The combined action was shown to be superior to the separate action of either agent alone. This indicates a potentiation effect between these two agents.

7. The mechanism of action through which powdered corncob, starch, and cellulose accomplish their disintegrating action is most likely the same. These agents swell when in contact with water and will rupture the tablets in which they are incorporated. To swell, however, it would appear that an agent must first absorb considerable amounts of moisture. Powdered corncob, starch, and cellulose-starch combinations were shown to absorb significant amounts of moisture. Cellulose absorbed less moisture than starch and corncob but more moisture than the control.

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Notes

Rapid Visual Assay for Penicillinase Concentrates

By FRANCES W. BOWMAN

Enzyme activity is measured by the time required to solubilize procaine penicillin G.

THE LITERATURE abounds with assay methods for enzyme penicillinase, but most are complicated and time consuming. Among these are the microbial assay of Bowman and Holdowsky (1) and various iodometric titrations (2–4). The manometric (5) and optical rotation (6) procedures are accurate but require special equipment. Pollock (7) suggested a rapid approximate assay based on the time required to decolorize a known quantity of iodine in the presence of sufficient penicillin substrate. Ghosh and Borkar (8) recently described an assay incorporating this idea.

A new and simple assay for penicillinase activity has been developed by using a different approach. Enzyme activity is measured by the time required to solubilize completely 300 mg. crystalline procaine penicillin G in pH 7.0 buffer solution. The procaine salt of penicillin G has a solubility in buffer of about 5 mg./ml.; but as quickly as the soluble portion is converted by the enzyme to penicilloic acid, more penicillin goes into solution until finally it is all dissolved.

No requirements of potency have been established for penicillinase concentrates, but for sterility testing of preparations containing penicillin (9) a solution of the enzyme should have at least 4000 Levy units per ml. One Levy unit of penicillinase inactivates 59.3 units (35.6 mcg. or 10^{-7} moles) of penicillin G in 1 hour when the substrate is in sufficient concentration to maintain a zero-order reaction. The test to be described can measure the penicillinase potency of solutions containing approximately 4000–40,000 Levy u./ml., and within this range the solubilization time is a linear function of the dose.

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EXPERIMENTAL

Reagents.—U.S.P. procaine penicillin G, Clark and Lubs 15% buffer, pH 7.0: potassium dihydrogen phosphate, 150 Gm.; 10 *N* sodium hydroxide, 47.9 ml., *q.s.* to 1 L. with distilled water. Distilled water. Penicillinase solutions. Equilibrate all reagents at 25° before use.

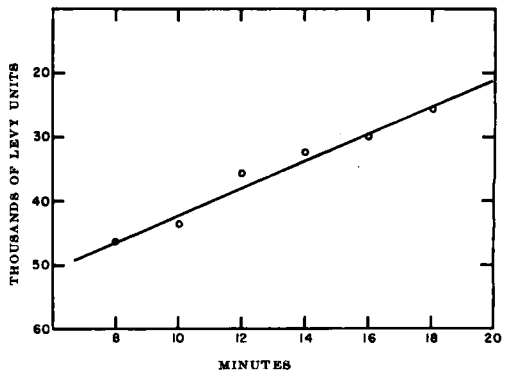

Fig. 1.—Solubilization time for 300 mg. procaine penicillin when tested with various amounts of penicillinase.

TABLE I.—PENICILLINASE ASSAY RESULTS BY TWO METHODS

Levy Units per ml.		Ratio
NaOH Titration Method	Solubilization Method	Col. 2/Col. 1, %
43,296	44,258	102.2
21,075	20,485	97.2
51,676	50,580	98.0
43,415	46,365	106.8
43,836	44,258	101.0
8,430	8,767	104.0
8,430	9,189	109.0
17,703	18,968	107.0
43,346	46,365	106.8
21,075	21,075	100.0
50,580	49,147	97.3
29,505	31,613	107.2
16,860	13,632	81.0
51,170	50,580	98.8
9,273	9,020	97.3
54,205	54,795	101.0
27,145	27,398	101.0
31,613	29,927	94.7
39,452	37,935	96.1
44,848	44,528	99.4
Average		100.1

Assay Procedure.—Suspend 300 mg. of procaine penicillin G in from 9.0–13.0 ml. water in a 20–ml. clear glass bottle with stopper. The volume of water depends on the volume of enzyme sample to be tested. Add 1.0 ml. of the pH 7.0 buffer. Add 1.0–5.0 ml. of the penicillinase test sample to bring the volume to 15.0 ml. and mix. Watch the cloudy suspension and note precisely the time required for it to become completely clear. (A rare small particle or two may be ignored if the solution is substantially clear.) If the time is less than 7 minutes, repeat the test using less enzyme. Samples requiring longer than 20 minutes when 5.0 ml. of enzyme solution is tested contain less than 4000 Levy u./ml. Calculate the Levy units in the sample as follows: Levy units = 63,100–2100 t where t is the solubilization time in minutes. (This relationship is shown graphically in Fig. 1.) Calculate the Levy units per ml. by dividing by the number of ml. of enzyme tested.

Assay of Low Potency Preparations.—Enzymes having very low activity may be assayed by modifying the method. Determine the time required for the enzyme sample to solubilize 300 mg. of procaine penicillin G. A number of hours may be necessary in some cases.

Dilute a previously standardized enzyme concentrate until a dilution is found that requires the same time as the sample to solubilize the same amount of penicillin. Estimate the potency of the unknown from this information. For example, 1.0 ml. of an enzyme solution took 25 hours to solubilize 300 mg. procaine penicillin G. Several dilutions of a stock penicillinase containing 8430 Levy u./ml. were tested by the same method. The dilution requiring 25 hours to solubilize the penicillin contained 422 Levy units per ml. Therefore, it was concluded that the unknown enzyme preparation also contained 422 Levy u./ml.

Application of Assay Method.—A number of penicillinase preparations were tested by the rapid visual method and by a sodium hydroxide titration method (10). The latter is based on the principle that penicilloic acid formed by penicillinase acting on the substrate penicillin may be titrated with sodium hydroxide (11). A solution of soluble sodium or potassium penicillin is adjusted to pH 7.0, the penicillinase (also adjusted to pH 7.0) added, and 0.1 N sodium hydroxide solution added dropwise from a buret, with stirring, to maintain pH 7.0. The reaction is allowed to proceed for 25 minutes and the rate of penicillin destruction calculated from the amount of sodium hydroxide consumed. The rate of inactivation is used to calculate the Levy units.

Results.—As shown in Table I the assays of penicillinase activity by the rapid visual method compare favorably with the results obtained by the sodium hydroxide titration method.

CONCLUSIONS

A simple rapid visual method for determining the enzyme activity of penicillinase concentrates has been presented. The method can be modified to get an approximate assay of low titer preparations. The procedure for enzyme concentrates is especially useful in screening enzyme yields during production runs. It also can be used to estimate the amount of a given enzyme solution to be used in various laboratory tests. When a more precise method with a limited working range is to be used to assign enzyme potency, this method may serve as a preliminary test to establish the approximate activity.

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