

PASSIVE TRANSFER OF COLD URTICARIA*

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THE occurrence of urticaria due to cold was described by Duke in 1925¹ as one of the manifestations of physical allergy and has since been reported by many authors. The studies of Lewis² suggested that the development of urticaria caused by such physical agents is mediated by H-substance similar in physiologic activity to histamine. Further evidence that a histamine-like substance is involved in the reaction was offered by Horton, Brown, and Roth³ who showed that development of cold urticaria released into the circulation a substance that produced tachycardia, lowered blood pressure, and increased secretion of free acid in the stomach. More recently antihistamine drugs have been reported to relieve the symptoms of cold allergy.^{4, 5}

Evidence of an antibody mechanism in cold urticaria is less conclusive. Antibodies reacting with isoantigens at temperatures near 0° C. are familiar in cold agglutinins and cold hemolysins. The presence of cold agglutinins in high concentration is occasionally manifested clinically by vasomotor symptoms, but not cold urticaria. Harris, Lewis, and Vaughan⁶ described cold urticaria associated with cold hemolysins in cases of syphilis. The serum of such a patient locally sensitized the normal skin of another syphilitic patient so that it reacted to cold with the development of urticaria. The skin-sensitizing substance, which they called dermolysin, was removed from the serum by adsorption with erythrocytes in the cold, suggesting it was identical with the cold hemolysin.

Cold urticaria has been reported in patients with cryoglobulinemia associated with various systemic diseases, although purpura and other types of skin sensitivity to cold are more frequent.⁷

The majority of patients with cold urticaria are otherwise healthy and do not have syphilis or diseases producing cryoglobulinemia. In a number of instances the sera of such patients have been reported to sensitize passively normal skin to cold, but a large number of negative reports have cast doubt on the occurrence of passive transfer of this type of reaction.^{8, 9} Walzer, in a review of the subject, concluded that the evidence of passive transfer of simple cold urticaria was questionable.⁸

The present report is of studies of a case of cold urticaria in an otherwise healthy patient whose serum readily transferred sensitivity to normal skin, with observations on the properties of the active factor in the serum and an unsuccessful attempt to isolate an antigen from chilled normal skin.

CASE REPORT

J. S., a 42-year-old man, was seen June 6, 1949, because of recurrent hives for 6 weeks on exposure to cold. There was a family history of allergy, the patient's brother and 2 of the patient's children having allergic rhinitis. His general health had been good in the past,

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although he had had hay fever in the grass and ragweed pollen seasons and nonseasonal rhinitis on contact with horses for many years. There was no history of syphilis. On several occasions he had noted a few scattered hives after a hot bath but he had no unusual reaction to cold before or during the preceding winter, part of which he spent in New York City.

In April, 1949, while in Florida, he noted an itching hive on his leg which progressed to an isolated purpuric spot 2 to 3 cm. in diameter and gradually faded over a period of 2 weeks. This was thought to be the bite or sting of an unidentified insect or jellyfish. During the 6 weeks after this occurrence, he noted on 3 occasions that his face became swollen and itchy after exposure to cold wind, and that his lip swelled when he held ice against it.

On June 4, 1949, he swam for a few minutes in cold ocean water near New York. Almost immediately after leaving the water, his entire skin surface became itchy and red, and in a few minutes he became weak and collapsed. After rest, Pyribenzamine, and whiskey, he gradually recovered but felt weak the remainder of the day. No hemoglobinuria was noted.

Physical examination on June 6 showed him to be well developed and well nourished, with a blood pressure of 155/110, but showed no other abnormal findings. On subsequent examinations the blood pressure was within normal limits. Dermographism was not demonstrable.

The blood Kline, Mazzini, and Wassermann reactions were negative; the blood group was O. Plasma proteins were 7.6 Gm. per 100 ml.; gamma globulin by the method of Wolfson, Cohn, Calvary, and Ichiba¹⁰ was 0.98 Gm. per 100 ml. The test for cold agglutinins was negative in dilutions of 1:10 to 1:320. The urine was normal. Skin tests with the principal allergens showed marked reactions to timothy pollen, ragweed pollen, horse dander, and house dust. Other inhalants and common foods showed no significant reactions. Intracutaneous tests with histamine acid phosphate gave normal reactions.

The existence of a high degree of cold allergy was confirmed by the observation that application of an ice cube to the skin of the forearm for a period of 10 seconds resulted in the formation of a distinct wheal.

