all of the reticulum cells showed the same intensity. Reticulum cells of the thymus were negative. Under certain conditions the Kupffer cells of the liver showed a strong reaction. The conditions responsible for this variation are being studied.

Summary. The 3-(5-Bromoindolyl)-β-D-glucopyranoside (I) was prepared in our laboratory for histochemical study of tissue β-glucosidase. The final reaction product following incubation of tissue sections was 5,5′-dibromoindigo which is highly chromogenic and precipitates in cells and tissue at the site of enzymic activity. The enzyme is readily destroyed in tissue by ferri-ferrocyanide and formalin, which is not the case with non-specific esterase or glucuronidase. Saccharo-1-4-lactone, which has an inhibitory effect on β-glucuronidase, does not affect β-glucosidase. "Substrate inhibition" can also be demonstrated. Optimum pH is 5.4. The β-glucosidase can be demonstrated best in the preputial gland where activity is high. The older acini of the gland showed more intense reaction. Reticulum cells within the villous stalks of the small intestine showed a good reaction. Reticulum cells of the spleen, lymph node and Kupffer cells showed a varied reaction. Extremely small amounts are present in the liver and kidney.

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Diagnosis of Human Allergy Utilizing Passive Skin-Sensitization in the Monkey *Macaca irus.* (27015)

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If blood serum from an actively allergic individual is injected into the skin of a nonallergic volunteer the skin of the recipient may become sensitized in the immediate area of the serum injection. This phenomenon is referred to as passive sensitization. The passively sensitized skin site will often exhibit an inflammatory reaction with wheal formation when injected with a minute amount of the substance(s) causing allergy in the serum donor. This particular direct local application of the phenomenon of passive cutaneous anaphylaxis (P.C.A.) in humans is referred to as the "Prausnitz-Küstner test" or the "P.-K. test" (1). The P.-K. test has been used extensively as a diagnostic aid in identifying the substances responsible for allergies, and is especially useful in allergies of infants, debilitated children and severely allergic older patients.

The P.-K. test presents the grave danger of transfer of serum hepatitis infection to the human volunteers. Because of this danger of infection we avoided the use of the technique in testing the sera of those factory employees showing signs of sensitivity to castorbean dust.

Ovary(2) has discussed the phenomenon of passive cutaneous anaphylaxis in guinea pigs and other lower animals. He suggested that a colloidal dye such as Evans Blue be injected

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intravenously along with the antigen, in order to make more visible the inflamed area resulting from antigen-antibody reaction. Dye leakage into the extravascular spaces (extravasation of dye) results from the increased permeability of the minute blood vessels in the inflamed areas. Layton and coworkers(3) have adapted this technique to the problem of detecting seed antigens of *Ricinus communis* (castorbean).

Tests with rabbit antisera in the skins of guinea pigs are not of themselves adequate criteria of allergenicity to humans, hence any conclusions relating to allergenicity must ultimately rely upon human reactions to the allergen or the use of human material in testing. Because of this essential criterion and the hazards involved in passive sensitization of human volunteers, the possibility of using monkeys was investigated.

Grove(4) attempted to sensitize the skins of chimpanzees and lower monkeys with serum from allergic humans and concluded that passive transfer to these primates was not feasible. Nevertheless, the need to determine the allergenicity of isolated castorbean antigens(5,6,7,) suggested attempts to produce passive sensitization by transfer of human serum reagins in another species of primate. The Philippine crab-eating monkey, *Macaca irus*, was a first choice because of its light skin, small size, gentle nature, relative abundance, and low price.

Preliminary tests conducted upon 4 female monkeys showed(8) that human reagins were bound in the monkey's skin but that not less than 24 hours nor more than 72 hours time should be allowed for attaining optimum reactivity in the sensitized sites. It was also found that extreme fear and physical struggle would inhibit wheal formation without affecting extravasation of intravenously injected Evans Blue dye. Light tranquilization with reserpine appeared to enhance reactivity in the monkey.

After demonstrating that human serum reagins would passively sensitize this species of monkey, an effort was made to standardize the techniques involved.

