2. Intraperitoneal injection of ellagic acid in mice produced significant sedation, ataxia, potentiation of sodium pentobarbital sleeping time, and protected the animals from death after electroconvulsive shock.

3. Intravenous injection of ellagic acid decreased blood pressure and elevated the T wave. Whereas heart and respiration rate initially increased then decreased.

4. *In vitro* studies indicated that ellagic acid appeared to have no apparent effect on activity of the duodenal segment and uterus of the rat.

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Keyphrases

Ellagic acid—*Juglans nigra*
Pharmacologic screening—ellagic acid
IR spectrophotometry—identity
UV spectrophotometry—identity

Influence of Dimethyl Sulfoxide (DMSO) on the Percutaneous Absorption of Salicylic Acid and Sodium Salicylate from Ointments

By JOSEPH M. STELZER, JR.*, JOHN L. COLAIZZI, and PAUL J. WURDACK

Dimethyl sulfoxide (DMSO), 15 percent by weight, was incorporated into selected ointment bases containing 10 percent salicylic acid or 11.6 percent sodium salicylate. Percutaneous absorption was studied by determining salicylate blood levels in New Zealand white rabbits at regular intervals for 8 hr. following application of the ointment to the shaved intact skin and confinement by a specially designed bandage. DMSO in hydrophilic ointment and hydrophilic petrolatum produced more rapid drug absorption and higher salicylate blood levels than the control systems. Polyethylene glycol ointment and a polyoxyethylene (20) stearyl ether gel with DMSO did not produce any significant change in the absorption pattern. The salicylate blood levels obtained from percutaneous absorption of sodium salicylate in hydrophilic ointment containing DMSO were lower than with control systems. In the case of hydrophilic petrolatum, there were no significant differences in absorption patterns of sodium salicylate with or without DMSO. Sodium salicylate did not appear to be absorbed from polyethylene glycol ointment whether or not DMSO was included.

AMONG FACTORS which influence percutaneous absorption, Barr (1) included such factors as the condition of the skin, the thermodynamic properties of the medicament, the effects of moisture, the effects of surfactants, the effects of pH, and the effects of the vehicle. He indicated that organic solvents such as ether, chloroform, benzene, and acetone penetrate the skin with ease and enhance the percutaneous absorption of a drug.

The object of the following research was to determine whether dimethyl sulfoxide (DMSO), a relatively nontoxic organic solvent, would alter the percutaneous absorption patterns of salicylic acid and sodium salicylate when incorporated

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* Fellow of the American Foundation for Pharmaceutical Education.

into hydrophilic ointment USP, polyethylene glycol (PEG) ointment USP, hydrophilic petrolatum USP, and a polyoxyethylene (20) stearyl ether[1] (PSE) gel system.

Biologically, DMSO has demonstrated a broad spectrum of activities including the enhancement of penetration through plant and animal membranes (2). DMSO has been suggested for possible use as an analgesic agent, anti-inflammatory adjunct, bacteriostatic agent, diuretic, tranquilizer, potentiator of other compounds, and penetrant carrier (3). DMSO was reported to facilitate the transport of sodium salicylate, sodium sulfadiazine, aminophylline, Evans blue dye, and sodium heparin across the mucous membrane of a dog's bladder (4). By recording the presence or absence of a physiologic reaction in adult humans after topical application, Stoughton and Fritsch (5) presented evidence that DMSO caused an increase in cutaneous absorption in the order of 25-fold for hexopyrronium ion in 20% DMSO, 25-fold increase for naphazoline hydrochloride in 50% DMSO, and a fivefold increase for fluocinolone acetonide in 25% DMSO. Kligman (2) concluded from his experimentation that if DMSO is to be clinically useful, quite concentrated solutions, perhaps 80 to 90% would be required to accelerate the rate of topical medicaments such as corticosteroids through the skin.

