Stability of Crystalline Sodium Penicillin G

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A study of solution stability of crystalline preparations of penicillin G indicated that pH and potency dropped rapidly when such solutions (5,000 units/ml.) were stored at either 15° or 24°C.; this behavior is in sharp contrast to the greater stability of earlier, cruder preparations. As this was probably due to the removal during the crystalline process of certain impurities having buffering capacity, a study was initiated to find some substance which would protect the solutions of crystalline penicillin without detriment to the stability of the dry powder when stored in open containers at 100°C.

TABLE 1

Buffer	5,000 units/ml. solution at 24°C.—potency losses*							Solid at 100°C.
	Initial pH	4 days		5 days		7 days		6 days
		% loss	pH	% loss	pH	% loss	pH	% loss
Sodium phosphate	7.2			12.7	6.95			98.6†
" bicarbonate	7.0			33.5	5.1			41.0†
" oleate	7.0			12.4	6.5			100.0†
" acetate	6.7			13.2	5.7			42.0†
" borate	6.7			65.5	4.6			100.0†
" succinate	6.6			21.2	5.8			97.2†
" tartrate	6.6			50.8	4.8			100.0†
" citrate	7.0			8.5	6.5			100.0†
" sulfanilate	5.2	78.4	4.9					7.8
" phthalate	6.1	35.1	4.9			73.7	4.8	1.4
" mandelate	6.1	71.0	4.5					6.3
" benzoate	5.7	60.4	4.6					8.1
" nicotinate	6.0	27.2	5.0			62.1	4.8	10.4
" salicylate	5.9	83.4	4.5					19.6
" sulfamate	6.0	85.7	4.6					18.0
" tartrate	6.1	71.1	4.6					23.9
" gluconate	6.0	81.4	4.5					20.0
" succinate	6.4	7.4	5.3			26.2	5.2	16.8

* Based on values obtained by the spectrophotometric method outlined by R. M. Herriott (*J. biol. Chem.*, 1946, **164**, 725–736).

† Samples were dried in such a manner as to yield amorphous mixtures; the others are all crystalline.

Table 1 indicates the results obtained using various buffers to the extent of 5 per cent by weight of the crystalline sodium penicillin co-dried to yield in some cases a crystalline mixture and in others an amorphous mixture, depending upon the procedure employed in the drying process.

For a more detailed study we chose sodium bicarbonate, sodium acetate, sodium succinate, and sodium citrate. The effect of temperature of storage, buffer concentrations, and starting batch material are clearly indicated in Table 2.

It appears that about 5 per cent by weight of penicillin of the buffers studied will protect solutions of crystalline penicillin for at least 4 days at 24°C. and for at least 7 days at 15°C. Those solutions which contain 1 per cent or less buffer exhibit lack of stability comparable to unbuffered solutions, losing over 50 per cent at 24°C. in 4 days.

When applied to plant batches, the average loss in potency[1] of solutions stored at 15°C. for 7 days for 10 unbuffered batches is 26 per cent, while the average loss of potency in these batches buffered with sodium citrate under the same conditions of storage is only 1 per cent.

[1] Based on values obtained by the iodometric method outlined by Joseph F. Alicino (*Ind. eng. Chem.* (Anal. ed.), 1946, **18**, 619).

TABLE 2

Buffer	Batch No.	Conc. buffer (%)	5,000 units/ml. solution—potency losses					Solid at 100°C.
			Initial pH	4 days—24°C.		7 days—15°C.		6 days
				% loss	pH	% loss	pH	% loss
Sodium bicarbonate	1	4.5	7.4	9.3	6.7	3.2	7.2	0.6
		2.25	7.1	10.8	5.8	9.7	6.1	0.1
		0.90	6.8	31.4	4.9	6.8	5.7	0.3
		0.43	6.4	58.9	4.5	15.2	4.8	2.1
Sodium acetate	1	10.0	6.4	3.4	5.7	0.3	5.9	5.0
		5.0	6.5	4.0	5.7	5.3	5.7	8.8
		1.0	6.3	43.4	4.7	7.4	5.3	6.8
		0.1	6.1	76.0	4.4	38.5	4.8	6.0
" succinate	1	10.0	6.3	4.2	5.9	1.7	5.6	3.5
		5.0	6.2	3.4	5.4	11.3	5.7	6.0
		1.0	6.0	62.0	4.6	19.3	5.3	7.0
		0.1	5.8	83.0	4.6	40.5	4.9	7.1
" citrate	1	10.0	6.6	+4.3	5.7	+1.6	6.4	0.3
		5.0	6.6	+6.3	5.3	2.1	6.1	+0.1
		1.0	6.5	65.1	4.4	2.2	5.2	5.9
		0.1	6.2	81.0	4.4	43.9	4.7	8.0
" citrate	2	5.0	6.5	6.2	5.2	+2.3	5.9	4.4
	3	5.0	6.6	+4.0	5.7	+1.3	6.1	14.6
	4	5.0	6.7	+10.1	5.6	1.3	6.0	1.1
	5	5.0	6.5	13.5	4.7	+0.3	5.8	2.4
	6	5.0	6.6	2.3	5.6	2.7	6.2	15.4

From the results obtained it would appear that the decomposition of sodium penicillin in solution is autocatalytic and that, once decomposition starts, it is difficult to control. The rate of inactivation increases rapidly with increases in temperature.

An Operative Approach to the Treatment of *Schistosomiasis mansoni* Infections

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With the finding of a new method for the simple removal of adult *Sch. mansoni* from experimentally infected animals (*1*), a series of interesting observations were made, among which were the following:

(1) The use of heparin simplified the removal of the adult worms from the blood vessels of the experimentally infected animal.

