References

1. PARKER, R. F., and LUCE, S. *J. Bact.*, **56**, 75 (1948).
2. GRUNBERG, E., SCHNITZER, R. J., and UNGER, C. *Yale J. Biol. Med.*, **20**, 479 (1948).
3. GRUNBERG, E., UNGER, C., and ELDRIDGE, D. *Ibid.*, **21**, 121 (1948).

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The Detection of Crystals Following Local Injection of Cortisone and Compound F into the Skin of Man

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In the course of investigative studies (*1*) with the local application and injection of four oxysteroids, a cortisone acetate,[1] a water soluble ester[1] of cortisone, Compound F free alcohol, and Compound F acetate,[1] it was found necessary to attempt to control some of the observations by the examination of crystals of these compounds in the skin.

In routine paraffin-fixed sections crystals of these steroids cannot be found. The purpose of such studies on crystals is to determine the morphologic distribution patterns of such crystals in relationship to possible local mechanisms (epidermis, dermis, etc.) and the persistence in relationship to possible persistent local therapeutic effects from local injections. Moreover, an effort should be made to determine whether the crystals remaining in the skin are the same as those injected. Finally, a study of these crystalline masses may serve to explain the tissue reactions produced in normal tissue by the local injections of suspensions of cortisone and Compound F.

In his study of skin irritations in animals, Ake Nilzen (*2*) in our department, together with Knut Schmidt-Nielsen, was able to detect crystals presumably of cortisone acetate in the skin of guinea pigs following local injection. Their technique, in brief, was the preparation of frozen sections without fixation of tissue. We have modified the technique of Nilzen and Schmidt-Nielsen in our recent experiments with cortisone acetate and Compound F.

The steroids used in the work reported here included only cortisone acetate, Compound F, and cholesterol (plate type only). The vehicles used included, first, the suspension mixture of benzyl alcohol and polyoxyethylene sorbitan monooleate, sodium carboxymethylcellulose; and, second, saline as the only suspending agent. It was necessary to use saline suspensions since the other vehicle had definite reactions on local injection (*1*).

At first, suspensions of the steroids in the two vehicles were observed under varying conditions such as room temperature, freezing, and heating to attempt to determine any changes in the size of the crystals. At the suggestion of Schmidt-Nielsen, we incubated crystals of cortisone with minced human tissue (skin and subcutaneous tissue) at 37° for 24 and 48 hr. There was no change in the size of the crystals. The crystals of Compound F acetate measured in our experiments 1.25 μ and are smaller than those of cortisone acetate, which varied in length from 6 to 9 μ (Fig. 1). The crystals of the free alcohol of Compound F are 30–45 μ. The heavy saline suspensions of cortisone acetate gave larger crystals than the suspension of cortisone acetate in benzyl alcohol and the other suspending agents.

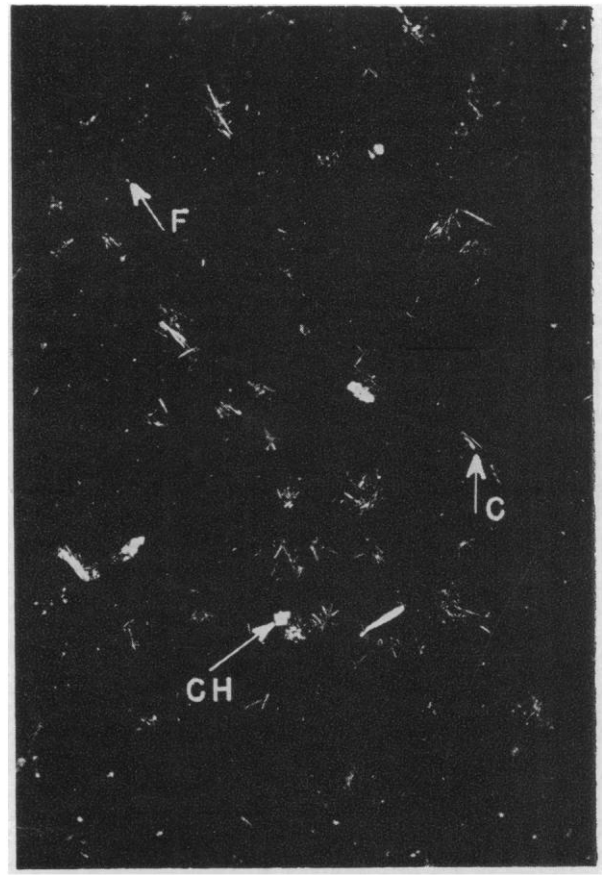

FIG. 1. Mixture of crystals of cortisone acetate (*C*), Compound F acetate (*F*), and cholesterol (*CH*). Vehicle, benzyl alcohol, polyoxyethylene sorbitan monooleate, and sodium carboxymethylcellulose; polarized light; approx ×100.