The mechanism of increased membrane permeability effected by DMSO is essentially unknown. Jacob *et al.* (4) has pointed out that the carrier mechanism brought about by DMSO might be related to the facts that it is a weak base and that it forms complexes with various substances. The attraction of DMSO for water should also be considered as a possible factor involved in altered membrane permeability in the presence of DMSO.

Dimethyl sulfoxide is relatively nontoxic. Block (6) indicates that it is nonirritating and nontoxic to skin of rabbits. Jacob and Rosenbaum (7) have summarized some of the known toxicologic information on DMSO. It has been reported that a topical application of 90 to 100% DMSO with occlusive covering to human skin resulted in a papulovesicular reaction after 1 hr. of occlusion which disappeared in a few hours. The vesiculating ceases abruptly when the concentration is reduced to 80% or lower (2). In later toxicity studies, Rubin and Barnett (8) noted unusual lenticular changes in animals tested. Changes in the lens were characterized by division of the lens into two zones, a central area simulating a lens nucleus and a peripheral area. These changes were noted after 90 days of dermal application of 4 ml. 100% DMSO/kg. of body weight/day in rabbits and swine. No such changes have been noted in humans (9, 10). Kleberger (11) applied DMSO to the skin of five human subjects in daily doses ranging from 30 to 800 ml. No change in lens structure was noted. He suggested that the refractive changes seen in animals might be due to the extremely large doses of DMSO received.

[1] Marketed as Brij 78, Atlas Chemical Industries, Inc., Wilmington, Del.

EXPERIMENTAL

Preparation of Ointments—Salicylic acid[2] and sodium salicylate,[2] previously reduced to fine powders in a ball mill, were each passed through a No. 80 mesh sieve and dried at 50° in a heated vacuum desiccator[3] for at least 48 hr. before use. The ointments prepared contained 10% (w/w) salicylic acid or 11.6%[4] (w/w) sodium salicylate and 15% (w/w) of medicinal grade dimethyl sulfoxide.[5] Each ingredient was accurately weighed and incorporated into the various ointment bases. Bases used were hydrophilic ointment USP XVII, hydrophilic petrolatum USP XVII, polyethylene glycol ointment USP XVII, and a polyoxyethylene (20) stearyl ether gel system.[6]

Test Animals—The test ointment containing DMSO was compared to a control ointment that was similar in all respects to the test ointment except that it did not contain DMSO. Each set of ointments was applied to two pairs of New Zealand white rabbits weighing between 2.8 and 3.6 kg. The rabbits were randomly selected without regard to sex. Each rabbit was used only four times and received the same ointment base in each test. Only one pair of rabbits was utilized during any one experimental day due to space and time limitations. The two ointments compared on that day differed with respect to the presence of DMSO in the one, and its absence in the other. Within each pair, the rabbits were alternated weekly. The rabbit receiving the test ointment for the first test run received the control ointment for the second run and *vice versa*. For two of the four experimental runs each rabbit received the test ointment with DMSO, while for the other two runs, the same rabbit received the control ointment without DMSO. A 7-day rest period ensued before reapplication of ointments. The animals were maintained on Purina rabbit chow and water *ad libitum* and housed individually in an animal room maintained at a temperature of approximately 20° and at a relative humidity of approximately 50%. Twenty-four hours prior to the application of the ointment to the

[2] Analytical reagent grade, Mallinckrodt Chemical Works, St. Louis, Mo.

[3] Model No. 68351, Precision Scientific Co., Chicago, Ill.

[4] 11.6% sodium salicylate is equivalent to 10% salicylic acid.

[5] Generously supplied by Crown Zellerbach Corp., Camas, Wash.

[6] The gel was prepared by adding 1 part water to 3 parts PSE. The water and PSE were separately heated to 70° on a steam bath. The two were then removed from the heat and added together with stirring until gel was formed.