(2) The number of adult worms recovered from the portal vein proper was far greater in animals heparinized before death than in those that had not been heparinized.

(3) A striking number of adult *Sch. mansoni* could be recovered from the livers of animals heparinized before death,

as compared with animals that had not been heparinized.

(4) It is possible to aspirate the portal vein of an heparinized rabbit and keep the animal alive.

The experience gained from working out the procedure for the removal of adult worms served as a basis for an attempt at applying a similar procedure to animals as a therapeutic measure. Thus, a group of animals infected with *Sch. mansoni* were subjected to portal vein aspiration under operating room technique. Five rabbits and two monkeys were operated on by the author and collaborators.[1] All animals received intravenous amytal anesthesia.

From these preliminary experiments a number of observations have been made and therapeutic possibilities envisioned:

(1) In monkeys with infections that closely simulate *Sch. mansoni* infections in humans, it appears entirely possible to aspirate the portal vein of the heparinized monkey and keep the animal alive.

(2) The lighter the infection in monkeys (and the more closely it approximates the mild human variety), the greater the percentage of recovery of adult worms, as determined by post-mortem examination when the animal was sacrificed following surgery.

(3) If heparin is effective in mobilizing the adult worms and localizing them in the portal vein and liver, it may be possible first to use heparin systemically and then anthelmintics directly into the portal vein to produce a large concentration of the drug where it could have a maximum effect on the offending worms.

Reference

1. Brandt, J. L., and Finch, E. P. *Proc. Soc. exp. Biol. Med.*, 1946, 61, 22-23.

Radiographs With C^{14}

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Since the discovery of C^{14} (*3*) there has been a temptation to use it to study the distribution of any desired organic substance in a chemical or biological system. To do this it was necessary only to incorporate C^{14} into the organic molecule and then prepare radiographs in the manner originally described by Marie Curie in her classical experiments with radium (*1*).

Unfortunately, the radiation from C^{14} is one of the weakest known. The β-particles emitted have an energy of only 90 kv., and the half-absorption thickness in aluminum is only 2.6 mg./cm.^{2}, or 0.01 mm. In this respect these rays are very similar to the β-rays of actinium, which have a half-thickness of 2.3 mg./cm.^{2} of aluminum and which, because of their weakness, have eluded detection until only recently (*2*). Thus, when C^{14} was available only from cyclotronic sources, it seemed hopeless to attempt to radiograph it. Now that strong sources are available from the Oak Ridge uranium pile, the situation has changed.

[1] Dr. S. B. Beaser, Boston, Massachusetts, and Maj. Richard Upjohn (MC), AUS.

Below we show some C^{14} radiographs obtained in some of our orienting experiments. In all our tests (except the very latest) 35-mm. Eastman "Super XX" film (panchromatic type B) was used. It was developed for 25 minutes at 20° C.

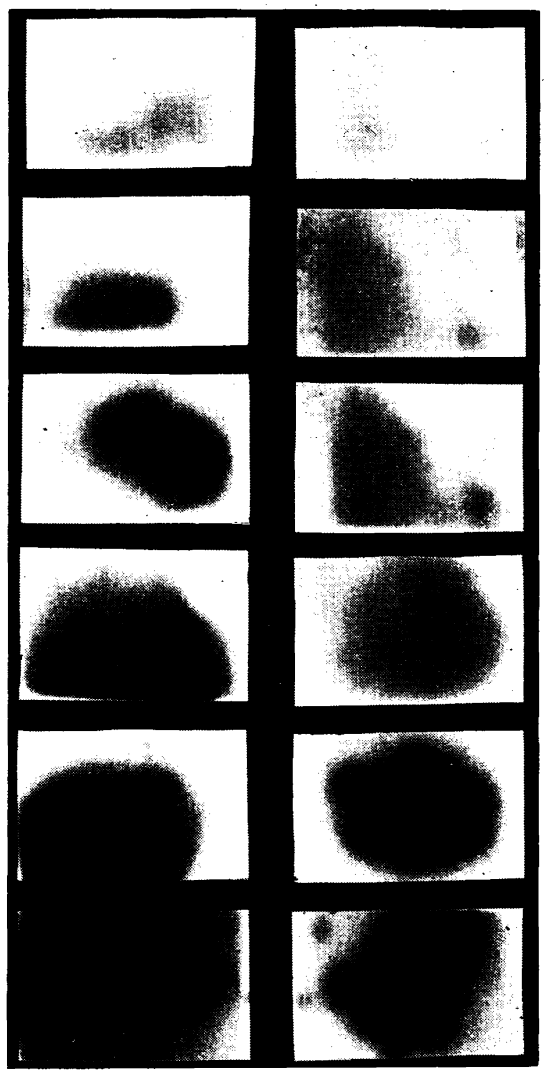

Fig. 1. Direct contact prints made from photographic film exposed to barium-1-radioacetate (left) and 9-radiodibenzanthracene (right).

in a tank, with intermittent agitation, using Eastman developer DK-20. The resolving power under these conditions is about 60 lines/mm.

In the first two series, shown in Fig. 1, about 30 mg. of barium-1-radioacetate and 9-radiodibenzanthracene crystals were placed directly in contact with the emulsion side of the film for varying periods of time. The specific activities of these compounds were, respectively, 266,000 and 3,020 disintegrations/minute and 1 mg. carbon. The time of irradiation, weight of substance, and integrated amount of irradiation are given in Table 1.

By studying Fig. 1 (actually, the original films show much