

latter is due to formation and discharge of potential differences across a semipermeable membrane, this process may be changed depending on the presence of certain electrolytes and it may be accelerated or even initiated on warming and slowed down or abolished by cooling the center of extrasystoles formation.

Conclusions. Coupled extrasystoles appear in dogs following epicardial or subepicardial application of hypertonic solutions

of sodium or barium chloride on circumscribed areas. Warming of the injected area increases the number and the rate of the extrasystoles or causes them to appear even if they were not present after the injection. Cooling the area of injection slows the rate of formation of extrasystoles or abolishes them completely.

These results can be explained satisfactorily by assuming a heterogenetic stimulus formation in the treated area.

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Inhibiting Effect of Methionine, Choline and Betaine on Rabbit's Susceptibility to Infection with Vaccinia.*

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In the course of experiments to determine what factors in a rabbit's diet are essential to virus multiplication it was observed that the subcutaneous injection of methionine increased the rabbit's resistance to dermal infection with vaccinia. Six experiments have been performed with methionine. The results of a typical experiment are shown in Table I. In this experiment and others described below rabbits weighing approximately 2 kg were used. The experimental rabbits received 300 mg of methionine subcutaneously daily for 2 days preceding and 5 days following vaccination. In other experiments the conditions of the experiment were varied as to the amount of methionine and the times at which it was given. These experiments showed that if the methionine was given 48 hr before vaccination, the resistance was increased 14 fold; if given immediately following vaccination, the increase was 10 fold; if delayed until 48 hr after vaccination the increase was 5 fold. In addition to these quantitative changes in resistance, the lesions of the animals whose resistance had been stimulated most by methionine were smaller and disappeared approximately 24 hr earlier

than did the ones of the control animals. Measurements of resistance were made by use of serial dilutions of vaccinia virus as described previously.¹ The experimental error in this method is considerably less than a 2-fold difference, hence the observed changes were highly significant.

Additional studies were then undertaken to determine what biological functional group of the methionine molecule was responsible for this effect. That it was probably not dependent on the generalized properties of the amino acids as such was indicated by the fact that glycine had no significant effect on susceptibility. Neither does the mere presence of sulfur in the molecule explain the action, for cystine did not duplicate the effect of methionine.

The chemical characteristic which seems most likely to be involved is the biologically labile methyl group. Choline and betaine share with methionine the property under proper circumstances of possessing an easily transferable methyl group,² and these substances were therefore investigated in a series of 5 experiments. When given 48 hr before

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¹ Sprunt, D. H., *J. Exp. Med.*, 1942, **75**, 297.

² du Vigneaud, V., *Biological Symposia*, 1941, **5**, 234.

TABLE I.
Effect of Methionine, Choline and Betaine on Susceptibility of Rabbit to Infection with Vaccinia as Indicated by the 50% Point.

Chemical used		No. of rabbits used	50% point log	Inc. resistance $\times$
Methionine	Controls	5	5.65	
	Exper.	5	4.40	18
Betaine	Controls	6	6.00	
	Exper.	6	5.28	5
Choline	Controls	6	5.40	
	Exper.	5	4.12	19

and continued until 5 days after vaccination, betaine showed little effect on the lesions for the first 2 days following inoculation of the virus; after this initial lag period, however, its action became manifest and resulted ultimately in a 5-fold increase in resistance. There was, however, considerable variation between rabbits, some showing little change from the controls and others a marked decrease in the number and size of the lesions. The results of a typical experiment with betaine are shown in Table I. The experimental rabbits were injected subcutaneously with 500 mg of betaine hydrochloride which was neutralized with soda bicarbonate. These injections were made at approximately 8-hr intervals. They were begun 48 hr before and continued for 5 days after vaccination. Choline also caused an increase in resistance (20-fold). The results of a typical experiment are shown in Table I. In this experiment 300 mg of choline chloride was given to the experimental rabbits at same intervals as the betaine in the above experiment. The lesions in the animal receiving the choline remained small throughout the

experiment. The effect of the choline, however, cannot be attributed definitely to a specific action for the animals treated with this substance did not eat as well as the controls and lost some weight. It is therefore conceivable but unlikely that the consequent increase in resistance may have been due to loss in weight for fasting has been shown previously to decrease susceptibility to vaccinia infection.¹ The degree of weight loss was not nearly so great in the rabbits receiving the choline (5%) as in those which were purposely fasted (18%) and in which increased resistance occurred.

The results obtained with 2 substances and to a less extent with a third containing biologically labile methyl group—methionine, choline and betaine—show that these substances inhibit dermal infection with vaccinia in the rabbits. Further investigations of this possibility are now under way. If the observed effects are proven for other viruses and other animals, it is possible that a chemotherapeutic agent effective against viral infections may be discovered among these or related chemical substances.