Study of the Degradation of Dexamethasone in Certain Pharmaceutical Preparations

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A chromatographic separation of the decomposition products of dexamethasone in tablet form was achieved by using thin-layer chromatography. Spectrophotometric and quantitative colorimetric analyses were run. A stable tablet formula has been suggested.

THE ANTI-INFLAMATORY corticosteroids represented by hydrocortisone and related synthetic analogs have gained an unchallenged position. The therapeutic effects of many steroids depend on their stability (1). It has been found that dexamethasone (Δ'9-fluoro-11β,17α,21-trihydroxy-16-methyl pregna-1,4-diene-3,20-dione) should be protected from light (2) and it has been proved that 50% oxidation of the α-ketol side chain occurs within 6–8 min. in the presence of a base catalyst (3). Dexamethasone has a marked oral potency in range of about 30 times that of hydrocortisone (4–7). The high oral potency is attributed to the protection from metabolic inactivation of the 17,21-dihydroxyacetone side chain by steric hindrance of 16-methyl substituents (8, 9). Identification and investigation of 16-methyl-9-fluoroprednisolone was reported to be carried out by infrared, ultraviolet spectra and triphenyltetrazolium chloride color reaction (10). It has been observed that the direct spectrophotometric analysis of a methanolic extract of dexamethasone tablets yield higher results than the triphenyltetrazolium chloride color assay on aged tablets. A study of the methods of analysis, degradation, and stability of dexamethasone tablets, leads to the finding of a stable tablet formula.

EXPERIMENTAL

Materials—(*a*) Dexamethasone (Roussel). The sample was purified by recrystallization from ether. Purity was confirmed by physicochemical means. m.p. 263°, infrared, ultraviolet absorption. (*b*) Polyvinylpyrrolidone (Kollidon-25, Badische Anilin and Soda Fabrik). (*c*) 2,3,5-Triphenyl tetrazolium chloride (Reanal-Hungary). (*d*) Talc, lactose, starch, magnesium stearate, Cellulose acetate phthalate, carboxymethylcellulose, acetone, ethyl alcohol, methyl alcohol, chloroform, sulfuric acid, Silica Gel G, all from the British Drug House and of analytical grade.

Procedure—*Thin-Layer Chromatography*—Methanolic and chloroformic extracts of dexamethasone tablets were spotted beside methanolic and chloroformic solutions of authentic sample (NF XII) of dexamethasone on 0.3 mm. thick Silica Gel G plates. The plates were developed with chloroform–methanol mixture (9:1). The plates were dried, sprayed either with triphenyltetrazolium chloride reagent (11) (Fig. 1) or sulfuric acid 50% in methanol, and then heated at 80° for 10 min. (12) (Fig. 2). The results obtained are illustrated in Figs. 1 and 2.

From Figs. 1 and 2 it is clear that the methanolic extract of the tablets showed two spots with sulfuric acid reagent; one of them is identical with the spot obtained from the chloroformic extract. On using the triphenyltetrazolium chloride reagent the methanolic extract gave only one spot which was found to be identical with that obtained by the chloroformic extract of the tablets and those of the authentic sample. Hence the lower spot proved to be nonextractable with chloroform.

Quantitative Determination of Standard Dexamethasone, Corresponding Spots, and Its Degradation Products—Dexamethasone authentic sample was assayed by direct ultraviolet absorption at 240 mμ as well as colorimetrically following the official

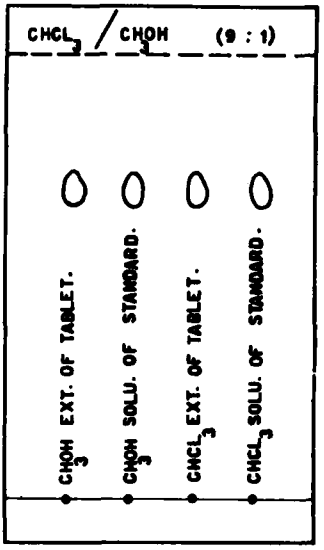

Fig. 1—Dexamethasone chromatograms sprayed with tetrazolium chloride reagent.

