THE USE OF SPECIFIC "LYMPHOCYTE" ANTISERA TO INHIBIT HYPERSENSITIVE REACTIONS OF THE "DELAYED" TYPE*

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The tuberculin and contact reactions may be regarded as prototypes for the class of "delayed" hypersensitive reactions, in which sensitivity appears not to be mediated by circulating antibody but rather by some type of sensitized cell (1, 2). There is extensive evidence, largely indirect, to suggest that the responsible cell may be the small lymphocyte of the blood (3–6). Humphrey and Inderbitzin (7, 8, 24) introduced the use of specific heteroantisera to destroy circulating lymphocytes as a technique for studying the participation of these cells in the tuberculin reaction; and more recently Wilhelm *et al.* (9) have applied the same method to the study of contact sensitivity. Both groups of workers have shown that these reactions are depressed by the injection of lymphocyte antiserum and have drawn the inference that lymphocytes are the circulating element mediating sensitivity.

Heteroantisera have been used since the first part of the century as more or less specific tools for destroying circulating cells of a given type. Anemia, agranulocytosis, and thrombocytopenia, produced in experimental animals by this means, are well known entities and have been extensively studied (for review, see reference 4). It is obviously appropriate that immunologically induced cell destruction be applied as a type of ablation experiment to the problem of elucidating the role of circulating cells of a given type in the pathogenesis of defined experimental lesions due to hypersensitivity. This possibility has thus far been most successfully realized in studies directed at determining the role of polymorphonuclear leukocytes and platelets in the Arthus reaction (10). The demonstration by this means that these cells play an essential role in the pathogenesis of the Arthus lesion is in accord with the findings in experiments in which less specific techniques such as x-irradiation or injection of nitrogen mustard were used to destroy cells. That lymphocyte antisera might be used for similar experi-

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ments was suggested by the finding that such sera produce transient lymphopenia on injection into the species from which the cells used for immunization were originally obtained (11–14). Lymphocyte antisera have been found to possess a direct toxic effect on lymphocytes *in vitro* (13) or to fix complement with these cells (12, 14).

It has become increasingly clear that several other important types of immunologic response are to be included in the class of "delayed" reactions, among them experimentally induced "auto-allergic" lesions affecting the nervous system, eye, testis, thyroid, and adrenal (15), the rejection of vascularized homografts of solid tissues (16, 17), and certain reactions to purified proteins (18, 19). Since the ablation experiment made possible by the use of specific cell antisera provides a direct method of attacking the problem of mechanism in these experimental situations, it seemed desirable to confirm and extend the published observations on the tuberculin and contact reactions and to complement these findings by investigating the effect of lymphocyte antisera on allergic encephalomyelitis, one of the experimental auto-allergies, on the rejection of skin homografts, and on the "delayed" reaction to diphtheria toxoid. The present paper is a report of such studies in guinea pigs, treated with sera prepared in rabbits immunized with guinea pig lymph nodes. As controls we studied passive cutaneous anaphylaxis and the Arthus response, reactions mediated by humoral antibody, and non-specific inflammatory lesions produced by turpentine and by irritant concentrations of 2,4-dinitrochlorobenzene applied to the epidermis.

Methods

Animals.—New Zealand albino female rabbits weighing about 2.5 kg were used for preparation of antisera. They were kept in groups in a large bin and fed Purina rabbit chow and water *ad lib*. For grafting experiments we used randomly bred, male, English short-haired guinea pigs obtained from Rockland Farms. For all other experiments, male guinea pigs of the Hartley strain, obtained from Tumblebrook Farm, were used. These animals were kept in cages in groups of 3 to 5 and fed Purina rabbit chow, water *ad lib*., and fresh greens daily. All guinea pigs weighed 400 to 600 gm at the time of experiment. The numbers assigned to individual guinea pigs in the Tables are arbitrary and do not always refer to the same animal.

Antisera.—Pooled normal guinea pig lymph nodes, freed of gross blood, fat, and connective tissue and washed with sterile physiologic saline, were ground in saline to form a 20 to 30 per cent suspension and incorporated into an adjuvant mixture containing 8.5 volumes mineral oil (bayol F),[1] 1.5 volumes arlacel A,[2] 10 volumes of tissue suspension, and killed tubercle bacilli[3] at a final concentration of 3 mg/ml. This mixture was injected into rabbits, usually 0.5 ml divided among toe-pad sites of the 4 extremities and another 0.5 or 1.0 ml subcutaneously at a single site on the back. First bleedings were taken at 3 and 4 weeks.

[1] Bayol F, a light technical oil obtained from Esso Standard Oil Company, Linden, New Jersey.

[2] Arlacel A, an emulsifying agent obtained from the Atlas Powder Co., Wilmington, Delaware.

[3] Heat-killed tubercle bacilli of the Jamaica 22 strain, obtained through the courtesy of Dr. Jules Freund.

Booster injections (0.5 or 1.0 ml of the same mixture subcutaneously) were given approximately at monthly intervals and the rabbits bled weekly between these injections. These bleedings represented individual samples of "antilymphocyte" antiserum (ALAS). For much of our work, pools of ALAS were prepared large enough to permit the repeating of experiments under essentially comparable conditions. Every type of experiment reported in the present paper was shown to give closely similar findings with at least 2 distinct ALAS serum pools or with crude globulin prepared from these by ammonium sulfate precipitation.

Similar "antipolymorphonuclear" antisera (APAS), serum pools, or globulin pools were prepared from rabbits immunized with adjuvant mixtures containing washed peritoneal exudate cells from guinea pigs injected 4 to 24 hours earlier with sodium caseinate (20) (85 to 95 per cent polymorphonuclear leukocytes, 5 to 15 per cent cells of the monocyte-macrophage type). Control sera were either pools of normal rabbit serum or of serum obtained from rabbits injected with adjuvant alone.

Merthiolate 1:10,000 was added to serum and globulin pools. These were stored at −20°C and thawed just before use. Any sera showing signs of contamination were discarded.

Absorption of ALAS Serum Pools.—Guinea pig blood leukocytes were prepared by mixing freshly drawn venous blood with an equal volume of 3 per cent dextran[4] in Hanks' solution containing 5.0 mg per cent heparin. After 30 minutes' sedimentation at room temperature, the supernatant, containing most of the white cells, was removed and centrifuged. $1\text{–}2 \times 10^8$ of these cells were added to 1 ml of an ALAS serum pool in 2 successive portions followed by incubation for 1 hour at 37°C and overnight at 4°C respectively. Volumes were noted, so that injected doses of absorbed and unabsorbed serum in comparative experiments were exactly comparable.

