activity, and failed to sensitize human erythrocytes to hemagglutination. Furthermore, HL antibodies were easily removed by adsorption with leptospiral cells containing HL antigen, which was contrary to the findings of Rothstein and Hiatt with their precipitinogen. It is possible that variance in adsorptive technics between different laboratories may account for such differences. In this work leptospirae used in adsorptions were freshly harvested, non-formalinized cells. The most striking divergence is the difference in precipitating abilities. The relationship of HL antigen to the antigens of Rothstein and Hiatt remains unclear, although the HL antigen shows more resemblance to S than to P antigen. It is probable that the HL antigen and ESS, as suggested by Rothstein and Hiatt(6), contain their S antigen, and that differences in characteristics reflect differences in methods of preparation. Further clarification of relationships between leptospiral antigens and their chemical natures must await investigations using purified preparations, and purification will be difficult until leptospirae can be cultivated in chemically-defined, non-protein media.

Summary. Use of HL antigen adsorbed onto erythrocytes, and *L. biflexa* repeatedly extracted with 50% ethanol, permits preparation of antisera containing only type-specific (agglutinating) or genus-specific (HL) antibodies. HL antigen, as extracted from *L. biflexa* with 50% ethanol, contained small quantities of type-specific antigen, which was capable of adsorbing onto rabbit and sheep erythrocytes along with the HL reactive antigen. However, the type-specific antigen was apparently not operative in the hemolytic reaction. HL antibodies would not precipitate HL reactive antigen, agglutinate leptospiral cells, nor fix complement in the presence of HL reactive antigen or *L. biflexa* cells. Preliminary studies indicate that HL antigen contains polysaccharide which is probably complexed with some low molecular weight nitrogenous material.

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Inhibition of Arthus Reaction During Protracted Systemic Anaphylaxis in Mice and Guinea Pigs.* (24013)

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During studies on anaphylaxis in mice sensitized with antigens incorporated in paraffin oil adjuvants, it was found that protracted anaphylactic shock elicited by intralabial injections of antigen inhibited Arthus reactions at sites of injection. Furthermore, when mice sensitized to both hemocyanin and human serum albumin were injected into the lip with

* These studies, begun at Public Health Research Inst., supported in part by grant from Am. Cancer Soc., upon recommendation of Com. on Growth of Nat. Research Council.

hemocyanin and intravenously with human serum albumin, Arthus reactions were scant or failed to occur. Similarly sensitized mice given only intralabial injections developed intense local reactions(1). Our observations were in accord with work of Bier *et al.*(2) who found that systemic antigen-antibody reactions in the rat alter its reactivity in passive cutaneous anaphylaxis (PCA) tests involving other antigen-antibody systems. Inhibition of PCA reactions after (intraperitoneal) injection of a nonrelated antigen and (intravenous) injection of its corresponding antibody was compatible with the hypothesis that complement (C′) or components of complement have a contributory role in the local reaction. Rats were chosen for these experiments because of low normal C′ levels permitting decomplementation by means which might prove lethal in the guinea pigs, and because of the purported refractoriness of rats to systemic anaphylactic shock. Further studies by Osler *et al.*(3) indicated that the amount of third component (C′3) of C′ present in the circulation is critical insofar as PCA reactions are concerned, but participation of some or all other components in the local reaction was not ruled out and was considered a probability. We have found that a severe protracted anaphylactic shock can be consistently produced in sensitized guinea pigs after subcutaneous eliciting injections of antigen(4). This observation made it possible to study systemic antigen-antibody reactions on local hypersensitivity in this species (which has relatively large amounts of circulating C′3).

In experiments to be described, mice and guinea pigs were sensitized with 2 unrelated antigens, and the effect of systemic anaphylactic shock upon Arthus reactions was observed. As in the mouse, anaphylactic shock in the guinea pig was accompanied by a decrease of Arthus sensitivity or reactivity.†

