

Hypersensitivity responses in monkeys

III. The influence of various tranquilizers and other drugs on immediate skin reactions

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The common use of "tranquilizers" in experimental hypersensitivity studies in monkeys, involving immediate skin reactions, posed the problem of the possible influence of these agents on skin reactivity. Acepromazine, a phenothiazine, was suspected as particularly likely to suppress skin reactivity. With the aid of Evans blue or Coomassie blue dye, the end point of skin reaction was determined from intradermal injections of serial dilutions of histamine, compound 48-80, Ascaris and human serum (in animals allergic to these antigens), Ascaris in monkeys passively sensitized with monkey Ascaris-reaginic serum, and ragweed in animals sensitized to human ragweed-reaginic serum. The titrations were then repeated at the same sitting after the animal was given an injection of either phencyclidine (Sernylan), acepromazine, reserpine, or pentobarbital. Pentobarbital caused no inhibitory effect on the skin test but was regarded as generally unsafe and unreliable. Reserpine gave inconsistent but long tranquilization and some diminution in skin reactions. Acepromazine resulted in consistent and sometimes profound decrease in skin response and not always good tranquilization. Phencyclidine made the monkeys very easy to handle and had no suppressive effect on the skin test.

Although we have not found it necessary to tranquilize* monkeys in most of our experiments relating to hypersensitivity, others have used drugs for this purpose, especially when a monkey is very excitable or work must be done around the mouth. Of those used, acepromazine,¹ phencyclidine (Sernylan),² reserpine,³ and pentobarbital have been frequently mentioned.⁴⁻⁷ Acepromazine is a phenothiazine, while phencyclidine belongs to an unusual class of analgesic

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*The term "tranquilize" or "tranquilizer" in this paper is used in the broad sense, indicating calming, and does not distinguish the mode of action, such as anesthesia, analgesia, etc.

and depressant or stimulant agents whose action differs according to the dose level and species of animal. We have suspected that at least one of these compounds, acepromazine, because of its phenothiazine structure, might be expected to cause diminished skin reactivity.

The present investigation was planned to determine any inhibiting or augmenting effect of acepromazine, phencyclidine, reserpine, and pentobarbital on titrated skin reactions in monkeys.

MATERIALS AND ANIMALS

Animals

Seven male *Macaca mulatta* monkeys and one female *Macaca speciosa* monkey, ranging in weight from 4.7 to 6.8 kilograms, were used in these experiments. The animals were restrained by tying the extremities to a board.

Tranquilizers

Tranquilizers used were: (1) acepromazine maleate,* injectable, 10 mg. per cubic centimeter, Ayerst Labs., dose: 1.1 to 3.3 mg. per kilogram, intramuscularly; (2) phencyclidine HCl† (Sernylan), 20 mg. per cubic centimeter, Parke, Davis & Co., dose: 0.75 to 0.9 mg. per kilogram, intramuscularly; (3) reserpine (Serpasil), 2.5 mg. per milliliter, Ciba Pharmaceutical Products, Inc., dose: 1.0 to 1.5 mg. per kilogram, intramuscularly, or 1.0 to 1.6 mg. per kilogram, intravenously, and (4) pentobarbital sodium (Nembutal Sodium), 50 mg. per milliliter, Abbott Laboratories, dose: 12.5 to 17.4 mg. per kilogram, intravenously.

Challenging materials

Histamine, 48-80, *Ascaris suis* extract, ragweed extract, normal human serum, human reaginic serum to ragweed, and monkey reaginic serum to *Ascaris* were prepared as outlined previously.⁸

Indicators

Evans blue and Coomassie blue dyes were used as described previously.⁸

PROCEDURES

Evans blue or Coomassie blue was given intravenously. About 6 to 15 minutes after dye injection, titrations were made on one side of the body with 0.1 ml. threefold serial dilutions of histamine and 48-80 and fivefold serial dilutions of antigens (*Ascaris* and human serum). Monkeys passively sensitized locally with human ragweed-reaginic serum in fivefold serial dilutions were challenged (usually in 24 hours) with ragweed extract 1:300 w/v locally; animals sensitized with monkey *Ascaris*-reaginic serum in fivefold serial dilutions were challenged locally with *Ascaris* extract, usually 1:25. Reactions were read in about 15 minutes and photographed. The drug under test was then administered in the doses indicated previously. Titrations were then repeated on the opposite side of the

*Available only for experimental use in animals.

