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Sensitivity Tests to Insulin in Patients with Local Skin Lesions from Insulin. (21067)

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(Introduced by J. H. Sandground.)

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A considerable number of diabetic individuals experience local skin reactions following the administration of insulin in the conventional manner. In previous years these were ascribed to chemical irritation arising either from the alcohol used for sterilization of syringes and needles, from the preservatives added in the manufacture of insulin or from the acidity of the earlier insulin preparations. At present, most workers consider these local manifestations as hypersensitivity to either the impurities within the insulin preparations, to the insulin protein itself or to the pancreatic protein of the species from which the hormone is derived. Recently Fabrykant and Ashe (10,10a) reported that skin changes at the site of insulin injections could be prevented by introducing the hormone into the deeper layers of the subcutaneous tissue.

The present investigation is concerned with sensitivity studies in a group of patients presenting local skin reactions while on insulin therapy in order to shed more light on the genesis of such manifestations.

Plan of study. Sixteen diabetic patients, whose local dermal reactions to insulin therapy consisted of nodules with or without erythema or stinging, were tested intradermally on the outer aspects of the arms with 0.02 cc of several insulin products in a variety of dilutions, as well as with commercial extract of beef and pork. Control tests were performed with the same quantity (0.02 cc) of Coca's diluting fluid. A group of 16 non-diabetic controls who were free from any skin affection, were similarly tested with the same materials and technic. The results were read 2, 5 and 10 minutes after the injection and were interpreted as negative, slight, moderate, marked or any combination of the last three, depending on the size of the wheal, the degree of erythema and the presence of pseudopods. For example, if the injected material did not produce a wheal greater than that from the control solution, the reaction was considered negative; if the wheal was only a little larger and a small area of erythema had developed, the reaction was termed slight; and if the wheal was more than twice the size produced by the original amount administered, with a considerable areola and the appearance of pseudopods, it was designated marked. A moderate reaction was one between these two. Some patients presented a transition from one type of reaction to another, and this was interpreted as slight-to-moderate or moderate-to-marked. We have considered as significant only the slight-to-moderate, moderate and

TABLE I. Skin Reactions of 16 Diabetic Patients with Local Response.

Product	Type of skin reaction: Negative	Slight	Slight-to-moderate	Moderate	Moderate-to-marked
Regular I (full)*	0	8	6	2	0
Regular I 1:10	7	9	0	0	0
Regular I 1:100	13	3	0	0	0
Prot. zinc I (full)	1	8	4	3	0
Prot. zinc I 1:10	11	5	0	0	0
Prot. zinc I 1:100	16	0	0	0	0
N.P.H. I (full)	1	7	6	2	0
N.P.H. I 1:10	7	9	0	0	0
N.P.H. I 1:100	15	1	0	0	0
Boiled regular I (full)	0	2	10	2	2
Beef protein	11	5	0	0	0
Pork "	11	2	1	1	1

* I = insulin; (full) = full strength.

moderate-to-marked reactions. In addition, passive transfer tests were carried out with the use of sera from five patients with nodules who showed significant positive skin-test reactions to insulin. The sera were injected in the amount of 0.1 cc into 2 sites on the backs of five acceptors. Twenty-four hours later, 0.02 cc of the respective insulin preparation used by each of the 5 donors was injected into the sensitized sites as well as into 2 adjacent nonsensitized control areas.

Results. From Table I, which records the skin test in patients in whom nodules were present, it may be seen that almost identical results were obtained with the use of regular, protamine zinc and NPH insulin. Two patients who demonstrated reactions to full-strength PZI showed only a slight-to-moderate reaction to regular and NPH insulin. There were 6 patients whose reactions to regular and NPH insulin were interpreted as slight-to-moderate, and of these, 4 presented the same reaction to PZI. Dilutions of 1:10 and 1:100 of these 3 types of insulin elicited no significant skin changes. In almost all the patients tested with regular insulin previously boiled for one hour, the skin reactions were more pronounced than when there had been no boiling. Beef protein in the usual testing strength solution gave no significant reaction, and only 3 patients exhibited slight-to-moderate or marked reactions to pork protein administered in the conventional dosage.

The results recorded in the control group composed of nondiabetics were analogous to those in the diabetic patients (Table II). With full-strength regular insulin a moderate-to-marked reaction was noted in two subjects, a moderate reaction in one and a slight-to-moderate reaction in 5. One patient presented a negative reaction to regular and NPH insulin but a moderate one to PZI, and another one a slight reaction to PZI and none at all to NPH insulin. In the group as a whole the reactions to boiled regular insulin were somewhat more pronounced. Pork protein elicited but a slight response in 5 patients and a slight-to-moderate reaction in one subject whereas beef protein demonstrated but a slight response in one.

The Prausnitz-Küstner passive transfer test performed with sera from 5 patients exhibiting the most intense skin reactions when tested with insulin, evoked a negative response in each case.

Discussion. The results of the insulin-sensitivity skin tests which we have recorded in a group of patients with nodules, stinging and areas of erythema due to insulin administered by the conventional method, were no different from those obtained from a control group of nondiabetics, non-users of insulin. Boiled insulin which has been recommended as a means of avoiding allergic reactions to this hormone (11) produced a larger wheal and area of erythema than the same quantity of insulin not subjected to boiling. The negative response to beef and the occasional positive skin reactions to pork protein solutions releases these substances from the responsibility in the

TABLE II. Skin Reactions of 16 Normal Nondiabetics.