The patient was advised to take a brief cold shower daily and Chlor-Trimeton Maleate, 4 mg. twice a day, and warned of the risk of swimming in cold water. Injections of ragweed pollen extract for hay fever were started.

He spent the summer in Rhode Island where he cautiously tried ocean bathing and found that wading in water below 60° F. (approximately 15° C.) caused itching of the skin of the legs, but that brief immersion in warmer water caused no reaction. He was next seen September 9, at which time repetition of the tests with ice showed a slight decrease in sensitivity, contact for 15 seconds producing a definite urticarial reaction.

On that date, the serum protein was 8.8 Gm. per 100 ml., albumin 6.63 and total globulin 2.1. Electrophoresis of the serum in the Tiselius apparatus showed no unusual components; the normal protein fractions were present in essentially normal amounts. As a test for cryoglobulin, serum was separated at 37° C., then chilled 7 days at 4° C. This did not cause a precipitate to form. A test for cold hemolysins by the method of Mac Kenzie gave no reaction.¹¹

During October he made a trip to Europe without noticeable symptoms and was again seen in November. At that time the skin reacted after contact with ice for 20 seconds. During December while in New York, he noted itching of the face, without swelling, after exposure to cold. On Jan. 4, 1950, whealing of the skin occurred only after 40 seconds contact with ice.

Effect of Cold on the Patient's Skin.—As previously noted, on June 6, application of an ice cube to the skin of the forearm for 10 seconds produced a definite urticarial wheal which developed within 8 minutes of the removal of the ice. Contact with ice for 3 to 5 seconds produced erythema with slight whealing. Exposure for 2 seconds had no visible effect. Periods of contact from 15 seconds to 1 minute produced increasingly marked whealing. Contact longer

than one minute caused no greater response. In all cases, the wheal was strictly limited to the area of contact, without pseudopod formation, but with a surrounding zone of erythema 2 to 3 cm. wide. When a site which had reacted was retested the following day, the reactivity was decreased, but exposure to ice for four minutes produced a maximal response.

A slight decrease in the cold sensitivity was demonstrable during the seven months of observation. On June 6, contact with ice for 10 seconds produced a definite hive. On September 9, contact for 15 seconds was effective, and on November 16, 20 seconds' exposure was required. On Jan. 4, 1950, contact with ice for 30 seconds produced only erythema of the skin, 40 seconds slight whealing and 60 seconds a definite wheal.

Effect of Antihistamine Drugs.—In seeking an effective palliative medication the effects of 3 antihistamine drugs were tested on different occasions: Chlor-Trimeton Maleate, 4 mg., and Neo-Antergan, 100 mg., orally, and Benadryl, 20 mg., intramuscularly. In each case the threshold of the whealing reaction to contact with ice was determined, the drug administered, and the tests with ice repeated 30 minutes after the oral medication or 15 minutes after intramuscular injection. In each case the reactions after medication were identical with the control observations made the same day, none of the 3 drugs producing a measureable change in reactivity.

Passive Transfer.—Into a site in the skin of the forearm of a normal person, 0.25 ml. of fresh serum obtained June 9 from the patient, J. S., was injected intracutaneously. Into a control site was injected the same amount of serum from another patient allergic to ragweed pollen but not to cold. Twenty-four hours later ice was applied to both sites for 6 minutes. Within 5 minutes of the removal of the ice, a definite wheal 2.5×3 cm., with surrounding erythema, but without pseudopod formation or itching, appeared at the site (Fig. 1) of injection of the serum of J. S. The control site showed no reaction. Sites made with both sera reacted to intracutaneous injection of 0.025 ml. of ragweed pollen extract, 1,000 protein nitrogen units per ml.

Similar urticarial reactions to cold were demonstrated with samples of serum drawn from the patient, J. S., on June 9, September 10, and September 22 in tests on 7 different normal individuals. The results obtained with these 3 samples of serum were essentially identical and these sera were used in tests described subsequently. Sera obtained on November 4 and November 16 were less active but produced definite wheals if sites were tested within 3 hours. Serum taken Jan. 4, 1950, caused definite whealing in 1 of 2 normal subjects tested 2 hours after injection of the serum.