TABLE I—BLOOD SALICYLATE CONCENTRATIONS OBTAINED FROM OINTMENTS CONTAINING SALICYLIC ACID[a]

Time, hr.	Hydrophilic Ointment, mg. % Test[b]	Hydrophilic Ointment, mg. % Control[c]	Hydrophilic Petrolatum, mg. % Test[b]	Hydrophilic Petrolatum, mg. % Control[c]
0.5	2.68 ± 0.83	0.75 ± 0.22	3.53 ± 0.51	0.55 ± 0.34
1	6.33 ± 1.89	2.38 ± 0.83	6.80 ± 0.53	1.01 ± 0.39
2	10.44 ± 2.42	3.91 ± 0.65	8.71 ± 0.49	1.63 ± 0.32
3	10.41 ± 1.61	5.49 ± 0.86	9.68 ± 0.80	2.50 ± 0.29
4	9.21 ± 1.54	5.45 ± 1.09	8.72 ± 0.31	3.32 ± 0.64
5	7.53 ± 2.27	5.81 ± 1.39	7.52 ± 0.96	3.38 ± 0.39
6	6.24 ± 2.21	4.23 ± 1.03	6.20 ± 0.97	3.56 ± 0.66
7	5.31 ± 2.07	4.06 ± 1.07	5.01 ± 0.84	3.73 ± 0.76
8	3.80 ± 1.46	2.80 ± 0.88	5.00 ± 0.74	3.82 ± 1.03
	PEG Ointment, mg. % Test[b]	PEG Ointment, mg. % Control[c]	PSE Gel, mg. % Test[b]	PSE Gel, mg. % Control[c]
0.5	0.34 ± 0.17	0.03 ± 0.03	1.18 ± 0.73	0.76 ± 0.36
1	0.09 ± 0.03	0.13 ± 0.05	1.89 ± 1.35	0.66 ± 0.29
2	0.31 ± 0.03	0.12 ± 0.05	2.54 ± 1.00	1.27 ± 0.57
3	0.47 ± 0.16	0.50 ± 0.14	2.51 ± 0.87	2.50 ± 1.19
4	0.83 ± 0.54	0.30 ± 0.07	2.66 ± 1.10	1.84 ± 0.87
5	0.69 ± 0.30	0.38 ± 0.22	2.11 ± 0.78	1.45 ± 0.69
6	1.14 ± 0.58	0.52 ± 0.10	2.38 ± 0.55	1.91 ± 0.69
7	1.08 ± 0.49	0.19 ± 0.07	1.36 ± 0.30	1.41 ± 0.43
8	0.92 ± 0.49	0.94 ± 0.36	1.50 ± 0.49	2.01 ± 0.92

[a] Average of eight determinations with standard error of the mean. [b] Contains 10% salicylic acid and 15% DMSO in ointment base. [c] Contains 10% salicylic acid in ointment base.

rabbit, hair was removed with an animal clipper[7] from the skin of the ears and the dorsal area between the forelegs and hind legs on both sides of the spine.

Application of Ointment—A specially prepared bandage restricted and controlled the area of contact between the ointment and the skin of the rabbit. The edges of an 8.35 × 14.7 cm. piece of aluminum foil were doubled over and flattened 1 cm. on each side to produce a rectangular plate measuring 6.35 × 12.7 cm. with a 1-cm. reinforced margin. An accurately weighed 5.00-g. sample of the selected ointment was uniformly spread over one surface of the plate whose opposite side was centered on an 8.9 × 20 cm. strip of adhesive tape. The entire assembly was then applied to the shaved dorsal skin of the rabbit and adjusted to conform to the contours of the area. To insure adequate contact between the ointment and the skin and to minimize contamination, the assembly was covered with two elastic bandages.[8] The bandage arrangement employed was adapted from Stolar *et al.* (12). The ointment remained in contact with the skin for the 8-hr. experimental period, during which time the rabbit did not receive food or water.

