

A METHOD OF SENSITIZING GUINEA PIGS TO HORSE SERUM THAT EVENTUATES IN 100 PER CENT MORTALITY AFTER CHALLENGING*

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ASSAYING the value of antihistaminics in protecting guinea pigs from anaphylactic shock would require fewer animals if a method of sensitizing were at hand that invariably led to such a titer of antibodies that the later injection of a challenging dose of the antigen, given after a suitable interval, would always be fatal. The usual methods of sensitizing guinea pigs to horse serum by the intravenous, intramuscular, or intraperitoneal injection of variable amounts of the antigen seldom lead to a fatal degree of sensitivity in 100 per cent of the guinea pigs. Table I, gathered from the literature, is a partial summary of various experiments, giving the number of control animals that died after the challenging dose of the antigen. Only in about one-half of the experiments did the controls prove 100 per cent fatally sensitive, despite the wide variations in the procedural details reported. It is perhaps significant that the 100 per cent fatal sensitivity was usually reported for the smaller control groups. This failure is easily compensated for by the use of more animals, but that would seem unnecessary if a method of sensitization could be devised that would render the guinea pig so sensitive that the ultimate injection of a challenging dose of the antigen would always be fatal. For this reason the present studies were initiated. This problem could, of course, be met by the use of passive sensitization, but for most laboratories this method would probably be more time consuming and less economical.

METHODS

Male guinea pigs weighing from 300 to 350 grams at the time of sensitization were employed in all the experiments. Since the injections of horse serum alone in varying doses, and by varying routes, did not permit the achievement of our purpose, we were led to try 3 other methods: (1) a procedure described by Freund and McDermott,⁹ which was originally recommended to us by Richards,²⁰ and which was an outgrowth of earlier work (see their bibliography); (2) the intrahepatic injection of the antigen¹; and (3) combinations of the first 2 methods. The method of intrahepatic sensitization was as follows: While an assistant firmly held the animal supine, a 1 inch, 26 gauge needle, attached to a 1/2 ml. syringe, was perpendicularly placed on the skin just to the right of the xyphoid process, and just below the lowest costosternal juncture. The upper end of the syringe was then tilted a

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TABLE I. MORTALITY RATE OF CONTROL GROUPS OF ACTIVITY SENSITIZED GUINEA PIGS IN ANAPHYLACTIC EXPERIMENTS;
A PARTIAL SUMMARY OF THE LITERATURE

NUMBER ANIMALS CONTROL GROUP	MORTALITY	PER CENT DEAD	WEIGHT RANGE (GRAMS)	ANTIGEN	DOSE AND ROUTE	SENSITIZING PERIOD* (DAYS)	SHOCKING DOSE	REFERENCES
--	--	90	--	Horse serum	0.1 c.c. S. C.	--	0.2 c.c. I. V.	Bernstein and Feinberg ²
30	24	80	--	Horse serum	0.1 c.c. S. C.	14	--	Ercoli and associates ⁷
"Large"	--	61	--	--	--	--	--	Feinberg and Bernsteins
14	14	100	400-500	Horse serum	0.1 ml. S. C.	12	0.5 c.c. I. V. penile vein	Friedlaender and Fried- laender ¹⁰
--	--	100	--	Sheep serum	2 x 0.5 c.c. after 48 hr. I. P.	15-21	0.1 c.c. I. V.	Halpern ¹¹
10	10	100	--	Sheep serum	2 x 0.5 c.c. after 48 hr. I. P.	15-21	0.1 c.c. I. V.	Halpern ¹²
3	3	100	--	Beef serum	0.5 c.c. S. C.	15	0.1 c.c. I. V.	Halpern ¹³
21	17	81	--	Sheep serum	0.5 c.c. I. P.	23-26	0.25 c.c. I. C.	Landau and Gay ¹⁴
(3 groups: 5, 8, 8)	37	86	--	Sheep serum	0.5 c.c. I. P.	21	0.25 c.c. I. C.	Landau and associates ¹⁵
12	12	100	--	Egg white	2.0 c.c. of 10 per cent I. P.	21	0.5 c.c. I. C.	Landau and associates ¹⁵
8	8	100	--	Horse serum	2 x 0.5 c.c. I. P. 48 hr. apart	21	0.5 c.c. I. C.	Mayer and associates ¹⁶
4	4	100	250-300	Horse serum	2 x 0.5 c.c. I. P. 48 hr.	21	1 ml. I. C.	Mayer ¹⁷
13	13	100	--	Horse serum	2 x 0.5 c.c. I. P. 48 hr.	21	1 ml. I. C.	Mayer ¹⁸
13	27	87	300-400	10 per cent horse serum	1.0 c.c. I. P.	about 14	0.5 c.c. I. V.	Reinhard and Seudis ¹⁹
52	43 (table)	83	300-400	10 per cent horse serum	1.0 c.c. I. P.	about 14	0.5 c.c. I. V. jugu- lar vein	Scudi and associates ²¹
10	9	90	--	Egg albumin	--	--	I. V. jugular vein	Selle ²²