Injections of 0.1 to 0.3 ml. of each of the first 3 samples of serum consistently produced sensitization, the size of the wheal varying with the amount used; even 0.1 ml. of a 1:10 dilution of fresh serum produced a distinct wheal. The reaction could be elicited 2 to 3 hours after the injection of serum, as soon as the irritation produced by the intracutaneous injection had subsided or (with serum less than 4 days old) 24 hours later. Application of ice for periods varying from 6 to 14 minutes produced similar reactions. In most tests contact

was for 10 minutes. Wheals appeared only after the skin again became warm. This could be hastened by placing the arm in a water bath at 35° C. or by applying the palm of the hand to the site, but neither procedure was essential.

In order to test the effect of stimulating the patient's skin on the sensitizing power of the serum, 2 samples of serum were taken on the same day before and after 9 large urticarial wheals were produced on the forearms by applying ice. The 2 samples were equally active in passively sensitizing normal skin.

When the same test subject was used for several tests, care was taken to use a different skin site on each occasion. However, the 2 subjects used for the largest number of tests over a period of weeks both became resistant to sensitization even with fresh serum that was highly active in the skin of other individuals. The nature of this change was not apparent. Serum from the most "resistant" test subject did not form a precipitate with the sensitive serum (J. S.) in ring tests, nor did the "resistant" serum lessen the sensitizing activity of serum J. S. when mixed with it in equal volumes and placed in the refrigerator overnight.



Fig. 1.—Passive transfer of cold urticaria. Upper site sensitized with 0.25 ml. of serum J. S., lower site with 0.25 ml. of serum from another ragweed-sensitive patient. After 24 hours, ice applied to both sites for 6 minutes. Wheal 2 × 3.5 cm. at sensitized site; no reaction at control site.

Inactivation of Serum by Age and Heat.—With freshly separated serum, reactions could be demonstrated by testing sites 24 hours after injection of the serum. Cooling the serum to 4° C. in a refrigerator did not affect its activity. After 4 days' storage at room temperature or in the refrigerator, the serum no longer gave distinct urticarial reactions if 24 hours elapsed between injection and testing with ice, but definite reactions could be elicited if the sites were tested 2 to 9 hours after injection. Serum stored in the refrigerator for 1 month or more showed only feeble activity.

Heating fresh serum one-half hour in a water bath at 56° C. affected its activity only slightly, but 4 hours at the same temperature destroyed its potency. Addition of 0.05 ml. of a 1:5 dilution of fresh pooled guinea pig serum to 0.15 ml. of heated serum did not restore its activity (Table I). Heating four hours at 56° C. also destroyed the activity of the serum in passively sensitizing normal skin to ragweed pollen extract.

TABLE I. EFFECT OF HEATING AT 56° C. ON COLD-SENSITIZING FACTOR

PREPARATION OF SERUM	REACTION IN SITE PREPARED WITH SERUM	REACTION IN SITE PREPARED WITH SERUM AND GUINEA PIG COMPLEMENT
Serum unheated	Wheal 1.6 × 2.5 cm.	Wheal 1.8 × 2.2 cm.
Serum heated ½ hour	Wheal 1.2 × 1.2 cm.	Wheal 1.6 × 1.6 cm.
Serum heated 4 hours	0	0

Exhaustion of Sites by Testing.—Passively sensitized skin sites, which had once reacted to application of cold, did not react to a second stimulation with ice after the first response had subsided. Exhaustion of the reaction to cold did not affect the reaction of sites to intracutaneous tests with ragweed pollen extract, 1,000 protein nitrogen units per ml., suggesting that the factor producing sensitization to cold was independent of the skin-sensitizing antibody for ragweed pollen.

In order to neutralize the skin-sensitizing antibody for ragweed pollen, a portion of serum was mixed with an equal volume of ragweed pollen extract, 5,000 protein nitrogen units per ml., and a corresponding control mixture of serum and saline prepared. Skin sites sensitized with the 2 mixtures both reacted to cold; a site prepared with the serum-ragweed mixture did not react to test with ragweed pollen extract, while a site sensitized with the control mixture did.

Attempts at Reversed Passive Transfer.—Reversal of the usual procedure of passive transfer by injecting the sensitive serum into previously chilled normal skin might be expected to produce a specific reaction, if cold liberated from normal skin a substance (antigen) reacting with the serum. Attempts at reversed passive transfer with the serum J. S. were made on 4 normal test subjects. In each case a portion of the skin of the forearm was chilled by applying ice for 10 minutes. As soon as the ice was removed, 0.05 to 0.15 ml. of the sensitive serum was injected into the chilled skin and an equal volume into uncooled skin. The results were similar in all 4 trials. Each intracutaneous injection of serum produced a slight wheal and redness, but there was no significant difference in the reactions produced in the chilled and normal areas.

