TABLE I.
Blood Sugar Values at 10-Minute Intervals After Oral Administration of Saccharine to Fasting Subjects.

Subject	10 min.	20 min.	30 min.	40 min.	50 min.
1	110*	98	105	104	102
2	91	98	98	93	97
3	110	107	99	95	101
4	97	95	91	99	97
5	107	108	108	98	104
6	105	102	104	102	99
7	105	106	105	107	109
8	101	97	98	105	106
9	95	96	94	98	95
10	103	101	100	98	95
Mean	102.4	100.8	100.2	99.9	100.5

* Blood sugar values expressed as % of control level.

Methods. The 10 subjects used were departmental personnel who were familiar with the purpose of the experiment and had no apprehension concerning the procedure. All were in good health. On the morning of the test, each subject appeared at the laboratory without breakfast and was made to lie quietly on a cot for 20 to 30 minutes before the first blood sample was taken. Two control blood samples were taken 10 minutes apart and after the second, 50 mg of saccharine in 80 ml of water was drunk over a period of 5 minutes. Ten minutes after the subject began drinking the saccharine solution, the third blood sample was drawn and 4 additional samples were taken at 10 minute intervals. Duplicate determinations of blood sugar were made on each sample by the micro method of Hagedorn and Jensen.[6]

Results and Discussion. In the accompanying table, the average of the 4 determinations on the first 2 blood samples is taken as the control value. All other values on each subject are then expressed as per cent of his own control. The greatest single variation from a control is 10% and the greatest mean variation for all subjects at any one time period after saccharine is 2.4%.

Why Kun and Horvath obtained consistent and significant lowering of blood sugar and all others, including ourselves, did not, we are unable to explain. The mean control value of all subjects was 97.8 mg%, which is a usual fasting value for the blood sugar as determined by the method used. Therefore, one could not say that other factors raised the blood sugar in our experiments and thus obscured a lowering due to the sweet taste, nor could one account for the absence of a hypoglycemic response in terms of an abnormally low initial blood sugar level.

Summary. Oral administration of saccharine to 10 normal subjects, fasted and at rest, did not influence the blood sugar during the subsequent 50 minutes.

[6] Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, **137**, 92.

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Enzymatic Inactivation of Serum Vasoconstrictor.*

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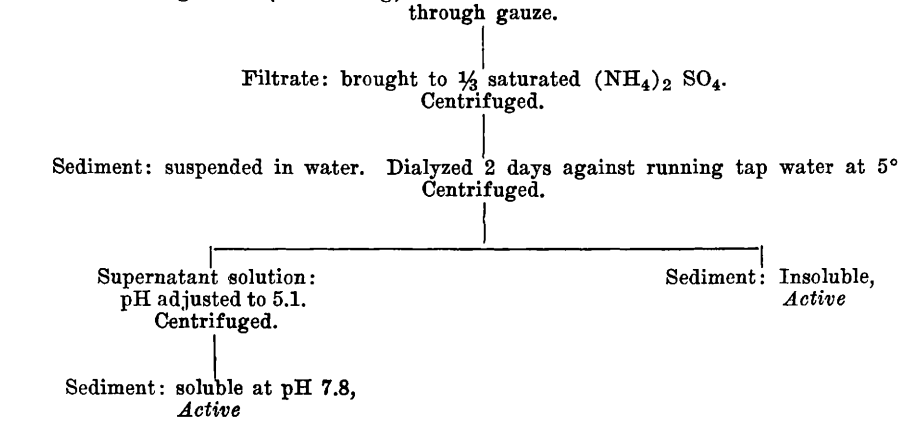
It has been the practice for many years to abolish the vasoconstrictor properties of serum or defibrinated blood to be used in the perfusion of isolated organs, by passing the blood through oxygenated lungs. The mechanism of this action is not known.

Our studies on the identification of the substance which causes the vasoconstriction led us to investigate the action of cell-free protein extracts from lung tissue on highly purified preparations of serum vasoconstrictor. The results of this study, which are reported in this paper, indicate that the

* A preliminary report of this paper was published in *Federation Proceedings*, 1948, **7**, 180.