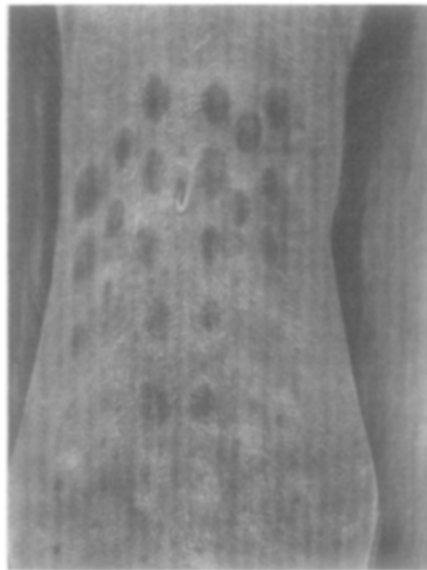


Fig. 1

On left side of monkey, from center toward side of abdomen, are titrations with *Ascaris*, 48-80, and histamine 6 minutes after Evans blue injected intravenously. On right side of monkey, from center toward the side of abdomen, are titrations with the same dilutions of *Ascaris*, 48-80, and histamine 2 hours after the Evans blue had been injected.

body after the following time intervals: acepromazine, intramuscularly, 25 to 60 minutes; phencyclidine, intramuscularly, 12 to 45 minutes; reserpine, intravenously, 43 to 59 minutes; reserpine, intramuscularly, 33 to 47 minutes; and pentobarbital, intravenously, 8 to 27 minutes.

There was no diminution of the intensity of reaction or titer without the use of tranquilizer when the same challenges were made as long as 2 hours after injection of Evans blue. Fig. 1 shows, on left side of the monkey, challenges with serial dilutions of histamine, 48-80, and *Ascaris* and, on the right side, the same challenges 69 minutes after the Evans blue injection.

When Coomassie blue was used as the indicator, a second injection was given before the challenge after administration of the tranquilizer.⁸

Photography was done as outlined previously.⁸

The exact degree of tranquilization is difficult to determine, but an approximate determination could be made by placing a plastic tube between the monkey's teeth. Failure to bite on the plastic usually indicated good effect.

RESULTS

Results are indicated in Table I. It is obvious that acepromazine results in diminution in titer or reactivity of all the challenging materials. Its tranquilization effect was somewhat inconsistent. Phencyclidine produced no change in skin-test reactions and made the monkeys very easy to handle. Reserpine seemed to inhibit the reactions slightly at times. It was not a useful tranquilizer be-

