Sustained-Release Formulation of Prednisolone Administered Orally to Man

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Abstract □ A new method of formulation of prednisolone phosphate into a sustained-release tablet is presented. The sustained-release tablet consists of a porous plastic matrix in which the corticosteroid is dispersed. In the gastrointestinal tract, the active ingredient is gradually leached from the matrix and absorbed systemically. The small plastic skeleton is insoluble and is passed with the feces. *In vitro* experiments demonstrated the prolonged action of prednisolone phosphate in this form, and in man the sustained-release formulation gives a more uniform blood level of prednisolone over a longer time than prednisolone alcohol in conventional compressed tablets. The evidence for this is based on plasma prednisolone estimations and reduction of plasma cortisol in normal subjects. The sustained-release formulation also avoids high peaks of plasma prednisolone. Collection of matrixes from the feces showed that the bulk of the administered dose was released into the gastrointestinal tract.

Keyphrases □ Prednisolone phosphate sustained-release tablets—formulation □ Polyvinyl chloride matrix—prednisolone phosphate tablets □ Plasma levels—prednisolone phosphate sustained-release, nonsustained-release tablets □ Adrenocortical suppression—prednisolone phosphate dosage form comparison □ UV spectrophotometry—analysis

The therapeutic efficacy of corticosteroids has been established by years of clinical experience. The potential hazards of corticosteroid therapy are well known and well documented; they occur with prolonged usage, usually at high dosage. Numerous other corticosteroids have been developed, giving equal therapeutic effects with reduced dosage. However, to a large extent, prednisolone, which is one of the earliest and simplest derivatives of cortisol, has remained a standard corticosteroid used when oral dosage is required.

Treatment with prednisolone requires the administration of frequent doses, which can lead to peaking of plasma steroid concentrations and uneven blood levels of the steroid throughout the treatment. Therefore, a study was made of the effects in healthy volunteer subjects of a new sustained-release formulation of prednisolone, with particular reference to plasma prednisolone levels and to effects on the pituitary–adrenal axis as shown by reduction of plasma cortisol levels. The degree and duration of these effects produced by administration of the sustained-release formulation of prednisolone were compared with similar criteria after administration of the same amounts of prednisolone as conventional compressed tablets.

EXPERIMENTAL

Materials—*Sustained-Release Formulation*—The principle of the sustained-release formulation[1] is that the water-soluble prednisolone disodium phosphate is dispersed within a polyvinyl chloride framework or mesh in a compressed tablet. On contact with the gastrointestinal secretions, the prednisolone is gradually leached from the pores of the matrix and is absorbed systemically. The small plastic skeleton of the tablet is insoluble and is passed with the feces. This method of sustained release has the advantage that the formulation can be modified to provide various release patterns in order to achieve the optimal pharmacological action of the water-soluble active ingredient.

[1] The Duretter *R* sustained-release formulation is the invention of A. B. Hässle, Göteborg, Sweden, in collaboration with the Galenical Department of the Royal Pharmaceutical Institute, Stockholm, Sweden.

Table I—Sustained-Release Prednisolone Tablet Formulations

Composition	Formulation A (Milligrams per Tablet)	Formulation B (Milligrams per Tablet)	Formulation C (Milligrams per Tablet)
Prednisolone disodium phosphate BP (equivalent to 7.5 mg. of anhydrous prednisolone alcohol)	10.5	10.5	10.5
Insoluble tablet matrix	193.0	290.2	368.0
Binding agent	26.0	31.9	60.0
Lubricant	0.5	2.4	2.0
Solubilizing agent	20.0	—	—
Tablet weight	250.0	335.0	440.5

The particles of polyvinyl chloride are held together for compression by a binding agent. The porosity of the tablet matrix, upon which the rate of release of prednisolone depends, may be increased by the addition to the formula of a soluble substance. In the present work, sucrose was used.

The general principles involved in the sustained-release formulation have been described (1–5), and this method of achieving sustained release has been used in a number of experimental and proprietary tablets (6–8).

Volunteer Subjects—Ten healthy volunteers took part in one or more of the *in vivo* studies (tablet egestion, plasma prednisolone, and cortisol estimations). Seven of these subjects were male, age 22–42 years, and weighed 64–93 kg.; three were female, age 22–38 years, and weighed 64–82 kg. All tests were carried out under medical supervision.

Methods—*Measurement of* In Vitro *Release of Prednisolone from Sustained-Release Tablets*—For each tablet formulation, 20 tablets were placed in a 100-ml. beaker of 5.5-cm. diameter; 35 ml. of distilled water was added at 37°. The contents were stirred gently so that the tablets were continuously in motion. Temperature was maintained at 37 ± 1°. After 1 hr. ± 1 min., the liquid was transferred into a 100-ml. volumetric flask. The tablets were washed twice with separate 5-ml. quantities of distilled water and the washings were transferred to the flask. The prednisolone content of the solution was determined by UV spectrophotometry. A further volume of 35 ml. of distilled water at 37° was added to the tablets in the beaker, and the procedure was repeated after intervals of 1 hr. for 12 hr. and thereafter at 24 and 30 hr.