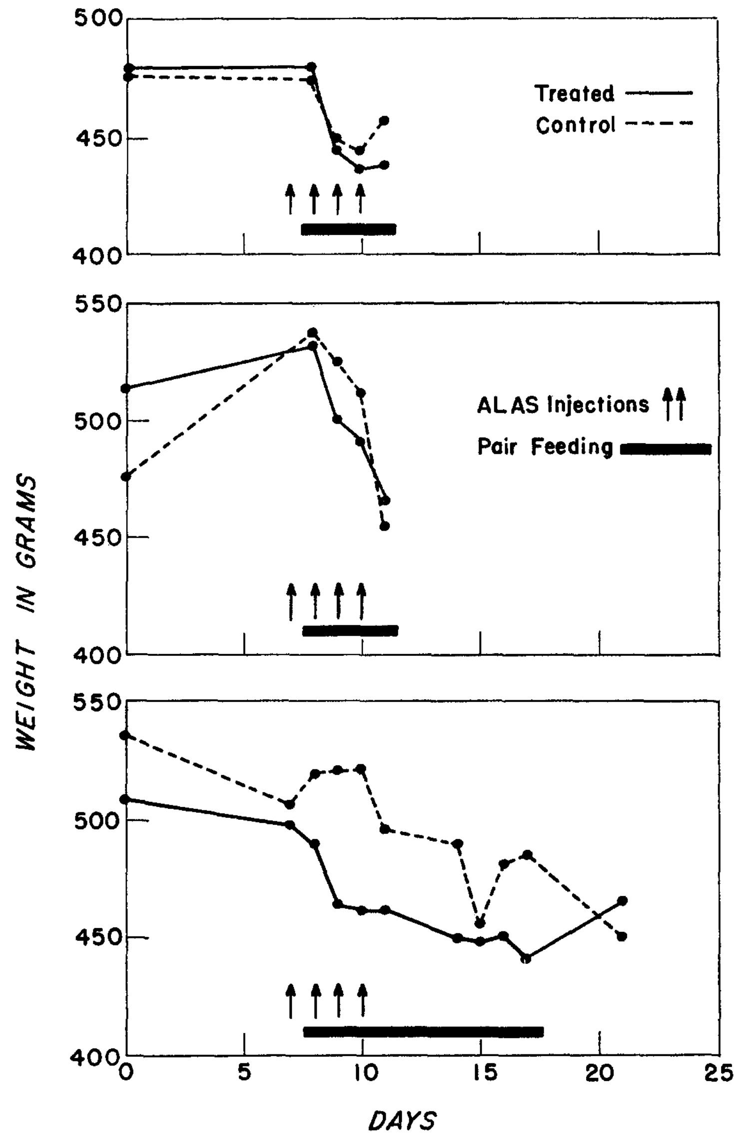
Injection of Sera.—In a few guinea pigs, ALAS or APAS were injected subcutaneously or intradermally. In experiments concerned with the effect of ALAS on various immunologic reactions, serum or globulin pools were always injected intraperitoneally. The usual effective doses were 3 to 4 ml ALAS and 0.5 to 1.0 ml APAS. In studying the various skin reactions, we usually found it necessary to give one dose of serum (or globulin) in the afternoon and a second the following morning at or shortly before the time of the test itself. The data presented in summary form in Tables II, VII, and X were obtained in animals injected in this manner. In experiments on allergic encephalitis and the rejection of skin homografts, serum was given repeatedly over several days. Treatment was continued for 4 days in all instances, since we found, in agreement with others (see reference 4), that the sera usually ceased to exert any demonstrable effect on longer administration.

Experiments were always performed on several (up to 10) guinea pigs at a time. Some received ALAS; others received control serum or remained untreated. In short term experiments, additional animals were treated with APAS. In long term experiments, more animals were started on ALAS injections than on the other sera, and those which died or presented severe diarrhea or weakness were discarded.

In short term experiments, some animals were tested for 2 different kinds of reaction at once. Such an experiment, in which the effect of ALAS or APAS on the tuberculin reaction and on PCA was studied in the same guinea pigs, is shown in Table III and Figs. 3 and 4. The findings in such animals did not differ from those in guinea pigs subjected to only a single type of test. Comparative observations in animals tested twice (*e.g.* with tuberculin), some days before and in conjunction with serum administration, agreed closely with findings obtained by comparing different guinea pigs treated with ALAS (or APAS) and control serum. The utility of repeated testing is illustrated in the experiment with absorbed and unabsorbed sera (Table IV).

Blood Counts.—Total erythrocyte, total and differential leukocyte, and total platelet counts

[4] Clinical dextran, batch No. 3M38–3M39, obtained from the Dextran Corporation, Yonkers, New York.



TEXT—FIG. 1. Effectiveness of pair feeding in two EAE experiments and one experiment on first set skin homografts. The average weights of three treated and three control animals are compared in each experiment.

were carried out as frequently as feasible on all the animals in each experiment. Sometimes red cell and platelet counts were omitted in alternate counts, or, in later experiments, omitted altogether. In all experiments, these counts were used as a guide in deciding the size of subsequent doses of serum. While eosinophils, basophils, and monocytes were counted, these counts

are omitted in the Tables, since they showed no significant or consistent change with the various types of treatment.

Quantitative Complement Determinations.—A series of guinea pigs were injected with ALAS or APAS and bled by cardiac puncture at frequent intervals over 24 to 48 hours. The individual sera were harvested within 2 hours and stored at $-20°C$. At a later date, all the sera from a given animal were titrated simultaneously for their content of complement by a modification of the method of Schwab *et al.* (21) with the use of veronal buffer containing optimal amounts of Ca^{++} and Mg^{++}.

Pair Feeding.—Guinea pigs treated repeatedly with some ALAS pools showed varying degrees of anorexia and weight loss. Therefore, in about half the long term experiments, all experimental animals were weighed daily and the controls were pair-fed as a group. The effectiveness of this procedure in maintaining the weight of the controls parallel with that of the test animals is shown in Text-fig. 1. There was no apparent difference in the findings in experiments in which pair feeding was carried out and other experiments in which it was omitted.

Sensitization and Testing.—Guinea pigs were made tuberculin-sensitive by an intradermal injection (0.1 ml over the sternum) of killed tubercle bacilli[3] in mineral oil (3 mg/ml). They were tested intracutaneously with old tuberculin (OT) in concentrations of 1:20, 1:100, and 1:500 given simultaneously 2 to 5 weeks after sensitization. The characteristic skin reactions were observed at 24 and 48 hours. They were red indurated reactions, frequently showing a central pale zone of necrosis with or without hemorrhage, maximal at about 24 hours.

Guinea pigs were sensitized (19) to diphtheria toxoid by an intradermal injection over the sternum (0.1 ml) of specific precipitate in complete adjuvant (8.5 volumes bayol F,[1] 1.5 volumes arlacel A,[2] 10.0 volumes of saline containing specific precipitate, and tubercle bacilli[3] at a final concentration of 3 mg/ml). The specific precipitate was prepared from highly purified diphtheria toxoid[5] precipitated in the zone of antibody excess with horse antitoxin[6] and washed 3 times. The 0.1 ml dose contained 3.0 μg of toxoid. All animals were tested at 6 to 9 days by intradermal injection of 3.0, 0.3, and 0.03 μg doses of the same toxoid sample in saline. At this time, they gave red, indurated skin reactions, maximal at approximately 24 hours and showing no hemorrhage or necrosis. No reaction of Arthus type preceded these "delayed" reactions. In parallel experiments with similarly prepared animals, no serum antibody could be demonstrated by active anaphylaxis or by PCA.

