of each tube was the equivalent of 10,000 units per 5000 cc of blood, which will produce *in vivo* a prolongation of coagulation lasting for about 4 hours.

Results. Table I shows the significant results. Repeat experiments were very closely confirmatory. Readings under 1.0 mg per 100 cc were called trace, a fractional reading being too inaccurate. As can be seen from the table, the amount of penetration of the blood clot under all conditions and with the different drugs was practically negligible. Heparin did not have any effect on the permeability of the blood clot.

Summary. The permeability of human blood clot to various sulfonamide drugs under conditions of increased temperature was determined in an *in vitro* study. Negligible penetration of the clot occurred even in the presence of temperatures up to 41°C. Heparin had no effect on the permeability of the blood clot.

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Evidence for the Existence of Two Antibodies for Crystalline Insulin.

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Marked insulin resistance was discovered in a 43-year-old female diabetic exhibiting severe urticaria following injections of crystalline and regular insulin. The patient was studied as follows: (1) Intravenous insulin-tolerance tests were carried out with commercial crystalline insulin and with a preparation of human insulin; (2) skin tests and passive-transfer tests were made; (3) the patient's serum was tested for the presence of an insulin-neutralizing antibody by mouse-assay.

This patient has been reported previously as a case of allergy to insulin.[1]

Materials and Methods. Serum from the patient was sterilized by filtration through a Seitz filter and stored at 4°C.

Lilly's u40 commercial solution of zinc-insulin crystals derived from beef and pork pancreas was used in all tests except as mentioned below and will be referred to as crystalline insulin.

[1] Yasuna, E., *J. Allergy*, 1940-41, **12**, 295.

Human insulin was prepared from human pancreatic tissue obtained at necropsy 3 to 12 hours postmortem. Acid-extraction was done as described by Somogyi, *et al.*,[2] and the final product contained 7 units per cc.

Assay of insulin was carried out in starved mice.[3] Each animal was injected intraäbdominally with 0.5 cc of a dilution of insulin, and then all the animals were placed together in a room maintained at 38°C. The number of mice showing symptoms of hypoglycemia at the end of 90 minutes was noted. A dose of 0.02 units caused hypoglycemic convulsions or coma in 90-100% of mice.

Tests for insulin-neutralizing antibody were made by mouse-assay by injecting a mixture of the patient's serum and insulin in a total volume of 0.5 cc. In these experiments control tests were also made using a normal human serum. There are two theoretical objections to these tests. The patient's blood sugar was elevated and the increased glucose present in the serum might have masked the physiological effect of the insulin. This appeared unlikely as the amount of glucose present was sufficient to cover only about 0.001 units of insulin when undiluted serum was used. A second objection was the possibility that normal human serum contained sufficient insulin to increase the incidence of hypoglycemic symptoms in mice receiving normal serum. However, tests in which mice received insulin contained in 0.5 cc of saline and 0.5 cc of undiluted normal serum showed that normal serum did not increase the incidence of symptoms but, on the contrary, delayed the onset of symptoms slightly.

Intravenous insulin-tolerance tests were carried out with the patient in the fasting state. Three weeks before the tests were done, the patient was desensitized with carefully graded doses of insulin. Subsequently she received daily doses of 40 to 230 units of insulin. At the time the tests were done, the relatively large doses of insulin were having no observable effect on the diabetic state. The patient was hospitalized for the entire period of observation and received a diet of 1400 calories. No adrenalin or glucose was given for reactions occurring during the tests.

Passive-transfer tests with the patient's serum were done in subjects who were satisfactory recipients in that they exhibited no local reaction to the endermal injection of insulin diluted 1:10. Normal skin sites were injected with 0.1 cc of serum, diluted and undiluted, and tested 24 to 48 hours later with 0.02 cc of a 1:10 dilu-

2 Somogyi, M., Doisy, E. A., and Shaffer, P. A., *J. Biol. Chem.*, 1924, **60**, 31.

3 *The Biological Standardization of Insulin*, C. H. 398, League of Nations Health Organization, Geneva, April, 1926.

tion of crystalline insulin. The appearance within 10 to 20 minutes of a definite wheal and erythema, with or without itching, indicated the presence of skin-sensitizing antibody in the injected serum. Mixtures of 0.05 cc of the patient's serum and 0.05 cc of various dilutions of crystalline insulin were also injected into normal skin sites which were subsequently tested for passive sensitization with injections of insulin diluted 1:10. The amount of insulin necessary to destroy the ability of the patient's serum to confer sensitivity on normal skin was determined in this way.

