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STABILIZED VASOCONSTRICTOR-ENZYME PREPARATION

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This invention relates to the protection of an enzyme preparation against the inhibiting effect of a vasoconstrictor and to the resulting protected or stabilized vasoconstrictor-enzyme preparation. Such a preparation is suitable for use in the treatment of inflammations of the mucous membranes of the respiratory tract. The protection is achieved by the incorporation of a proteolytic enzyme substrate as, for example, gelatin, hydrolyzed gelatin, and collagen preparations in a vasoconstrictor-enzyme solution.

Solutions containing both proteolytic enzymes and vasoconstrictors have previously been proposed for nasal medicants. However, such solutions have been found impracticable, because of the lack of stability of the enzymes in the solutions. The vasoconstrictor compounds apparently have an inhibiting effect upon the activity of the enzyme. Such inhibition appears to be a competitive inhibition caused by the structural similarity of the vasoconstrictor to an amino acid or peptide. The enzyme cannot distinguish between the vasoconstrictor and a protein, polypeptide, or peptide, which are the normal substrates for these enzymes. The enzyme attaches itself to the vasoconstrictor as it would to a normal substrate and cannot release itself because it cannot break the chemical bond to which it is attached.

Due to such difficulties, it has been the usual procedure to administer the enzyme and the vasoconstrictor in separate solutions. This is repeated every hour or so, as required by the patient. The enzyme solution must be made up daily and stored in the refrigerator. An alternative procedure which may be used to treat infections of the respiratory tract with enzymes is to dissolve the enzyme in a vasoconstrictor solution and use it immediately. This is inconvenient to the patient because it is necessary to make up the solution many times each day.

An object of the present invention is to provide a solution containing an enzyme, a vasoconstrictor, and a stabilizer, or protecting agent which will stabilize the enzyme in the solution against the inhibiting effect of the vasoconstrictor for a reasonable length of time, so that it may be utilized throughout a normal course of treatment without making up a fresh solution.

It has been found that the addition of from about 1 to about 5% of gelatin, partially hydrolyzed gelatin, collagen or equivalent substrate, will provide a substantial protection of the enzyme of an enzyme-vasoconstrictor solution from the inhibiting action of the vasoconstrictor for a period of about five days, even at room temperature. The composition of the invention, therefore, comprises a solution of a proteolytic enzyme, a vasoconstrictor, and a stabilizing quantity of a substrate for such enzyme.

For purposes of the invention, U.S.P. gelatin or other equivalent gelatin material is quite satisfactory. Partially hydrolyzed materials suitable as substrates may be produced by treating gelatin or collagen by any of a number of well-known methods. For example, a partially hydrolyzed gelatin suitable for purposes of the invention may be prepared by dissolving 100 grams gelatin

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in 600 ml. of water and heating it in an autoclave for 60 minutes at 15 lbs. pressure. The solution is then cooled and diluted to 1000 ml. with water. This is given merely by way of example and is not to be considered as limiting the scope of the invention.

The vasoconstrictor solutions used in conjunction with the enzymes are those commonly used by the medical profession, and may comprise, for example, aqueous solutions containing 0.25% phenylephrine, 0.25% ephedrine, or 1% tuamine. These preparations usually contain other substances in addition to the vasoconstrictor, such as preservatives, stabilizers, salts, and buffers. Specific examples of typical solutions are as follows:

Phenylephrine solution:

- 15 0.002% merthiolate or phenyl mercuric nitrate
- 0.2% sodium bisulfite
- 0.25% phenylephrine HCl
- M/15 Sorensen's phosphate buffer, pH 6.5 to pH 7.5

20 Ephedrine solution:

- 0.25% ephedrine HCl
- Other ingredients as above

Tuamine solution:

- 25 1% tuamine sulphate
- 0.002% phenyl mercuric nitrate
- 0.9% sodium chloride
- M/15 Sorensen's phosphate buffer, pH 6.5 to pH 7.5

The enzymes utilized in the composition may be any of the well-known commercial proteolytic enzyme preparations. These are usually marketed and stored in dry form, in which they keep indefinitely. Examples of suitable materials are commercial preparations of crystalline trypsin and chymotrypsin.