Guinea pigs were sensitized to 2,4-dinitrochlorobenzene by repeated applications of a 10 per cent solution of this compound in acetone to various areas of the skin of one flank. By 2 to 3 weeks, they reacted to tests with an 0.1 per cent solution of dinitrochlorobenzene with definite redness lasting 48 hours or longer, with or without palpable induration. All animals were tested with 1.0, 0.1, 0.01 per cent solutions of the allergen in acetone, a single drop of each concentration being placed simultaneously on a selected area of clean skin on the untreated flank; and the reactions were read at 24 hours.

Experimental allergic encephalomyelitis was produced by injecting guinea pigs with mixtures of bovine white matter or spinal cord (or a petroleum ether extract of bovine tissue) plus Freund adjuvant, as a single dose of 0.1 ml intradermally over the sternum (see reference 15). The animals were observed daily for neurological symptoms. They were sacrificed either on the day when symptoms first appeared or, in most experiments, at a fixed time following inoculation.

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[5] Diphtheria toxoid No. MS998, kindly provided by Dr. C. G. Pope of the Wellcome Research Laboratories, prepared from twice recrystallized diphtheria toxin.

[6] Antitoxin No. 437 (1200 units per ml), obtained through the courtesy of Miss Louise Wyman of the Massachusetts Department of Public Health, Division of Biologic Laboratories, Boston.

Full thickness grafts of flank skin, scraped free of adherent fat or muscle were placed on an exposed site on the lateral thoracic surface and held in place without sutures by vaseline gauze, a gauze compression pad, and a light plaster cast. All animals received an autograft as well as a first set homograft, and many an autograft and first and second set homografts at the same time. All first set grafts were well vascularized by the 2nd or 3rd day after grafting; and the epidermis had fully regenerated by the 4th to the 6th day. Second set grafts were placed 12 to 19 days (in most, 14 and 15 days) after previous grafting. A large proportion of these failed to become vascularized and remained white and ischemic.

Passive cutaneous anaphylaxis (PCA) was studied in normal guinea pigs sensitized with simultaneous intradermal injections in the flank of 0.04, 0.2, and 1.0 μg N of rabbit anti-ovalbumin in 0.1 ml. They were challenged at 18 hours by intravenous injection of 1 mg of crystalline ovalbumin and Evans blue; and the reactions (blue spots) measured at 15 to 20 minutes. Reversed passive Arthus reactions were induced by injecting normal guinea pigs with 2 to 3 mg of bovine serum albumin intravenously and immediately thereafter injecting intradermally doses of specific rabbit antibody (0.02 mg and 0.1 mg N). The reactions (edema, erythema, central hemorrhage) were read at 2 hours.

Non-specific inflammatory reactions were produced in normal guinea pigs by the intradermal injection of turpentine, diluted in olive oil to concentrations of 1:5, 1:20, and 1:100. The reactions looked like tuberculin reactions, showing a central zone of necrosis and induration and erythema at 24 hours and persisting to 48 hours or longer. Application of one drop of 10 per cent 2,4-dinitrochlorobenzene in acetone to the normal guinea pig skin produced, at 24 hours, a slightly indurated red area about 10 mm in diameter.

All reactions in all experiments were observed in the gross and studied histologically in paraffin or celloidin sections stained with hematoxylin and eosin. A few sample reactions of each class from ALAS-treated and control guinea pigs were examined after staining with methyl green–pyronine.

RESULTS

In vitro Properties of ALAS and APAS.—Several ALAS produced agglutination of normal guinea pig lymph node cells (1 per cent suspension) at a titer of 16–32; some of these sera gave a positive ring test with an extract of the same cells (obtained by repeated freezing and thawing in 4 volumes of physiologic saline). Conversely APAS gave agglutination titers of 16 to 64 against polymorphonuclear leukocytes (washed suspensions containing 80 to 90 per cent polymorphonuclears, obtained from the peritoneal cavity 24 hours after an injection of caseinate) and strongly positive ring tests with extracts of these cells. The reactions of ALAS and APAS with heterologous antigens were minimal. Complement fixation was not obtained in either system.

Local and General Effects of ALAS and APAS Injection.—Subcutaneous injection of 4 ml ALAS produced a brawny, pink edema of the body wall lasting more than 24 hours. Histologically (Fig. 1B) there was edema and extensive infiltration of polymorphonuclear leukocytes throughout the subcutaneous fat and connective tissue and between the deeper muscle layers. In some areas, many nuclear fragments were seen but the type of cell damaged could not be identified. Intradermal injection of 0.1 ml ALAS produced a smaller but essentially comparable lesion.

Intraperitoneal injection of ALAS in most guinea pigs produced transient anorexia. With larger doses (3 to 5 ml), some animals showed diarrhea lasting up to 6 hours, weakness, and occasionally death. The weight loss in guinea pigs given repeated ALAS injections is illustrated in Text-fig. 1. Certain samples of ALAS appeared essentially non-toxic, though still effective in lowering the lymphocyte count and inhibiting the various reactions tested. In animals autopsied 24 hours after one or two injections of ALAS, there were collections of lymphocytes and histiocytes about medium sized veins in the lung (Fig 1A) but the spleen and nodes appeared normal. In animals given repeated injections over several days, all lymph nodes examined appeared depleted including nodes draining the inoculation site in encephalomyelitis experiments or the graft site in animals receiving skin homografts. Depletion was judged by a decrease

TABLE I

Effect of Antilymphocyte Serum on Various Types of Blood Cells

Cell type	No. of guinea pigs with count:		
	Increased	Decreased	Unchanged
Erythrocytes	6	17	27
Platelets	10	6	15
Polymorphonuclears	29	4	12
Lymphocytes	3, 6	13, 14	1, 12

Results in 50 guinea pigs receiving single or multiple injections of 2 different ALAS serum pools and one ALAS globulin pool. For lymphocytes, the first figure represents animals given single or double doses of ALAS; the second, those given multiple doses over several days.

in node size compared with animals given control sera and a marked decrease in the number of follicles and of small lymphocytes (Fig. 2). There was also hypospermatogenesis. All other viscera remained normal. No histologic change could be identified in animals given one or more doses of APAS.