Measurement of Prednisolone Remaining in Egested Sustained-Release Tablets—The matrix of the sustained-release tablet was composed of polyvinyl chloride and did not disintegrate in the intestine. Tablet matrixes, therefore, were collected in the feces, washed, and assayed for residual prednisolone content by UV spectrophotometry after each individual tablet was ground up.

Table II—*In Vitro* Release of Prednisolone from Sustained-Release Tablet Formulations

Formulation	Cumulative Percentage Prednisolone Released 1 hr.	2 hr.	4 hr.	6 hr.
A	42.4	60.3	82.6	95.1
B	32.0	45.2	61.2	72.6
C	6.2	13.4	29.7	44.3

Table III—*In Vivo* Release of Prednisolone from Sustained-Release Tablets as Indicated by Percentage of Prednisolone Content Retained in Tablet Matrix

	Formulation A, Subjects				Formulation B, Subjects				Formulation C, Subject
	1	2	3	4	1	2	3	4	4
Percentage prednisolone content retained in sustained-release tablet matrix	3.9	10.8	21.5	27.3	14.9	28.5	33.0	52.4	60.3
	5.7	9.3	22.5	36.0	14.4	15.5	29.0	55.3	84.0
	3.5	7.2	7.1	12.0	11.7	N.C.	28.0	56.5	75.6
	5.1	8.3	8.3	17.3	13.6	N.C.	35.0	44.5	69.1
	3.5	6.1	6.9	14.1	13.7	N.C.	43.0	56.7	N.C.
	3.9	10.3	8.9	32.4	13.9	N.C.	42.5	N.C.	N.C.
	3.1	N.C.[a]	N.C.	N.C.	15.3	N.C.	34.0	N.C.	N.C.
	3.6	N.C.	N.C.	N.C.	15.5	N.C.	30.0	N.C.	N.C.
					12.9	N.C.	N.C.	N.C.	N.C.
					17.7	N.C.	N.C.	N.C.	N.C.
					13.3	N.C.	N.C.	N.C.	N.C.
					15.6	N.C.	N.C.	N.C.	N.C.
Mean	4.0	8.7	12.5	23.2	14.4	22.0	34.3	53.1	72.5
±*SE*	0.31	0.46	3.01	4.12	0.46	—	2.02	2.27	—
Number of tablets taken	8	8	8	8	12	12	12	12	12
Number of tablets recovered	8	6	6	6	12	2	8	5	4

[a] N.C. = not collected.

Plasma Prednisolone Estimations—A 20-ml. sample of venous blood was taken from each subject before administration of the prednisolone and at specific time intervals (1, 2, 3, 5, 7, 9, 12, and 24 hr.) after the corticosteroids had been taken. Blood samples were heparinized after collection and centrifuged to separate the plasma. Five milliliters of heparinized plasma was extracted with 25 ml. of methylene chloride; the extract was washed first with 2 ml. of 0.1 *N* sodium hydroxide, followed by 0.1 *N* acetic acid, and finally with water. The extract was evaporated to dryness, dissolved in a small volume of a mixture of methanol and methylene chloride, and applied to Whatman No. 1 chromatography paper. Chromatograms were run for 4 hr. in a benzene–methanol–water (100:50:50) system (9). The prednisolone area was located by UV light and eluted with methanol from the chromatogram. The eluate was taken to dryness and redissolved in 0.5 ml. of methylene chloride, and the steroid was estimated. The phenylhydrazine–sulfuric acid reagent (10) was used according to the technique of Peterson *et al.* (11). Standard amounts of prednisolone were taken through the whole procedure.

This method for measuring plasma prednisolone concentrations

Table IV—Plasma Prednisolone Levels (mcg./100 ml.) in Six Subjects after Taking 90-mg. Equivalent of Prednisolone Alcohol in a Single Dose of 12 Sustained-Release Tablets (7.5 mg.) in Formulation A, B, or C or 18 Conventional Compressed Tablets (5 mg.)

