HORSE-SERUM SKIN TESTS

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With the widespread use of therapeutic sera at the present time the possibility of severe serum reactions must be kept in mind, and skin testing of the patient with a small amount of serum prior to the injection of the therapeutic dose is a frequent procedure, undertaken with the object of predicting sensitivity to the serum protein. In this investigation an attempt has been made to find what type of skin reaction may be expected on carrying out intracutaneous tests with horse serum in the human subject and to estimate their practical value for the purpose mentioned.

Part I. Skin testing with horse serum

Lewis (1924) estimated that 25% of normal people show slight, and 5% marked whealing of the skin to mechanical stimuli, and Grow & Herman (1936), skin-testing 150 normal individuals with thirteen of the common allergens, found that 55·5% reacted positively to one or more allergens.

Figures of skin-sensitive response to horse serum in normal individuals vary from 5% (Tuft, 1932) to 50% in children and 74% in adults (Park, 1924), both investigators using 0·02 c.c. of 1 : 10 normal serum; while Hooker (1924), retesting sixteen patients who were skin-negative prior to intravenous serum therapy, reports a positive reaction of the immediate type in each case.

Longcope & Rackemann (1918) first described in detail the reaction to intracutaneous horse-serum injection and differentiated the immediate traumatic reaction produced equally by controls from the subsequent true reaction of sensitivity. Kellett & Wright (1930) reported a delayed type of reaction to 0·2 c.c. normal horse serum injected in a subject previously untreated with serum. The immediate reaction was normal, but on the sixth and seventh days four distinct crops of urticaria were noted. Repeating the injection 16 days later in the opposite arm, to test for general sensitivity, they noted an accelerated urticarial reaction within 18 hr.

Investigation

The object of this investigation was to show the incidence and type of skin reaction which occurs in the normal and sensitized human subject following intracutaneous injection of normal horse serum.

Material used. Normal horse serum, containing no antiseptic, obtained in 1 c.c. ampoules from the Belmont Laboratories (L.C.C.).

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Method adopted. Intracutaneous injection of 0·1 c.c. undiluted serum on the anterior surface of the forearm.

Reading of results. Test reactions were watched for 20 min. after injection, inspected 1, 3 and in some cases 6 hr. later and daily up to the sixteenth day. Intermediate reactions were seen as reported.

Subjects. Patients admitted to hospital for treatment of diphtheria, measles and scarlet fever. Bearing in mind the fact that an acute exanthem may cause temporary suppression of skin reactivity (Lewis, 1924; Westwater, 1935), patients were only considered to be normal individuals, and so included as such in this series, two or more weeks after the onset of rash of measles or scarlet fever.

Control tests with normal saline were made at the onset, but this was abandoned, to avoid multiplicity of injections, as little difficulty was experienced in distinguishing the normal reaction to horse serum (equivalent to the control) from the reaction of sensitivity.

Types of skin reactions observed

(1) In the normal, traumatic reaction produced by the intracutaneous injection of 0·1 c.c. normal horse serum the wheal retains its original size ($\frac{1}{4}$ in. diameter) and disappears within from 5 to 20 min. after injection. The wheal may be surrounded by a flare of erythema of varying intensity, having an irregular edge and spreading to a diameter of 1 or 2 in. This erythematous zone usually persists after the wheal has subsided and may be apparent several hours after injection.

(2) The immediate type of sensitive reaction usually manifests itself 10–15 min. after injection by an extension of the original wheal, which becomes firmer and loses its defined edge. Pseudopodia-like processes are protruded from the margin of the wheal in about 30 % of subjects 10–20 min. after injection and later become absorbed in a spreading oedema. In cases showing marked sensitivity subcutaneous oedema, comparable to a mild degree of the Arthus phenomenon, may extend over a wide area, reaching a diameter of several inches, surrounded by a ring of erythema and lasting 2–3 days. Crops of urticaria, appearing at the edge of the oedematous area and extending peripherally on successive occasions, occurred in 2 % of normal and 14 % of sensitive individuals in this series. The flare of erythema at the onset of the reaction is of deeper intensity and covers a wider area than in the normal reaction, usually fading 3 or 4 hr. after injection.

(3) Secondary or delayed reactions may occur at the site of the skin test after the primary reaction has subsided. These are of a transient nature and take the form of erythema, frequently of the punctate type, oedema or urticaria. This skin-serum reaction is comparable with the general reaction which may follow serum therapy, appearing 2–12 days after the skin-test injection, and was noted in 14 % normal and 20 % sensitive subjects in this investigation.