Effect of ALAS and APAS on Blood Cells.—The total leukocyte count in normal guinea pigs varied from 8,000 to 15,000, approximately 50 per cent being "lymphocytes". In actively sensitized animals the counts were somewhat higher. The effect of single or multiple injections of ALAS on the blood count in 50 representative guinea pigs is summarized in Table I. The lymphocytes decreased, usually to less than 3000/mm^3, in most animals given ALAS once or twice, even at low to moderate doses (1 to 2 ml). Of guinea pigs given larger doses repeatedly, half to two-thirds showed a clear-cut, prolonged lowering of the lymphocyte count. Counts below 1000/mm^3 were frequently attained in experiments involving passive sensitization (Arthus, PCA) or mild active sensitization (contact) but rarely in experiments with vigorous active sensitization (tuberculin, encephalomyelitis, grafting). Reactive leukocytosis frequently

accompanied ALAS injection but was not necessarily correlated with the fall in lymphocytes. It was more common in encephalomyelitis and grafting experiments. There was no significant effect of ALAS treatment on the levels of erythrocytes or platelets. The findings in individual guinea pigs given ALAS once or repeatedly are illustrated in subsequent tables. It should be noted here that antiserum, administered in the late afternoon, gave a low count the following morning; administered at noon or earlier, it gave a low count the same afternoon. ALAS treatment tended to lose its effectiveness over several days. In long term experiments, counts below $3000/mm^3$ were considered to represent effective lymphopenia, especially if maintained for 2 or more days. This level was selected on the basis that an appreciable decrease of the tuberculin reaction occurred in guinea pigs with less than 4000 $lymphocytes/mm^3$.

TABLE II

Effect of Specific Lymphocyte or Polymorphonuclear Antiserum on the Tuberculin Reaction

Treatment	No. of animals	Average reaction to OT*		
		1:20	1:100	1:500
Anti-lymphocyte serum	11	14 ery or ±	8 ery or ±	3 ery
Anti-polymorphonuclear serum	3	20 ++ (11)	15+ (4)	10 ±
Control serum or none	11	19 ++ to +++ (2–11)	14+ to ++ (0–4)	8 ± to +

* Figure represents average diameter of induration in mm at 24 hours. Thickness of induration is graded subjectively as 0 to +++, erythema as ery. Diameter of necrosis or hemorrhage given in parentheses.

APAS, even in doses of 0.5 ml, was highly effective in lowering the granulocyte count, usually to levels well below $1000/mm^3$. It did not affect other blood elements. The various control sera, in particular sera from rabbits given adjuvant alone, failed to affect the blood count of injected guinea pigs in any way.

Effect of ALAS and APAS on Complement.—In 11 normal guinea pigs given single doses of 1.5 to 4.0 ml ALAS and 6 given 1.0 to 2.0 ml APAS, quantitative complement titers decreased, at some time in the first 24 hours, to values ranging between one-half and two-thirds the preinjection titers. At no time did the values fall below half the control level, even in animals with highly significant falls in lymphocyte or polymorphonuclear count.

Effect of ALAS and APAS on Specific Immunologic Reactions of "Delayed" Type.—The *tuberculin reaction* was markedly reduced or abolished in guinea pigs treated with two doses of ALAS administered 1 day before and approximately 1 hour before the tuberculin test (Table II). A clear-cut reduction of induration was seen in 8 of 11 tested animals whereas all controls showed vigorous reactions, with induration close to the average value. Necrosis and

TABLE III

Protocol and Data of Single Comparative Experiment on Effect of ALAS and APAS on Tuberculin Reaction and on PCA

Day	Time	Guinea* pig	Manipulation	Result				
				RBC	WBC	Platelets	Poly	Lympho
							per cent	*per cent*
1	1500	1	Blood count	4.7	9,800	102,000	57	41
		2	" "	6.3	14,400	274,000	58	37
		3	" "	5.4	14,800	160,000	60	39
	1600	1	Inject ALAS, 3 ml					
		2	" APAS, 0.5 ml					
		3	" AS‡, 4 ml					
2	900	1	Blood count	3.8	13,300	220,000	88	9
		2	" "	3.7	12,600	195,000	36	62
		3	" "	4.9	16,400	74,000	58	40
	1200	1	Inject ALAS, 3 ml					
		2	" APAS, 1 ml					
		3	" AS,‡ 4 ml					
	1300	1	Skin test all with: OT					
		2	1:20, 100, 500					
		3	Sensitize all for PCA with: antibody 1.0, 0.2, 0.04 μg N					
3	900	1	Blood count	4.0	23,800	115,000	87	11
		2	" "	5.4	8,400	118,000	6	94
		3	" "	4.4	27,800	135,000	70	29
				OT 1:20		1:100	1:500	
	1300	1	Read tuberculin reactions§	8±		0	0	
		2		20++ (11)		13+ (4)	10 ±	
		3		19+++ (8)		12++ (2)	8 +	
				1.0 μg Ab N		0.2	0.04	
		1	Elicit‖ and read PCA	22 x 20		19 x 18	13 x 13	
		2		22 x 19		15 x 15	10 x 10	
		3		20 x 17		13 x 13	9 x 9	

* Guinea pigs sensitized with adjuvant 3 weeks earlier.

‡ AS, anti-adjuvant serum.

§ Induration, diameter (in mm) and thickness (subjective). Diameter of necrosis and hemorrhage in parentheses.

‖ Injection of 1.0 mg Ea plus Evans blue intravenously.

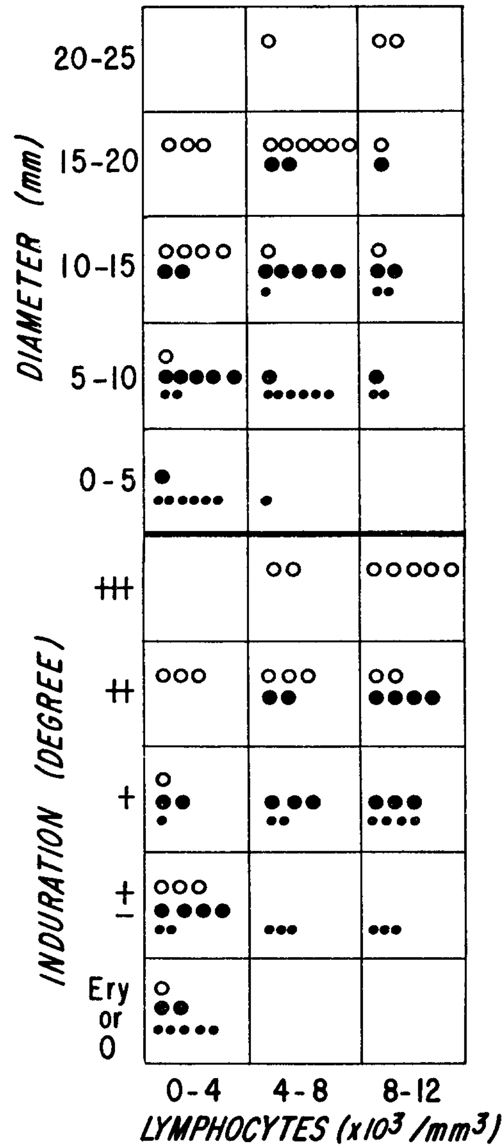
hemorrhage were absent in all but one of the test animals. No alteration was seen in a small number of guinea pigs treated with APAS. The use of serum from rabbits immunized with complete adjuvant did not affect the reactions. The protocol and the data obtained in a representative experiment of this type, in which tuberculin reactions and PCA were studied together, are shown in Table III: The gross appearance of the reactions at 24 hours is shown in Fig. 3, and their histologic character in Fig. 4.