Procedure—Blood samples were withdrawn and tested for blood salicylate concentration. One-half milliliter of blood was withdrawn from the marginal ear vein of the rabbit at the following time periods: prior to application of ointment, 0.5 hr. after ointment application, and at hourly intervals for 8 hr. after application of the ointment. The 0.5 ml. of blood was collected in a syringe[9] containing 0.1 ml. of heparin USP,[10] 5,000 units/ml. This blood and heparin mixture was added to 5.0 ml. of the combined protein precipitant-color reagent in a centrifuge tube and analyzed for salicylate content according to the method described by Trinder (13). After centrifugation and filtration the colorimetric analysis for salicylate was performed at a wavelength setting of 540 mμ using a Spectronic "20"[11] spectrophotometer. The absorbance reading obtained from the blood sample withdrawn prior to application was considered the zero reading and was subtracted from the other readings to account for any absorbance contributed by any constituents in the blood other than the salicylic acid. The salicylate content of the sample was obtained from a standard curve previously prepared by treating 0.1 ml. of heparin and 0.5-ml. quantities of sodium salicylate solutions containing the equivalent of 10, 20, 30, 40, and 50 mg. % of salicylic acid, with 5.0 ml. of color reagent.

Statistical inferences drawn concerning the results obtained in this study were based upon a randomized blocks design analysis of variance at a 95% level of significance, using two treatments and four blocks (14).

RESULTS

DMSO, 15% by weight, was incorporated into the four selected ointment bases (Table I) containing 10% (w/w) salicylic acid.

A stiff, white ointment resulted when hydrophilic ointment was combined with 10% salicylic acid. The addition of 15% DMSO altered the consistency, producing a slightly softer ointment. Figure 1 illustrates the altered percutaneous absorption pattern of salicylic acid obtained upon the addition of DMSO to hydrophilic ointment. The statistical analysis of the results illustrated in Fig. 1 showed that the salicylate blood levels obtained with the ointments containing DMSO were significantly higher than with the controls for the determinations made 1, 2, 3, and 4 hr. following application of ointment. Statistical analysis of the data illustrated in Figs. 2 and 3 indicates that the addition of DMSO in a 15% (w/w) concentration produced no significant change in the absorption pattern of salicylic

[7] Model No. A-2 with size 40 head, John Oster Manufacturing Co., Milwaukee, Wisc.
[8] Readi-flex bandages generously supplied by Parke, Davis & Co., Detroit, Mich.
[9] Glaspak disposable tuberculin syringe, Becton, Dickinson, and Co., Rutherford, N. Y.
[10] Generously supplied by the Upjohn Co., Kalamazoo, Mich.
[11] Bausch and Lomb, Rochester, N. Y.

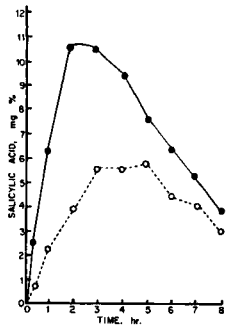

Fig. 1—Effect of DMSO on percutaneous absorption of 10% salicylic acid from hydrophilic ointment. Key: ●—, 15% DMSO added; ○- - -, control.

acid when added to PEG ointment or to PSE gel, both containing 10% salicylic acid. PEG ointment with 10% salicylic acid was a stiff, glossy white ointment that softened slightly when 15% DMSO was added. When 10% salicylic acid was combined with the PSE gel system, the gel liquified. After standing undisturbed for 15 min. the system again congealed. The addition of 15% DMSO caused the gel to liquify again and at room temperature it remained in this condition. This resultant liquid system, when refrigerated at 10–15°, congealed.

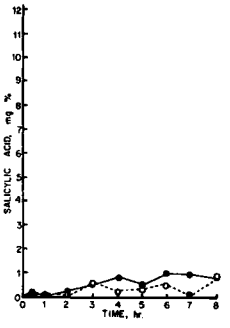

Fig. 2—Effect of DMSO on percutaneous absorption of 10% salicylic acid from polyethylene glycol ointment. Key: ●—, 15% DMSO added; ○- - -, control.