Methods and materials. Adult male Hartley guinea pigs and adult male albino mice were used. Guinea pigs were sensitized by single subcutaneous or intracutaneous injections of 2.25-2.5 mg of egg albumin (Ea) or by both this amount of Ea and 0.025-0.05 ml of washed packed sheep erythrocytes (SRBC) incorporated together in paraffin oil adjuvant without mycobacteria. One group of mice was injected with both 1 mg of human serum albumin (HuSA) and 0.08 mg N of Busycon hemocyanin (BH) emulsified together in complete adjuvants containing 0.2 mg of *M. tuberculosis*. Other mice were sensitized with both chicken egg albumin (Ea, 0.5 mg) and the BH incorporated into one dose with complete adjuvants. For details of preparation of antigens in paraffin oil adjuvants see(6). For testing local sensitivity in mice, a 30 gauge needle was used for injections into the lip(7) or footpad(8). The volume of fluid injected was 0.05 ml in the lip and 0.03 ml in the footpad. Intralabial injections were made into the dry mucous membrane of the free edge of the upper lip, the needle entering parallel with the skin. Reading of labial reactions were made as follows: 1+ = swelling noted by comparison with uninjected lip; 2+ = swelling obvious without comparison; 3+ = swelling of lip, cheek, and upper "snout"; 4+ = resembles profile of moosehead. (For details on intraperitoneal immunization and intralabial testing of mice, see 7). Rectal temperatures of mice and guinea pigs were recorded before and after eliciting injections

TABLE I. Arthus Reaction and Systemic Anaphylaxis after Intralabial Injection of Sensitized Mice.

Antigen*	Days after last inj.	Anaphylactic death†	Labial Arthus‡
Horse serum	7– 8	1/30	15/29
Human serum albumin	9–13	10/20	3/11
Hemocyanin	7–16	1/10	8/ 9
Horse serum globulin	13	0/ 6	6/ 6
Human serum globulin	18	0/15	15/15
Egg albumin	37	1/ 4	3/ 3

* Sensitization consisted of 2 intraper. injections of antigen 3 wk apart. Mice were given eliciting injections of homologous antigen (0.05 ml of HS, 3-5 mg of HuSA, 0.8 mg N of BH, 8-9 mg of HGG, 8.25 mg of HuGG and 0.5 mg of Ea).

† Numerator is No. of mice dead of anaphylactic shock within 24 hr; denominator is No. of mice challenged.

‡ Reading of 2+ or greater at 4 hr after eliciting injection.

† Described in part in abstract: *Excerpta Medica*, July 1958(5).

TABLE II. Inhibition of Arthus Reactions in Mice Sensitized to 2 Unrelated Antigens.

Sensitizing antigens* Mice sensitized to Busycon hemocyanin and	Route of challenge† Lip	I.V.	Anaphylactic death‡ 1 hr	4 hr	24 hr	Anaphylactic symptoms§ 1 hr	Arthus‖ 4 hr
Human serum albumin	BH	HuSA	1/ 9	2/ 9	2/ 9	5/ 8	1/ 7
Idem	"	None	0/ 5	0/ 5	0/ 5	0/ 5	5/ 5
Egg albumin	"	Ea (.1 mg)	2/ 5	2/ 5	2/ 5	1/ 3	0/ 3
Idem	"	Ea (.05 ")	3/10	3/10	3/10	6/ 7	0/ 7
"		None	0/10	0/10	0/10	2/10	7/10

* Both antigens were incorporated in complete adjuvants containing 0.2 mg *M. tuberculosis* and inj. by intraper. route. Mice were challenged 16 days after a second inj. (HuSA-BH) or 21-22 days after a single inj. (Ea-BH).

† Intrav. inj. immediately preceded intralabial inj. of .08 mg N of BH.

‡ Numerator is No. of deaths until hour listed; denominator is total No. of mice challenged. This is accumulative; thus, the figure in 24 hr column is total of mice which died in 24 hr period.

§ Dyspnea and/or bristling of fur and/or collapse.

‖ Reaction of 2+ or greater.

TABLE III. Anaphylactic Syndrome and Arthus Reaction in 16 Mice Sensitized to Busycon Hemocyanin and Egg Albumin and Injected Intralabially with Hemocyanin.