*The interval between the administration of the sensitizing dose and the challenging dose.
Lacuna (--) indicate the absence of data in the original references.

I.P. = Intraperitoneally.

I.V. = Intravenously.

I.C. = Intracardially.

S.C. = Subcutaneously.

short distance toward the guinea pig's left hind leg so the needle was pointed somewhat laterally and slightly cephalad. The point was then quickly thrust through the skin and muscle into the liver. Its presence therein was indicated by failure to withdraw blood or fluid upon pulling back on the plunger, a peculiar feeling of resistance as the upper end of the syringe was slightly and gently oscillated, and a moderate resistance to the injection. These sensations, as well as the direction in which to insert the needle, are best learned by trial injections into the livers of dead animals with open abdomens. It might be added that, at best, one can never be absolutely sure the needle is in the liver. An occasional animal has been lost during the first day or two after sensitization as a result of hemorrhage or infection. Attempts at injections through the right, lower, and lateral aspect of the thoracic cage led too often to pulmonary complications or intrathoracic injections. The dose injected was 0.05 ml. and if 2 doses were given, the needle was withdrawn from the liver and muscle, and reinserted for the second 0.05 ml. Obviously, sudden and excessive changes in the direction of the axis of the syringe while the needle is in the liver can cause serious or fatal hemorrhage.

The adjuvant employed in this study was killed tubercle bacilli (human 37RV strain made available by our associate, Dr. Philip Eisman), and the vehicles were Aquaphor and paraffin oil, as recommended by Freund and McDermott.⁹ Table II gives the proportion of the various materials present when horse serum alone was not employed. It will be noted that the original formula of Freund and McDermott was modified for the intrahepatic injections.

TABLE II. FORMULAE OF ANTIGENIC MATERIAL

TOTAL DOSE (ML.)	EXPERIMENT*	HORSE SERUM (ML.)	AQUAFOR (ML.)	PARAFFIN OIL (ML.)	T. B. ORGANISM (MG.)
0.50	Section B, group II	0.125	0.125	0.25	0.5
0.50	Section B, group III	0.125	0.125	0.25	None
0.05	Sections A & C, group II	0.0165	0.0165	0.0165	0.064
0.05	Sections A & C, group III	0.0165	0.0165	0.0165	None

*Indicates the group in Table III receiving the formula noted.

RESULTS

Table III summarizes the results. The entries are in chronological order. It is difficult to explain why the first 5 groups injected with horse serum alone, given intrahepatically in 2 doses, proved 100 per cent fatally sensitive, whereas survivors were encountered in all subsequent groups but one. Survivors were also encountered in the groups sensitized intramuscularly by the original method of Freund and McDermott. The results would indicate, however, that their formula, with or without tubercular organisms, would yield 100 per cent fatal sensitivity if given intrahepatically or intraperitoneally in 2 doses of 0.05 ml. each.