Subject	Sex	Zero	1 hr.	2 hr.	3 hr.	5 hr.	7 hr.	9 hr.	12 hr.	24 hr.
			Sustained-Release Prednisolone (Formulation A)							
			Blood Level, mcg./100 ml.							
3	M	0	28	—	82	62	44	22	14	0
6	F	0	82	—	104	90	76	64	30	6
5	F	0	58	—	64	63	51	38	30	4
4	M	0	44	—	54	20	22	12	8	16
7	M	0	63	—	70	64	33	24	10	5
1	M	0	50	—	29	—[a]	20	10	0	0
Mean		—	54.2	—	67.3	59.8	41.0	28.3	15.3	5.2
±*SE*		—	7.5	—	10.4	11.2	8.6	8.2	5.0	2.4
			Sustained-Release Prednisolone (Formulation B)							
			Blood Level, mcg./100 ml.							
3	M	0	48	—	49	44	—	37	48	4
6	F	0	46	—	62	71	54	39	33	16
5	F	0	44	—	38	22	45	38	28	0
4	M	0	53	—	44	34	20	19	3	4
7	M	0	69	—	44	56	29	0	0	0
1	M	0	21	—	36	36	28	29	14	5
Mean		—	46.8	—	45.5	43.8	35.2	27.8	21.0	4.8
±*SE*		—	6.4	—	3.8	7.3	5.6	5.7	7.6	2.4
			Sustained-Release Prednisolone (Formulation C)							
			Blood Level, mcg./100 ml.							
7	M	0	21	—	17	17	26	13	—	11
			Prednisolone in Conventional Compressed Tablets							
3	M	0	123	104	81	45	29	—	1	0
6	F	0	160	118	106	50	41	—	3	1
5	F	0	165	109	126	71	43	—	—	0
4	M	0	118	96	72	36	30	15	7	0
7	M	0	92	93	71	28	8	3	0	0
1	M	0	148	102	83	56	33	—	2	0
Mean		—	134.3	103.7	89.8	47.7	30.7	9.0	2.6	0.17
±*SE*		—	11.5	3.7	8.9	6.2	5.1	6.0	1.2	—

[a] Technical difficulties were experienced in the extraction of this sample.

Table V—Plasma Cortisol Levels (mcg./100 ml.) in Six Subjects after Taking 90-mg. Equivalent of Prednisolone Alcohol in a Single Dose of 12 Sustained-Release Tablets (7.5 mg.) in Formulation A, B, or C or 18 Conventional Compressed Tablets (5 mg.)

Subject	Sex	Control	Blood Levels (mcg./100 ml.) of Cortisol							
			1 hr.	2 hr.	3 hr.	5 hr.	7 hr.	9 hr.	12 hr.	24 hr.
Sustained-Release Prednisolone (Formulation A)										
3	M	11.7	5.1	—	1.7	0.3	2.0	0.6	1.4	3.1
4	F	15.1	4.3	—	1.1	0.0	0.0	0.0	0.0	3.5
5	F	9.0	4.7	—	3.0	1.7	1.7	1.7	2.0	4.3
6	M	11.3	3.2	—	2.6	1.3	0.0	1.7	0.0	8.1
7	M	8.9	2.7	—	0.5	1.1	0.0	0.0	0.3	1.4
8	M	12.3	3.3	—	1.8	1.3	0.5	0.0	0.0	7.7
Mean		11.4	3.90	—	1.80	0.95	0.70	0.66	0.60	4.7
±*SE*		0.86	0.39	—	0.37	0.26	0.24	0.34	0.39	0.11
Sustained-Release Prednisolone (Formulation B)										
3	M	16.3	4.4	—	1.6	2.5	2.2	2.2	1.9	15.9
4	F	30.0	14.3	—	5.4	5.0	2.5	4.3	2.5	3.9
5	F	10.6	5.0	—	3.2	2.9	2.6	2.6	2.4	2.4
6	M	23.9	6.6	—	3.8	2.1	3.8	2.4	2.9	26.7
7	M	12.3	6.8	—	4.2	3.5	4.5	4.8	4.2	7.7
8	M	28.0	6.0	—	1.7	3.0	1.3	1.0	0	15.7
Mean		20.10	7.10	—	3.36	3.10	2.80	2.80	2.30	12.00
±*SE*		3.4	1.5	—	0.60	0.43	0.47	0.57	0.47	3.72
Sustained-Release Prednisolone (Formulation C)										
7	M	17.5	7.0	—	2.1	0.7	0.4	0.7	—	5.6
Prednisolone in Conventional Compressed Tablets										
3	M	18.6	6.1	3.9	2.5	2.1	1.8	—	—	16.1
4	F	24.5	5.8	5.2	6.4	3.0	2.4	—	—	4.8
5	F	15.0	5.3	4.7	3.8	3.1	1.9	—	—	8.8
6	M	7.3	3.5	2.7	2.4	4.6	—	—	—	20.4
7	M	8.3	3.7	4.0	4.0	3.0	—	—	—	11.3
8	M	26.9	11.2	6.5	3.5	3.1	3.8	—	—	28.1
Mean		16.70	15.90	4.50	3.80	3.20	2.40	—	—	14.90
±*SE*		3.20	1.10	0.50	0.57	0.33	0.38	—	—	3.46

was used by Jenkins and Sampson (12); it measures the level following the oral administration of 90 mg. of prednisolone but is not sensitive enough to determine the concentrations achieved following normal therapeutic doses (5–15 mg.).