Figure 4 indicates that a more rapid drug absorption and higher salicylate blood levels were achieved when 15% DMSO was added to hydrophilic petrolatum containing 10% salicylic acid. The statistical analysis of the study involving the percutaneous absorption of salicylic acid from hydrophilic petrolatum showed that the blood levels obtained with the ointments containing DMSO were significantly higher than with the controls up to and including the determinations performed 5 hr. after initial ointment application. The absorption was approximately four times greater than that obtained when no DMSO was present. Hydrophilic petrolatum

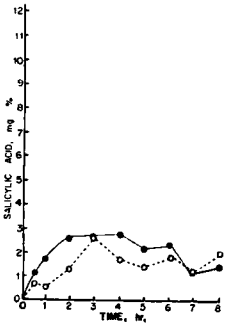

Fig. 3—Effect of DMSO on percutaneous absorption of 10% salicylic acid from a polyoxyethylene (20) stearyl ether gel. Key: ●—, 15% DMSO added; ○- - -, control.

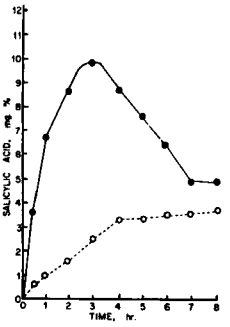

Fig. 4—Effect of DMSO on percutaneous absorption of 10% salicylic acid from hydrophilic petrolatum. Key: ●—, 15% DMSO added; ○- - -, control.

containing 10% salicylic acid formed a smooth, translucent ointment. Upon addition of 15% DMSO no change in the ointment consistency was noticed. After standing for 24 hr., droplets of DMSO appeared on the surface of the ointment. The droplets of liquid could be reincorporated into the ointment by additional mixing.

Hydrophilic ointment and hydrophilic petrolatum both containing 11.6% sodium salicylate and 15% DMSO, were compared to control ointments without DMSO, as indicated in Table II. The blood level patterns obtained in these studies with sodium salicylate are shown in Fig. 5 (hydrophilic ointment) and Fig. 6 (hydrophilic petrolatum). Statistical analysis showed that DMSO appeared to significantly decrease percutaneous absorption of sodium salicylate from hydrophilic ointment after about 5 hr. from the time of ointment application. The results of the study with hydrophilic petrolatum showed no statistically significant differences between the ointments containing DMSO and the controls. The preparations containing the hydrophilic ointment as the base were opaque white and had the same smooth consistency. The two sodium salicylate ointments prepared with hydrophilic petrolatum were smooth and white with no noticeable change in consistency resulting from the addition of 15% DMSO.

A stiff, glossy white ointment resulted when sodium salicylate in an 11.6% (w/w) concentration was added to polyethylene glycol ointment. The addition of 15% DMSO caused a slight softening of the ointment. Sodium salicylate did not appear to be significantly absorbed from polyethylene glycol ointment whether or not DMSO was present.

DISCUSSION

A statistical analysis of the results of this study indicated that DMSO functioned as a penetrant carrier, enhancing the percutaneous absorption of salicylic acid from both hydrophilic ointment and hydrophilic petrolatum. DMSO appeared, however, to have a reverse effect on the percutaneous absorption of sodium salicylate from hydrophilic ointment. In the case of sodium salicylate, the salicylate blood levels observed when DMSO was incorporated into the hydrophilic systems were lower than with the control ointment. Figures 2 and 3 indicate that DMSO appeared to produce only a slight increase in the percutaneous absorption pattern of salicylic acid from polyethylene glycol ointment and the polyoxyethylene (20) stearyl ether gel, but this increase was not found to be statistically significant. In addition, the slight

TABLE II—BLOOD SALICYLATE CONCENTRATIONS OBTAINED FROM OINTMENTS CONTAINING SODIUM SALICYLATE[a]