Since the activity of sensitized sites had been shown to be exhausted by one reaction to cold, the sites made in 2 test subjects in the attempt at reversed passive transfer were retested 5½ hours later after the irritation caused by the serum injections had subsided. The test and control sites reacted equally, indicating that the activity of the serum had not been exhausted by injection into the chilled skin (Table II).

TABLE II. ATTEMPT AT REVERSED PASSIVE TRANSFER

SENSITIVE SERUM 0.15 ML. INJECTED INTO	IMMEDIATE REACTION	REACTION OF SITE TO ICE 5½ HOURS LATER
Skin chilled with ice for 10 minutes	±	Wheal 1.6 to 1.6 cm.
Skin not chilled	±	Wheal 1.6 × 1.6 cm.

Electrophoretic Fractionation of Serum.—Ten milliliters of undiluted serum adjusted to pH 7.4 with phosphate buffer was subjected to electrophoresis in a Tiselius cell. After the components were separated, 4 samples (0.5 to 0.8 ml. each) were withdrawn corresponding to (1) pure albumin, (2) albumin and alpha globulin, (3) beta and gamma globulin, and (4) pure gamma globulin. Merthiolate was added to each sample to a strength of 1:10,000. Sites were prepared in the skin of 3 test subjects by intracutaneous injection of 0.1 or 0.2 ml. of each fraction. On testing with ice, none of these sites reacted. Sites similarly prepared with the residual material from the electrophoretic cell showed definite reactions, showing that the activity had not been destroyed by the procedure.

The same serum was again run in the Tiselius cell and somewhat larger amounts (1 to 2 ml.) of the same 4 fractions removed. The protein content of the albumin fraction was found to be 0.34 Gm. per 100 ml., that of the albumin and alpha globulin fraction 2.3, and that of the gamma globulin fraction 0.23. The entire third fraction (beta and gamma globulin) was used in passive transfer tests and none remained for analysis. As with the previously obtained fractions, sites prepared in normal skin by intracutaneous injection of each fraction did not respond to chilling with ice. After testing with ice, the sites were retested with 0.025 ml. of low ragweed pollen extract (1,000 units/ml.). As reported by Newell, Sterling, Oxnam, Burden, and Krejci¹² only the sites prepared with gamma globulin reacted to pollen extract.

Addition of 0.05 ml. of fresh pooled guinea pig serum 1:5 to 0.15 ml. of each fraction failed to restore the property of sensitizing to cold. In the case of the albumin and gamma globulin fractions, the addition of 0.05 ml. of fresh normal human serum to 0.15 ml. of electrophoretic fraction was also tried, and likewise failed to restore activity.

A mixture of 0.05 ml. of each of the 4 fractions sensitized normal skin so that a 1.2 cm. wheal was produced by stimulation with ice 3 hours later. A mixture of 0.1 ml. of the second fraction (albumin and alpha globulin) with an equal volume of the third fraction (beta and gamma globulin) gave a stronger reaction with a 1.5 cm. wheal. Mixtures of other combinations (pure albumin with beta and gamma globulin; albumin with pure gamma globulin; albumin and alpha globulin with pure gamma globulin) gave negative results (Table III). The amount of material available did not permit testing all possible combinations.

Because of the marked differences in protein content of the 3 fractions analyzed and the lack of an analysis of the other fraction, definite conclusions as to which of the protein components of the serum were involved in the

reaction are impossible. However, it appeared that transfer of cold sensitivity depended upon the presence of more than 1 component, in contrast to the transfer of ragweed pollen sensitivity which was accomplished with pure gamma globulin.

TABLE III. PASSIVE TRANSFER OF COLD SENSITIVITY BY ELECTROPHORETIC FRACTIONS OF SERUM

FRACTION	PROTEIN COMPONENTS	PROTEIN CONTENT GM./100 ML.	REACTION OF SENSITIZED SITE TO CHILLING
1	Albumin	0.34	0
2	Albumin + alpha	2.3	0
3	Beta + gamma	---	0
4	Gamma globulin	0.23	0
1 + 3	Albumin, beta and gamma globulin	---	0
1 + 4	Albumin, gamma	0.28	0
2 + 3	Albumin, alpha, beta, gamma	---	Wheal 1.5 × 1.5 cm.
2 + 4	Albumin, alpha, gamma	1.7	0
1-2-3 + 4	Albumin, alpha, beta, gamma	---	Wheal 1.2 × 1.2 cm.
Residue in cell	---	---	Wheal 1.6 × 1.9 cm.
Original serum			Wheal 2.5 × 3 cm.