The protected vasoconstrictor-enzyme solutions are preferably prepared by dissolving the stabilizers, i.e. the enzyme substrates, in the vasoconstrictor solutions before the enzymes are added. The enzymes are then dissolved in the vasoconstrictor containing the stabilizer. Typical amounts of the dry enzyme preparations to be added to the vasoconstrictor solution are, for example, from 10 to 100 mg. to 100 ml. of solution. However, it will be understood that this amount can be widely varied, depending upon the enzyme activity desired.

In the following examples of practice, the enzyme activity is reported in trypsin or chymotrypsin units per milliliter. The enzymes were assayed by a modification of Anson's method (Journal of General Physiology, volume 22, page 79, 1938). One trypsin unit is that amount of crystalline trypsin which upon incubation at $25.0 \pm 0.1^\circ \text{C}$. for 10 minutes with a suitable preparation of pure hemoglobin will cause the decomposition of the hemoglobin to such an extent that the amount of hydroxyl substance liberated will, upon reaction with Folin Ciocalteu phenol reagent result in the formation of a colored solution of equal intensity to that resulting from the reaction of 5.52×10^{-6} millimoles of tyrosine with Folin Ciocalteu reagent. 1000 trypsin units of activity are equivalent to 1 mg. of tyrosine. The chymotrypsin unit is similar to the trypsin unit except the chymotrypsin assay uses casein as the substrate instead of hemoglobin and the incubation is carried out for 20 minutes at $35.5^\circ \text{C} \pm 0.1^\circ \text{C}$.

EXAMPLE 1.—THE EFFECT OF GELATIN ON THE PHENYLEPHRINE INHIBITION OF TRYPSIN

An aqueous phenylephrine solution having the analysis given above was divided into three 100 ml. portions. To the second and third portions 1% and 5% of gelatin was added, respectively, and was dissolved therein. About 40 mg. of trypsin was then dissolved in each of the three phenylephrine solutions. This amount was sufficient

theoretically to provide a trypsin activity of about 840 units per ml. The same amount of trypsin was also dissolved in a 100 ml. portion of water. Assays were performed one hour after solution of the enzyme. This was taken as zero time. The results were recorded in trypsin units per milliliter. The solutions were then stored at room temperature in covered containers without further protection and samples for trypsin assays were taken at intervals. The results are recorded in Table I with the percentage degrees in activity shown in brackets.

Table I

THE EFFECT OF GELATIN ON THE PHENYLEPHRINE INHIBITION OF TRYPSIN

Diluent	Trypsin Activity in Units/Ml.				
	0 hrs.	1 Day	2 Days	5 Days	8 Days
Water.....	736	488 (-36.9)	400 (-45.6)	248 (-66.2)	152 ¹ (-79.3)
Phenylephrine.....	773	293 (-62.1)	293 (-62.1)	139 (-82)	97 (-87.5)
1% gelatin in Phenylephrine.....	1,005	888 (-11.1)	892 (-10.8)	834 (-16.3)	751 (-24.2)
5% gelatin in Phenylephrine.....	837	877 (+4.8)	868 (+3.6)	844 (+0.8)	831 (-0.7)

¹ 12 days.

The inhibiting effect of phenylephrine on trypsin is shown by the fact that whereas the enzyme in water alone lost 79.3% of its activity in 12 days, trypsin in phenylephrine solution lost 87.5% of its activity in 8 days. This was decreased to 24.2% when 1% gelatin was added to the phenylephrine solution and to 0.7% when 5% gelatin was present.

Specific embodiments of the invention as disclosed in Example 1 constitute the phenylephrine-trypsin solutions stabilized with 1% and 5% of gelatin.