Temp. before inj., °C	Amt of Ea inj. I.V.,* mg	Reactions, 1 hr Anaph.†	Temp., °C	Arthus‡	Reactions, 4 hr Anaph.†	Temp., °C	Arthus‡
38.8	.3	BF, D	32.9	0	C	34.4	0
37.3	.1	N	37.5	0	N	38.1	0
37.9	.1	BF, D	31.9	0	BF, D	34.6	0
37.5	.1	N	36.5	0	N	37.5	0
38.3	.05	Hy	35.2	±	N	37.1	0
38.3	.05	BF, D	32.5	±	BF, D	33.9	0
38.4	.05	D	34.8	1	N	38.0	1
38.5	.05	N	37.5	2	N	37.7	1
38.6	.05	BF, D, C	32.9	0	N	37.5	0
38.0	.05	BF, WG	36.3	±	N	37.8	1
39.0	.05	WG	35.5	1	N	37.9	0
38.3	.0	N	38.8	1	N	38.4	3
38.5	.0	N	38.7	2	N	39.5	3
38.9	.0	N	39.1	3	N	39.1	2
37.9	.0	N	37.8	1	N	38.6	1
38.0	.0	N	38.2	3	N	37.7	2

* Eliciting injections 21-22 days after sensitization. Hemocyanin inj.: .08 mg N.

† BF, bristling of fur; D, dyspnea; Hy, hyperexcited; WG, wobbly gait; C, collapse; N, apparently normal.

‡ Reactions ± to 4+ described in methods.

of antigens. TRI-R‡ electronic thermometers were used with appropriate probes for the 2 species of animals.

Results. Experiments in mice: It was observed in preceding studies that mice sensitized with various antigens in paraffin oil adjuvants frequently died of anaphylactic shock after an intralabial injection for testing Arthus sensitivity(7). Mice suffering ultimately lethal or severe anaphylactic shock had scant or no Arthus reactions in the lip. Table I shows some of the results of intralabial injections of homologous antigen into sensitized mice. When the antigen caused a high percentage of severe systemic reactions (HuSA) there were few Arthus reactions of more than 2+ intensity in surviving mice; when the antigen did not induce severe systemic reactions (BH), intense Arthus reactions were demonstrable in a large percentage of mice. Furthermore, mice in readily apparent shock did not react at labial sites of injection.

‡ Model TML, TRI-R Instrument Co., Long Island City. Probe for rectal temperatures in mice TP-5; for rectal temperatures in guinea pigs, TP-1B.

TABLE IV. Anaphylaxis and Arthus Reaction in 17 Mice Sensitized to Busycon Hemocyanin and Egg Albumin and Injected into Foot Pad with Hemocyanin.

I.V. inj. of Ea*	Reactions, 1 hr			Reactions, 4 hr		
	Anaph.†	Temp., °C	Arthus‡	Anaph.†	Temp., °C	Arthus‡
.05 mg	BF, WG	32.5	0	N	37.8	0
	N	37.3	±	N	38.1	2
	BF, WG	33.9	0	N	38.4	2
	N	35.6	1	N	38.2	1
	D	35.9	0	N	38.6	0
	N	37.7	1	N	38.0	1
	N	38.4	1	N	38.6	1
.0 mg	N	39.2	2	N	38.0	3
	N	38.5	1	N	36.7	2
	N	39.0	3	N	37.7	4
	N	39.5	2	N	37.7	3
	N	39.1	1	N	38.0	3
	Mild BF & D	38.7	2	N	36.1	2
	Idem	36.4	1	N	37.0	3
	N	38.2	1	N	37.5	3
	N	38.7	2	N	37.9	3
	N	39.2	1	N	37.9	4

* Eliciting inj. 1 wk after mice were used for intralabial testing. Mice inj. at that time with BH and Ea were challenged with BH and Ea; mice inj. at that time with BH only were challenged only with BH. BH dose: .03 mg N.

† BF, bristling of fur; D, dyspnea; WG, wobbly gait; N, apparently normal.

‡ Reactions ± to 4+ corresponding to increasing amounts of edema (the reaction in feet is described in reference 8).