When the primary test gives a sensitive reaction the skin-serum reaction tends to occur early (accelerated type), is more intense, explosive urticaria and oedema developing rather than simple erythema.

In order to estimate the incidence of skin sensitivity to normal horse serum two series of cases were tested.

Series A: 150 subjects having no history of allergy or previous serum injection.

Series B: 100 individuals who had received previous serum injection.

In series A (150 normal cases) reaction of sensitivity occurred in 30 %. These reactions were of a mild type, erythema being at its height 15 min. after injection and oedema reaching a diameter of $\frac{1}{2}$–1 in. in 3 hr., then subsiding. "Pseudopodia" appeared at the onset in 35·5 % of these cases and urticaria in 2 %. In 87 % the duration was less than 3 hr., and only 2 % lasted longer than 24 hr. The highest percentage of sensitive reactions occurred in the 10–15 year age group.

In series B (100 sensitized cases) reaction of sensitivity occurred in 88 %. Reactions were more intense and of longer duration than in series A, erythema (2 × 3 in.) persisting in some cases until the fourth day, while oedema up to 3 × 4 in. reached its height in 3–24 hr. and was occasionally surrounded by a ring of erythema. "Pseudopodia" were noted during the first hour in 30·7 % of sensitive cases and urticaria in 14 %, the latter frequently occurring in crops, each one more peripheral to the site of injection. Duration of reaction was under 3 hr. in only 24 % of cases, 36·4 % persisting for longer than 24 hr. The highest percentage of sensitive reactions was found to occur in the age group of over 15 years.

Skin sensitivity may persist for many years after serum injection; two cases in series B, which had been treated for diphtheria 20 years prior to the skin test, showed reactions of marked sensitivity.

The incidence and degree of skin sensitivity, as demonstrated by the intracutaneous injection of horse serum, thus shows a definite increase where the subject has been sensitized by previous serum injection.

To confirm this statement thirty-five normal cases, ten of whom had shown primary skin sensitivity of a mild degree, were retested 2 weeks to 3 months after therapeutic serum had been received. Sensitive skin reactions of the oedematous type were noted in every case.

The amount of serum received and the length of time since its administration do not seem to be factors in determining the degree of the skin sensitivity, which evidently depends on individual power to produce antibodies.

A final series of tests was carried out in a normal subject to find the result of repeated skin tests. The primary test with 0·2 c.c. normal horse serum injected in the left forearm gave a normal immediate reaction and a skin-serum reaction lasting from the seventh to the tenth day. The latter consisted of an area of punctuate erythema 3 × 4 in. in diameter, accompanied by transient severe crops of urticaria, each appearing in a new area more peripheral to

the site of injection. Secondary tests were made 2 weeks later using 0·1 c.c. horse serum in each forearm. Similar immediate reactions of sensitivity were noted on both arms, lasting 30 hr. The original wheal spread by means of "pseudopodia", apparent 15 min. after injection, and an extending ovoid area of oedema, tender to the touch, reached its height (4 × 8 in.) in 12 hr., then gradually subsided. No urticaria developed, and no subsequent reaction appeared. For the final tests 2 months later 0·1 c.c. horse serum was injected in the left and a similar amount of concentrated diphtheria antitoxin in the right forearm. Again similar immediate reactions of sensitivity developed on both arms but were less intense. "Pseudopodia" were absent, oedema ($2\frac{1}{2} \times 5$ in.) reached its height 9 hr. after injection and was surrounded by a ring of punctuate erythema. The reaction subsided within 30 hr. but was followed by the development of peripheral crops of urticaria at 30 and 48 hr. in the left and 48 hr. in the right arm. General skin sensitivity and the presence of skin antibodies produced by the intracutaneous injection of 0·2 c.c. normal horse serum was thus demonstrated by an immediate skin-sensitive reaction in the opposite arm 2 weeks later and was still apparent after 2 months.

Part II. The value of skin tests in the prognosis of serum reaction

Introduction. Park (1913) used horse serum skin tests in an attempt "to disclose dangerous liability to anaphylaxis". A number of cases previously injected with antitoxic serum were tested 2–3 weeks later and "they regularly developed an immediate reaction in the skin at the point of intracutaneous injection". After a second injection of serum 50% of these cases showed fever and general urticaria within a few hours but none of the reactions was severe. Fifty cases of diphtheria were tested on admission, and three showed immediate skin reactions. All were given 10,000 units of antitoxic serum and the three who were previously skin sensitive developed fever and urticaria within 12 hr. but had no alarming symptoms. There is no mention of occurrence of serum reaction in the other forty-seven cases.