EXAMPLE 2.—THE EFFECT OF GELATIN, ARGinine, GLYCEROL, AND PARTIALLY HYDROLYZED GELATIN ON THE INHIBITION OF TRYPSIN ACTIVITY CAUSED BY PHENYLEPHRINE, EPHEDRINE, AND TUAMINE

Solutions of trypsin were prepared by the addition of the enzyme to aqueous phenylephrine, ephedrine and tuamine solutions with and without enzyme stabilizers or protectors. The vasoconstrictor solutions had the analysis set forth above. Gelatin, hydrolyzed gelatin, glycerol, and arginine were utilized as stabilizers of the enzyme. Glycerol was utilized because many biological materials are stabilized by marketing them in 50% glycerol. The amino acid arginine was included because it was thought the effect of hydrolyzed gelatin might be due to the free amino acids. Arginine was selected because it is one of the necessary amino acid constituents of a protein which enables trypsin to act on the protein. Assays were carried out at zero hours and after 5 days of storage at room temperature. The results are recorded in Table II.

Table II

THE EFFECT OF GELATIN, ARGinine, GLYCEROL, HYDROLYZED GELATIN ON INHIBITION OF TRYPSIN ACTIVITY

Vasoconstrictor Solution	Protector or Stabilizer	Enzyme Activity in Units/Ml.		Percent Decrease in Enzyme Activity
		0 Hrs.	5 Days	
Phenylephrine.....		872	91	-87.2
Do.....	5% gelatin.....	848	704	-17.0
Do.....	1% arginine.....	756	172	-77.1
Do.....	50% glycerol.....	772	480	-37.8
Do.....	5% hydrolyzed gelatin.....	756	660	-12.7
Tuamine.....		680	128	-81.2
Do.....	5% gelatin.....	796	1,001	+25.5
Ephedrine.....		452	28	-94.0
Do.....	5% gelatin.....	596	320	-46.3

It will be observed from Table II that gelatin and hydrolyzed gelatin stabilized the trypsin and protected it

against phenylephrine, tuamine and ephedrine inhibition. 50% glycerol was only partially effective, and 1% arginine was only slightly effective against the phenylephrine inhibition.

Specific embodiments of the invention as disclosed in Example 2 constitute the phenylephrine-trypsin solutions stabilized with 5% gelatin and with 5% hydrolyzed gelatin, the tuamine-trypsin solution stabilized with 5% gelatin, and the ephedrine-trypsin solution stabilized with 5% gelatin.

EXAMPLE 3.—THE EFFECT OF GELATIN, ARGinine, AND GLYCEROL ON THE INHIBITION OF CHYMOTRYPSIN ACTIVITY CAUSED BY PHENYLEPHRINE, TUAMINE, AND EPHEDRINE

The conditions and materials of this example were substantially the same as in Example 2, except that chymotrypsin was used instead of trypsin as the enzyme. The amount of chymotrypsin was sufficient initially to provide a solution having 6400 chymotrypsin units per milliliter. The results are recorded in Table III.

Table III

THE EFFECT OF GELATIN, ARGinine, AND GLYCEROL ON INHIBITIONS OF CHYMOTRYPSIN ACTIVITY

Vasoconstrictor Solution	Protector or Stabilizer	Enzyme Activity in Units/Ml.		Percent Decrease in Enzyme Activity
		0 Hrs.	5 Days	
Phenylephrine.....		1,870	120	-93.6
Do.....	5% gelatin.....	6,400	3,760	-41.2
Do.....	1% arginine.....	2,420	0	-100
Do.....	50% glycerol.....	2,390	0	-100
Tuamine.....		5,590	5,020	+5.9
Do.....	5% gelatin.....	7,150	7,040	-1.3
Ephedrine.....		5,890	0	-100
Do.....	5% gelatin.....	6,240	720	-88.5

Specific embodiments of the invention as disclosed in Example 3 constitute the phenylephrine-chymotrypsin solution stabilized with 5% gelatin, the tuamine-chymotrypsin solution stabilized with 5% gelatin, and the ephedrine-chymotrypsin solution stabilized with 5% gelatin.