We investigated this inhibition in mice sensitized with 2 unrelated antigens incorporated in complete adjuvants. Table II shows that a systemic antigen-antibody reaction can also interfere with local reactions involving another antigen-antibody system. Intravenous injections of Ea or HuSA in mice sensitized to these antigens and hemocyanin have an inhibitory effect on the labial Arthus reaction to hemocyanin. The systemic antigen-antibody reaction under these conditions elicited a typical shock syndrome. The mice in shock had dyspnea, bristling of fur, and a fall in rectal temperature. Table III shows the relationship of shock symptoms to labial Arthus reactions in these mice. Degree of shock and degree of inhibition of local reactions were apparently related. One week after the intralabial tests, similar experiments were undertaken using the same mice, which were this time tested in the footpad with BH. Similar results were observed, *i.e.* local reactions were scant or did not occur in mice showing shock symptoms due to the systemic reaction to Ea (Table IV).

Experiments in guinea pigs. Table V shows results of subcutaneous injection of varying amounts of Ea into guinea pigs sensitized to Ea 25-32 days previously. The guinea pigs which received doses of antigen capable of eliciting severe protracted anaphylactic shock did not have Arthus reactions at sites of subcutaneous injection. Five of 7 guinea pigs injected with 1 to 5 mg of Ea had distinct local reactions. No guinea pigs injected with this amount of Ea subcutaneously showed any signs of anaphylactic shock.

Table VI shows that the results in guinea pigs sensitized to 2 antigens are like those in the mouse. At 4 hours there was complete inhibition of the Arthus reaction to Ea in guinea pigs showing shock symptoms. Twenty-four hours after the challenging injections of SRBC, there were reactions in the skin of guinea pigs which had suffered the anaphylactic shock. However, these differed grossly from reactions in guinea pigs not injected with sheep erythrocytes. The late reactions were characterized by a uniform purple color; there was no noticeable edema. The 24-hour responses in control animals were typical Arthus reactions; *i.e.* there was redness and swelling, with signs of necrosis in the center of the lesions.

Discussion. The mechanism of inhibition of local anaphylactic reactions by a systemic antigen-antibody reaction cannot be elucidated on the basis of evidence thus far available. Our experimental results are in harmony with at least 2 explanations which are not mutually exclusive:

(1) Local anaphylactic reactions depend on at least 3 factors, antigen, antibody, and complement or a component or components of complement. Complement removed by a systemic antigen-antibody reaction may not be available for a contributory role in the local reaction. This is the hypothesis of Bier and Osler(2,3) with regard to inhibition of passive cutaneous anaphylaxis in the rat. They emphasized, however, that their recent studies

TABLE V. Arthus Reactions and Systemic Anaphylaxis after Subcutaneous Injection of Sensitized Guinea Pigs.*

Amt of antigen in chall. dose	Anaphylactic reactions‡ 3-7 hr	24 hr	Arthus reactions§ 3-7 hr	24 hr
100 mg	† (3½)		0	
	† (4)		i.d.h.	
	Sev.	†	0	i.d.h.
	† (3)		±	
	Sev.	Sev.	0	0
50	Sev.	0	0	0
	"	Mild	0	0
	"	†	0	† NR
	† (1½)			
	Sev.	†	0	† NR
	"	†	0	0
20	Mild	0	0	0
	"	0	P10×10	RS40×45×2
	Mod.	Mod.	0	0
5	0	0	0	0
	0	0	PS40×40×SS	RS50×50×SS
	0	0	NeS15×15×SS	PS40×50×SS
	0	0	R10×10	PS40×50×SS
	0	0	0	0
1	0	0	P10×10	P10×10
	0	0	P10×10	0

* Guinea pigs were sensitized by single subcut. inj. of 2.5 mg of egg albumin in incomplete adjuvant.

‡ Sev. = severe; Mod. = moderate; 0 = no anaphylactic symptoms; † = death. Figure in parentheses denotes hour of death.

§ i.d.h. = ill defined hemorrhagic reaction; ± = doubtful reaction; P = pinkness; R = redness; Ne = necrosis; S = swelling; SS = slight swelling; NR = not read; 0 = no reaction. Dimensions of lesions are in mm; thus 40×45×2 = reaction 40 mm by 45 mm, raised 2 mm.

did not provide unequivocal proof as to the absolute requirement for C′ in initiation of PCA(2). Serum C′ may merely serve to potentiate the PCA reaction by "intensifying" the interaction of antigen with antibody.

(2) Systemic antigen-antibody reactions cause a series of physiological changes which include alteration of capillary permeability and fall in blood pressure. There may be interference with antigen-antibody union at the local site and/or inhibition of results of that union, *i.e.* interference with participation of cellular or humoral factors from the circulation.