Text-fig. 2 shows that the suppressive effect of ALAS on both the diameter and thickness of skin induration 24 hours after the test was clearly related to the circulating lymphocyte count during the evolution of the reaction. That this relationship was specific is suggested by the fact that absorption of ALAS with blood leukocytes reduced simultaneously its effectiveness in lowering the lymphocyte count and in suppressing the tuberculin reaction (Table IV). The suppressive affect of a single dose of lymphocyte antiserum on the tuberculin reaction was clearly related to the time at which it was administered (Table V). Even as late as 6 hours following the intradermal injection of tuberculin, a large dose of ALAS produced an appreciable reduction in the reaction.

Histologic study indicated that lymphocyte antiserum affected the tuberculin reaction by producing a great diminution in the actual amount of cellular inflammation, both in the deep dermis and in the region just beneath the epidermis (Fig. 4). In uninjected controls and in animals injected with several types of control serum, the principal reacting cells were lymphocytes and histiocytes, present both as perivascular accumulations throughout the deep dermis and the subcutaneous fat and muscle and diffusely in the dermal connective tissue. In animals given lymphocyte antiserum there was a striking relative reduction in these cells and an increase in the number of polymorphonuclear cells. In these instances, the few mononuclear cells in the deep dermis or subdermal fat were found to be clumped, frequently in or next to small vessels, partly pyknotic or fragmented, and always accompanied by a number of polymorphonuclears. There was little cellular invasion of the surrounding connective tissue. Plasma cells, which were present in considerable numbers in the controls, were entirely absent in these reactions.

The *"delayed" reaction to purified diphtheria toxoid* was reduced or completely suppressed in guinea pigs treated with ALAS. Table VI shows an illustrative experiment. The suppressive effect was clearly correlated with the degree of suppression of the circulating lymphocytes. Some reduction of the reaction was observed in 3 of 6 guinea pigs given APAS; but in these animals the lymphocyte level was below 4000 per mm^3. At 48 hours after testing, reactions appeared at the previously negative sites in several of the ALAS guinea pigs.

The *contact reaction* of sensitized guinea pigs tested with various concentrations of 2,4-dinitrochlorobenzene, was sharply reduced by treatment with ALAS (Table VII). In 6 of 7 treated animals there was no visible reaction



TEXT—FIG. 2. Relation of 24 hour tuberculin reactions, in guinea pigs treated with two doses of ALAS, APAS, or control serum, to level of circulating lymphocytes. Only animals are included in whom reliable and relatively constant blood lymphocyte counts were obtained at the time of testing and of reading the test. The diameter and the thickness of induration are shown separately. Old tuberculin: O 1:20, ● 1:100, • 1:500.

TABLE IV

Effect of Absorbing Antilymphocyte Serum with Blood Leukocytes on Its Ability to Suppress the Tuberculin Reaction

Guinea pig*	Serum	Number of doses‡	First test	Second test						Leukocyte counts at time of 24 hour reading‖		
			24 hour reactions§ to	24 hours reactions§ to			48 hour reactions§ to					
			1:100 OT	OT 1:20	1:100	1:500	OT 1:20	1:100	1:500	Total WBC	Poly	Lympho
											per cent	*per cent*
1	Absorbed	2	12+	18+	13e	5e	15++	11+	6±	14,700	58	34
2	Unabsorbed	2	14+	0	0	0	11+	6±	2e	17,900	71	22
3	Absorbed	2	11+	13++	8+	5e	10+	8±	3e	13,700	58	37
4	Unabsorbed	2	12+	0	0	0	10±	0	0	17,800	73	21
5	Absorbed	1	13+	15+-++	10±	6e	14+	11±	7e	20,300	80	18
6	Unabsorbed	1	13+	10±	6e	0	10±	5e	0	12,300	86	7
7	Absorbed	1	16+	14++	11±	8e	13++	10+	6e	17,000	71	24
8	Unabsorbed	1	15+	12±	8e	0	13+-++	8±	3e	28,500	82	15
9	Absorbed	1	11+	16+-++	10±	5e	11+	7±	7e	33,500	75	19
10	Unabsorbed	1	12+	16++	10+	4±	12+	7±	4e	24,000	70	22
11	None	—	11+	15++	11+	7±	—	—	—	—	—	—
12	None	—	14+	16++	12+	8±	—	—	—	—	—	—

* Sensitized guinea pigs tested with OT 1:100, retested 24 to 48 hours later with 3 concentrations of OT and treated with serum.

‡ Single dose: given simultaneously with second test. 2 doses: one given 18 hours before second test, second given simultaneously with it.

§ Reactions are given as diameter (in mm) and thickness (subjective) of induration.

‖ The average count before injection of serum in these animals was 19,700 leukocytes, with 56 per cent polys and 38 per cent lymphocytes.

to 0.1 per cent dinitrochlorobenzene, and in 4 of 7 no induration was palpable even in reactions to 1.0 per cent allergen.

Histologically, the reaction in controls was seen to consist of infiltration of lymphocytes and histiocytes in the upper dermis and invasion and destruction of epidermis by these cells. In reactions to 1.0 per cent allergen, there was superimposed non-specific necrosis of epidermis in a limited area. In the treated

TABLE V

Relation of Time of Administration of Single Dose of Lymphocyte Antiserum to Suppressive Effect on the Tuberculin Reaction

Lymphocyte antiserum 2 to 4 ml dose at:	Reactions at 24 hrs.*			Inflammation seen histologically
	OT 1:20	1:100	1:500	
−18 hrs.	17+++	13++	10+	+++
−3 "	16+-++	13±	9±	+++
	8 pale	0	0	++
0 "	25+(16)	16±	0	+
	0	0	0	±
6 "	17 pale (12)	5±	0	+-++
	23++(15)	17+(11)	11±	+++
24 "	17++(5)	13±	11 ery	++++
	20+++(6)	11+(4)	7±	++++
None (controls)	27+++(5)	20++	3+	++++
	23+++	17++	9+	++++
	21+++(11)	15++(4)	12+	+++
	12++	9+	7±	+++

* Readings are given for individual guinea pigs (two in most experimental groups and 4 controls). See Table II for meaning of symbols.

animals the non-specific epidermal damage was unaltered, but there was a striking reduction in the cellular infiltration of dermis and epidermis and in the specific destruction of the latter.

As in the case of the tuberculin reaction, suppression of the contact reaction by lymphocyte antiserum was quite apparent with doses of serum which did not completely remove lymphocytes from the circulation. The 6 guinea pigs which showed a clear-cut effect all had lymphocyte counts below 3000/mm^3 at the time of skin testing and the reading of the reaction.

Experimental allergic encephalomyelitis presents in guinea pigs (22) as weight loss, paralysis of extremities and sphincters, and ataxia. The onset, in control

animals of the present experiments, varied from the 11th to the 17th day following inoculation. Histologically, meningitis and disseminated inflammatory and demyelinative foci, consisting almost exclusivelv of mononuclear cells were found in the brain, brain stem, and spinal cord.