Received August 23, 1967, from the Drug Research and Control Center, The Egyptian Organization For Pharmaceuticals, Chemicals and Medical Appliances, Cairo, U.A.R.
Accepted for publication November 7, 1967.
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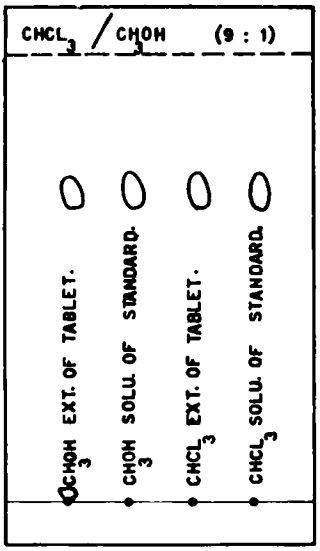

Fig. 2—Dexamethasone chromatograms sprayed with sulfuric acid reagent.

method (13). The tablets were similarly treated when the direct spectrophotometric determination for a methanolic extract gave higher results than the colorimetric method. The chloroformic extract of the same batch of tablets gave the same result as the colorimetric method when assayed spectrophotometrically. Elution of the spots corresponding to the authentic dexamethasone was effected from the plates with methanol. The methanolic eluate was assayed spectrophotometrically for its dexamethasone content. At the same time, the eluate of the lower spot was similarly assayed.

The results obtained are shown in Table I.

Stability of Dexamethasone in Tablet—Preparation of the tablets: four different samples of dexamethasone tablets of the following general formula were prepared: dexamethasone, 0.5 mg., lactose, 88 mg., starch, 15 mg., talc, 3 mg., magnesium stearate, 1.5 mg.

Sample 1: Prepared by the slugging method (14). Sample 2: Prepared by the wet granulation method (16) using 20% solution of polyvinylpyrrolidone in alcohol as a binding agent. Sample 3: Prepared by the wet granulation method using 3% solution of cellulose acetate phthalate in acetone as a binding agent. Sample 4: Prepared by the wet granulation method using 3% mucilage of carboxymethylcellulose in water as a binding agent.

An amount of lactose equivalent to the polyvinylpyrrolidone, cellulose acetate phthalate and carboxymethylcellulose used was reduced from the weight of lactose in the general formula. All tablets were compressed to give a hardness of 3 kg. using a single-punch Korsch machine.

Quantitative Analysis—The four mentioned tablets were assayed using the official colorimetric method every 20 days. Shelf storage at 25° at 60% relative humidity, temperature of 40°, 60°, and humidity of 75% and 100% at 25° temperature were chosen as different factors to compare their stability so as to choose the best tablet formula for making the most stable tablet.

TABLE I—PERCENTAGE OF DEXAMETHASONE IN DIFFERENT ELUATES

Eluate	Ultraviolet Absorption at 240 μ	Dexamethasone, %
Methanolic dexamethasone standard	1.100	100
Methanolic extract of tablets[a]	1.290	117
Methanolic extract of upper spots	0.840	76.3
Methanolic extract of lower spots	0.458	39.09

[a] Direct methanolic extract from tablets.

The results obtained are shown in Table II.

Rate of Degradation—The rates of degradation of the four tablets were found to be a pseudo first-order reaction. Figure 3 shows the relation between the log concentration of dexamethasone and time. The rate of K_{25} and the half-life (16) of the four tablets are shown in Table III.

Observations—(*a*) The high result in the total methanolic tablet extract is due to the decomposition product which absorbs heavily at that wavelength. (*b*) The summation of the absorption of the two spots is equal to the absorption of the direct methanolic extract.

RESULTS AND DISCUSSION

According to the chromatographic analysis the dexamethasone tablet, prepared either by the slugging or the wet granulation method, on aging even at room temperature (shelf) suffered from cleavage of the α-ketol side chain group. This was indicated by the fact that the lower spot was only visible with the sulfuric acid reagent and gave no color with the triphenyltetrazolium chloride reagent which is specific for this group. Hence the direct spectophotmetric determination of a methanolic extract gave higher results than the colorimetric method.

