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Techniques for Evaluating Dermal Irritation

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Synopsis—The use of Sulphan Blue (sold as Alphazurinine 2 G) is an aid in visualizing the response of rabbit's skin to mild irritants. In as short a period as one hour skin changes may be observed. Sulphan Blue can also be used as an aid in the visualization of those materials which induce photosensitivity in the guinea pig.

Introduction

In developing a preparation to be applied to the skin the cosmetic manufacturer must take into consideration the factors of public acceptance and safety. In most cases the elegance of the finished product is the deciding influence in its promotion. The possibility of any irritating effects that this product may have on the skin, either because of its final composition or because of one of its many components, is of equal importance. Since the use of these products is widespread and the usage directions are very general, it is of great importance that the introduction of a new preparation shall produce no irritation even in one individual in ten thousand.

In order to check the innocuousness of a new preparation animal test methods are utilized in the laboratory. Patch testing techniques employ the rabbit or guinea pig as the animal of choice. The time of application of the test samples is purposely lengthened to magnify the extent of reaction that may occur.

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The present animal test methods available to most laboratories have proven useful in eliminating those ingredients and products that have been found to be moderate to severe irritants. Those preparations which may produce only a very slight irritation, in an occasional animal, or to such a slight degree that it may be overlooked, must be evaluated by some method which shows greater sensitivity than the methods now available. The most important criterion is to be able to predict, from laboratory observations, the low order of sensitivity that may occur in the general public.

The skin of the rabbit responds to the application of irritants through changes in its blood supply at the site of treatment. These responses are characterized by erythema, which involves vasodilation at the site of application, edema, which is an excessive accumulation of fluid in the tissue spaces, and necrosis, which is an actual damage of the cellular structure of the skin if the insult is sufficiently strong or prolonged. The determination of the mildest form of erythema, oftentimes very difficult to evaluate objectively, is an important observation.

Several methods have been reported in the literature which were used to increase the sensitivity of the erythema response. The basis of this increased reaction is the injection of a substance which permits greater visualization of the site and therefore magnifies the reaction obtained. This response is dependent upon substances, such as vital dyes, collecting in areas where vasodilation has occurred.

Tainter *et al.* (1) and Hoppe *et al.* (2) indicated many years ago that Trypan Blue would be effective in this respect, and recently Finkelstein *et al.* (3) have also used this material. Other dyes that have been used are Evans Blue, Kiton Green, and Pontamine Sky Blue. Most recently Brown and Clarke (4) have shown that Sulphan Blue is a dye which could be used as an aid in the visual assessment of mild erythema. In this laboratory a 6% solution of Sulphan Blue (sold as Alphazurinine 2 G by National Aniline Div. of Allied Chemical Co.) was prepared in sterile, distilled water and injected intravenously at a dose level of 1 ml per rabbit. Sulphan ue Blhas the distinct advantage of coloring the skin within minutes after it is injected and completely disappearing from the skin area within four hours after injection.

Methods and Results

The patch test is performed by first removing the hair from the back of the rabbit with an electric clipper. The test material is applied to a 2.5 × 2.5 cm Webril patch (The Kendall Co.), which is in turn placed

on the clipped backs and held in place with Blenderm Surgical Tape (Minnesota Mining & Manufacturing Co.). Test substances were utilized which, under normal conditions, either caused no visible reaction after being applied to the skin for 24 hours or produced the mildest type of erythema. An injection of Sulphan Blue into a rabbit whose response was borderline (Fig. 1) produced a definite area of change in the skin directly beneath the patch (Fig. 2) In order to determine if the time necessary for the formation of the primary erythema could be decreased, patches were removed after four hours (Fig. 3) and one hour of contact (Fig. 5). At these application times the test materials did not show a visual erythema at the site of application. However, an injection of Sulphan Blue into these animals (Figs. 4 and 6) showed a definite increase in the intensity of the dye at the site of product application. This indicated that changes were occurring in the skin, as evidenced by the concentration of the dye; this, in turn, could be correlated with mild erythema which could not be seen without the aid of the injected dye.

Vinson and Borselli (5) recently outlined a method for developing photosensitization in the guinea pig. It was felt that there was a need to re-examine this method to, *i*, determine if the procedure and response could be duplicated using strains of animals other than the closed strain which was employed by the authors, and, *ii*, whether the use of Sulphan Blue could increase the sensitivity of the test.

Guinea pigs were purchased from three commercial breeders, and the procedures followed were essentially those described in the published paper (5). Groups of five animals of both sexes each received 0.05 ml of a 2% solution in alcohol of either Bithionol or Temasept II. After application of the test material to the clipped cervical region, the animals were exposed for 15 minutes to a No. 275 Westinghouse Ultraviolet bulb at a distance of 46 cm. This procedure was repeated daily for five consecutive days. After the last treatment the animals were allowed to remain untreated for seven days. Finally, 0.05 ml of a 0.1% solution of either test agent in olive oil was applied to the same group of animals that had previously received this material. The animals were then placed under the ultraviolet light for 15 minutes and skin readings recorded 24 hours later.

Following the initial application of the test materials in absolute alcohol no erythema was evident either in those animals receiving the Bithionol or those receiving the Temasept II. Ultraviolet irradiation, after these same animals had received 0.05 ml of a 0.1% solution of the

test agent in olive oil, did cause mild erythema in those animals which had been treated with Temasept II. After the skin responses had been recorded, each animal received an intraperitoneal injection of 1 ml of a 6% solution of Sulphan Blue. Within ten minutes after the injection the dye had accumulated at the sites of application of the Bithionol but not at the sites of application of the Temasept II. Since the response reported was obtained in animals from three different sources it may be concluded that the method of Vinson and Borselli can be utilized to screen products which may induce photosensitivity.

Summary

Sulphan Blue should be considered as a useful adjunct in evaluating those materials which produce a minimal degree of vasodilation which is normally not readily discernible in rabbit patch tests or the guinea pig photosensitivity tests.

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Figure 1. Borderline reaction.

Figure 2. Borderline reaction following Sulphan Blue injection

Figure 3. Erythema after four hours

Figure 4. Erythema after four hours following Sulphan Blue injection

Figure 5. Erythema after one hour

Figure 6. Erythema after one hour following Sulphan Blue injection