TABLE VI

Effect of Specific Cell Antisera on the Delayed Reaction to Diphtheria Toxoid

Guinea pig	Globulin pool*	Leukocytes per mm³ blood						Reactions‡ to toxoid		
		−24 hrs.		0 hrs.		24 hrs.				
		Poly	Lympho	Poly	Lympho	Poly	Lympho	3 μg	0.3 μg	0.03 μg
1	ALAS	6,250	10,250	11,750	5,150	4,500	3,000	0	0	0
2	"	10,950	7,650	10,000	2,250	6,200	1,950	0	0	0
3	"	4,950	8,750	18,850	3,100	9,150	3,000	7	4	0
4	"	9,100	8,550	6,650	2,400	12,850	3,300	0	0	0
5	"	9,950	12,950	26,800	2,950	10,450	2,500	11	3	0
6	"	11,400	5,800	30,650	5,850	21,550	5,450	14	10	6
7	"	2,450	17,450	12,400	6,700	3,950	14,450	15	10	5
8	APAS	4,500	5,100	0	3,300	200	2,700	15	10	5
9	"	4,300	7,000	0	4,450	0	2,800	12	6	2
10	"	3,100	7,100	0	3,250	100	3,000	16	12	4
17	"	7,450	8,050	2,550	11,700	300	4,600	24	17	11
18	"	5,900	7,200	2,450	11,175	250	6,100	21	15	7
19	"	4,650	7,500	3,025	8,600	100	4,250	20	13	7
11	Control	1,500	10,100	8,200	6,200	10,500	5,600	21	15	7
12	"	10,250	14,700	8,200	5,650	6,650	6,150	21	15	8
13	None							21	17	10
14	"							23	18	15
15	"							25	17	—
16	"							21	15	—

* Doses of ALAS (2 ml), APAS (1 ml), or control (normal rabbit globulin pool, 2 ml) injected 24, 18, 8 and 1 hour before skin testing and 7 and 15 hours after

‡ Average diameter of induration 24 hours after testing.

Ten experiments were carried out in which lymphocyte antiserum or control serum was injected daily over 4 days during the incubation period. In 5 of these experiments, animals receiving control serum or untreated were matched and pair-fed with animals receiving the test serum. One experiment is omitted in the following discussion, as the animals suffered an intercurrent infectious illness. Data from the remaining nine experiments are presented in Table VIII, animals also being omitted which died or were sacrificed before the 12th day. In 8 of the 9 experiments, lymphocyte antiserum resulted in a pronounced

delay in the onset of the disease or possibly, in some cases, in its complete prevention. In animals sacrificed within a few days following termination of the antiserum injections, there was a clear-cut reduction in severity of the disease observed. Histologically, there was no difference in the encephalomyelitis in treated animals and controls, other than the difference in intensity already noted.

Of the 22 treated animals shown in Table VIII, 14 showed reduction of the circulating lymphocyte count below $3000/mm^3$ for 1 or more days. Encephalomyelitis was not notably milder or slower in onset in these animals than in other animals which received lymphocyte antiserum.

In the present experiments, *rejection of first set skin homografts* was first recognizable in the gross as a darkening of the color and some thickening,

TABLE VII

Effect of Specific Cell Antisera on the Contact Allergic Reaction

Treatment	No. of animals	Reaction* to 2,4-dinitrochlorobenzene		
		1.0 per cent	0.1 per cent	0.01 per cent
Lymphocyte antiserum	7	10 ery or ±	2 ery	0
Polymorphonuclear antiserum	5	15+ to ++	8±	1 ery
Control serum or none	4	14+ to ++	7 ery or ±	0

* See Table II for meaning of symbols.

apparent occasionally as early as the 5th day. In 18 animals in which a definite time could be assigned to this early change, the average onset of rejection was the 8th day. In animals treated with lymphocyte antiserum, the average onset of visible rejection, in 23 cases in which a definite time could be assigned, was at 10 days; *i.e.*, somewhat delayed. Microscopically, the reaction began, in most specimens from untreated guinea pigs, as early as the 5th day with a massive infiltration of lymphocytes and histiocytes in the upper dermis, rapidly followed by invasion of the epidermis and follicles and their destruction. In treated animals (Table IX), there was a delay in the appearance of the cellular infiltrate in a considerable proportion of grafts. Even as late as 9 to 12 days, the infiltrative and destructive process was less advanced in such grafts than in controls (Fig. 5). There was little correlation between retardation of rejection and effective reduction of the blood lymphocyte count.

A large proportion of *second set skin homografts* were white and ischemic when examined in the gross 3 to 5 days after grafting. This was equally the case in controls and in animals which received lymphocyte antiserum. Histologically, there was cellular infiltration of the graft bed and ischemic necrosis of the entire graft. However, in many of the treated animals examined microscopically,

TABLE VIII

Effect of Treatment with Lymphocyte Antiserum on Day of Onset and Severity of Experimental Allergic Encephalomyelitis

Experiment	Period of treatment	Day terminated	Day of appearance of disease		Severity of encephalomyelitis*	
			Treated animals	Controls	Treated animals	Controls
	days					
I	6–9	Onset‡	20, —	14, 15, 15		
II§	7–10	22	16, 21, —	16, 17, 17	+(+++), ±(++), 0(+)	++(+++), ++(++), +(++)
III§	7–10	14	14, —, —	12, 12, 12	+(+), 0(+), 0(0)	+++(+++), ++(+++), ++(+)
IV§	10–13	Onset‡	16, 16	11, 12, 12		
V§	10–13	17	14, 14, 14	13, 15, 15	++(++), ++(0), +(+)	+++(++), ++(++), ±(0)
VI	7–10	14	14	11, 12	++(++)	+++(+++), +++(+++)
VII	9–11	14	—	14	0 (0)	+++(+++)
VIII§	7–10	14	14, —, —, —	13, 14, 14	0(+), ±(±), 0(0), 0(0)	+++(+++), +++(+++), +(+)
IX	7–10	15	—, —, —	12, 15	0(+), 0(0), 0(0)	++(++), +(+)

* Severity of clinical signs. Histologic estimate of severity in parentheses.

‡ Each animal autopsied at onset of neurological signs.

§ Pair feeding.

the second set graft showed partial regeneration of follicular and surface epidermis comparable to that seen in the accompanying autograft. From this finding it could be inferred that there was a less complete degree of ischemia in these grafts. A small proportion of second sets grafts became vascularized, and were

TABLE IX

Effect of Lymphocyte Antiserum on Rejection of Skin Homografts

Day on which graft was examined	Treatment*	No. of animals‡	Degree of rejection (histological)		
			0 or +	++	+++
	A. First set grafts				
2–4	Controls	7	6	1	0
	Ineffective	6	6	0	0
	Effective	3	3	0	0
5–7	Controls	14	6	8	0
	Ineffective	4	3	1	0
	Effective	10	9	1	0
9–12	Controls	7	1	0	6
	Ineffective	10	3	3	4
	Effective	8	2	3	3
	B. Second set grafts				
2–3	Controls	7	5	2	0
	Ineffective	7	7	0	0
	Effective	2	2	0	0
4–6	Controls	13	5	5	3
	Ineffective	6	3	1	2
	Effective	9	9	0	0

* Effective treatment means reduction in circulating lymphocytes below 3000/mm^3 for 1 or more days.