The tablets formulated with polyvinylpyrrolidone as a binding agent showed the greater stability against different humidity and temperature factors and the longest half-life time.

SUMMARY

(*a*) Tablets formulated by slugging or wet

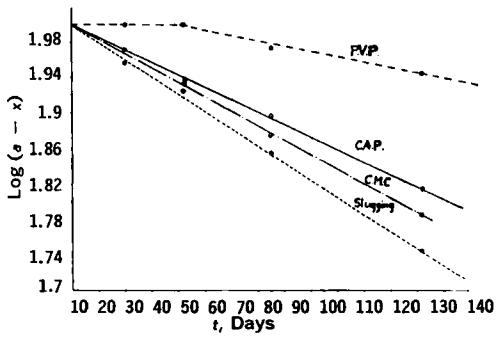

Fig. 3—Relation between the logarithmic concentration of dexamethasone in tablets using different binding agents and time.

TABLE II—STABILITY OF DEXAMETHASONE TABLET AT DIFFERENT TEMPERATURES AND HUMIDITY

Sample No.	Time/Day	Dexamethasone % Shelf[a]	100% Humidity[b]	75% Humidity[b]	40° C.[c]	60° C.[c]
1	0	100				
	20	90.6	85.3	83.9	100	92.3
	42	83.9	55.9	67.1	97.9	92.3
	77	72.6	52.4	55.9	90.9	81.8
	133	55.9				
2	0	100				
	20	99.8	96.4	95.2	95.5	94.6
	42	99.8	94.6	95.2	94.6	94.6
	77	93.8	92.1	93.8	94.6	93.8
	133	88.1				
3	0	100				
	20	93.5	93.5	92.0	79.9	79.9
	42	86.5	78.4	79.9	79.9	76.1
	77	79.3	61.3	74.6	75.3	67.8
	133	65.8				
4	0	100				
	20	94	98.4	87.5	96.9	90.7
	42	86.7	76.9	77.5	90.7	84.6
	77	75.4	61.5	92.4	78.4	69.2
	133	61.4				

[a] at 25° and 60% relative humidity. [b] at 25°. [c] 60% relative humidity.

TABLE III—HALF-LIFE OF THE FOUR DEXAMETHASONE TABLETS

Sample No.	Time/Day	$(a-x)$ %[a]	Log $(a-x)$[a]	Log $\left(\frac{a}{a-x}\right)$[a]	K_{2s}[a]	Half-life, Days
1	0	100	2.0	0.0	0.00436	159
	20	90.6	1.9571	0.0429	0.00436	159
	42	83.9	1.9238	0.0762	0.00436	159
	77	72.0	1.8573	0.1427	0.00436	159
	133	55.9	1.7474	0.2526	0.00436	159
2	0	100	2.0	0.0	0.00139	542
	20	100	2.0	0.0	0.00139	542
	42	100	2.0	0.0	0.00139	542
	77	93.8	1.9722	0.0278	0.00139	542
	133	88.1	1.9450	0.0550	0.00139	542
3	0	100	2.0	0.0	0.00314	220
	20	93.5	1.9708	0.0292	0.00314	220
	42	86.5	1.9370	0.0630	0.00314	220
	77	79.3	1.8993	0.1007	0.00314	220
	133	65.8	1.8182	0.1818	0.00314	220
4	0	100	2.0	0.0	0.00366	189
	20	94	1.9731	0.0269	0.00366	189
	42	86.7	1.9380	0.0620	0.00366	189
	77	75.4	1.8774	0.1226	0.00366	189
	133	61.4	1.7882	0.2118	0.00366	189

[a] a = initial concentration %, x = degradation %, K_{2s} = integrated rate equation.

granulation method and different binding agent except with polyvinylpyrrolidone showed instability toward different humidity and temperature factors.

(*b*) The TLC analysis of the methanolic extract of the tablets showed two spots; one of them is identical with the authentic dexamethasone spot. Meanwhile the other spot could not be localized on using triphenyltetrazolium chloride reagent as a spray which indicates the cleavage of the keto side-chain group.