The large variations in the 0-hour activity of the solutions shown in Example 3 can be explained by the fact that "0-hour" designates the time of the first analysis which is, in fact, about an hour after the materials are first mixed. During this first hour the inhibition of the enzyme by phenylephrine is already well advanced. Thus in the first experiment where phenylephrine alone is present the activity drops from 6400 to 1870 in the first hour. When 5% gelatin is added, complete protection is provided. The other additives, glycerol and arginine, are only partially effective at the 0-hour analysis and are totally ineffective at the end of 5 days. In the case of tuamine, 5% gelatin provides a higher initial assay—7150 instead of 6400—which would be the theoretical amount. This rise in enzyme activity is at present unexplainable. It is surprising that although tuamine does not substantially inhibit the activity of chymotrypsin, the presence of gelatin in the solution does cause both an increase in the zero activity and in the 5-day activity. Ephedrine caused

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a complete destruction of the chymotrypsin activity in 5 days. Gelatin decreased this inhibition both at the 0-hour and 5-day period.

It will be apparent from the foregoing that a method has been provided for protecting the enzyme activity of a vasoconstrictor-enzyme preparation against the inhibiting effect of the vasoconstrictor, and that stabilized vasoconstrictor-enzyme preparations have been provided which will retain their activity to a satisfactory extent over a period of at least 5 days.

We claim:

1. A therapeutic composition comprising an aqueous solution containing a minor but effective quantity for nasal medication of a vasoconstrictor compound selected from the group consisting of phenylephrine, ephedrine, tuamine, and the nontoxic water-soluble inorganic salts thereof, from about 10 to about 100 mg. per 100 ml. of solution of a proteolytic enzyme selected from the group consisting of trypsin and chymotrypsin, and a quantity of an enzyme substrate sufficient to substantially stabilize the enzyme activity and protect said enzyme from inhibition by said vasoconstrictor compound so that said enzyme is effective for a period of at least 5 days, said enzyme substrate being selected from the group consisting of gelatin, hydrolyzed gelatin, and collagen.

2. A therapeutic composition comprising an aqueous solution containing a minor but effective quantity for nasal medication of a vasoconstrictor compound selected from the class consisting of phenylephrine, ephedrine, tuamine, and the nontoxic water-soluble inorganic salts thereof, from about 10 to about 100 mg. per 100 ml. of solution of a proteolytic enzyme selected from the group consisting of trypsin and chymotrypsin, and from about 1% to about 5% of a substrate for said enzyme selected from the group consisting of gelatin, hydrolyzed gelatin, and collagen.

3. A therapeutic composition comprising an aqueous solution containing about 0.25% of a vasoconstrictor compound selected from the group consisting of phenylephrine and its nontoxic water-soluble inorganic salts, from about 10 to about 100 mg. per 100 ml. of solution of a proteolytic enzyme selected from the group consisting of trypsin and chymotrypsin, and from about 1.0 to about 5.0% of a substrate for said enzyme selected from

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the group consisting of gelatin, hydrolyzed gelatin, and collagen.

4. A therapeutic composition comprising an aqueous solution containing about 0.25% of a vasoconstrictor compound selected from the group consisting of ephedrine and its nontoxic water-soluble inorganic salts, from about 10 to about 100 mg. per 100 ml. of solution of a proteolytic enzyme selected from the group consisting of trypsin and chymotrypsin, and from about 1.0 to about 5.0% of a substrate for said enzyme selected from the group consisting of gelatin, hydrolyzed gelatin, and collagen.

5. A therapeutic composition comprising an aqueous solution containing about 1% of a vasoconstrictor compound selected from the group consisting of tuamine and its nontoxic water-soluble inorganic salts, from about 10 to about 100 mg. per ml. of solution of a proteolytic enzyme selected from the group consisting of trypsin and chymotrypsin, and from about 1.0 to about 5.0% of a substrate for said enzyme selected from the group consisting of gelatin, hydrolyzed gelatin, and collagen.

6. A method for preparing an aqueous vasoconstrictor-enzyme preparation exhibiting enzyme activity over a period of at least 5 days, comprising: dissolving from about 1% to about 5% of an enzyme substrate selected from the group consisting of gelatin, hydrolyzed gelatin, and collagen in an aqueous solution containing a minor but effective amount for nasal medication of a vasoconstrictor compound selected from the class consisting of phenylephrine, ephedrine, tuamine, and the nontoxic water-soluble inorganic salts thereof, and then mixing from about 10 to about 100 mg. per ml. of solution of a proteolytic enzyme selected from the group consisting of trypsin and chymotrypsin with the resulting solution.

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