The following materials were injected intradermally into the normal skin of man: (1) vehicles without the steroid; (2) cortisone acetate suspensions, 25 mg/cc; (3) Compound F suspension, 25 mg/cc; (4) cholesterol, 25 mg/cc.

Injections of cortisone acetate and Compound F were done also with the Hypospray jet injection apparatus. Subsequently, injections of the steroids were made into pathologic skin and into normal skin which,

[1] Supplied by Augustus Gibson, Merck & Co., Inc., Rahway, N. J.

after the injection of the steroid, was subjected to various forms of local irritation. Biopsies were taken of skin from a few minutes to 4 months after injection.

Scalpel or punch biopsies were taken, in many instances without local anesthesia. The tissue was fixed in 10% neutral formaldehyde (calcium hydroxide) for varying intervals, then frozen sections were made. The following staining techniques were used: hematoxylin-eosin, oil blue N, Sudan III, Sudan IV, Schiff's periodic reagent, Giemsa, trichrome, van Gieson, and tetrazolium salts. The sections were mounted in glycerin jelly. A series of sections was also done with the vacuum freeze-drying technique, but no crystals were observed in the paraffin-imbedded sections. Experiments are now under way with other imbedding agents which are water-soluble. The sections were examined under ordinary light, polarized light, phase contrast, and ultraviolet light through a Wood's filter. For additional studies on the analysis of the local tissue reactions to locally injected steroids, routine paraffin tissue sections with a variety of staining techniques were also made.

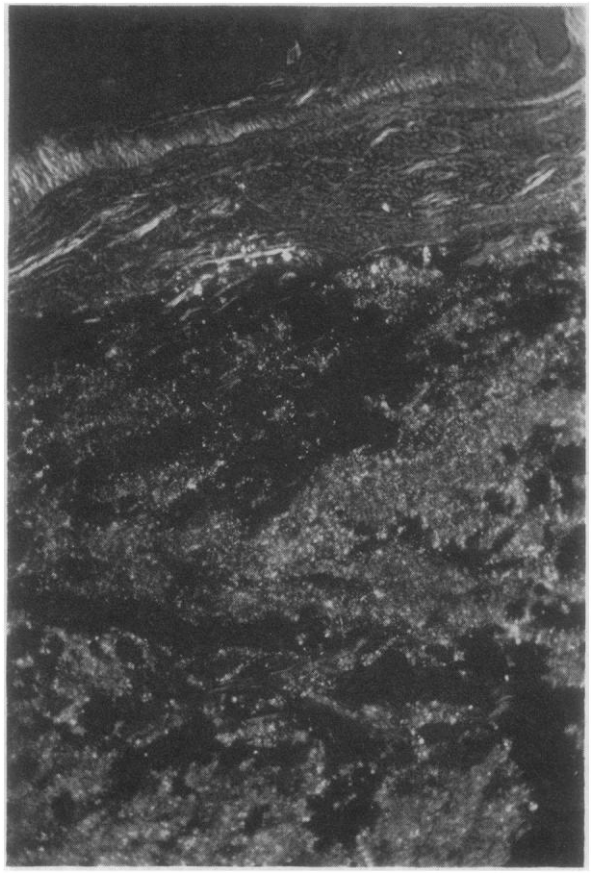

FIG. 2. Showing depth of crystal deposits of Compound F acetate in the skin of man 5 days after injection. Unstained section, approx × 100; polarized light.

After local injections, the insoluble crystals of cortisone acetate and Compound F are clearly visible in these unstained sections. The crystals are observed in large masses relatively deep in the skin even after superficial injections (Fig. 2). In superficial injections made with the Hypospray jet injection apparatus a broader band of crystal deposit was found. Experiments are under way at present to attempt to produce some intra-epidermal localization of crystals. In most instances the crystal size appeared to be the same as the size of an *in vitro* suspension. In some instances large birefringent crystals were observed from 5 min to 24 hr after the local injection of cortisone acetate. It is not entirely clear at present just what they represent. Except for this observation, it is apparent that there is no obvious change in these insoluble crystals as they persist in the skin. There is no proof, for example, that in the skin of man cortisone acetate can change into Compound F. Injections of the vehicle alone produced no crystals. With our present techniques, it was possible to identify crystals only when they occurred in large masses, not as individual crystals or portions of crystals. No fluorescence of crystals was observed under ultraviolet light.

For the staining techniques, Giemsa stains appeared to make the crystals more prominent by contrast staining of neighborhood tissue, both with cortisone and Compound F. Hematoxylin-eosin contrast staining was also effective. Experiments have been initiated recently with Frazer at the Environmental Health Center for electron microscopic examination of scrapings from the injection site after varying intervals of time. As additional controls of the local injection of crystals, chemical analyses of the local tissue for steroids are being done at the Merck Institute.

Following superficial injection, crystals of the steroid may persist in the skin for some months, especially when injected with the vehicle containing benzyl alcohol and polyoxyethylene sorbitan monooleate, sodium carboxymethylcellulose. Saline suspensions of the steroids do not persist as long. In pathologic skin, the crystals also do not persist as long.