Time, hr.	Hydrophilic Ointment, mg. %—Test[b]	Hydrophilic Ointment, mg. %—Control[c]	Hydrophilic Petrolatum, mg. %—Test[b]	Hydrophilic Petrolatum, mg. %—Control[c]
0.5	0.20 ± 0.20	0.19 ± 0.12	0.13 ± 0.13	0 ± 0
1	0.40 ± 0.40	0.44 ± 0.16	0 ± 0	0 ± 0
2	0.19 ± 0.12	0.56 ± 0.21	0.13 ± 0.07	0.33 ± 0.2
3	0.44 ± 0.16	1.20 ± 0.48	0.13 ± 0.13	0 ± 0
4	0.51 ± 0.28	2.23 ± 0.34	0.29 ± 0.21	0.61 ± 0.15
5	0.66 ± 0.27	2.95 ± 0.63	0.41 ± 0.19	1.40 ± 0.39
6	0.91 ± 0.19	3.19 ± 0.25	0.19 ± 0.12	1.46 ± 0.33
7	0.88 ± 0.05	3.39 ± 0.23	0.74 ± 0.22	2.09 ± 0.48
8	1.38 ± 0.33	4.03 ± 0.47	1.11 ± 0.43	2.83 ± 0.56

[a] Average of eight determinations with standard error of the mean. [b] Contains 11.6% sodium salicylate and 15% DMSO in ointment base. [c] Contains 11.6% sodium salicylate in ointment base.

decrease that resulted in the percutaneous absorption pattern when sodium salicylate was incorporated with DMSO and hydrophilic petrolatum was found not to be statistically significant. DMSO appeared to have no effect on sodium salicylate absorption from polyethylene glycol ointment.

The 10% salicylic acid in the ointment systems was completely solubilized by the quantity of DMSO added to the ointment, but only about one-third of the quantity of 11.6% sodium salicylate was solubilized in the same volume of DMSO. The addition of DMSO resulted in significantly increased absorption for both hydrophilic ointment and hydrophilic petrolatum which might be attributed to the ability of DMSO to solubilize salicylic acid and then serve as a penetrant carrier for it.

Salicylic acid, which is lipid-soluble, penetrates more readily through the skin than the nonlipid-soluble sodium salicylate (15). Skin membrane permeability is thought to be determined largely by the lipid-protein structure of the cellular membrane. It is known that various organic solvents penetrate the skin with ease and enhance percutaneous absorption of lipid-soluble drugs (16). Solvents can increase permeability by solubilizing lipoidal materials of the cell wall (15). This solubilization may be induced by DMSO, thereby facilitating the passage of both the DMSO and dissolved drugs. Substances that are lipid-soluble penetrate the cell wall because of its lipoidal nature, on the other hand the uptake of water by cell membrane protein provides entry for water-soluble substances (17). Dimethyl sulfoxide is extremely hygroscopic and causes dehydration of the skin. This dehydration may free the skin membrane of water, permitting a more rapid absorption of the lipid-soluble salicylic acid through the membrane. However, dehydration would produce a reverse effect on water-soluble sodium salicylate since the sodium salicylate would have a tendency to remain in the water which was extracted from the skin into the ointment base, and thus not pass through the membrane. Kligman (2) indicated that DMSO will cross the dermal barrier rapidly and in high concentrations. Substitution of DMSO for bound water in the protein barrier, thereby causing reversible configuration changes of these proteins, was suggested by Rammler and Zaffaroni (18) as a possible mode of action. This displacement of protein-bound water could possibly further account for the fact that DMSO caused a reduction, rather than an increase, in the percutaneous absorption of sodium salicylate from hydrophilic ointment as observed in this study.

