Chick Embryo Technique for the Evaluation of Absorption of Radioactive Sodium Iodide from Ointment Bases

By JOHN S. RUGGIERO and DONALD M. SKAUEN

In view of the lack of agreement pertaining to the absorption of ingredients from ointment bases, an investigation was conducted to develop a procedure which would be suitable for this type of evaluation. A new method was developed which measures the percentage of absorption of radioactive sodium iodide by 11-day-old chick embryos. Penetration of the isotope was measured from the ointment base through the shell membrane and chorioallantoic membrane of the chick embryo to their thyroid glands.

EVER SINCE man first used a vehicle as a diluent for applying medication, a search has been conducted to find the "ideal ointment base" which could be used to give maximum release and penetration of the active ingredients. Unfortunately, much of the information that has been gathered in this field has led to confusion with many contradictory statements appearing in the literature. For example, Macht (1) states that lanolin and lard are unsatisfactory for absorption through the skin while Fayaud (2) claims that lanolin is best adapted for use as a base to be absorbed through the skin. According to Beeler (3), washable ointment bases permit the best penetration of medicaments, while Busse (4) claims that absorption through the skin depends exclusively on the nature of the substance which is incorporated and that the base makes little difference in the total absorption.

During the past half century many ingenious methods have been proposed to evaluate ointment bases according to their ability to release incorporated ingredients. In general, these methods may be classified either as *in vitro* or *in vivo* procedures. The *in vitro* methods (5–20) can contribute to our knowledge of the release of ingredients under certain specific conditions, but cannot be correlated with *in vivo* efficacy. On the other hand, although *in vivo* methods (21–36) are more applicable, they are usually limited by the unavailability of large numbers of experimental animals which are frequently needed to yield meaningful results.

Many of the aforementioned techniques have been superseded by radioisotopic procedures (37–48). These techniques offer a number of distinct advantages, especially since radioisotopes behave the same as their stable counterparts in chemical and biological processes. Also methods for their detection are so very sensitive, making it possible to test for minute quantities of the isotope.

Perhaps the major difficulty in attempting to develop a procedure for evaluating absorption lies in the fact that no animal possesses skin quite like that of man and, therefore, any correlations which have been made from experiments conducted *in vitro* or *in vivo* are necessarily limited. True, animal and human skin have many and important differences, however, experience indicates certain fields in which animal skin may be used to predict human skin reactions. Neuhauser (49), in seeking a form of keratin which would support the growth of dermatophytes, found a very good correlation between the physical similarities of human epidermis and the shell membrane of the avian egg. Hunter and Smith (50) have previously developed a technique that utilizes chick embryos for evaluating the release of antibiotics from various ointment bases through the shell membrane and chorioallantoic membrane. According to such a procedure, absorption would only be evaluated via the transepidermal route (51) since there are no appendages present to provide for transfollicular absorption. It is interesting to note, however, that even when absorption takes place through hair follicles and other appendages found in hairy animals, the absorbed ingredients must pass through at least one layer of epidermis, which is somewhat analogous to the thin tissue system of the chick embryo.

Radioactive isotopes were incorporated into ointment bases and chick embryos were employed as test animals in an attempt to develop a new technique for evaluating the efficiency of a base to release its active ingredients.

EXPERIMENTAL

The procedure that was developed utilizes radioactive sodium iodide (I^{131}), measuring the penetration of the isotope through the shell membrane and

Received June 17, 1960, from the Research Laboratories, University of Connecticut, School of Pharmacy, Storrs.
Accepted for publication June 16, 1961.
Presented to the Scientific Section, A.PH.A., Los Angeles meeting, April 1958.

chorioallantoic membrane to the thyroid gland of chick embryos (Fig. 1).

Following preliminary experiments indicating the degree of absorption of radioactive sodium iodide by chick embryos of various ages, it was decided that 11-day-old embryos which had been incubated with the isotope for 48 hours would be most suitable for use in evaluating the release of ingredients.

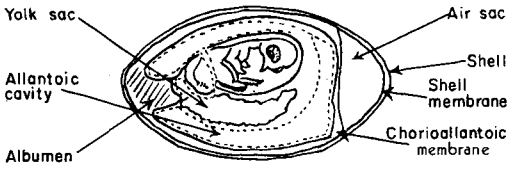

Fig. 1.—Schematic drawing of a chick embryo.

Preparation of the Ointment.—The ointment bases which were to be evaluated were prepared by carefully pipetting a calculated amount of radioisotope onto a known weight of ointment base that had been smeared onto a watch glass. After allowing this mixture to dry, the ointment was thoroughly mixed to insure uniform dispersion of the isotope.

Application of the Ointment.—Small glass rings were utilized in the application and standardization of the ointment in order to assure the fact that the ointment would come into contact with the same area of shell membrane in each embryo employed. These rings were cut from 10-mm. Pyrex tubing by a rotating carborundum wheel. The height of the rings was established by means of an adjustable stop which permitted little variation in height.