Detection of crystals of steroids on local injection is a very important tool, especially with Compound F, since this steroid, unlike cortisone acetate in our investigations (*3, 4*), has a definite effect on various types of local inflammatory processes in the skin of man. One recent important phase has been the local effect of Compound F on lymphoma tissue. This is limited rather sharply to the area of injection. The study of frozen sections for crystals provides a definite help for the observations of the pattern of distribution of crystal masses. The superficial or deep deposit of crystals and the mass of crystals may influence the locus of the inhibitory reaction produced. There is a rough correlation between the degree of inhibition and the amount of Compound F present. The Hypospray jet injection apparatus provides for large masses of Compound F locally. It has not been possible, with our techniques, as indicated above, to determine whether these insoluble crystals change into

other compounds before solution. With the study of such crystal masses, it should be possible to determine the effects of various enzyme systems which may make for changes in the rate of solution. The persistence of the crystal deposits because of slow solution suggests that local therapeutic results may also persist for some time. This is borne out by our recent studies.

References

1. GOLDMAN, L., THOMPSON, R. G., and TRICE, E. R. *Arch. Dermatol. u. Syphilis*, **65**, 177 (1952).
2. NILZEN, A. *J. Investigative Dermatol.*, **18**, 7 (1952).
3. GOLDMAN, L. *J. Am. Med. Assoc.*, **149**, 265 (1952).
4. GOLDMAN, L., PRESTON, R., and ROCKWELL, E. *J. Investigative Dermatol.*, **18**, 89 (1952).

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Comments and Communications

Scientific Communication

JACQUES AVIAS' letter (SCIENCE, **115**, 250 [1952]) persuades me to state my own conviction that access to world literature is the next practical problem to be attacked in the art of scientific communication.

Nuclear Science Abstracts points the way. Every article published should be abstracted promptly at a central institute. Abstracts should be given serial numbers, each number unique. Authors and abstractors should list the words under which the article should be indexed, using single words for rare subjects, but using one or several modifying words for usual subjects. The ideal should be a bulky alphabetical subject index leading to the word (concept) desired, however deeply embedded, and without recourse to the title. The index of the *Encyclopaedia Britannica* is something on the order I have in mind, but it does not have enough modifiers on the index words to lead one directly enough to the few places where that word is used in the connection the researcher desires. The art of such indexing would have to be developed.

At present books are indexed by title, and there is no way of knowing from the indexing what the several chapters contain. It would be worth while to give each chapter an abstract, with its own unique number.

The power of such an index would be enormous. It would be attainable because the reference for every index heading would be only the bare abstract number, not the entire journal, volume, page, and year.

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The Teaching of Specific Dynamic Action

THE phenomenon of specific dynamic action seems to be a source of misunderstanding and perplexity to a great many students and to many teachers of biochemistry and physiology. I would like to suggest that the reason is the way the subject is usually presented by lecturers and in textbooks.

The student is likely to be told that the ingestion of protein equivalent to 100 kcal gives rise to 130 kcal. He may be told that carbohydrate and fat also have specific dynamic action, but in smaller degree. He will probably be told that, to allow for this, extra kilocalories must be added to the food intake that meets basal and activity requirements. An obvious way to think about these statements is that, if kilocalories are required to release those of the food, their release in turn must depend upon the expenditure of other kilocalories. How, then, can the effect ever be overcome? How can there be caloric equilibrium, or positive balance, or growth, and why do we not waste away because we eat? This seems to be a common complication of thought concerning specific dynamic action, and one can hardly believe that in such cases it is given credence.

Specific dynamic action as a topic merits only brief consideration in a general or elementary course, but the more briefly a subject is treated, the greater is the necessity of presenting the essentials clearly. This applies whether the course provides the student's only encounter with the subject, or whether it serves as preparation for more advanced study. The important thing in this particular case is that specific dynamic action has been demonstrated, and what it is and what it implies should be outlined. The following proposal provides a means of doing this without leading to perplexity. It depends upon stressing the relationship of specific dynamic action to the basal metabolism, which is more important than its relationship to the food.

When food is given to an animal under basal conditions, there is a subsequent period when the metabolic rate is higher, even though the same conditions are maintained. The length of this period and the degree of elevation above the basal depend principally upon the kind and quantity of the food. For any one food or food mixture the total increment is proportionate to the amount fed. Foods high in protein have the greatest effect. It follows that under such conditions, if the caloric value of the food given does not exceed the basal expenditure plus the increment, there will be negative caloric balance.

Quantitatively, the specific dynamic action is the energy increment to the basal which results from food utilization. The importance of the relationship of specific dynamic action to the basal metabolism must be realized for a proper understanding of the phenomenon. This is developed in the following example:

Suppose that an individual under basal conditions expends 60 kcal/hr. He is given food with a caloric