Results. Intravenous Insulin-tolerance Tests. The patient's fasting blood sugar taken on numerous occasions varied between 330 and 380 mg/100 cc. A single intravenous injection of 30 units of crystalline insulin caused a slight rise in the blood sugar followed by a fall to a point slightly below the fasting level. There was no reaction to this injection.

On the following day, 30 units of human insulin were injected intravenously. A marked allergic reaction occurred within 15 minutes characterized by severe generalized urticaria and a feeling of constriction in the throat. It was identical with previous allergic reactions following injection of crystalline or regular insulin. After the urticaria subsided the patient felt well until about 90 minutes after the test was started. She then began to feel shaky and weak. A blood sugar done at this time was 59 mg/100 cc. Two days later, in order to show that the patient was still resistant to crystalline insulin, 120 units were given subcutaneously over a period of 8 hours. At the end of this time, the blood sugar was 395 mg/100 cc, somewhat higher than the usual fasting level.

The two insulin-tolerance tests are illustrated in the chart.

Tests for Insulin-neutralizing Antibody. Five mice were injected with 0.5 cc of the patient's serum to which had been added 0.02 units of crystalline insulin. No mice in this group showed any symptoms in 90 minutes. Four of 5 mice in a control test developed convulsions, and the fifth developed mild symptoms. In a second test, 8 mice were injected with 0.5 cc of the patient's serum diluted 1:10 to which were added 0.04 units of crystalline insulin. In this group, only 4 mice (50%) showed symptoms. This dose of insulin was twice the convulsive dose. In the control test with 5 mice, using normal serum instead of the patient's serum, all developed convulsions within 40 minutes. No insulin-neutralizing effect was demonstrable when human instead of crystalline insulin was used in mouse-tests. When the skin-sensitizing antibody in the patient's serum was destroyed by heating to 57°C for 2 hours, the neutralizing

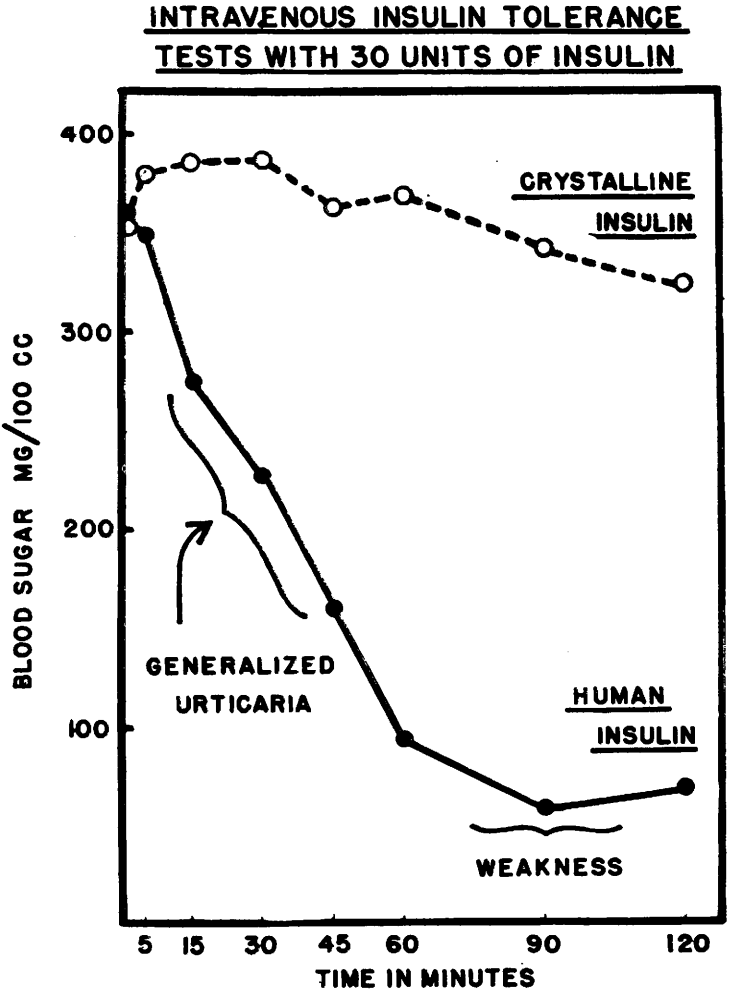

FIG. 1.

activity for crystalline insulin was still clearly demonstrable by the mouse-test.