Adsorption of Active Material From Serum With Skin Cells.—A piece of fresh normal human skin excised in the course of a surgical operation was frozen with carbon dioxide and cut into sections 20 micra thick with a microtome. To approximately 0.5 c.c. of such sections was added 1.0 ml. of sterile saline solution and the mixture allowed to stand overnight in the refrigerator. After centrifugalization the supernatant fluid was removed and 0.5 ml. of serum J. S. added to the packed skin cells. Another portion of the serum was added to an equal volume of washed packed Type O red cells. Both tubes were shaken and again placed in the refrigerator at 4° C. for 24 hours. At that time, the sera were removed by centrifugalization at 0° C., and 0.15 ml. of each sample injected intracutaneously in each of 2 normal persons. Control sites were prepared simultaneously with 0.15 ml. of untreated serum J. S. After 3 hours, the sites were tested by applying ice for 10 minutes. The sites prepared with serum “adsorbed” with skin cells showed slight redness but no wheals, those prepared with serum “adsorbed” with red cells and with untreated serum showed definite urticarial reactions, indicating that the sensitizing factor had been fixed by excised skin cells in the cold, but not by Type O red cells (Table IV). When tested 24 hours later with ragweed pollen

TABLE IV. ADSORPTION OF SENSITIZING FACTOR BY EXCISED SKIN

SERUM USED TO PREPARE SKIN SITE (0.2 ML.)	REACTION OF SITE TO CHILLING 3 HOURS LATER
Untreated serum	Wheal 2.2 × 2.0 cm.
Serum adsorbed with skin cells	No wheal
Serum adsorbed with Type O red cells	Wheal 2.2 × 2.8 cm.

antigen (0.025 ml. of extract containing 1,000 protein nitrogen units per ml.) all sites reacted essentially equally, indicating that the skin-sensitizing antibody for ragweed was not adsorbed in vitro by skin cells.

Attempt to Isolate Antigen From Frozen Skin.—If an antigen were liberated from normal skin by cold and reacted with the patient's serum, it might be expected to be present in the saline which had been used to wash the frozen skin cells in the adsorption experiment described in the preceding section. As a test for such an antigen, skin sites prepared by the injection of 0.1 ml. of serum J. S. and control sites injected with an equal amount of normal serum were tested after 4 hours by intracutaneous injection of 0.025 ml. of the supernatant fluid obtained after washing the skin cells with saline. This material produced a slight (0.4 cm.) wheal in both sites. The reaction in the site prepared with serum J. S. was no greater than that in the control site. There was no evidence that freezing normal skin liberated an antigen which would react with the serum J. S. at body temperature.

DISCUSSION

The patient had typical and severe cold urticaria with the generalized reaction to bathing in cold water described by Brown and Horton.¹³ The degree of sensitivity to cold was unusual, contact with ice for 10 seconds producing an urticarial wheal. In most cases with similar symptoms contact with ice for 4 to 10 minutes is necessary to produce a similar reaction. During the period of 7 months of observation, the patient's sensitivity appeared to decrease definitely but remained marked. In June, 1949, contact with ice for 10 seconds produced a definite wheal, while in January, 1950, contact for 40 seconds was necessary. Whether this change was spontaneous or resulted from daily cold baths during most of the period was not determined. Antihistaminic drugs (Chlor-Trimeton Malcate, 4 mg., Neo-Antergan, 100 mg., by mouth, and Benadryl, 20 mg., intramuscularly) did not affect the reaction. Their inefficacy, in contrast to previous reports,^{4, 5} may have been due to the extreme sensitivity of this patient.

There was no evidence of syphilis, cold hemolysins, cold agglutinins, or cryoglobulinemia. The onset of symptoms within a few weeks after a lesion which was presumed to be the bite or sting of an unidentified insect or jellyfish is of interest. Such a temporal relationship to a sting or bite has been reported in cases of urticarial reaction to sunlight,^{17, 18} but not in cold urticaria. The possibility of a causal relationship between the bite and physical allergy remains uncertain.

The present case was unusual not only in the degree of sensitivity to contact with ice, but also in the marked activity of the patient's serum in sensitizing normal skin to cold. Such passive sensitization is only occasionally demonstrable, usually of brief duration (2 to 5 hours) and the reactions small.⁸ With the sera obtained in June and September, 1949, wheals 2 to 3 cm. in diameter were demonstrable on testing sites 24 hours after sensitization. Samples of serum obtained in November, 1949, and January, 1950, were definitely less active. All samples of serum decreased in potency when stored at room temperature or at 4° C. for 4 days or more.