The rings were filled with ointment in much the same manner as one would fill an ointment jar. The rings were placed upon a piece of parchment paper and held firmly with a forcep while the ointment was added by means of a microspatula. It was possible to transfer the filled rings to the shell membrane of the embryos by first gently sliding the tip of a microspatula underneath the ring and then carefully pushing the ring from the spatula with the aid of a pair of forceps. Due to the delicate nature of the shell membrane, extra caution was employed when depositing the ring on the membrane so that trauma would not result and influence the degree of absorption.

The ointment was applied to the shell membrane by lowering the ring through a suitable opening which was made by piercing the shell at the air sac at a point where the shell membrane conveniently divides into two layers (Fig. 1). After application of the ointment, the opening in the air sac was sealed with Scotch Tape and the embryos were returned to an incubator at 37.5° where they were allowed to remain for 48 hours.

Determination of the Amount of Radioactivity Applied.—Usually three rings containing the ointment (more could be used depending on the total number of cases involved) were set aside from each batch of ointment used in order to ascertain the relative amount of radioactivity that was applied in each case. The amount of radioactivity in the rings, selected at random, was determined by dissolving the ointment in a suitable solvent, and then assaying various aliquots of the solutions. The result of averaging the radioactivity indicated in each of the samples was chosen to represent the relative amount of radioactivity applied in each case.

Determination of Amount of Radioactivity Absorbed by the Thyroid Glands.—After the radioactive ointment had remained in contact with the shell membrane for 48 hours, the eggs were removed from the incubator. The embryo was separated from the egg, taking care not to contaminate the embryo with any excess ointment that remained. The thyroid glands were viewed through a stereoscopic Bausch and Lomb microscope and removed with the aid of watchmaker's forceps. The glands were macerated with 10% sodium hydroxide solution, dried, and assayed for radioactivity.

Calculation of the Percentage of Absorption.—The percentage of radioactivity that was absorbed in each case was calculated by dividing the amount of radioactivity found in each thyroid by the amount of radioactivity that was applied. The percentage of absorption indicated by each of the embryos in the group was then averaged and reported as the percentage of absorption of radioactivity for a particular ointment base. A sample form showing all of the pertinent data is illustrated in Table I.

TABLE I.—EXAMPLE OF STANDARD FORM AND RESULTS OBTAINED FOR A TYPICAL OINTMENT BASE[a]

Embryo No.	C.p.m. in Thyroid above Background	Percentage of Applied Radioactivity in Thyroid
1	50.0	0.33
2	16.8	0.12
3	35.6	0.23
4	23.2	0.15
5	18.8	0.12
6	37.4	0.24
7	22.6	0.15
8	27.4	0.18
9	31.2	0.20
10	10.0	0.07
11	33.8	0.22
12	29.2	0.19
13	14.0	0.09
	0.176 ± 0.075 (mean % absorbed)	

[a] Amount applied, average of three standard rings, 15,360 c.p.m.; duration, 48 hours; base, Almay emulsion base (product of Almay, Inc.); background count, 22 c.p.m.

SUMMARY

A method is presented for determining the efficiency of ointment bases to release sodium iodide. The method utilizes the thin tissue system provided by the shell membrane and chorioallantoic membrane of 11-day-old chick embryos and the ability of the thyroid gland to absorb and concentrate radioactive sodium iodide. Such a system provides a rather simple, inexpensive, and rapid analysis of absorption.

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Evaluation of the Absorption of Radioactive Sodium Iodide from Various Ointment Bases by Means of a Chick Embryo Technique

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Forty-seven ointment bases were evaluated for their ability to release radioactive sodium iodide when evaluated by a chick embryo technique. In general, ointments of the hydrophilic type indicated better release of the isotope than did bases of the absorption or oleaginous type.

A PREVIOUS REPORT (1) illustrates the development of a chick embryo technique which permits the investigator to evaluate the ability of various ointment bases to release radioactive sodium iodide. This chick embryo technique is employed here to determine the release of radioactive sodium iodide from different ointment bases. Limited studies were also conducted to evaluate the effect of surface-active agents and of varying quantities of water on the degree of absorption that might take place from ointment bases.

Received June 17, 1960, from the Research Laboratories, University of Connecticut, School of Pharmacy, Storrs.
Accepted for publication June 16, 1961.
Presented to the Scientific Section, A.PH.A., Los Angeles meeting, April 1958.

EXPERIMENTAL

Various ointment bases were selected as representatives of the three major categories of ointment bases as listed by Robinson (2), namely; those which are oleaginous or water repellent; those which absorb water, but are greasy and nonwashable; and those which are water miscible or water soluble.

Ointment Bases Selected.—*Oleaginous Bases.*—petrolatum U.S.P. XV; white petrolatum U.S.P. XV; yellow ointment U.S.P. XV; white ointment U.S.P. XV; lard N.F. X; Domolene, product of Dome Chemicals Inc.; Plastibase, product of E. R. Squibb and Sons; Spry, product of Lever Brothers, Inc.; Singiser base No. 200 (3); Singiser base No. 225 (3); Singiser base No. 425 (3); Singiser base No. 625 (3); 15% Epolene in liquid petrolatum, Epolene is a product of Eastman Chemical Products, Inc.