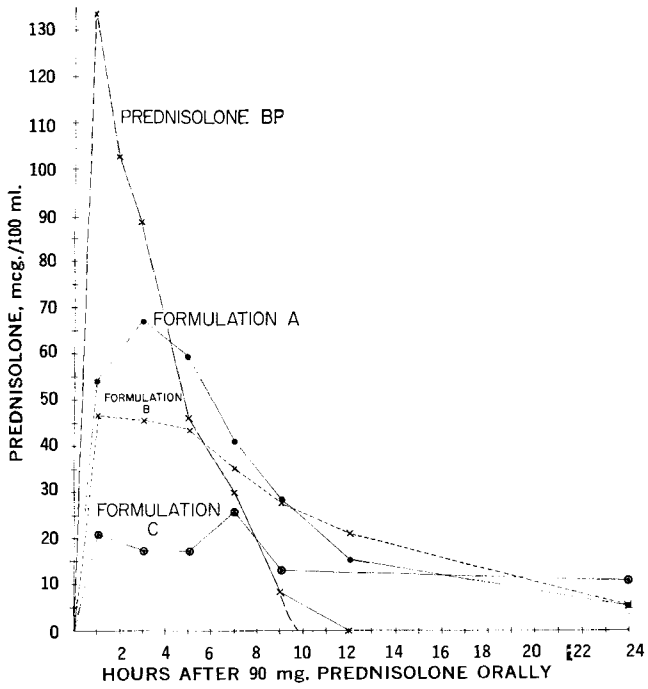

Figure 1—*Comparative plasma prednisolone levels in six subjects after taking 90-mg. equivalent of prednisolone alcohol in three sustained-release formulations, A, B, or C, and conventional compressed tablets. Key:* ×—×, *prednisolone BP compressed tablets;* ●—●, *Formulation A;* ×- - -×, *Formulation B; and* ⊙—⊙, *Formulation C.*

Plasma Cortisol Estimations—A fluorometric technique (13) was used to measure cortisol in plasma without interference from cortisone or other metabolites.

RESULTS

Effect of Formulation on *In Vitro* and *In Vivo* Release of Prednisolone—Three sustained-release tablet formulations (A, B, and C) were investigated in this study; their compositions are shown in Table I. *In vitro* release rates of prednisolone were determined for each formulation (Table II). It was apparent that the *in vitro* release rates of prednisolone from sustained-release tablet Formulation C would be too slow, since the matrix would be past the ileocecal junction within 6 hr., and that the choice of an acceptable tablet probably lay between Formulations A and B.

In the *in vivo* studies, four volunteers each took eight sustained-release tablets of Formulation A and at a later time 12 sustained-release tablets of Formulation B while one subject took 12 tablets of Formulation C. Egested tablet matrixes were collected from their feces. A total of 32 tablets of Formulation A was taken, of which 26 were egested; 48 tablets of Formulation B were taken, of which 27 were egested; and 12 tablets of Formulation C were taken, of which four were egested. In these and other related studies, there was evidence that some of the matrixes were present in the feces as small particles; it was not possible to collect these and measure their prednisolone content with any degree of accuracy. This accounts for the discrepancy of some matrixes uncollected in the present study.

The amount of residual prednisolone in each recovered matrix is shown in Table III. Results are expressed as percentages of the original prednisolone content of the tablets. The amount of prednisolone remaining in the recovered tablet matrixes of Formulation C was 72.5%, and that of Formulation B was 28.0 ± 2.35 (*SE*) %. Formulation A had the smallest residuum of prednisolone: 11.5 ± 2.48 (*SE*) %.

Plasma Prednisolone Levels in Man following Administration of Sustained-Release Tablets—The concentration of prednisolone in plasma (mcg./100 ml.) at specific intervals following the oral administration in six subjects of 12 sustained-release tablets at one time (equivalent to 90 mg. of prednisolone alcohol) of Formulation A, B, or C, or of 18 conventional compressed tablets, each of 5 mg

