Table 1. Results from replicate assays for the anhydrous morphine content of ammonium chloride and morphine mixture B.P.

Sample	Anhydrous morphine content by proposed method (% w/v)		Anhydrous morphine content by B.P. 1980 method (% w/v)	
(i)	Mean	0·0053	Mean	0·0048
	Range	0·0051–0·0053	Range	0·0045–0·0050
		n = 4		n = 4
(ii)	Mean	0·0052	Mean	0·0052
	Range	0·0053–0·0050	Range	0·0047–0·0058
		n = 5		n = 4
(iii)	Mean	0·0049		
	Range	0·0048–0·0050		n.d.
		n = 4		
(iv)	Mean	0·0052		
	Range	n = 3		n.d.

Key:
(i) Mixture made in the laboratory from individual components.
(ii) Mixture purchased from local pharmacy and made from individual components.
(iii) Mixture purchased from a local pharmacy and made from a commercially available concentrate.
(iv) Mixture purchased from a local pharmacy and made from a commercially available concentrate.
n.d. Not done.

in the dark for 15 min, after which 12 ml of dilute ammonia solution were added and the volume made to 50 ml with water. The extinction of a 4 cm layer of this solution was measured at the maximum at about 442 nm, using as a blank a solution prepared in the same manner and at the same time, but replacing the sodium nitrite solution with water. The content of anhydrous morphine was determined by reference to a calibration curve prepared from suitable portions of an accurately prepared 0·008% w/v solution of morphine in 0·1 M HCl, each being diluted to 20·0 ml with 0·1 M HCl and using the method described above commencing at the addition of sodium nitrite solution.

The anhydrous morphine contents of the samples examined are given in Table 1; replicate determinations were performed on all samples. Mixture (i) was prepared from a chloroform and morphine tincture with a known anhydrous morphine content and gave 100·9% recovery of anhydrous morphine.

The method described is simple, quantitatively reproducible (Table 1) and requires less than 90 min to perform. The present British Pharmacopoeial limits for content of anhydrous morphine in ammonium chloride and morphine mixture are 0·0040–0·0066% w/v and we suggest that with the improvement in accuracy and reproducibility of the proposed over the existing method these limits could be narrowed to 0·0045–0·0060% w/v anhydrous morphine content.

REFERENCES

Adamson, D. C. M., Handisyde, F. P. (1946) Quart. J. Pharm. 19: 350

Helliwell, K., Sanders, L. (1978) Pharm. J. 220: 224

Helliwell, K., Game, P. (1980) J. Pharm. Pharmacol. 32: 649–650

Radulescu, D. (1905) Bul. Soc. Stinte Bucuresti 14: 602

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Liposomes—A selective drug delivery system for the topical route of administration: gel dosage form

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Most research efforts with liposomes have been involved with the investigation of their use as drug carriers to particular target organs in either in vitro conditions or after oral or parenteral administration (Gregoriadis 1979; Papahadjopoulos 1979). Only one report (Mezei & Gulasekharam 1980) has related to the topical use of liposomes. In that study triamcinolone acetonide was encapsulated into liposomes and applied to rabbit skin in a 'lotion' form which provided a more favourable drug disposition than the conventional ointment. We have now tested the disposition of triamcinolone after topical application in liposomal and 'free' form incorporated in a hydrocolloid gel vehicle and applied to rabbit skin.

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Materials and methods

Preparation of liposomes: DL-α-Dipalmitoyl phosphatidylcholine, cholesterol (Sigma Chemical Company, St Louis, Mo.), and triamcinolone [2-^{14}C]acetonide (New England Nuclear, Boston, Mass.) (1·1:0·5:0·5 molar ratio) were dissolved in chloroform–methanol (2:1). The solvent was evaporated under vacuum until a smooth, dry lipid film was observed. Calcium chloride (8 mM) solution was added and the mixture was vigorously stirred for 10–20 min and then allowed to stand at room temperature (20 °C) for 1 h.