Passive-transfer Tests. Skin-sensitizing antibodies were consistently demonstrable in all of 10 specimens of serum obtained from the patient over a period of 6 months. Sensitization of normal skin sites could still be obtained with most of these sera when they were diluted 1:16, 1:32, and, in some tests, 1:64. Mixtures containing 0.05 cc of serum and 0.04 units of crystalline insulin made up to a volume of 0.1 cc, were capable of sensitizing normal skin, but the addition of 0.4 units to 0.05 cc of serum destroyed the skin-sensitizing activity. The skin-sensitizing activity of the serum was also destroyed when

the serum was heated to 57°C for 2 hours. Skin-sensitizing antibodies were demonstrable in unheated serum when human, instead of crystalline, insulin was used for testing the sensitized sites.

Discussion. Allergy to insulin in the absence of resistance has been noted clinically, as well as resistance to insulin in the absence of allergy. Thus the two conditions can exist independently of each other. The data obtained from this patient are consistent with this and establish the following points: the patient was resistant to crystalline insulin but not to human insulin; the patient reacted allergically to both crystalline and human insulin and skin-sensitizing antibody was demonstrable for both insulins; the patient's serum contained an antibody for insulin, which protected mice from a lethal dose of crystalline insulin but failed to protect against human insulin; heating the patient's serum destroyed the skin-sensitizing antibody without destroying the insulin-neutralizing antibody. These findings indicate that there were two antibodies present in the patient's serum, an allergic antibody which was heat-labile and conferred sensitivity on normal skin, and an insulin-neutralizing antibody which was heat-stable and was capable of destroying the physiological effect of crystalline insulin. The presence of two antibodies for ragweed extract has been demonstrated in the serum of treated patients who are sensitive to ragweed by Loveless.[4] Two rough calculations can be made of the amount of the insulin-neutralizing antibody contained in the patient's total plasma volume, assuming this to be 2500 cc. In terms of insulin neutralized the figure is more than 2000 units by the endermal tests involving antibody-neutralization, and 1300 by the mouse-tests. From the quantitative point of view, both tests were crude, but the fact that the two figures are of the same order of magnitude suggests that the same antibody was active in both tests. It appears probable that the insulin-neutralizing antibody accounted for the patient's resistance to crystalline insulin. However, this antibody was not, strictly speaking, an antihormone because the patient responded well to human insulin. This suggests that the part of the molecule which served as antigen for the neutralizing antibody was characteristic for insulin derived from beef and pork pancreas and was not the part of the molecule which acted physiologically as insulin. The patient could perhaps be successfully treated if an insulin immunologically unrelated to beef and pork insulin were used. Human insulin was not available in quantities sufficient for treatment. The fact that the patient responded allergically to both crystalline and human insulin indicates

[4] Loveless, M. H., *J. Immunol.*, 1940, **38**, 25.

that the allergic antibody was alone responsible for the patient's skin sensitivity, generalized urticaria and other allergic symptoms, and was entirely unrelated to the patient's resistance to insulin.

Conclusions. Evidence is presented indicating the presence of two antibodies for crystalline insulin in the serum of a patient who was both allergic and resistant to crystalline insulin. It was also shown that the patient was allergic but not resistant to a preparation of human insulin. The relation of the two antibodies to the patient's response to crystalline and human insulin is discussed.

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Collodion-Agglutination Test in Human Tuberculosis.

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In human tuberculosis the precipitin-reaction, even utilizing a highly purified and reactive antigen, has been found positive in only about 12% of cases.[1] It was thought that the collodion-agglutination technic[2] would demonstrate precipitins in a much higher percentage of tuberculous humans. Previous reports concerning this latter method in human and experimental tuberculosis had suggested its accuracy and extreme delicacy.[3, 4]

The technic as outlined by Cannon and Marshall[2] was rigidly adhered to in all details. As antigens, Old Tuberculin and a purified tuberculoprotein were used. The protein had been previously shown to be a superior antigen, *in vitro*, to Old Tuberculin.[1] For adsorption, 1 cc of OT in 19 cc of water was allowed to stand overnight with 20 cc of a dense suspension of collodion particles. For adsorption of the TPT, 20 mg of this was substituted for the 1 cc of OT, and the pH of the solution was adjusted to 5.8 with sodium-acetate acetic-acid buffer.

Over a period of several months, coated particles prepared in this manner were repeatedly tested with tuberculous human and rabbit sera, and normal human and rabbit sera. Some undoubtedly true and specific reactions were obtained in the animal sera, better reactions

1 Seibert, F. B., *Am. Rev. Tuberculosis*, 1930, **21**, 370.

2 Cannon, P. R., and Marshall, C. E., *J. Immun.*, 1940, **38**, 365.

3 Weir, J. M., personal communication.

4 Weir, J. M., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 47.