TABLE I.
Preparation of Vasoconstrictor Inactivating Enzyme.



mechanism by which the lungs inactivate the vasoconstrictor substance may be enzymatic.

Materials and Methods. Serum vasoconstrictor substance was purified as described.[1]

Lung extracts were prepared from beef lungs obtained from the slaughter house on the same day the animal was killed. The lung tissue was ground twice and then extracted for one hour at 5° with 2 volumes of cold saline. The mixture was filtered through gauze. Fractionation of the filtrate followed the scheme summarized in Table I.

Dog lungs were treated similarly.

Beef lungs obtained one day after being removed from the animal yielded preparations with only slight activity.

Dog lungs which were chilled rapidly after removal and kept frozen for 3 months gave satisfactory preparations.

The inactivation studies were carried out as follows: Test tubes containing 0.2 ml of a standard serum vasoconstrictor solution (purified material containing 1 mg/ml) 0.2 to 0.5 ml of enzyme solution, and 1.2 ml of 0.05 M phosphate buffer (K_2HPO_4/NaH_2PO_4=9) made up to a total volume of 2.0 ml, were incubated at 37°. 0.1 ml aliquots were withdrawn after varying periods of time, diluted to 1.0 ml, and then assayed in the perfused rabbit ear preparation against a substrate control in the manner described.[1] Substrate controls have never been observed to lose activity. Enzyme controls exerted vasoconstrictor effects representing only about 3% of the initial activity of the incubation mixture.

Results. Under the conditions employed (pH 7.4 to 7.6) the observed inactivation reaction appeared to follow a first order course (Table II).

The effect of pH has been examined at pH 7.2, 7.6, and 8.0. A marked decrease in the rate was observed with decreasing pH, the reaction proceeding about twice as rapidly at pH 8 as at pH 7.2.

The enzyme was found to be heat labile; heating at 60° for 3 minutes resulted in 50% inactivation. Preparations lost activity even when kept cold. The losses have been variable in both chilled and frozen solutions, but, in general, about half the activity was lost in 5 days at 5° C. Keeping the enzyme suspended in 35% saturated ammonium sulfate did not enhance the stability. As

TABLE II.
Inactivation of Serum Vasoconstrictor by Enzyme from Dog Lung. 1.7 mg Protein, 0.1 mg (75 Units) Purified Serum Vasoconstrictor per ml. pH 7.5. 37°C.

Time in hr	% inactivation	$K = \frac{1}{t} \log_{10} \frac{a}{a-x}$
1½	38	23×10^{-4}
3	63	24
4½	69	19
6	77	18

[1] Rapport, M. M., Green, A. A., and Page, I. H., *J. Biol. Chem.*, 1948, **174**, 735.

can be seen from Table I, active enzyme preparations were found in both soluble and insoluble protein fractions. The activity per mg of protein and the stability of the insoluble fraction was usually slightly greater than that of the soluble one. With beef lungs, about 1/3 of the total activity was found in the soluble fraction; with dog lungs, a smaller fraction of the total activity was soluble.

Summary. Saline extraction of ground lung tissue (beef or dog) followed by ammonium sulfate fractionation, dialysis, and isoelectric precipitation at pH 5.1 yields a protein extract which catalyzes the inactivation of a purified preparation of serum vasoconstrictor.

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Anticonvulsant Properties of 5,5-Phenyl Thienyl Hydantoin in Comparison with Dilantin and Mesantoin.*†‡

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Dilantin (Diphenylhydantoin, U.S.P.) has been successfully employed for a decade in the treatment of grand mal and psychomotor epilepsy. In the past 2 years, Mesantoin (3-methyl, 5,5-phenyl ethyl hydantoin) has been used for the same purposes.[1-4] Still another congener, 5,5-phenyl thienyl hydantoin (Lilly 00079), has recently been used clinically with encouraging results.[5] A number of previous communications have dealt with the ability of Dilantin, Mesantoin and other congeneric substituted hydantoins, some of which have had initial therapeutic trial, to protect against or modify experimental seizures.[6-16] Comparable data on 5,5-phenyl

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