Osler *et al.*, pointing out that analogies made between *in vitro* and *in vivo* systems are subject to criticism, look at PCA as the final step in a series of events corresponding to an *in vivo* C′ fixation reaction in the presence of a large excess of C′ supplied by the circulating blood(3). However, anaphylactic type reactions differ in several respects. It should be noted that Mongar and Schild find that C′ is not a factor in anaphylactic contraction of smooth muscle or in release of histamine(9). The Arthus reaction is more complex and more lasting than PCA, involving cellular elements from blood. Complement may be less important in the Arthus reaction than in PCA. There are several points which argue against the concept of C′ having a major role in eliciting Arthus reactions. Mice sensitized with various antigens in complete adjuvants show strong Arthus reactions after intralabial injection of homologous antigen, despite the low level of circulating complement in mice (1 to 2 $C'H_{50}$ units/ml, personal communication, J. A. Bauer) limited by very low titers of C′2 and C′3(10). Guinea pigs have large amounts of circulating C′ and are difficult to decomplement by injection of antigen-antibody combinations or zymosan (personal communication,

TABLE VI. Inhibition of Arthus Reactions to Egg Albumin in Guinea Pigs Sensitized to Both Sheep Erythrocytes and Egg Albumin. Subcutaneous injection of sheep erythrocytes and intracutaneous injection of egg albumin.

Guinea pig	Subcut. inj. SRBC	Anaphylactic reactions*			Arthus reactions‡		
		2 hr	4 hr	24 hr	2 hr	4 hr	24 hr
§	0.25 ml of 10% susp.	†(2½)			P 10×10		
§		Not read	BF,D↓	†(25)	Not read	0	Pl 15×18
§		BF,D	"	R	0	0	Pl 20×15
§		"	"	R	0	0	Pl 10×10
‖		"	BF,D	R	0	0	Pl 15×18
‖		"	D	R	0	0	0
‖		"	†(4)		0		
§	None	0	0	0	RS15×15×2	PS18×18×SS	RS30×30×SS
§		0	0	0	RS20×20×2	PS25×20×SS	P25×25
§		0	0	0	R15×18×3	PS15×15×SS	PS40×40×SS
§		0	0	0	PS10×10×3	PS25×20×3	RS35×35×SS
‖		0	0	0	PS12×12×SS	RS20×20×2	RS 8× 8×SS
‖		0	0	0	PS25×25×5	PS30×30×3	RS40×40×SS
‖		0	0	0	PS25×25×5	PS20×25×2	P30×30

* Anaphylactic reactions—BF = bristly fur; D = dyspnea, † = dead (figure in parentheses denotes hour of death); ↓ = hypothermia (temperature 37° or below); R = apparent recovery; 0 = apparently unaffected.

‡ Arthus reactions—Guinea pigs were inj. intradermally with 0.1 ml of 1% crystalline egg albumin. Dimensions are in mm; thus 25×25×2 denotes a reaction 25 mm long by 25 mm wide, raised 2 mm. Pl = purple; R = redness; P = pinkness; S = swelling; SS = slight swelling; 0 = no reaction.

§ Sensitized by 1 inj. of egg albumin and sheep erythrocytes in incomplete adjuvant given intradermally.

‖ Sensitized by 1 inj. of egg albumin and sheep erythrocytes in incomplete adjuvant given subcut.

J. A. Bauer). However, inhibition of Arthus reactions in our guinea pigs during anaphylactic shock would require complete or almost complete decomplementation if C′ were a critical factor. Experiments concerned with the mechanism of inhibition of the Arthus reaction are in progress.

Summary. Arthus reactivity was diminished or abolished in mice and guinea pigs showing protracted anaphylactic shock. Mice were sensitized to 2 unrelated antigens, then injected with one of these antigens into the lip or footpad and with the other antigen intravenously. The reaction in the lip or footpad was scant or did not occur. Guinea pigs in protracted anaphylactic shock after subcutaneous injection of antigen did not react locally 4 hours after skin tests with an unrelated antigen to which they were also sensitive. Reactions were observable 24 hours after intracutaneous injections; these differed grossly from those found in control guinea pigs not injected with subcutaneous shocking doses of antigen.

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