injection of 400 mg. of sodium amytal, the blood sugar fell from 150 mg. % to 94 mg. % in 30 minutes. A rapid increase in the injection rate of glucose was possible, 12 gm. per hour being given 2 hours later with a blood sugar of 130 mg. %. In another experiment on the same dog insulin was given at the rate of 1.21 units, and 1.9 gm. of glucose per hour. In 20 minutes after the intravenous injection of 2 cc. of nembutal, the blood sugar had fallen from 140 mg. % to 110 and in 50 minutes to 88 mg. %. A fall of 40 mg. % in blood sugar in 30 minutes has also been demonstrated after nembutal in one of the 9.5 kg. dogs.

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Urea as a Solvent in Preparation of Antigen Extracts.

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The development of the study of allergy in recent years, and particularly the search for the etiologic factors, has focused attention upon numerous protein containing substances. These include foods, pollens, molds, epidermal structures, orris root, house dust and such less common factors as the scales on sand flies or the stinging organ of bees, wasps, etc. The substance suspected may be tested on the patient in various ways: (a) by direct application of it to the skin, to the conjunctivae, or if a food, it may be ingested; (b) the juice may be expressed from certain of the substances and used by application to a scratch on the skin, or by intracutaneous injection. This method is naturally limited in its application. The ideal method is one which would concentrate the active substance, change its physical or chemical composition little or none, and one which could be used on all types of substances. By analogy with immunologic phenomena, and in view of the evidence which has accumulated in the field of allergy, one is led to believe that the active allergic agent is protein or closely attached thereto.

Proteins in general are rarely water soluble to any extent; this is especially true after they have undergone the procedures necessary to separate them from the various substances mentioned above. The simplest extraction methods employ a solution of NaCl in various concentrations. Alkalies, notably a bicarbonate or hydroxide, or a neutral phosphate buffer solution, find most general use. Recently a

glucose extract has been advocated. The multiplicity of methods indicates that none is entirely satisfactory. This fact led to the present attempt to utilize the peculiar solvent action of strong urea solution in the preparation of these and other active extracts.

Spiro[1] was the first to describe the solvent action for proteins of strong urea solutions. Ramsden[2] studied this property in more detail. Although a saturated or strong urea solution is necessary for rapid solution the preparation may then be greatly diluted. Urea is a neutral, relatively inactive and natural compound in the organism, well suited for injection. It is true[3] that it denatures proteins. Whether this denaturation is of the same type as suffered by alkali-treated proteins is not known. The relative influence of various denaturing agents upon antigens has not been carefully examined. Acetone[5] and heat[4, 5] denatured serum albumins are grossly altered immunologically. We hope to examine the immunological changes induced in proteins by solution in strong urea.

The urea extracts were prepared by triturating the original desiccated and powdered product or a dried simple extract of it with urea crystals and water sufficient to make a 50% urea solution. Five grams of powder were extracted with 50 or 100 cc. of the strong urea solution. Sterilization was effected by filtration through a bacteria-restraining filter, with some difficulty in the case of several of the extracts. It is probable that the bactericidal effect of strong urea solutions[6] may make this further treatment unnecessary.

Many protein extracts form, at refrigerator temperature, an irreversible precipitate, or they will do so when sufficiently diluted with a neutral isotonic solution. The lower concentration is usually necessary in skin testing. Thus far urea extracts have failed to show such undesirable changes. Certain extracts give a high percentage of positive reactions which can rarely be correlated with clinical sensitivity. There is evidence that the urea solutions may be free from these.

Fifty-five patients, presenting allergy-like complaints, were tested by the scratch (1:20 dilution) or intracutaneous (1:1,000 dilution) method, with a urea extract of several foods,* chicken feathers and a false ragweed pollen. The same patients were tested with conventional stock allergens—foods, pollens, epidermals, etc. Forty-

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[3] Hopkins, F. G., *Nature*, 1930, **126**, 329, 383.

[4] Obermayer, F., and Pick, E. P., *Wein. Klin. Woch.*, 1906, **19**, 327.

[5] Miller, B. F., *J. Exp. Med.*, 1933, **58**, 625.

[6] Foulger, J. H., and Foshay, L., *J. Lab. and Clin. Med.*, 1935, **20**, 1113.

* Bean (lima), beef, celery, flaxseed, lamb, potato, rye and tomato.

three patients (78%) reacted to no stock allergen and to none of the urea extracts. Seven patients reacted to stock allergens, but to none of the urea solutions, and these reactions did not include a sensitivity to any of the foods, etc., used in making urea extracts.

These results are still consistent with specificity of the new extracts. Five patients (9.1% of 55) reacted to one or more of these extracts and, as indicated, also to stock allergens. Nine reactions, or an average of approximately 2 per reacting patient, were obtained. But 5 urea extracts† (½ of those employed) demonstrated any sensitivity. It is manifestly unfair to emphasize a comparison of these reactions with those obtained to stock allergens on the same 5 patients. These latter reactions, however, are of value as presumptive evidence in favor of allergy. The clinical complaints emphasized by the 5 patients were referable to the respiratory tract. All reactions obtained are potential causes of such symptoms.

Summary and Conclusions. Protein constituents of foods, pollens, epidermals and many special substances are important factors in allergy. The extraction of these components is a necessary feature in their study. The ideal extractive would be one that is easy to use, is generally applicable to all substances, produces a potent concentrated extract, which will remain in solution, modifies the original protein little or none, and, most important of all, one which will yield extracts that are capable of detecting the maximum of clinical sensitivities. If these ideals are met it is probable that the present problem of non-specific skin reactions would become insignificant.

Urea solutions do modify the original protein constituent: the degree and direction of this denaturation is still being investigated. Such extracts are easy to make, permit of considerable concentration; have found general application and seem to give few, if any, reactions on non-allergic individuals as judged by a series of 55 patients tested with 10 urea extracts. In the total of 550 tests, but 9 reactions (1.6%) were obtained and these were given by 5 of the 10 extracts. The 5 patients giving these were potential allergic patients, as their clinical conditions belonged to that group and each patient gave one or more reactions to stock allergens. There was absolute agreement—all negative—between the special and stock extracts on 43 patients. Further study by different workers is necessary before the method should be urged in substitution for the conventional ones now in widespread use.

† Reactions were obtained to bean, celery, rye, tomato, chicken feathers and false ragweed.