As discussed by Stolar *et al.* (12), the type of ointment base does exert a very significant influence on the extent of percutaneous absorption of both salicylic acid and sodium salicylate. Higuchi (19) suggested that activity coefficient plays a major role in absorption. Drugs held firmly by the vehicle, such as when the drug forms a complex with the vehicle, exhibit a low activity coefficient. Therefore the rate of release from such a drug-vehicle combination is slow. An example of this phenomenon is provided by the complexation of salicylic acid with PEG ointment thus providing a reservoir which releases the drug very slowly (20). The data of this present study indicated that DMSO apparently has little effect upon the release of salicylic acid from this complex with PEG polymers. The negligible results obtained with sodium salicylate and PEG ointment might also be due to similar type complexation. The PSE gel systems

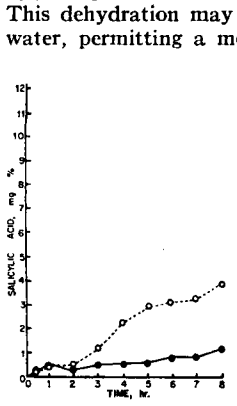

Fig. 5—Effect of DMSO on percutaneous absorption of 11.6% sodium salicylate from hydrophilic ointment. Key: ●—, 15% DMSO added; ○- - -, control.

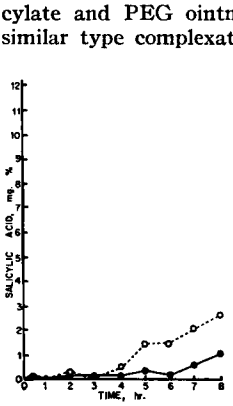

Fig. 6—Effect of DMSO on percutaneous absorption of 11.6% sodium salicylate from hydrophilic petrolatum. Key: ●—, 15% DMSO added; ○- - -, control.

responded in a somewhat similar manner as the PEG ointments. Relatively low salicylate blood levels were obtained with this gel, but it appeared that the blood levels obtained were somewhat higher than with PEG ointment. This may be due to a less intense complexation occurring in the case of the surfactant polymer than in the case of PEG.

SUMMARY

1. Dimethyl sulfoxide, in a 15% concentration, enhanced the percutaneous absorption of salicylic acid from hydrophilic ointment USP XVII and hydrophilic petrolatum USP XVII.

2. Dimethyl sulfoxide, in a 15% concentration, hindered the percutaneous absorption of sodium salicylate from hydrophilic ointment USP XVII.

3. Salicylic acid is slowly released from polyethylene glycol ointment USP XVII and a polyoxyethylene (20) stearyl ether gel system.

4. Dimethyl sulfoxide, in a 15% concentration, had little effect upon the release of salicylic acid or sodium salicylate from polyethylene glycol ointment USP XVII.

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Keyphrases

Salicylic acid, sodium salicylate absorption—ointments
Dimethyl sulfoxide effect—salicylic acid, sodium salicylate absorption
Ointment preparation—salicylic acid, sodium salicylate
Colorimetric analysis—spectrophotometer

Analysis of Steroids VIII

Determination of Conjugated Ketosteroids in Pharmaceutical Preparations Using the Sodium Borohydride Method

By SÁNDOR GÖRÖG

A simple and rapid ultraviolet spectrophotometric method has been developed for the determination of Δ^4-3-keto- and $\Delta^{1,4}$-3-ketosteroids in pharmaceutical preparations. The method consists of the reduction of the C-3 carbonyl group with sodium borohydride, followed by the determination of the decrease of absorbance due to the reduction measured by differential spectrophotometry. Other active components or excipients of the preparation whose absorbances are not changed by sodium borohydride do not interfere with the determination.

Δ^4-3-KETO AND $\Delta^{1,4}$-3-KETO bonding systems very often occur in various kinds of steroid drugs. Their simplest determination is carried out spectrophotometrically. In some cases the direct spectrophotometric measurement can be done without any difficulties with the aid of the intensive absorption band at about 241 mμ, log $\epsilon = 4.2$ (1).

The spectrophotometric determination is disturbed either by the spectra of other components present in the pharmaceutical preparation or by the excipients if they have light absorption. In

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