Experimental. Development of a Diagnostic Procedure. Sera were available from blood samples drawn 15 months earlier from employees who were exhibiting symptoms of allergy during a castorbean processing run. These patients had been referred to local general medical practitioners, but not to allergy specialists, and were diagnosed as probably allergic to castorbean meal. The blood serum samples were taken 4 days after the appearance of symptoms, and had been preserved by freezing and storage at $-10°C$.

A sample of partially purified, heat-stabile, water-soluble castorbean seed protein was available from a joint project with Stanford Research Institute and was coded CB-S.R.I.(3,6). This antigenic material was dissolved in physiological saline solution to give a solution containing approximately 10 mg of protein per ml. of solution.

Five male and 5 female monkeys of the species *Macaca irus* were purchased from a local animal importer. These animals weighed from 2 to 3 lbs and appeared to be free of infection and skin lesions.

Procedure: The hair was clipped from the abdomen, upper arms, thighs, cheeks, chin and eyebrows of each animal. The bare skin was wiped with 80% ethanol and while yet damp the abdomen was marked off with a red wax pencil into 10 areas, 5 on either side of the midline, to give a total of 19 ventral sites on each animal. With this arrangement 9 serum samples could be tested in duplicate against one antigen on the light ventral skin surfaces of each monkey.

Each site was injected intradermally with 0.05 to 0.1 ml. of undiluted human serum using a No. 27 needle and a 1-ml. tuberculin syringe. One or more sera from individuals known to be nonallergic were always included as negative controls. After the sensitizing injections the monkeys were returned to their cages, and 24 hours were allowed to elapse before conducting the local skin tests for sensitivity. The local skin tests were conducted in a manner similar to that described by Prausnitz and Küstner(1) for tests on passively sensitized humans; a minute drop of the selected saline solution of antigen was injected intradermally into the sensitized site at or near the the orginal needle mark. Only one antigen or antigenic fraction was tested in an individual monkey because of diffusion of the antigen from the site of injection thereby causing wheal formation in nearby sensitized sites.

FIG. 1. Passive cutaneous anaphylaxis in *Macaca irus* sensitized with sera from allergic humans. Extravasation of Evans Blue in sensitive sites. No reaction in control sites (B.R.); see sketch in Fig. 2.

In certain very calm monkeys it was observed that wheal formation occurred in the orbital and cheek sites 10 minutes after injection of 1 mg of CB-S.R.I. intradermally into one of the abdominal sites. A similar but more immediate multiple-site reaction was obtained by injecting 0.1 mg of the antigenic material into the heart or preferably into a saphenous vein. Multiple-site reactions to an injection of the allergenic material were easily read and interpreted when the colloidal dye, Evans Blue, had been injected intravenously 5 to 10 minutes prior to challenge with the allergenic material. With monkeys weighing 2 to 3 lb 1.5 to 2.0 ml of 0.5% solution of Evans Blue in physiological saline was injected directly into a saphenous vein, surgically exposed under local anesthesia with adrenaline-free procaine which did not affect the P.C.A. reactions.

Results. A photograph of a multiple-site passive reaction to castorbean seed protein is shown in Fig. 1. The blue spots are the result of extravasation of the colloidal dye due to localized increase in vascular permeability induced by the castor allergen-antibody reaction (histamine release) in the sensitized sites. This phenomenon is comparable to the P.C.A. reaction(2) in the guinea pig passively sensitized with rabbit anti-castor serum(3). Fig. 2 is a sketch giving the code to Fig. 1. The same type of reaction without the dye is shown in the photograph (Fig. 3) of large wheals in sensitized sites (K. H. serum) on the eyelids of a monkey tested by intradermal injection of CB-S.R.I. into sites on the abdomen. Those abdominal sites which had been injected with sera from castorbean-allergic individuals exhibited the wheal formation characteristic of the corresponding Prausnitz-Küstner reaction in passively sensitized humans. The observations are tabulated in Table 1.