Toomey & August (1930), using diphtheria antitoxic serum for skin testing, injected 780 cases prior to therapeutic serum injection. Of 659 negative skin reactions 11·4% had subsequent serum reactions and of the 121 positive reactions 13·1% developed serum reaction. They concluded that no definite relationship could be established between the result of skin testing and reaction to subsequent injection of antitoxic serum and questioned the value of skin tests in determining susceptibility to therapeutic serum. In this series no distinction is made between those patients who may have received previous serum injection and the so-called normal reactors.

Investigation

This investigation was carried out to find whether any relation could be proven between the presence of specific antibodies in the skin cells, as demon-

strated by an immediate reaction of sensitivity to the intracutaneous injection of horse serum and subsequent development of general serum reaction.

Material used and methods of testing were similar to those described in Part I of this paper.

Subjects tested were 200 cases of diphtheria (180 children and 20 adults). Skin tests were made immediately prior to therapeutic serum injection and watched for 20 min. to half an hour, readings being taken again at intervals of 1 and 3 hr. and thereafter as necessary. Sensitivity, if present, was usually apparent 10–20 min. after injection of test material (0·1 c.c. normal horse serum).

Results. Of 200 cases skin tested 24·5 % later developed general serum reaction.

Primary skin tests were normal in 61 % of the 200, and of these cases 22·1 % developed serum reaction, while in the skin sensitive group (39 %) the incidence of serum reactions was 28·2 %, an increase of 6 %.

Since previous serum injection raises the incidence both of skin sensitivity and of serum reaction, the cases have been divided into group A, 165 normal individuals having no history of previous serum injection, and group B, thirty-five subjects sensitized by previous serum injection. The results, as summarized in the following table, show that in cases receiving a first injection of serum there is an increase of 4·2 % in the incidence of serum reaction where primary skin sensitivity was present, but in reinjected cases, although skin sensitivity was demonstrated in 91·4 %, there was actually a lower incidence of serum reaction than amongst the primary normal skin reactors:

	Primary normal skin test	Serum reaction	Primary normal skin test	Serum reaction
Group A	119 (72·1 %)	26 (21·9 %)	46 (27·9 %)	12 (26·1 %)
Group B	3 (8·6 %)	1 (33·3 %)	32 (91·4 %)	10 (31·3 %)
Total	122 (61·0 %)	27 (22·1 %)	78 (39·0 %)	22 (28·2 %)

No difference was noted in the relation between skin test results and development of serum reaction when dosage rates were compared or in the age groups under 15 years, but in twelve adults with normal primary skin tests no serum reactions occurred, whereas of the eight adults presenting sensitive skin reactions, four subsequently developed serum reaction, none of which was severe. Neither length of time since previous serum injection nor severity of the skin sensitive reaction were found to be factors in this attempt to correlate skin sensitivity with later serum reaction.

In eight cases of this series additional skin tests were made using 0·1 c.c. of diphtheria antitoxic serum to ascertain whether parallel results would be obtained with the two test solutions, but results were not consistent in every instance. In six of these subjects similar results were obtained, but in the remaining two, both severe cases of diphtheria, the horse-serum tests were negative while the antitoxic serum gave mild reactions of sensitivity. One of these developed general urticaria on the tenth day.

SUMMARY AND CONCLUSIONS

An investigation is described which shows the type of skin reaction which may be expected from the intracutaneous injection of 0·1 c.c. normal horse serum in the normal and sensitized human subject. 150 normal individuals and 100 who had received previous serum injection were skin-tested. Of the normal subjects 30% showed a reaction of skin sensitivity, while 88% of the sensitized group gave a sensitive response, the reactions in the latter being more intense and of longer duration than those of the normal group. It is also shown that general skin sensitivity, demonstrated by a sensitive skin reaction in the opposite arm 2 weeks later, can be produced by intracutaneous injection of 0·2 c.c. of normal horse serum.

The value of specific skin tests in the prognosis of serum reaction is considered and a series of tests on 200 diphtheria cases is recorded. Since it has been shown that diphtheria has no appreciable effect in diminishing skin sensitivity (Westwater, 1935) the present series probably gives an accurate estimation of serum skin sensitivity even in the presence of acute diphtheritic infection.

The incidence of serum reaction was found to be only 6% higher in those cases which gave a sensitive skin reaction to horse serum prior to therapeutic serum injection than in those showing a normal response. It is therefore concluded that the demonstration of skin sensitivity is of no value in predicting the subsequent development of general serum reaction, and skin testing with horse serum, although it may demonstrate the presence of specific antibodies in the skin cells, is not an index of general sensitivity, which depends upon individual reactivity and power to produce antibodies following injection of foreign serum.

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