‡ Only animals included in which autografts took well and in which grafts not detached or hemorrhagic.

then infiltrated by mononuclear cells and rejected in the manner of a first set graft. This type of rejection was not seen in treated animals. The findings in both classes of rejection are presented in Table IX.

Effect of ALAS and APAS on Specific Immunologic Reactions Mediated by Antibody.—ALAS and APAS were completely without effect *on passive cutaneous anaphylactic reactions* (Table X, Fig. 3B). If anything, the reactions in animals given these sera were somewhat larger than in the controls, though the circulating lymphocytes were reduced markedly in 4 of the 6 animals given

anti-lymphocyte serum and polymorphonuclears were almost completely suppressed in all those given APAS. Histologically, the intra- and subcutaneous edema seen at the two higher doses of anti-ovalbumin was unaffected by either serum. However, the diffuse scattering of polymorphonuclear seen in controls at a dose of 1.0 μg of antibody N was abolished by APAS treatment.

The reversed passive Arthus reaction was only slightly decreased in guinea pigs given ALAS (Table X), in spite of reduction of the lymphocyte count below 1500/mm^3 in all treated animals. APAS in adequate dosage suppressed this reaction nearly completely, as shown earlier by Humphrey (10). The histologic findings of deep edema and massive polymorphonuclear exudation at 2 hours were similarly suppressed (Fig. 6). This effect was closely parallel

TABLE X

Effect of Lymphocyte and Polymorphonuclear Antisera upon Passive Cutaneous Anaphylaxis and the Reversed Passive Arthus Reaction

Treatment	Passive cutaneous anaphylaxis				Reversed passive Arthus reaction		
	No. of animals	Reaction (mm)* at sites sensitized with anti-Ea N			No. of animals	Reaction (mm)‡ at sites sensitized with ant-BSA N	
		1.0 μg	0.2 μg	0.04 μg		0.1 mg	0.02 mg
Lymphocyte antiserum	6	22	19	13	7	13 ++ (4)	6 ± to +
Polymorphonuclear antiserum	4	26	21	14	7	8 0 to + (2)	3 0 to ±
Control serum or none	4	21	17	10	5	16 +++ (5)	13 + (2)

* Diameter of blue spot.

‡ Edema graded as 0 to +++. Diameter of hemorrhage given in parentheses.

to the reduction in circulating granulocytes. Most of the test sites in APAS-treated animals increased in size very slowly and presented at 6 hours Arthus reactions comparable in the gross to those seen at 2 hours in the controls.

Effect of ALAS and APAS on Non-Specific, Inflammatory Reactions.—Intradermal *injection of turpentine* produced non-specific damage of the skin and secondary inflammation. Histologically, there was complete necrosis of a central area of dermis and of adjacent follicle surface epithelium. This area was filled with necrotic polymorphonuclear cells and with invading histiocytes; a central area sometimes remained completely acellular. The adjacent connective tissue and fat were heavily infiltrated with both histiocytes and polymorphonuclear cells, the reaction being very limited in extent. ALAS reduced the gross inflammatory response in 9 of 11 treated guinea pigs to a moderate degree. The necrosis remained unaltered. Histologically, the only change found was a reduction in the extent of the inflammation. APAS produced no change recogniz-

able in ths gross but did result in a decreased number of polymorphonuclear leukocytes in the necrotic zone.

Application of concentrated 2,4-dinitrochlorobenzene to the skin produced superficial erythema and induration. Histologically this was seen as necrosis of the epidermis and superficial dermis with a mild histiocytic response similar to that about the zone of turpentine necrosis. The dead epidermis was invaded by polymorphonuclears. As in the turpentine case, the induration was moderately or markedly reduced in 8 of 9 animals treated with ALAS and was grossly unaffected by APAS in 6 additional animals, though there was a reduction in the number of leukocytes in the dead epidermis.

DISCUSSION

Lymphocyte antiserum (ALAS) exerted a suppressive effect on each type of "delayed" hypersensitive reaction presumed to be mediated by lymphoid cells. The tuberculin and contact reactions were nearly completely abolished; in this our findings fully confirm the observations of Humphrey and Inderbitzin (7, 8) and Wilhelm *et al.* (9). The "delayed" reaction to purified protein (diphtheria toxoid) was also largely or completely suppressed. Allergic encephalomyelitis showed a decrease in both incidence and severity and a marked delay in onset. There was a similar delay in the rejection of first set skin homografts. However, the antiserum had no effect on PCA and the reversed passive Arthus reaction, both mediated by circulating antibody. Its effect on the rejection of second set skin homografts was relatively slight, a result in good accord with the possibility that this reaction may be mediated in part by humoral antibody (23). Non-specific reactions to dinitrochlorobenzene and turpentine, in which some secondary mononuclear infiltration is present, were moderately decreased by ALAS treatment. These effects, all relatively clear-cut, may be contrasted with the failure of APAS (antipolymorphonuclear antiserum) to affect any of the reactions studied except the Arthus and, to a limited extent, PCA.

At the same time, injection of ALAS produced direct destruction of circulating mononuclear cells (lymphocytes and/or monocytes), lymph node depletion (apparently secondary to the loss of circulating cells, since the nodes remained normal in short term experiments), a decrease of complement, and a variable degree of general toxicity. The lowering of the circulating level of "lymphocytes" by ALAS appeared to be highly specific, as numerous determinations failed to reveal any consistent change in the level of erythrocytes, platelets or polymorphonuclear leukocytes. We did not attempt to distinguish effects of the antiserum on the different categories of lymphocytes found in the circulation or on circulating monocytes. Our use of the term "lymphocyte" must therefore be understood to take in these other cell types in addition to the small lymphocyte.

The data suggest strongly that the observed effect of ALAS on delayed

reactions was determined by its specific effect on circulating "lymphocytes," since a decrease in the one was correlated with a decrease in the other in each case where it was looked for except allergic encephalomyelitis. In this our findings agree with those already reported by others (9). Humphrey's observation that antiserum specific for macrophages had no inhibitory effect on the tuberculin reaction (24) points more precisely to the lymphocyte as the specific cell concerned in immunologic reactions of this type, as does the fact that absorption of ALAS with blood leukocytes removed both its lymphopenia-inducing power and its ability to suppress the tuberculin reaction. However one cannot exclude the possibility that, in long term experiments, the observed effect was in part determined by lymph node depletion. The effect clearly was not brought about by a lowering of complement titer, since APAS injection affected the circulating complement as much as did ALAS. Nor was it due to a general toxicity of ALAS, since some ALAS preparations were relatively non-toxic (a point also noted by Humphrey, 24) and ALAS absorbed with blood leukocytes was as toxic as the unabsorbed globulin yet ineffective in reducing the tuberculin reaction. The theoretical possibility that some other non-specific quality of ALAS injection, *e.g.* a stressing effect mediated by the adrenal, determined its effectiveness would seem to be ruled out by the correlation of decreased reactions with lymphopenia and the result of the absorption experiment. Certain of our controls were done only for the case of tuberculin sensitivity; the extension of arguments based on these to the other reactions studied must remain inferential.