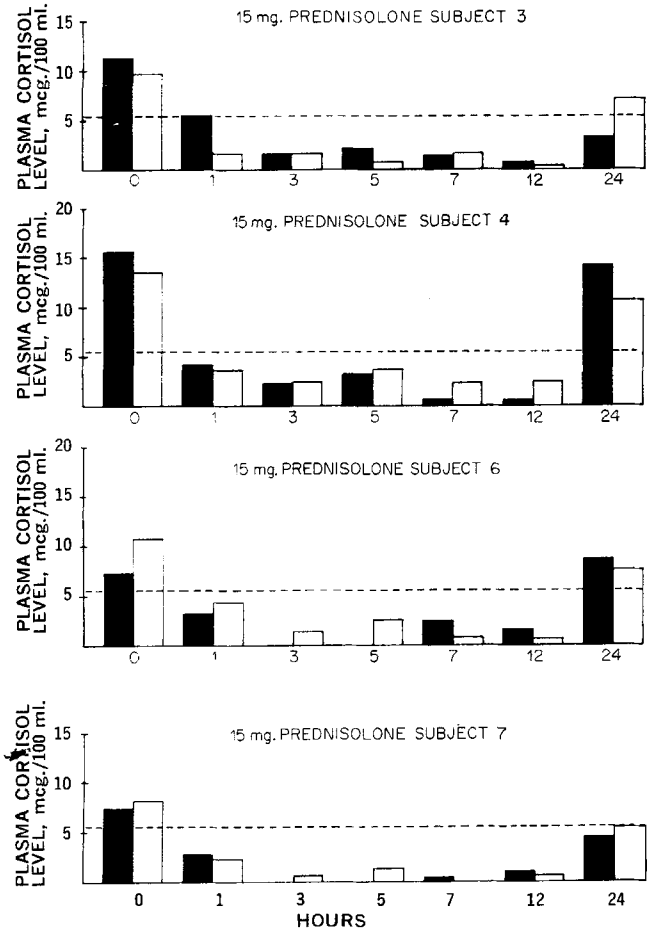

Figure 2—*Plasma cortisol levels (mcg./100 ml.) in four subjects after taking 15-mg. equivalent of prednisolone alcohol in sustained-release tablets (black columns) or conventional compressed tablets (open columns). The broken line at 5.5 mcg./100 ml. represents the lower limit for the range of normal blood cortisol levels.*

prednisolone alcohol, is shown in Table IV. Each subject received both sustained-release Formulations A and B, and one subject also received Formulation C. The conventional compressed tablet and the three sustained-release treatments were given in random sequence. An interval of 14 days was allowed between administrations to permit full recovery of adrenocortical function. The large dose of prednisolone was chosen so that levels in the blood could be measured readily by the method used.

Comparative plasma prednisolone levels produced by the three tablet formulations (sustained-release and conventional compressed tablets) are shown in Table IV. The data are presented graphically in Fig. 1, from which it can be seen that Formulation A gave the most desirable blood levels.

The peaking of plasma prednisolone shown for 3 hr. after conventional tablet administration was almost absent with sustained-release Formulation A. But the sustained-release formulation produced an appreciable plasma prednisolone level for longer than conventional compressed tablets; the increase in duration was greater than twofold. The two calculated regression lines so obtained for Formulation A were $r = -0.786$, $m = -0.0578$, and $c = 1.881$, and for conventional tablets $r = -0.9216$, $m = -0.099$, and $c = 2.116$. These differed significantly in gradient ($t = 4.43$; $p < 0.001$). It is apparent, therefore, that the difference in blood levels with time with these two formulations are statistically different.

Plasma Cortisol Levels in Man following Administration of Sustained-Release Tablets—It was expected that the high dose of prednisolone (equivalent to 90 mg. prednisolone alcohol) taken in the previous study would depress the pituitary release of corticotrophin and suppress the adrenocortical secretion. The blood samples taken from the volunteers in the previous study on plasma prednisolone levels were, therefore, also examined for their cortisol