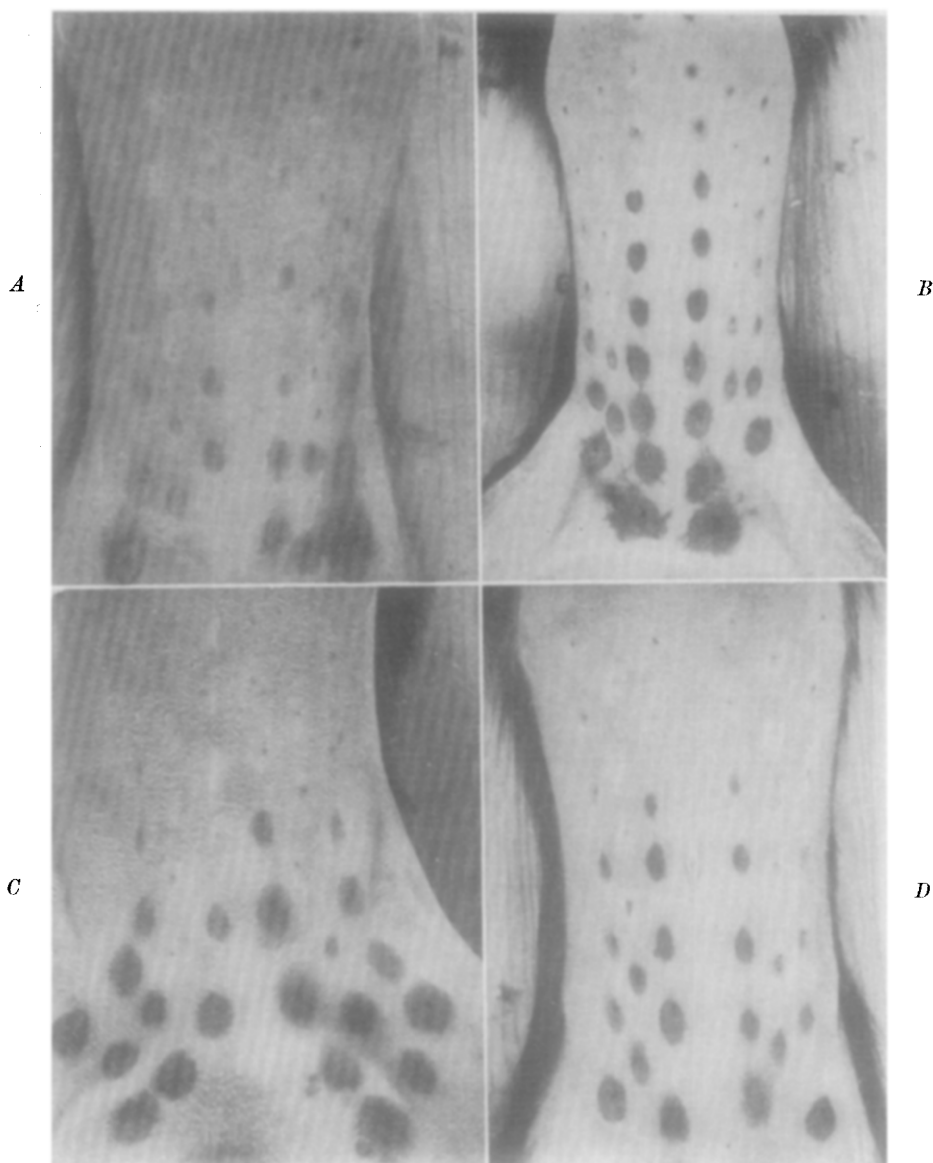
Table I. *Effect of tranquilizer on skin reactivity*