The urticarial reactions elicited in the patient's skin and in passively sensitized normal skin by cold resembled, in general, those produced in sensitive individuals by material antigens. The resemblance suggests that the reaction might be mediated by an antibody present in the serum. The possibility of such sensitizing antibody was perhaps greater since the patient was known to be allergic to ragweed and grass pollens. The thermolability of the cold-sensitizing factor in the serum corresponded to that of the skin-sensitizing antibody for ragweed, both being destroyed by heating the serum 4 hours at 56° C. However, the reactions to cold and to ragweed pollen were shown to be unrelated in exhaustion and neutralization tests.

Since the reaction could be demonstrated on normal skin, it might be supposed that the antigen was liberated from normal skin by cold, a mechanism similar to that suggested in some cases of urticaria produced by ultraviolet light.^{14, 15} However, attempts to demonstrate an antigen in an extract of excised frozen normal skin which would react with the serum at body temperature were unsuccessful, as were attempts at reversed passive transfer.

Unlike ultraviolet light,¹⁶ cold is not known to change the specificity of antigens, and in the case of cold hemolysis and cold agglutination, low temperatures are presumed to facilitate the reaction between antibody and a pre-existing antigen in the red cells, rather than produce a new antigen. Previous chilling of the red cells does not cause them to react with the cold hemolysin or cold agglutinin when added to it at body temperature. The reactions of the present serum were consistent with those of an antibody reacting at temperatures near 0° C. with an antigen present in normal human skin. The analogy to a cold hemolysin was furthered by the observation that the skin-sensitizing substance was adsorbed from the serum by skin cells in the cold, as cold hemolysins are adsorbed by red cells under similar conditions. However, the serum of this patient differed from that described by Harris, Lewis and Vaughan,⁸ which produced both cold hemolysis and cold urticaria, in that no cold hemolysin was present and the factor transferring cold urticaria was not adsorbed by red cells in the cold.

The concept of an antigen fixed in the skin cells might also explain the observation that neither the wheals produced on the patient's skin or those on passively sensitized skin showed the pseudopod formation which almost invariably occurs in wheals of comparable size produced by ordinary dissolved antigens.

The interpretation of the passive transfer tests with electrophoretic fractions of the serums is not clear, but the results strongly suggested that more than one protein component of the serum was involved in the passive sensitization of normal skin. Other antibodies which have been studied by electrophoresis have been found to move as a single component. Since complement was not apparently involved in the reaction, the presence of 2 (or more) components necessary for the reaction might be taken to suggest that both antigen and antibody were contained in the serum. No conclusive evidence on this point was obtained, but there was no precipitation or other evidence of antigen-antibody reaction when the serum was chilled in the absence of skin cells.

SUMMARY

1. A case of marked cold allergy manifested by urticarial reactions of the skin to contact with ice for 10 seconds is described. The reaction was not affected by ordinary doses of antihistamine drugs.

2. The patient was allergic to pollens and horse danders, but apparently free of other diseases, and the serum did not contain cold agglutins, cold hemolysins, or cryoglobulins. The electrophoretic pattern of the serum was normal.

3. Injection of the serum intracutaneously into normal skin produced passive local sensitization demonstrable by an urticarial reaction to contact with ice. The passive sensitization was exhausted by one reaction to cold. Stimulation of the skin of the patient with ice did not affect the sensitizing power of the serum.

4. The skin-sensitizing activity of the serum was only slightly affected by heating one-half hour at 56° C. but was destroyed by heating 4 hours at this temperature. Addition of guinea pig complement to the heated serum did not restore activity.

5. The sensitizing material in the serum was adsorbed by excised skin cells at 4° C., but not by Type O red cells.

6. Attempts to demonstrate reversed passive transfer and to isolate an antigen liberated from normal skin by cold which would react with the serum were unsuccessful.

7. Four fractions were separated from the serum by electrophoresis, containing respectively (1) albumin, (2) albumin and alpha globulin, (3) beta and gamma globulin, and (4) gamma globulin. None of these fractions passively sensitized normal skin to cold and addition of complement did not render them active. Mixtures of all 4 fractions or of the second and third fractions sensitized normal skin, suggesting that the presence of more than one protein component was necessary to produce sensitization.

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