Table 1. The effect of liposomal encapsulation of drug disposition.[a]

	Triamcinolone ng g^{-1} tissue			
	Control (gel) form		Liposomal (gel) form	
	Mean	s.d.[b]	Mean	s.d.[b]
Skin surface[c]	1 166 200	134 900	1 084 600	213 700
Epidermis	114 585 **	42 400	566 000**	233 200
Dermis	29 075 *	11 600	89 200 *	41 100
Subcutaneous tissue	2 065	1 430	2 200	910
Thalamic Region[d]	180 **	24·3	77 **	8·3
Brain[d]	112	5·8	132	21·3
Blood[e]	5 *	1·0	3 *	0·7
Liver	162	33·4	140	51·0
Spleen	68	16·9	126	45·0
Lung	30 *	3·1	91 *	38·1
Heart	56	11·7	68	36·5

[a] Drug Disposition was measured after five-day treatment with 0·1% triamicinolone [2-^{14}C]-acetonide.
[b] Standard deviation with n = 8.
[c] The data here expressed the total amount of drug unabsorbed into or through the skin.
[d] The thalamus, hypothalamus were dissected, without using microscopes or other special devices, and referred to as thalamic region; the remaining part of the brain is referred to as brain.
[e] The concentration here is triamcinolone ng ml^{-1} blood.
* The degree of significance of the difference: $P < 0·05$; ** $P < 0·001$.

The liposomal preparation was centrifuged at 22000 *g* for 15 min at 20 °C. The supernatant was decanted and the pellet was resuspended in 5·0 ml of 8 mM $CaCl_2$ solution. This procedure was repeated twice. The residue of the last centrifugation was resuspended in 1·5 ml of 8 mM $CaCl_2$ and a 10 µl aliquot was counted in 10 ml of Bray's solution. The volume of this preparation was adjusted by adding the required volume of 8 mM $CaCl_2$ such that 0·3 ml of the sample contained 0·6 mg (0·2%) triamcinolone acetonide with 2 µCi radioactivity. This preparation was incorporated into an equal volume of a hydrocolloid gel (K-Y Sterile Lubricant, Johnson and Johnson, Canada, Ltd), by trituration.

For the control product the drug was in a 'free' form in the same vehicle; i.e. the drug and the lipid components of liposomes, DL-α-dipalmitoyl phosphatidylcholine and cholesterol were dissolved in absolute ethanol and the solution was incorporated in the hydrocolloid gel so that the concentration (0·1%) and the radioactivity (1 µCi/0·3 ml) of the triamcinolone was the same as in the liposomal preparation.

The methods for the treatment of animals and for the determination of drug disposition in the skin, internal organs and in urine were as described by Mezei & Gulasekharam (1980).

Results and discussion

Treatment with the liposomal gel form provided a concentration of triamcinolone acetonide approximately five times higher in the epidermis and three times higher in the dermis, than that of the control form (Table 1). However, the treatment with the liposomal form resulted in a significantly ($P < 0·05$) lower drug concentration in the blood than did that with the control form indicating a higher percutaneous absorption of drug with the control form. The finding was supported by the urinary data (Fig. 1). The disposition of the drug in other organs is also shown in Table 1.

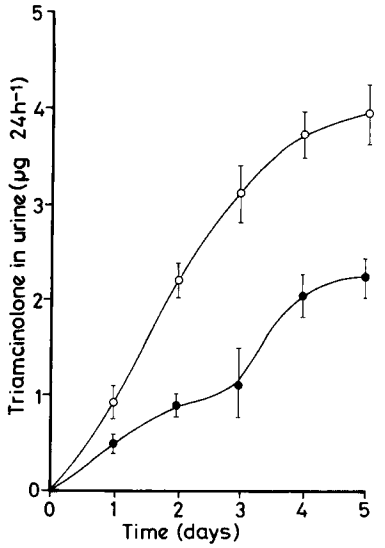

FIG. 1. Urinary excretion of triamcinolone [2-^{14}C]acetonide applied topically in 'free': ○, and in liposomal form: ●. The bars I indicate ± standard deviation with n = 8.

The results presented in Table 1 and Fig. 1, and also those reported earlier, when the liposomes were applied in a 'lotion form' (Mezei & Gulasekharam 1980), are definite indications that the liposomal form has potential as a selective drug delivery system for cutaneous application.

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REFERENCES

Gregoriadis, G. (1979) in: Gregoriadis, G. (ed.) Drug Carriers in Biology and Medicine. Academic Press, New York

Mezei, M., Gulasekharam, V. (1980) Life Sci. 26: 1473–1477

Papahadjopoulos, D. (1979) in: Hess, H. J. (ed.) Annual Reports in Medicinal Chemistry, Academic Press, New York, vol. 14, pp 250–260