

Production of Local Skin Reactivity by Passive Transfer of Anti-Protein Sera.

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As reported recently, the serum of guinea pigs rendered hypersensitive to certain chemicals contains antibodies which can transfer to the skin of normal guinea pigs reactivity towards the chemical and towards protein conjugates of the incitant.¹ Sera prepared against proteins have been tested in analogous manner with the idea that the principle might be of wider applicability. This is actually the case. Positive results were first seen with serum from guinea pigs injected repeatedly in the skin with horse serum (0.1 cc of 1:5 dilution in saline); more active sera were frequently secured by a method devised by Dr. Jules Freund as having a substantial adjuvant effect with several antigenic materials,² namely, injection under the skin of an emulsion of horse serum, Aquaphor, and killed tubercle bacilli in paraffin oil, the animals being bled 4 or preferably 6 weeks or longer afterwards. When skin sites were prepared in the manner of a Prausnitz-Küstner transfer with 0.15 to 0.05 cc of selected sera, and after an interval of 10 hours to several days the corresponding antigen was injected into the sensitized sites, or subcutaneously at a distance, the skin over and contiguous to these sites gave specific reactions of the early type with distinct erythema and oedema (Table I), as described for the drug hypersensitivity sera.¹

With a given serum, the extent of the reaction varies markedly with the particular recipient. Thus the reaction of recipient A although typical in its course was in its extent characteristic of the best acceptors; on the average, diameters of 2.5 to 3 cm were attained. The erythema mostly recedes in

less than an hour, while the oedema continues to increase for a time before slowly regressing. There may occur some resurgence in color between the 4th and 6th hour. On the following day the skin usually appears normal, but faint, rarely moderate, reactions have been noted at times, chiefly with anti-protein sera.

While the immune sera can often be diluted up to 8- or 12-fold, relatively large amounts of antigen seem preferable for subcutaneous injection. With the latter method of testing, the onset of the skin reactions varied between 10 and 30 minutes, apparently principally dependent upon the attainment of a sufficient concentration of the antigen in the reactive skin area.

Like effects were also seen with selected guinea pig anti-ragweed (*Ambrosia elatior*) sera, obtained after injection of pollen incorporated in an emulsion of the Freund type² or by repeated intracutaneous injection of extracts precipitated with alum (Caulfeild³), the recipient guinea pigs being tested with the usual "5%" extracts in Coca's fluid (phenol omitted) of defatted pollen, which were injected locally or subcutaneously (Table II). The intensity of the reactions, which started within 4 to 15 minutes, was rather more variable in individual recipients than with other types of sera, possibly indicative of low antibody content. Under the same experimental conditions a few attempted transfers of human reaginic (low ragweed) sera to guinea pig skin have been unsuccessful.

Positive skin transfer effects, elicited by tuberculin, were encountered with sera from guinea pigs which were injected with killed tubercle bacilli in paraffin oil and at the same time with a conjugated antigen. This reaction is being studied and will be described later.

In further experiments it was found that ordinary precipitin sera produced in rabbits

¹ Landsteiner, K., and Chase, M. W., *Proc. Soc. Exp. Biol. and Med.*, 1942, **49**, 688.

² Freund, J., and McDermott, K., *Proc. Soc. Exp. Biol. and Med.*, 1942, **49**, 548; Freund, J., paper read April 2, 1942, before the American Association of Immunologists in Boston, Mass.

³ Caulfeild, A. H. W., *J. Allergy*, 1936, **7**, 451.

TABLE I.*

Local Sensitization of Normal Skin by Sera from Guinea Pigs Immunized with Horse Serum. The recipients (albino guinea pigs) were prepared with 0.15 cc amounts of immune and normal sera injected intracutaneously on separate halves of the back.

Recipient A			Recipient B		
2 days later, 0.5 cc horse serum inj. subcut. on belly			1 day later, intracut. inj. of 0.1 cc horse serum† into same sites		
Reactions on injected sites			Reactions on injected sites		
Time	Anti-horse serum No. 1	Normal G.P. serum	Time	Anti-horse serum No. 2	Normal G.P. serum
13'	A few very faint pink spots about locus of antibody injection	nil	0'	10x10 wheal, almost colorless	10x10 al.cls. wheal
17'	Many pink spots over area 50x35	"	2'	Wheal has gray-blue hue	
25'	Confluent patches, giving pink, elevated area 50x35, with marginal pseudopodia; tiny pink dots beyond margin	"	8'	Pink pseudopodia extending outward from wheal	
31'	60x50 pink, markedly elevated	"	10'	21x21 pink, elevated centrally; uneven, spreading margin	12x10 faintly pink, elevated
38'	Color fading, oedema unchanged	"	18'	24x24 pink, elevated; center markedly elevated	No change
190' over-night	Colorless; oedema receding	"	30'	28x28 color receding, still swollen	" "
	Negative	"	over-night	23x21 faintly brownish pink color	13 faint brownish pink color

* In this and the following tables, the reaction areas are described as to size (cross diameters given in mm), erythema, and oedema.

