point of view urine must be considered as a supersaturated solution of extremely complex composition. The electrolytes and nonelectrolytes in the urine of a healthy person remain in solution at a much higher concentration than their solubility in pure water would indicate; this is due to the presence of protective colloids. This fact was stated by Lichtwitz some time ago (*4, 5*). Ord (*6*) and Rainey (*7*) had already observed that crystals formed from solutions containing hydrophilic colloids differed pronouncedly in their morphology and properties from those derived from pure aqueous solutions. To Ebstein (*2*) must go the credit for having been the first to draw attention (in 1884) to the importance of colloids in kidney stone formation. Since then much research pertaining to the etiology of stones has referred to an "unbalance" of colloids and crystalloids, but there is a serious lack of infor-