Product	Type of skin reaction: Negative	Slight	Slight-to-moderate	Moderate	Moderate-to-marked
Regular I (full)*	1	7	5	1	2
Regular I 1:10	11	4	0	1	0
Regular I 1:100	15	0	1	0	0
Prot. zinc I (full)	0	9	6	0	1
Prot. zinc I 1:10	14	2	0	0	0
Prot. zinc I 1:100	16	0	0	0	0
N.P.H. I (full)	3	7	3	2	1
N.P.H. I 1:10	11	4	1	0	0
N.P.H. I 1:100	15	1	0	0	0
Boiled regular I (full)	0	7	4	2	3
Beef protein	15	1	0	0	0
Pork "	10	5	1	0	0

* I = insulin; (full) = full strength.

production of local dermal reactions to insulin.

It is well known that positive skin tests do not necessarily indicate clinical sensitivity and that the typical response of the skin to injury, manifesting itself as an area of erythema with wheal formation, is the same whether it be the result of a nonspecific or of a true immunologic reaction(12).

There is a considerable mass of experimental and clinical evidence to prove that insulin can produce anaphylactic phenomena in animals and hypersensitive reactions in man. Antibodies to insulin have been reported(1,6-8,14,15), and positive results to skin test elicited with crystalline insulin as well as with modified insulin products(1-9). In a few instances passive transfer of reagins has also been accomplished(1,6-8). Since these immunologic studies were performed in animals or in humans with generalized allergic reactions to insulin (even though some of these individuals were reported to have manifested also local reactions) they have no bearing on our observations, which concern patients with skin reactions confined solely to the injected areas. It is therefore highly significant that we were unable to demonstrate any difference in the frequency and magnitude of positive skin-test reactions to various insulin products as manifested by the two groups studied namely, patients presenting local dermal reactions to insulin and the controls, who used no insulin at all.

Noteworthy too is our failure to obtain positive passive transfer tests in the five patients with a maximal response to skin testing with insulin, since in such patients one would expect positive transfer phenomena if their local reactions were truly allergic in nature. Although the group here studied may be considered too small for statistical analysis, the data clearly indicate that skin reactions to insulin of a degree sufficient to be interpreted as moderate or marked are undoubtedly due to some nonspecific mechanism and not to an antigen-antibody interaction.

Summary. 1. Sixteen diabetic patients exhibiting local skin reactions to insulin as nodules with or without erythema or stinging were tested intradermally with various insulin products, beef and pork protein. 2. Sixteen nondiabetics not using insulin were similarly tested with the same substances. 3. Passive transfer tests with the sera of 5 patients manifesting local reactions to insulin were performed. 4. Skin reactions noted in patients exhibiting local reactions to insulin were no different from those obtained in the control group of nondiabetics, non-users of insulin. 5. The Prausnitz-Küstner passive transfer phenomenon was absent in all 5 instances. 6. It is concluded that local nodules with or without stinging or erythema observed in patients receiving insulin therapy are not due to any antigen-antibody mechanism.

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Study of Ducrey's Bacillus and Recognition of a Gram-Positive Smooth Phase.* (21068)

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In the current and generally accepted classification system, *H. ducreyi* appears to have a rather definite taxonomic position in the genus Hemophilus(1). A review of the published studies lends little support to this view, and reveals that the accumulated knowledge of this bacterium is exceedingly limited (2). The lack of basic information appears to be due primarily to the difficulties which have attended the cultivation of Ducrey's bacillus. *H. ducreyi* is the recognized cause of chancroid. Most investigators agreed that the causative microorganism is extremely difficult, if not impossible, to isolate from primary chancroidal lesions. Primary lesions are invariably contaminated with a variety of bacterial species and this together with the fastidious growth requirements of *H. ducreyi* appear to make identification of this organism impossible by present-day procedures(3). The well-known characteristics of Ducrey's bacillus seem to have resulted from studies of cultures which were obtained from bubo material. The successful isolation of *H. ducreyi* from aspirated bubo fluid has been reported on numerous occasions(4,5). In all investigations the importance of incorporating whole blood in culture media has been emphasized. It has been determined that a growth factor necessary for the Hemophilus group is also required for *H. ducreyi* cultivation(6). Previous bacteriologic procedures have not permitted detailed characterization of Ducrey's bacillus in respect to colonial morphology, or to its physiologic or serologic behavior. The bacteriologist, therefore, with limited means of identification, can offer little assistance in the diagnosis of chancroid as a disease entity. The diagnosis of chancroid, therefore, is dependent upon clinical impressions which are not yet clearly defined. The development of new methods and procedures for the cultivation and identification of Ducrey's bacillus could offer a partial solution to this diagnostic problem. The successful accomplishment of these objectives should eventually help to explain some of the unanswered questions pertaining to epidemiologic and immunologic aspects of the disease.

The present report deals with a description of our experimental methods and procedures applied to the study of Ducrey's bacillus.

Experimental. Stock *H. ducreyi* cultures were received from Dr. R. B. Dienst, Medical College of Georgia; Dr. J. D. Thayer, Venereal Disease Experimental Laboratory, Chapel Hill, N. C. and from the Lederle Laboratories.

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