Stabilization of Trypsin in Ointment Bases

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Calcium has been used to stabilize trypsin in a polyethylene glycol ointment base. The samples were kept at room temperature and at 37° for a period of 6 months. Results of assays on these samples are reported. Eighty per cent of the trypsin activity was retained in the presence of calcium, including the samples stored at 37° for 6 months.

THE USE of proteolytic enzymes to remove necrotic tissue often present in burns, amputation stumps, wounds, and many other conditions is becoming more widespread. Incorporation of the enzyme into an ointment base provides a convenient method for use. Packaging is also simplified, since it is less expensive to put the preparation in one container than to package buffer and enzyme separately. An ointment is ready for immediate use, but when separate solutions are provided, the enzyme must be dissolved in the buffer before use.

The stabilization of the enzyme in the ointment base is of the utmost importance. Trypsin has been shown to be stable for several months in a Carbowax base ointment at 4° or less (1). However, at room temperatures proteolytic activity is lost. Calcium^{2+} (2, 3) has been used to stabilize trypsin. By incorporating calcium^{2+} into an ointment base, the proteolytic activity may be preserved for long periods of time at room temperature and at 37°.

EXPERIMENTAL

Ointments were prepared using polyethylene glycol ointment U.S.P. Fifty milligrams of noncrystalline trypsin isolated from beef pancreas was dissolved in one ml. of water and added to 27 Gm. of the ointment. This trypsin assayed 2000 Armour units/mg. Calcium chloride dihydrate was added in 1 ml. of water. After careful mixing of the ointment, the pH was adjusted to 6.7–6.8 using 0.1 *N* sodium hydroxide or 0.1 *N* hydrochloric acid. A pH meter was used for this adjustment. Buffers were not used because their influence on the stability of trypsin is not known. Water was added to bring the weight of each ointment to 30 Gm.

One set of ointments was stored at room temperature, another at 37°, and a third, which contained no calcium, at 3°. Table I lists the amount of calcium chloride dihydrate added and the proteolytic activity retained in the various ointments.

Assays were conducted using the hemoglobin method of Kunitz (4). For the assay of each sample, 2 levels of ointment were run in duplicate. Values given are the average per cent of proteolytic activity retained for each ointment after the time lapse indicated. Results agreed within ±10%. The reference standard was a highly purified crystalline trypsin which assayed 4000 Armour units/mg. It was standardized against tyrosine several times during the experimental work. The ointment kept in the refrigerator was used as the method control sample.

TABLE I.—PER CENT TRYPSIN ACTIVITY RETAINED

Temp., ° C.	Calcium Chloride Dihydrate, mg./30 Gm.	2 wk.	5 wk.	26 wk.
3	0.0	97.0	102.0	98.0
RT	0.0	80.0	71.0	63.0
RT	200.0	100.0	96.0	80.0
RT	400.0	100.0	99.0	83.0
37	0.0	81.0	51.0	32.0
37	200.0	95.0	80.0	80.0
37	400.0	95.0	85.0	82.0

Samples for assay were weighed, dissolved in water, and pipetted into the assay medium. A blank was prepared for both levels of each ointment by adding 5% trichloroacetic acid to the sample immediately after the ointment in water was added to the hemoglobin.

DISCUSSION AND SUMMARY

Results in Table I show that in the presence of calcium, trypsin losses are greatly reduced. There are slightly lower losses with the higher level of calcium, but the method of assay is not accurate enough to make this significant. However, there is definitely a difference between the samples containing calcium and those containing no calcium at both room temperature and at 37° after 5 weeks, or longer.

Twenty per cent loss after 6 months with calcium present is almost within the error of assay. The use of a 10–20% overage in the original preparation of the ointments would bring the results within labeled potency.

A method of stabilization of trypsin in an ointment base has been reported. It may be useful for further studies on stabilizing trypsin or other enzymes in ointments.

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