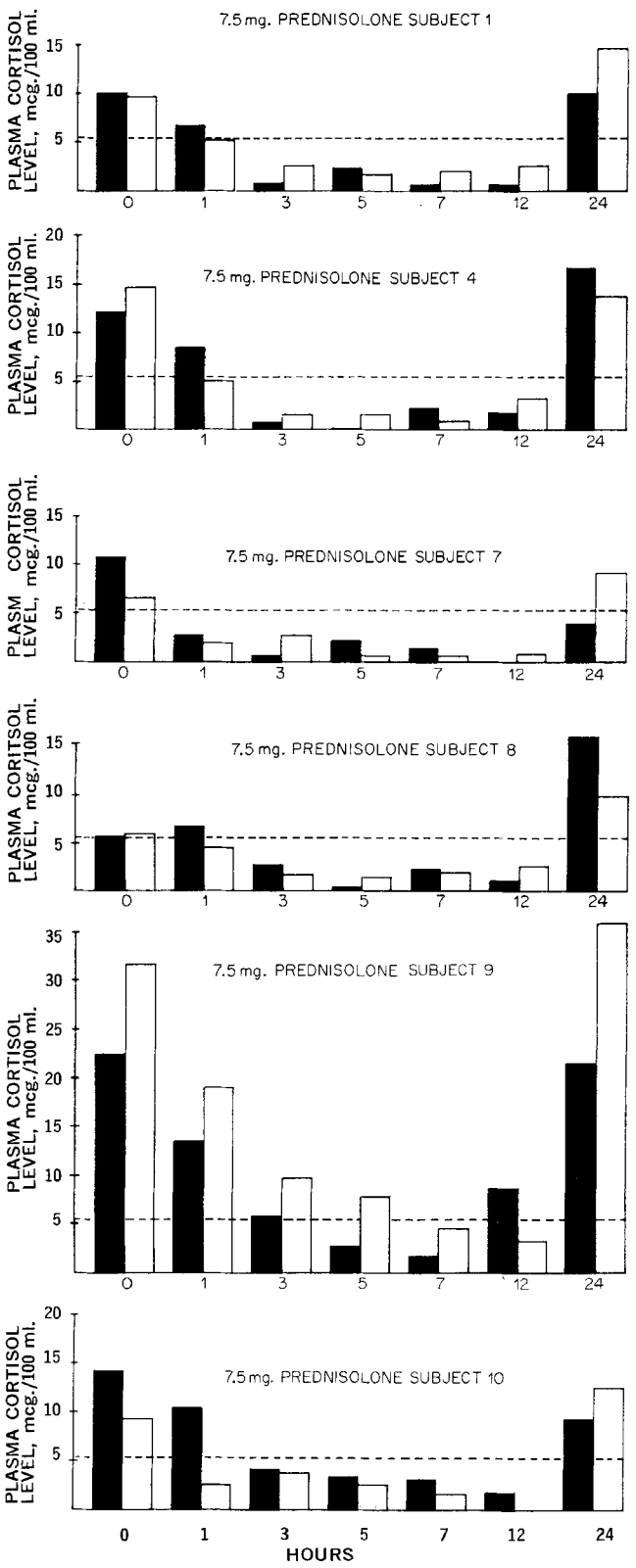

Figure 3—*Plasma cortisol levels (mcg./100 ml.) in six subjects after taking 7.5-mg. equivalent of prednisolone alcohol in sustained-release tablet formulation (black columns) or conventional compressed tablets (open columns). The broken line at 5.5 mcg./100 ml. represents the lower limit for the range of normal blood cortisol levels.*

level to determine the degree and duration of suppression produced by the two tablet formulations (sustained-release and conventional compressed tablets). Table V shows the plasma cortisol level for

each of the six subjects at specific times after taking 90 mg. of prednisolone in each of the three sustained-release formulations and after the same dose of conventional compressed tablets.

Blood cortisol levels of below 5.5 mcg./100 ml. were taken as evidence of adrenocortical suppression[2]. The range of normal blood cortisol levels is 5.5–23.5 mcg./100 ml.; wide individual and day-to-day variations within these limits were considered acceptable.

All four tablet formulations of prednisolone suppressed the adrenocortical secretion, as evidenced by a fall in plasma cortisol levels, in all six subjects. Plasma cortisol levels at 24 hr. were lower with the sustained-release formulations than corresponding levels with the conventional tablets in the six subjects. A very high dose of 90 mg. was given and, in consequence, a high level of prednisolone was maintained for a long time with the sustained-release preparation. The longer duration of adrenocortical suppression with the sustained-release tablet was thus a result of its prolonged action and supports the other evidence for such an action.

In these studies, the doses of prednisolone given were so enormous that it was unreasonable to correlate plasma prednisolone levels with the degree of cortisol suppression. It was, therefore, decided that the relative adrenocortical suppressant effects of a sustained-release formulation and conventional compressed prednisolone tablets should be investigated. The suppressant effects of Formulation A, as the most promising sustained-release preparation, were compared with conventional compressed tablets at therapeutic or near therapeutic dose levels (*i.e.*, 15 and 7.5 mg. prednisolone alcohol equivalents) at specific times after ingestion of the corticosteroid.

The results of these two latter studies on four and six subjects, respectively, are presented graphically in Figs. 2 and 3. Comparative results for the two formulations were drawn as histograms, showing plasma cortisol levels for the individual subjects.

At the 15-mg. level, both formulations of prednisolone suppressed adrenocortical secretion, as shown by the fall in plasma cortisol levels (Fig. 2). At 24 hr., Subject 3 showed adrenocortical suppression with the sustained-release preparation but not with the conventional tablets; Subjects 4 and 6 showed plasma cortisol levels within the normal range (5.5–23.5 mcg./100 ml.) for both formulations, while Subject 7 showed adrenocortical suppression with both formulations at 24 hr.

At the 7.5-mg. dosage level (Fig. 3), both formulations suppressed adrenocortical secretion as evidenced by a fall in plasma cortisol levels in all six subjects. However, at 24 hr., only one subject (Fig. 3, Subject 7) showed a blood cortisol level below 5.5 mcg./100 ml. and this was with the sustained-release preparation.

Adrenocortical suppression caused by therapeutic or near therapeutic doses of the sustained-release preparation was no greater than that produced by the same doses of prednisolone administered in conventional compressed tablets, but it was of longer duration.

DISCUSSION

A great disadvantage with conventional methods of preparing oral dosage forms has been that many of the drugs often rapidly attain a high blood concentration; side effects are, therefore, common when dosage is increased only slightly above the normal level. Thus, one goal of current pharmacological and pharmaceutical research is to produce a more even and prolonged absorption and blood level of drugs. In some instances, this can be achieved by formulating the active substance into oral preparations giving sustained release; peaking of drug plasma levels are avoided and the incidence of side effects can be reduced (1, 3, 6).

Studies of drug-release rates from plastic matrixes have been made by a number of investigators. In particular, techniques for the investigation of factors influencing *in vitro* release rates have been described (14–17), and comprehensive investigations of *in vivo* blood levels of drugs administered in sustained-release tablets of the plastic matrix type have been made (18). It has been shown that the average effective blood level of a drug administered by either a conventional compressed tablet or by a sustained-release tablet was approximately the same. However, while the conventional tablet produced variations from nil to twice the average level, the sustained-release tablet administered three times a day maintained the blood level of the drug within $\pm 20\%$ of the average throughout a 24-hr. period, and within $\pm 50\%$ of the average when taken twice a day.