Monkey	Experiment No.	Date	Tranquilizer dose (mg./Kg.)	Indicator	
				Type	Dose (mg./Kg.)
<i>Acepromazine maleate</i>					
3	15	4/17/68		CB	20.0
		4/17/68	3.3 intramuscular	CB	20.0
5	12	4/5/68		EB	3.5
		4/5/68	2.2 intramuscular		
6	6	3/22/68		EB	4.6
		3/22/68	2.2 intramuscular		
7	9	3/13/68		EB	4.2
		3/13/68	1.65 intramuscular		
8	3	3/8/68	1.05 intramuscular	EB	4.2
	7	4/2/68		CB	20.0
		4/2/68	2.2 intramuscular	CB	20.0
10	1	4/25/68		EB	4.2
		4/25/68	2.2 intramuscular		
11	2-24	5/1/68		EB	3.5
		5/1/68	2.2 intramuscular		
<i>Reserpine</i>					
3	14	4/12/68		EB	3.5
		4/12/68	1.0 intramuscular		
4	14	4/16/68		EB	3.3
		4/16/68	1.5 intramuscular		
7	16	4/24/68		EB	4.0
		4/24/68	1.0 intravenous		
10	3-24	5/10/68		EB	4.2
		5/10/68	1.6 intravenous		
11	4-24	5/15/68		EB	3.5
		5/15/68	1.0 intravenous		1.0
<i>Pentobarbital sodium</i>					
4	19	5/3/68		EB	3.3
		5/3/68	12.5 intravenous		
6	9	5/8/68		EB	4.6
		5/8/68	17.4 intravenous		
<i>Phencyclidine hydrochloride</i>					
3	11	3/13/68	0.9 intramuscular	CB	20.0
4	11	3/15/68	0.9 intramuscular	CB	20.0
4	12	3/28/68		EB	3.3
		3/28/68	0.75 intramuscular		
6	7	3/27/68		EB	4.6
		3/27/68	0.75 intramuscular		
7	11	4/3/68		CB	20.0
		4/3/68	0.75 intramuscular	CB	20.0
8	4	3/13/68	0.9 intramuscular	CB	20.0
11	1-24	4/25/68		EB	3.5
		4/25/68	0.75 intramuscular		

The effect of the different drugs on skin reactivity from different types of challenges is number of experiments, particularly those with antigen challenge and passive transfer, are not entirely in agreement with those in the table. Abbreviations are as follows: CB = Coomassie blue; reaginic antibody (to ragweed); MAb = monkey reaginic antibody (to *Ascaris*). Dilutions are a -0 follows a number, it signifies that there was no reaction at this dilution.

<i>Histamine</i>	<i>48-80</i>	<i>Antigen</i>		<i>Passive transfer antigen</i>		
		<i>Type</i>	<i>End point</i>	<i>Reagin</i>	<i>Concentration</i>	<i>End point</i>
2,700T	100T	As	390T			
300T	100T	As	78T			
				MAb	As 25	125
				MAb	As 25	1
900T		As	390T			
100T		As	78T			
900T		As	1,953T			
		HS	625			
100T-0		As	15T			
		HS	1			
100T						
900T	100T					
100T-0	33T-0					
2,700T	100T	As	390T	HAb	Rag 300	125
100T-0	33T-0	As	78T	HAb	Rag 300	1
2,700T	100T			HAb	Rag 300	125
2,700T	100T			HAb	Rag 300	25
900T	100T	As	78T			
300T	33T	As	78T			
900T	100T	As	390T			
900T	100T	As	78T			
2,700T	300T	As	78T			
2,700T	100T	As	78T			
900T	33T	As	78T	HAb	Rag 300	625
300T	33T	As	3T	HAb	Rag 300	25
2,700T	100T	As	78T	HAb	Rag 300	625
2,700T	100T	As	78T	HAb	Rag 300	125
900T	100T	As	78T			
900T	100T	As	78T			
2,700T	100T	As	1,953T			
2,700T	100T	As	1,953T			
900T		As	1,953T			
300T		HS	5			
		As	78T			
900T	100T	As	78T			
900T	100T	As	78T			
2,700T	100T	As	390T			
2,700T	100T	As	390T			
900T	100T	As	390T			
900T	100T	As	78T			
900T		HS	125			
2,700T	100T	As	78T	HAb	Rag 300	125
2,700T	100T	As	78T	HAb	Rag 300	125

recorded in terms of the weakest solution giving a visible reaction. It should be noted that a included here because part of the data was used in the preceding paper,⁸ and these gave results EB = Evans blue; As = Ascaris; HS = normal human serum; Rag = ragweed; HAb = human expressed as reciprocal and T signifies 1,000's. Thus 300 = 1:300, and 900T = 1:900,000. When