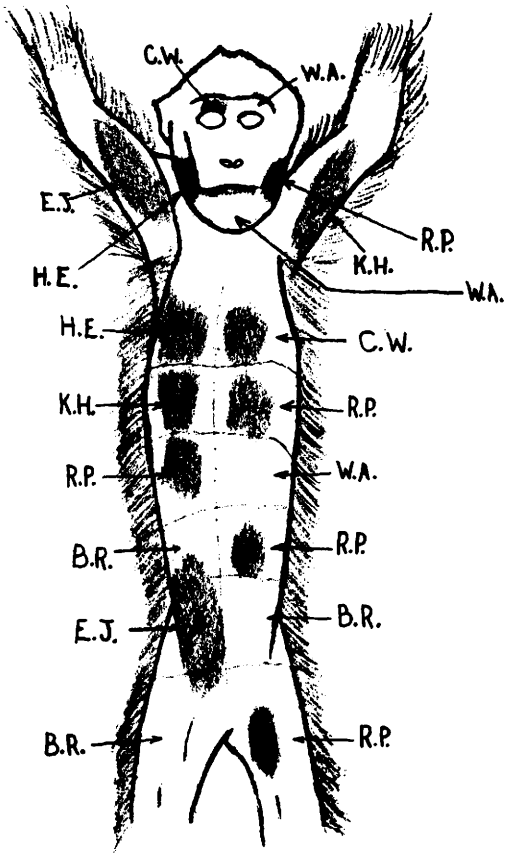

Fig. 2. Initials of donors identifying sources of human sera giving positive passive cutaneous anaphylaxis in *Macaca irus* challenged intravenously with castorbean antigens. See photograph, Fig. 1. (K. H. is not an employee, but is the wife of a nonallergic employee. She reacts to dust from his work clothing).

Fig. 3. Large wheals in sensitized sites on upper evelids of *Macaca irus*.

TABLE I. Passive Skin Testing of Human Sera in *Macaca irus* for Allergy to *Ricinus communis* (Castorbean)

Human serum donor	Symptoms	Reaction in passively sensitized monkeys	
		Wheal formation in direct skin tests (6 monkeys)	Extravasation of Iv.* injected Evans Blue (4 monkeys)
1. W.A.	asthma**	negative 6/6	negative 4/4
2. A.C.	Henoch's purpura**	" 6/6	" 4/4
3. R.P.	asthma, rhinitis	positive 6/6	positive 4/4
4. W.R.	asthma, conjunctivitis	" 6/6	" 4/4
5. K.H.	acute asthma	" 6/6	" 4/4
6. M.H.	asthma	" 6/6	" 4/4
7. C.W.	"	" 6/6	" 4/4
8. H.E.	"	" 6/6	" 4/4
9. C.H.	hay fever, asthma	" 4/4	" 2/2
10. B.R.	none (control)***	negative 6/6	negative 4/4
11. E.J.	hay fever, asthma	positive 6/6	positive 4/4
12. H.S.	burning nose	negative 4/4	negative 2/2
13. E.F.	none (control)***	" 4/4	" 2/2
14. J.S.	" " ***	" 4/4	" 2/2

* Intravenous injection.

** These employees may be sensitive to some castor constituent not present in CB-S.R.I.

*** Sera collected Nov. 1960; all other sera were collected Dec. 1959-Jan. 1960.

After the studies on monkeys, we made a screening study by skin tests of 107 personnel of a castorbean processing factory (in cooperation with a licensed physician) and confirmed the results of the monkey tests. The results of the clinical tests will be presented later.

Summary. Blood sera from patients suffering from castorbean-allergy will sensitize the skin of the Philippine crab-eating monkey, *Macaca irus*. The sensitized sites react to castorbean proteins, producing wheals and/or blanching characteristic of similar passive transfer tests (P.-K. tests) in nonallergic human volunteers. Skin reactions in the monkey are more easily interpreted when the colloidal dye Evans Blue is injected intravenously 5 or 10 minutes prior to challenge with the suspected allergenic material. The passive transfer of human serum antibodies into the skin of *Macaca irus* appears to be useful as a method which may be applied in lieu of the Prausnitz-Küstner test (on human volunteers) to avoid the grave risk of infection with serum hepatitis.

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