Storage life of prostaglandin E_2 in ethanol and saline

Few reports on the stability of prostaglandins in solution have been published. Karim, Devlin & Hillier (1968) measured the biological activity of saline solutions containing 100 ng/ml E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ kept at various pH values at room temperature. The PGE compounds showed 25–40% loss of biological activity after 60 days at pH 5–7, whilst the PGF compounds were still fully active after 182 days. Andersen (1969) reported 5–20% conversion to PGA_1, measured spectrophotometrically, in methanolic solutions of PGE_1 after one month at 5–10°. Now that the compounds are undergoing clinical trials, a more detailed investigation of their stability in different vehicles is necessary.

Ampoules containing 390 μg PGE_2 in 1 ml of 95% ethanol have been assayed spectrophotometrically at intervals over six months by measuring the chromophore developing at 278 nm after alkali treatment (Nugteren, Beerthuis & van Dorp, 1966). Both PGE_2, which does not itself absorb in the ultraviolet region, and PGA_2 (λ_{max} MeOH 217 nm) are converted to PGB_2 (λ_{max} MeOH 278 nm) on treatment with alkali. This assay therefore gives a measure of PGE_2 and of any PGA_2 formed by spontaneous dehydration.

After 4 weeks at 4° there was no detectable loss of total $E_2 + A_2$ and after six months at $-20°$ <10% loss had occurred. At no time did the loss in potency of any individual ampoule exceed 11%. The average loss measured during this period was 5–6%. One ampoule which had been stored at $-20°$ for 24 weeks was analysed by thin-layer chromatography on a silica gel G plate developed in chloroform–methanol–acetic acid–water (90:8:1:0·8). Pure PGE_2 and PGA_2 were run as markers; they were visualized by spraying with phosphomolybdic acid in ethanol and warming. The bands corresponding to PGE_2 and PGA_2 in the test sample were eluted and assayed by spectrophotometry. 378 μg (97%) was present in the PGE band and approximately 13 μg (3%) was present in the PGA band. One further ampoule stored as above was dried, taken up in saline and assayed for biological activity on the rat fundus strip against a fresh solution of PGE_2. This showed 75% potency.

For intravenous infusion, the above ampoules are usually diluted with sterile isotonic saline (pH 5–6) to give solutions containing 7·5 μg PGE_2/ml or 5 μg PGE_2/ml which are kept at 4°. Attempts were made to assess the storage life of these saline solutions. Aqueous solutions of PGE_2 show a chromophore with λ_{max} 283 nm after alkali treatment, compared with 278 nm in ethanolic solution. ϵ_{max} is also lower and takes longer to develop under aqueous conditions; conversion to PGB is incomplete (Nugteren & van Dorp, personal communication). Direct measurement of PGE content by alkali treatment and spectrophotometric measurement of the developing chromophore gives anomolous results in saline solutions, so that solutions of PGE_2 in saline were acidified to pH 3 with 2M citric acid, extracted with 2 volumes of diethyl ether and the dried extract dissolved in methanol. The methanolic solution was assayed by spectrophotometry and an aliquot analysed by thin-layer chromatography as described earlier. After ten days at 4°, 10% of the prostaglandin recovered from a 100 μg/ml solution was present as PGA. The biological activity of this solution was 70–80% (Table 1). After 6 months' storage under these conditions, one third of the prostaglandin recovered from a 7·5 μg/ml solution following extraction and thin-layer chromatography was present as PGA. Bioassay of this solution showed 50% potency. An unidentified chromophore (λ_{max} NaCl 256 nm, λ_{max} 0·5N KOH 296 nm) was separated from PGE and PGA by thin-layer chromatography and isolated.

The discrepancy between the results obtained by spectrophotometry and those from bioassay arises possibly because the spectrophotometric method measures μg quanti-

Table 1. *Composition and biological potency of solutions of PGE_2 after storage* (All values are quoted with reference to original concentrations.) The biological activity was determined on the rat fundus strip. Saline solutions of PGE_2 were extracted before spectrophotometric assay.

Concn µg/ml	Storage conditions: Vehicle	°C	Days	Biological potency (%) (approximate)	Spectrophotometric assay % PGE + PGA: % Total (PGE + PGA)	After t.l.c.: PGE	After t.l.c.: PGA
390	95% ethanol	−20	182	75	94	97	3
7·5	0·9% NaCl	4	182	50	68	54	19
						34	10
100	0·9% NaCl	4	10	75	—	43	5

ties of pure compounds, whereas bioassay measures biological activity (in ng) rather than chemical composition. Bioassay is of value, however, since it indicates that after storage in ethanol, most of the biological activity remains.

It therefore appears that the potency of PGE solutions for clinical use deteriorates on storage in saline solution. This can be prevented by preparing these compounds as concentrated alcoholic solutions in ampoules. These can be kept for long periods at −20° and diluted with sterile isotonic saline as required within 24 h of use. Similar conclusions about the stability and storage of PGE compounds have been reached by Dr. D. H. Nugteren and Professor D. A. van Dorp (personal communication), who recommend that PGE_2 can be kept in ethanolic solutions for 1 year at −15° and in aqueous solution for a maximum of 1 week at 0° or 1 month at −15°.

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