**Fig. 2**

Representative results showing titrations with challenging substances before (left side of monkey) and after (right side of monkey) the administration of the following drugs: *A*, Acepromazine, Animal 11. From outside toward midline are ragweed injections (1:300) of sites passively sensitized with serial dilutions of human reagin, injections of 48-80 and histamine. *B*, Phencyclidine hydrochloride, Animal 7. From outside toward midline, histamine, 48-80, and *Ascaris*. *C*, Reserpine, Animal 4. From outside toward midline, histamine, 48-80, and *Ascaris*. *D*, Pentobarbital, Animal 6. From outside to midline, histamine, 48-80, and *Ascaris*.

cause its onset was slow and effect prolonged so as to make it unsafe to place the recovering monkey in the same cage with another untranquilized monkey. Reserpine also seemed inconsistent in its tranquilization. Pentobarbital had to be given intravenously; it caused no inhibition, did not provide a reproducible effect on the central nervous system, and its margin of safety was small. Fig. 2 shows photographs typical of representative experiments of the effect of different drugs on skin titrations.

While doing passive transfer tests with ragweed and ragweed-sensitive human serum, it was observed that, at times, there were reactions occurring at the noninjected passive transfer sites having the larger amounts of reagin (Fig. 3). When the challenging concentration of ragweed was reduced to 1:1,000, these remote reactions were not obtained.

DISCUSSION

It is advantageous, but not always necessary, to tranquilize monkeys when giving intravenous or intracutaneous injections, especially when a number of such injections are given in one experiment. There is particular concern about the danger of fatal B virus infections from animal bites. A tranquilizer should be safe, consistent, and lasting in its action and should not influence the reactions. Layton's⁴ failure to note reduced reactivity of the monkey skin can be explained in large part by the fact that in his experiments he usually used optimal amounts of antibody and antigen. As will be noted from our figures, at

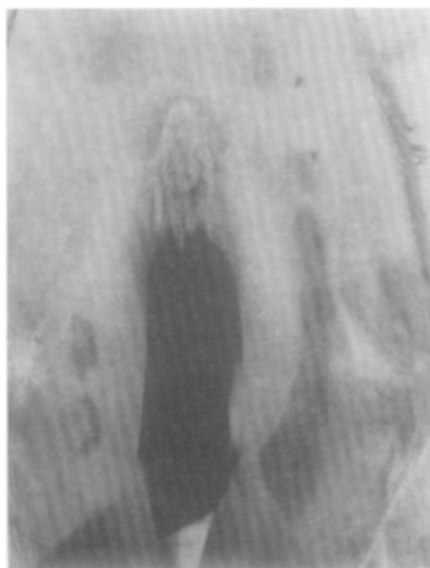


Fig. 3

Left side of monkey, reactions from intradermal injections of ragweed 1:300 in sites passively sensitized with serial dilutions of human ragweed-reaginic serum. Right side of monkey, flare-up of two sites sensitized but not injected.

the point where conditions for reactions are optimal (strongest antigen and undiluted reaginic serum), the reactions after acepromazine are virtually identical with those before the tranquilizer. However, when quantitative studies were made by serial dilution of antibody or serial dilution of antigen or other challenging substance, there remains no question about the depressing effect of this drug on immediate skin reactivity. The variation in sensitivity, from time to time, to *Ascaris* in some animals (without tranquilizer) may be due in part to desensitization from repeated injections.

Although it caused no diminution of skin reactivity, in our hands pentobarbital was not very satisfactory, as the intravenous dose was too critical. The effectiveness of intramuscular injection was inconsistent. Reserpine was also unsatisfactory because of late onset and long duration of tranquilization. It appeared to have a moderate but inconsistent effect in diminishing the skin reactivity. Phencyclidine (Sernylan) proved to be the most satisfactory of the compounds investigated. The animals were awake, would respond to stimuli to a diminished degree, and were quite docile. The skin reactivity was unchanged. This drug has been said to lack antihistaminic action,² but its effects on skin tests appear not to have been reported previously. For conclusive opinions as to the relative nervous system effects of these drugs, more experiments with many animals and dose-response data would be required.

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