TABLE III. SECTION A. MALE GUINEA PIGS SENSITIZED BY INTRAHEPATIC INJECTIONS

NUMBER OF GUINEA PIGS	SENSITIZING DOSE (ML.)	INTERVAL IN DAYS*	CHALLENGING DOSE (ML.)	ROUTE OF INJECTION	MORTALITY	PER CENT MORTALITY
<i>Group I: Horse Serum Alone</i>						
11	0.05	30	0.50	I. C.	7/11	64
9	0.05	21	0.50	I. C.	8/9	89
8	2 × (0.05)	30	0.50	I. C.	8/8	100
6	2 × (0.05)	21	0.50	I. C.	6/6	100
9	2 × (0.05)	22	0.50	I. V.	9/9	100
12	2 × (0.05)	21	0.50	I. V.	12/12	100
11	2 × (0.05)	21	0.50	I. V.	11/11	100
10	2 × (0.05)	21	0.50	I. V.	8/10	80
9	2 × (0.05)	21	0.50	I. V.	6/9	66
11	2 × (0.05)	21	0.50	I. V.	11/11	100
15	2 × (0.05)	21	0.50	I. V.	12/15	80
10	2 × (0.05)	21	0.50	I. V.	7/10	70
9	2 × (0.05)	21	0.50	I. V.	6/9	67
14	2 × (0.05)	21	0.50	I. V.	13/14	93
<i>Group II: Horse Serum + T. B.</i>						
15	2 × (0.05)	20	0.50	I. V.	15/15	100
17	2 × (0.05)	20	0.50	I. V.	17/17	100
19	2 × (0.05)	21	0.50	I. V.	19/19	100
<i>Group III: Horse Serum + Vehicles but no T. B.</i>						
24	2 × (0.05)	21	0.50	I. V.	24/24	100
SECTION B. MALE GUINEA PIGS SENSITIZED BY INTRAMUSCULAR INJECTIONS						
<i>Group II: Horse Serum + T. B.</i>						
8	0.50	21	0.50	I. C.	8/8	100
11	0.50	21	0.50	I. V.	8/11	73
11	0.50	21	0.50	I. V.	11/11	100
12	0.50	21	0.50	I. V.	10/12	83
26	2 × (0.05)	21	0.50	I. V.	22/26	84.6
<i>Group III: Horse Serum + Vehicles but no T. B.</i>						
19	0.50	21	0.50	I. C.	18/19	95
19	0.50	22	0.50	I. C.	11/19	58
18	0.50	16	0.50	I. V.	8/18	44
15	0.50	21	0.50	I. V.	15/15	100
13	0.50	21	0.50	I. V.	10/13	77
13	0.50	21	0.50	I. V.	13/13	100
SECTION C. MALE GUINEA PIGS SENSITIZED BY INTRAPERITONEAL INJECTIONS						
<i>Group II: Horse Serum + T. B.</i>						
18	2 × (0.05)	21	0.50	I. V.	18/18	100
<i>Group III: Horse Serum + Vehicles but no T. B.</i>						
19	2 × (0.05)	21	0.50	I. V.	19/19	100

*Refers to days elapsing after sensitizing before challenging.

I.C. = Intracardiac

I.V. = Intravenous

T.B. = Killed tubercle bacilla

Table IV summarizes the protective action exerted by 2 antihistaminics, 2-phenylbenzylaminomethyl imidazoline hydrochloride (Antistine), and N,N-dimethyl-N'-benzyl-N'- α -pyridyl ethylenediamine monohydrochloride (Pyri-benzamine), against fatal anaphylactic shock in guinea pigs sensitized by the modification of Freund and McDermott's method.