Evidence from the literature suggests that the formulation of corticosteroids in sustained-release tablets may reduce the incidence of side effects. Studies, both in dogs and in man (19), showed that a very simple sustained-release formulation of prednisolone was less likely to depress the pituitary–adrenal axis than was an equal dosage of prednisolone formulated as a conventional compressed tablet. Also, although the plasma 17-hydroxycorticosteroid levels did not reach as high a peak with the sustained-release preparation as with the conventional tablet, they were more uniform and of greater duration. Furthermore, these authors reported that the total amount of 17-hydroxycorticosteroid that was available to the human subjects in the 24-hr. period following the administration of the sustained-action dosage form was equivalent to that available following administration of conventional compressed tablets of the steroid at the same dosage.

A number of workers investigated the possibilities of intermittent corticosteroid dosage with the object of minimizing side effects (20–22). Dubois and Adler (23), while accepting the advantages of alternate-day administration, advocated the use of a long-acting preparation of methylprednisolone in daily doses in the treatment of rheumatoid arthritis and other collagen disorders. The long-acting preparation was more effective and had a lower incidence of side effects than did intermittent administration of methylprednisolone in conventional tablets.

The results of the present study suggest that prednisolone in this form should have a reduced incidence of side effects. Obviously, this is a matter for clinical determination; however, the main indication to support this suggestion is that following the administration of sustained-release tablets to volunteers at a dosage equivalent to 90 mg. of prednisolone alcohol, there was an appreciable and relatively uniform plasma prednisolone level for significantly longer than with equivalent dosage given in conventional compressed tablet form. The peaking of plasma prednisolone level shown for 3 hr. after conventional tablet administration was almost absent with the sustained-release formulation. Furthermore, adrenocortical suppression caused by therapeutic or near therapeutic doses of the sustained-release preparation was no greater than that produced by the same dose of prednisolone given in conventional tablets. Release of the prednisolone from the plastic matrix was demonstrated by the initial egestion studies.

Initial clinical trials (24) in patients with rheumatoid arthritis or asthma have suggested that a reduced dosage of prednisolone in sustained-release form would control symptoms as well as conventional doses in compressed tablet form.

REFERENCES

(1) J. Sjögren, *Farm. Revy.*, **58,** 233(1959).

(2) J. Sjögren, *Dan. Tidsskr. Farm.*, **34,** 189(1960).

(3) J. Sjögren and L.-E. Fryklöf, *Farm. Revy.*, **59,** 171(1960).

(4) J. Sjögren and I. Östholm, *J. Pharm. Pharmacol.*, **13,** 496 (1961).

(5) J. Sjögren and M. Ervik, *Acta Pharm. Suecica*, **1,** 219(1964).

(6) R. Sannerstedt, *Acta Med. Scand.*, **167,** 245(1960).

(7) G. Cramér, E. Varnauskas, and L. Werkö, *ibid.*, **173,** 511 (1963).

(8) L. Lundholm and N. Svedmyr, *Acta Pharmacol. Toxicol.*, **20,** 65(1963).

(9) I. E. Bush, *J. Biochem.*, **50,** 370(1952).

(10) C. C. Porter and R. H. Silber, *J. Biol. Chem.*, **185,** 201(1950).

(11) R. E. Peterson, A. Karrer, and S. L. Guerra, *Anal. Chem.*, **29,** 144(1957).

(12) J. S. Jenkins and P. A. Sampson, *Brit. Med. J.*, **2,** 205(1967).

(13) J. Spencer-Peet, J. R. Daly, and V. Smith, *J. Endocrinol.*, **31,** 235(1965).

(14) S. J. Desai, A. P. Simonelli, and W. I. Higuchi, *J. Pharm. Sci.*, **54,** 1459(1965).

(15) S. J. Desai, P. Singh, P. Simonelli, and W. I. Higuchi, *ibid.*, **55,** 1224(1966).

(16) *Ibid.*, **55,** 1230(1966).

(17) *Ibid.*, **55,** 1235(1966).

(18) A. Brändstrom and J. Sjögren, *Acta Pharm. Suecica*, **4,** 157 (1967).

[2] This figure is the lower limit accepted at St. George's Hospital.

(19) J. G. Wagner, O. S. Carpenter, and E. J. Collins, *J. Pharmacol. Exp. Ther.*, **129**, 101(1960).

(20) J. G. Harter, W. J. Reddy, and G. W. Thorn, *New Engl. J. Med.*, **269**, 591(1963).

(21) S. D. Grant, P. H. Forsham, and V. C. DiRaimondo, *ibid.*, **273**, 115(1965).

(22) G. L. Ackerman and C. M. Nolan, *ibid.*, **278**, 405(1968).

(23) E. L. Dubois and D. C. Adler, *Curr. Ther. Res.*, **5**, 43(1963).

(24) B. Lake, personal communication, 1969.