† Smaller doses are adequate in testing.

TABLE II.

Local Sensitization of Guinea Pig Skin by Guinea Pig Anti-ragweed Serum.

Recipient C.

1 day after the serum injections shown, intracutaneous injections of 0.025 cc "5%" extract of low ragweed pollen into same sites.

Preparation of sites		
Time	Guinea pig anti-ragweed serum (0.15 cc)	Control serum: Serum from G.P. sensitized to citraconic anhydride (0.15 cc)
8'	18x18 very faintly pink	nil
12'	20x18 pink	"
35'	23x21, pink, markedly elevated	"
ca. 45'	Color fading, oedema persistent	"
overnight	Practically normal	"

can be employed for local sensitization of guinea pig skin, and with these some of the largest and most pronounced reactions have been observed (transient, marked blue (cyanotic) discoloration over the central part of the area, urticaria-like welts); however, powerful antisera have had to be diluted for the preparatory skin injection in order that typical reactions could be elicited subsequently in the skin. The reason for this may be found to lie in a zone phenomenon or interference by a systemic reaction attributable to that

portion of the antibody which escapes from the skin site into the circulation. The rabbit immune sera tested, all with positive skin transfer effects, have been: horse serum precipitin (Table III), anti-hen ovalbumin, anti-"picryl ovalbumin" (tested vs. picrylated casein), anti-pneumococcus III (vs. pneumococcus III polysaccharide*). A horse anti-

* The author is indebted to Drs. O. T. Avery and W. F. Goebel for making the polysaccharide available.

TABLE III.

Local Sensitization of Guinea Pig Skin by Rabbit and Guinea Pig Antisera (Prepared Against Horse Serum).

Recipient D.

On the day following the intracutaneous injections indicated, 0.5 cc horse serum was injected subcutaneously on the belly.

Time	Preparation of sites			Normal G.P. serum .15 cc undiluted
	Rabbit anti-horse serum .15 cc 1:8 dil. in saline	Guinea pig anti-horse serum No. 3 .15 cc undil.		
37'	25x25 pink, markedly swollen	Trace		neg.
44'	26x26 pink, markedly swollen	19x16 pink, markedly elevated		"
66'	color fading, markedly elevated	24x23, color slightly faded, well elevated		"
overnight	Practically normal	Practically normal		"

pneumococcus III serum did not show the effect.

It may be mentioned that no reactions were obtained in the skin of rabbits (on the ear or the back) in sites injected with rabbit or guinea pig immune sera.

It would seem probable that the reactions described are due to anaphylactic antibodies; so far it can be said merely that in guinea pigs which had received guinea pig antisera intraperitoneally lethal shock could be induced not only in the case of horse serum but also of ragweed.[†]

By testing several like antisera simultaneously in one recipient, the antigen being given subcutaneously, it is possible to evaluate their comparative strengths.

† Passive anaphylactic transfer with guinea pig anti-ragweed serum was demonstrated following intraperitoneal injection (6 cc) into a normal guinea pig of 330 g weight, typical shock and death ensuing within 4 minutes following intravenous injection of 0.5 cc "5%" extract on the next day. The extract was not at all toxic for a normal control.

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Alterations in Response to Changing Body Temperature Following Artificial Fever and Chilling.

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During investigations upon the temperature control of birds, modifications of both panting and shivering temperature thresholds due to artificial fever and chilling were studied. Both young and adult domestic fowls (*Gallus domesticus*) were first placed in an ice chamber in which the temperature averaged about 10°C, and then in a heating blanket in which the temperature averaged about 55°C. After a suitable recovery period, the order of the

exposure was reversed, *i.e.*, the same bird was first warmed and then chilled. During these changes in body temperature, changes in breathing rate were recorded. Temperatures were recorded from sensitive thermometers placed to a constant depth in the cloaca and under the skin. The first muscular tremors preceding continuous shivering were detected by a stethoscope placed upon the muscles of the limbs and breast.

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Eighty minutes were required to lower the temperature of a typical bird (Table I) one