Even very high doses of the antihistaminics protected few animals but the animals that died usually survived much longer than the controls.⁶

DISCUSSION

Richards originally recommended the method of Freund and McDermott because up to that time it had never failed, in his laboratory, to yield a potentially fatal level of antibody. In our hands this was not always the case, and more recently Richards²⁰ has also noted exceptions (Table III, Section B, Group II). The method of intrahepatic injections was initially employed on the assumption that a direct delivery of the antigen to such a large concentration of reticulo-endothelial elements as possessed by the liver might lead to a higher concentration of antibodies, especially in the blood itself. The injection of only a single dose intrahepatically (Table III, Section A, Group I, first 2 experiments) failed to yield the required level of antibody. Presumably a minimal dose is required, as has been shown by Coulson and Stevens³⁻⁵ (as well as others), but once attained, further increases in the volume of a single injection do not materially increase the production of antibody. The injection intrahepatically or intraperitoneally of the formula of Freund and McDermott (with minor modifications) led to a 100 per cent potentially fatal level of antibody (Table III, Section A, Group II; Section C, Group II). The success of the method would not appear to validate the assumption that led to its trial. The injection intrahepatically or intraperitoneally of the same formula without the organisms also caused a potentially fatal degree of sensitivity (Table III, Section A, Group III; Section C, Group III). If, in additional experi-

TABLE IV. EFFECT OF ANTISTINE HCL AND PYRIBENZAMINE HCL ON ANAPHYLACTIC SHOCK IN MALE GUINEA PIGS (ADMINISTERED I. P. 30 MINUTES BEFORE ANTIGEN)

NUMBER OF PIGS	ANTISTINE HCL			HORSE SERUM (DOSE C.C.)	ROUTE	NUMBER OF DEATHS	PER CENT MORTALITY
	DOSE MG./KG.	CONCENTRATION PER CENT	ROUTE				
10	28	2	I. P.	0.50	I. V.	10/10	100
8	42	2	I. P.	0.50	I. V.	5/8	62.5
PYRIBENZAMINE HCL							
10	14	2	I. P.	0.50	I. V.	10/10	100
9	21	2	I. P.	0.50	I. V.	6/9	66.6
CONTROL GROUP							
6	--	--	--	0.50	I. V.	6/6	100

ments, these same results were obtained, it would indicate the intrahepatic and intraperitoneal administrations of the antigen were superior to the intramuscular and intravenous in respect to the titer of antibodies produced in the blood. This might be due to the more rapid access of the injected antigen to the liver via the portal circulation, but this speculation might be difficult to establish experimentally.

The antihistaminics protected from death only about one-third of the animals sensitized by the intrahepatic injection of a modified Freund and McDermott formula (Table IV). This might be due to one or both of the following factors: (1) substances other than histamine are released which threaten the life of the animal in anaphylactic shock, (2) such tremendous quantities of histamine are released by the injection of the challenging dose that cardio-

vascular collapse results. It has, of course, been repeatedly demonstrated that antihistaminics will only partially antagonize the hypotensive effects of large doses of histamine.

The value of the method described for assaying the activity of antihistaminics in anaphylactic shock is debatable. The method assures a potentially fatal titer of antibody but it does not permit adjudging, without precipitin tests, how many multiples of a minimally fatal titer may be present. The animals dying despite the antihistaminic did survive much longer than the controls (from 2 to over 70 times as long) and the use of the survival time might allow a more accurate comparison of the activities of 2 potent antihistaminics. The method might prove useful in detecting the action of potential therapeutic agents that interfere with, or prevent, the antigen-antibody reaction, but at present we know of no such drugs.

SUMMARY

Various methods of sensitizing guinea pigs to horse serum were employed in the hope of finding one that would invariably produce a potentially fatal level of antibody in 100 per cent of the animals. The only successful method involved intrahepatic or intraperitoneal injections of 2×0.05 ml. of horse serum alone, or with killed tubercle organisms. The details of this new method have been described. Even large doses of antihistaminics offered but partial protection against anaphylactic shock to animals so sensitized. This might be additional evidence that released histamine does not alone account for the symptoms of anaphylactic shock, but the possibility was not excluded that so much histamine was liberated that cardiovascular collapse resulted, since it is known that antihistamines only partially antagonize the hypotensive actions of higher doses of histamine.

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