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Behavior of Erythrocytes in Phosphate Buffer Systems

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Abstract □ Hemolytic behavior of human erythrocytes in sodium and potassium phosphate buffer systems was investigated. The data were used to calculate *hemolytic i* values for the buffer components at various pH values. The experimental *i* values were lower than those predicted by a theoretical equation, and the deviations were attributed to changes in pH causing alterations in the permeability of the red cell membrane to the anions and/or cations in solution or changes in the red cell contents. Alkaline solutions appear to be more favorable environments for the red blood cell under the conditions studied. The increased osmotic fragility (low *hemolytic i* values) at lower pH values was attributed to an increase in the osmotic activity of the cell contents and subsequent movement of water into the cell.

Keyphrases □ Erythrocytes, hemolysis—phosphate buffer solutions □ Isotonic coefficients, phosphate buffer solutions—erythrocytes hemolysis □ Hemolysis curves—phosphate buffer solutions □ Osmotic fragility, erythrocytes—low pH effect

Phosphate buffer systems are used in parenteral solutions primarily to stabilize the active ingredient against chemical degradation. Those buffer systems employed should normally have as low a buffer capacity as possible so that body buffer systems will not be significantly disturbed when the solution is injected. Phosphate buffers are routinely used in the compounding of intravenous solutions when adjustments between pH 6 and 8 are needed.

The purposes of this investigation were to study the effect of phosphate buffering agents on red blood cells and to determine tonicity values based on measurements of fragility of human red cells in various phosphate buffer systems. The hemolytic method was employed, and isotonic coefficients were calculated by comparison of standard hemolysis curves obtained for human blood in aqueous saline solutions and those obtained from experiments using sodium and potassium phosphate buffer solutions. Experiments were designed to determine isotonic coefficients of buffer components at various pH values.

EXPERIMENTAL

Collection of Blood—The blood samples used for all experiments were obtained from the forearm veins of a 22-year-old male Caucasian donor. Fresh blood samples were used in all experiments. Approximately 10 ml. of blood was obtained from the donor and placed in a 50-ml. round-bottom flask containing 10–15 glass beads. The flask was rotated gently for about 5 min. and then the blood was decanted into a 50-ml. conical flask and aerated by swirling the flask gently for about 5 min.

Preparation of Buffer Solutions and Determination of pH—All chemicals employed were reagent grade quality, and distilled water was used to prepare all solutions. Stock solutions were prepared (approximately 0.133 *M*); from these, quantitative amounts were taken in the desired ratios and diluted with water to produce 50-ml. samples. The pH was checked using a pH meter (Corning model 7).

Quantitative Determination of Percent Hemolysis—The hemolytic method was used in each experiment to determine the extent of hemolysis of erythrocytes in the phosphate buffer solutions. This method is a quantitative one, being based on the fact that a hypotonic solution liberates oxyhemoglobin in direct proportion to the number of cells hemolyzed. Into each of two test tubes were transferred 5 ml. of the standard sodium chloride solution (0.06, 0.062 ... 0.07, 0.072 *M*) and 5 ml. of the buffer system being tested. After the test tubes were brought to a constant temperature by being placed in a water bath (37 ± 0.5°), 0.05 ml. of blood was pipeted into each tube. The tubes were then inverted several times to ensure thorough mixing and allowed to remain 45 min. at 37°. After centrifuging, the absorbance of the supernatant liquid was measured using a Klett-Summerson photoelectric colorimeter equipped with a No. 54 filter.

To find the percent hemolysis, the absorbance readings were divided by the absorbance readings for 0.05 ml. of blood in 5 ml. of distilled water (standard for 100% hemolysis) and multiplied by 100. A blank, made by placing 0.05 ml. of blood in 5 ml. of 0.9% sodium chloride solution, was used to cancel any light absorbance inherent to the blood sample. Both the standard and the blank were subjected to the same conditions of standing for 45 min. at 37° followed by centrifuging.

Calculation of *i* Values—Through the use of the hemolytic method, concentrations of sodium chloride and the buffer solutions giving the same degree of hemolysis could be determined. Knowledge of these concentrations made it possible to calculate isotonic coefficients (*i* values) through the use of the following equation:

$$\begin{pmatrix} i \text{ value for} \\ \text{NaCl in water} \end{pmatrix} \begin{pmatrix} \text{molar concentration of} \\ \text{NaCl causing} \\ 25\% \text{ hemoly is} \end{pmatrix} = \begin{pmatrix} i \text{ value of} \\ \text{buffer components} \\ \text{in solution} \end{pmatrix} \begin{pmatrix} \text{molar concentration} \\ \text{of buffer solution} \\ \text{causing } 25\% \\ \text{hemolysis} \end{pmatrix} \quad \text{(Eq. 1)}$$

The value of *i* for sodium chloride was taken as 1.86, which is the accepted *i* value for 0.154 *M* (0.9%) sodium chloride in water (1).

Curves showing the degree of hemolysis in sodium chloride-water solutions and phosphate buffer solutions were plotted on