(*c*) Direct spectrophotometric determination of different degradation products separated by TLC or direct extraction from tablets confirmed the colorimetric assay.

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Keyphrases

Dexamethasone tablets—stability
Degradation rate—dexamethasone tablets
Half-life—dexamethasone tablets
Thick-layer chromatography—separation
TLC—analysis
UV spectrophotometry—analysis
Colorimetric analysis

Problem in the Estimation of Drugs in Biologic Tissue
Recovery of Phenol Red from the GI Tract

By STUART FELDMAN and MILO GIBALDI

Recovery of phenol red from homogenates of rat intestinal tissue, using aqueous extraction procedures, was near quantitative. However, significantly poorer recovery was observed after incubation of phenol red with intact intestinal sacs. It appears likely that phenol red binds to tissue or mucosa when in intimate contact, and the bound material is relatively resistant to aqueous extraction. The relevance of this phenomenon to tissue assays is considered.

THE CLASSIC approach to the determination of drug concentration in tissue involves the extraction of the compounds from homogenates. The general principles of this approach have been set forth by Brodie *et al.* (1). Accordingly, experimental standards are based on the recoveries of known amounts of drug from tissue homogenates. The assumption is made that the extent of recovery of standard quantities from the homogenate is equivalent to the recovery from *in vivo* tissue after drug uptake. The authors have recently observed anomalies in the recovery of phenol red from the gastrointestinal tracts of intact rats, rat intestinal sacs, and tissue homogenates, which suggest that the usual assumption of equivalent recovery may lead to significant error.

EXPERIMENTAL

Materials—Phenol red, trichloroacetic acid, and sodium hydroxide were obtained from Fisher Scientific Company as certified reagent grade.

Methods—Male Sprague-Dawley descent rats (Blue Spruce Farms, Altamont, N. Y.) weighing 140–180 Gm. were fasted 24 hr. The animals were then sacrified by decapitation, and the stomach and small intestine were removed. The small intestine was divided into three segments of equal length.

Tissue Homogenates—The stomach and each intestinal segment were homogenized individually for 5 min. in an Eberbach homogenizer with a minimum quantity of distilled water (approximately 3 ml.). The homogenates were placed in Nalgene tubes and 0.5 ml. of a 70 mg. % phenol red solution was added to each tube. The tubes were then agitated slowly for 1 hr. at 37° in a gyrotory water bath shaker.

Gastric Pouch and Intestinal Sacs—Each segment was ligated at both ends and 0.5 ml. of a 70 mg. % phenol red solution was injected in the pouch or sac through the ligated end by means of a 1-ml. tuberculin syringe and blunt needle. No loss of phenol red was observed through the ligatures.

Each segment was then placed in 20 ml. of Ringer's solution in a culture tube. The tubes were agitated for 1 hr. at 37°. The sac or pouch was then removed and homogenized as described above. The serosal fluid was also retained for assay.

Assay—The assay procedure was essentially that of Reynell and Spray (2). Each tissue segment (or homogenate thereof) was homogenized for 5 min. with 1 ml. of 1 *N* sodium hydroxide and about 5 ml. of distilled water. The resulting homogenate was brought to 30 ml. volume with distilled water, centrifuged, and filtered through a Büchner funnel. A 10-ml. aliquot was taken and 1 ml. of a 30% w/v trichloroacetic acid solution was added to the aliquot to precipitate proteins. After centrifugation 5 ml. of the supernatant was removed and 1 ml. of a 1 *N* sodium hydroxide solution was added to develop the color to maximum intensity. The solution was further diluted, passed through a Millipore filter (0.45 μ pore size), and assayed with a Beckman DB-G spectrophotometer at 560 mμ.

RESULTS

Table I shows the recovery of phenol red after addition to homogenates of various gastrointestinal segments. Recovery ranged from 93% to 103% with little difference between homogenates of different segments. The average recovery of phenol

Received February 1, 1968, from the Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214
Accepted for publication March 8, 1968.
This work was supported by grant 50-1077A from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service, Bethesda, Md.