The suppression of delayed reactions by immunologically induced lymphopenia agrees well with results obtained with less specific methods of inducing lymphopenia, in particular the use of x-irradiation and cortisone. It is well established that x-irradiation reduces temporarily the ability of sensitive animals to develop the tuberculin reaction (25) or the contact allergic reaction (26, 27), the suppressive effect being well correlated with the degree of lymphopenia produced. Irradiation of lymph nodes draining the site of inoculation tends to suppress the development of allergic encephalomyelitis (28); and homograft rejection is also depressed by irradiation (29), these effects not having been specifically linked to lymphopenia. Similarly cortisone and ACTH depress the tuberculin response (30–32), allergic encephalomyelitis (33, 34), and homograft rejection (35, 36), but appear not to affect the reaction of contact allergy (37–39). The mechanism in these cases has been generally assumed to be complex, since cortisone and x-irradiation have other, unrelated physiologic effects which may play a role in their suppressive effect on delayed reactions (40–42); cortisone, for example, profoundly alters vascular permeability, a factor with an important influence on the development of local inflammation.

On the basis of the findings in the present investigation and in the earlier studies cited, it seems proper to conclude that circulating lymphocytes or closely

related mononuclear cells are essential to the genesis of "delayed" hypersensitive reactions. It remains to be determined whether these cells participate in delayed reactions as the primary element reacting with antigen or by responding secondarily to an unknown primary event. The finding that lymphocyte antiserum reduced to a moderate degree non-specific mononuclear inflammation secondary to dermal or epidermal necrosis produced by turpentine or concentrated dinitrochlorobenzene favors the second possibility. Nevertheless, several points, both in our data and in work already well established, support the view that in delayed reactions the lymphoid cell is itself the specific primary reactant. The degree of suppression by the antiserum was much greater in the case of specific immunologic reactions than in the non-specific reactions studied. Not only was cellular inflammation reduced or abolished but necrosis was also frequently prevented. This could be the case only if necrosis were secondary to the local accumulation of mononuclear cells rather than the reverse. The success of passive transfer experiments with living "sensitized" mononuclear cells in each of the types of response with which we are concerned (43–46) strongly suggests a primary role for these cells in the genesis of the hypersensitive lesions. The view that these cells are the immunologic element reacting with antigen at a test site receives support from the fact that transfer of "sensitized" cells directly to a site containing antigen results in the typical reaction. Thus, in tuberculin sensitivity, comparable reactions are obtained whether sensitive cells are injected intraperitoneally or intravenously and antigen intradermally (43, 47), cells intradermally and antigen systemically (47), or a mixture of cells and antigen intradermally (48, 49). Similarly, "reversed" lesions may be produced by injecting living lymphoid cells of animals sensitized by skin homografts into the skin of the donor animal (50) or by injecting cells of animals sensitized with myelin and adjuvant into the subarachnoid space of normal animals; *i.e.*, in direct contact with the antigen-containing nervous tissue (51). Detailed histopathologic study of several categories of delayed reaction (5) also suggests strongly that the first change in each case is the accumulation around small vessels of hematogenous mononuclear cells.

The most striking aspect of our experimental results is that suppression of the circulating "lymphocytes" resulted in suppression of *all* the gross and microscopic features of each type of "delayed" reaction studied, not merely of the cellular infiltration. These included cellular infiltration (in all reactions), vasodilatation as manifested by erythema (in all skin reactions), invasive-destruction by the infiltrating cells of myelin (in allergic encephalomyelitis) or epidermis (in the contact reaction and in first set homografts), gross necrosis (in the tuberculin reaction), and the appearance of plasma cells. It follows that all of these other features *must* be secondary to the local reaction of antigen with sensitized "lymphocytes." Indeed it is clear that the cellular reaction may occur without these secondary features, as in the "delayed" reaction to diph-

theria toxoid, which consists of perivascular infiltration of mononuclear cells and some vasodilatation (19) occurring in the complete absence of gross or microscopic necrosis or of invasive destruction of other cellular elements. The findings also emphasize the essential identity of the different "delayed" reactions studied, an identity implied as well by their histologic character and the results of passive transfer experiments.

While it was quite easy to remove essentially all the polymorphonuclears from the blood stream by treatment with APAS, the lymphocyte count rarely fell below 1000 per mm^3 in animals injected with ALAS. This is not surprising since the blood lymphocytes make up such a small part of the body's total supply of these cells (52) and since tremendous numbers of new lymphocytes are constantly being introduced into the circulation (53). Gowans (53) has shown that, even when the total output of thoracic duct cells is discarded, a supply of new lymphocytes is continually available. There can be no question that ALAS did destroy lymphocytes, as shown by the lowered lymphocyte count, by *in vitro* observations (agglutination, precipitation of extracts), and by the finding of lymphocyte clumping and fragmentation at sites of intradermal or subcutaneous ALAS injection. There was no evidence that ALAS produced primary destruction of lymph node cells, though it has been shown (54) that hetero-antisera comparable to ours may localize in lymphoid tissue. The edema seen after local ALAS injection and the general toxicity with frequent marked leukocytosis which followed intraperitoneal ALAS injection may well have been secondary to the wholesale local or general destruction of lymphocytes and have interest as possible models for secondary changes in the local and general tuberculin reactions.

SUMMARY

Rabbit antisera against normal guinea pig lymph node, when injected into guinea pigs, produced transient depression of the level of blood lymphocytes. It had no effect on other circulating cellular elements. Repeated injection over several days produced lymphopenia, which became progressively less marked with continued treatment, and clear-cut depletion of small lymphocytes in lymph nodes, whether draining an inoculation site or remote. In guinea pigs treated with lymphocyte antiserum, there was marked suppression of the tuberculin and contact allergic reactions and the "delayed" skin reaction to purified diphtheria toxoid, and a relative suppression of allergic encephalomyelitis and the rejection of first set skin homografts. There was a slight effect on second set graft rejection and no effect on PCA or the reversed passive Arthus reaction. Non-specific reactions to intradermal turpentine or to concentrated dinitrochlorobenzene placed on the skin were moderately reduced. The suppression of these reactions (except allergic encephalomyelitis) was closely correlated with the degree of lymphopenia. Lymphocyte antiserum absorbed with normal

blood white cells lost both its lymphopenic effect and its ability to suppress the tuberculin reaction. It is tentatively concluded that a circulating mononuclear cell, probably the small lymphocyte, is the primary reactant in the various types of delayed hypersensitive reactions.

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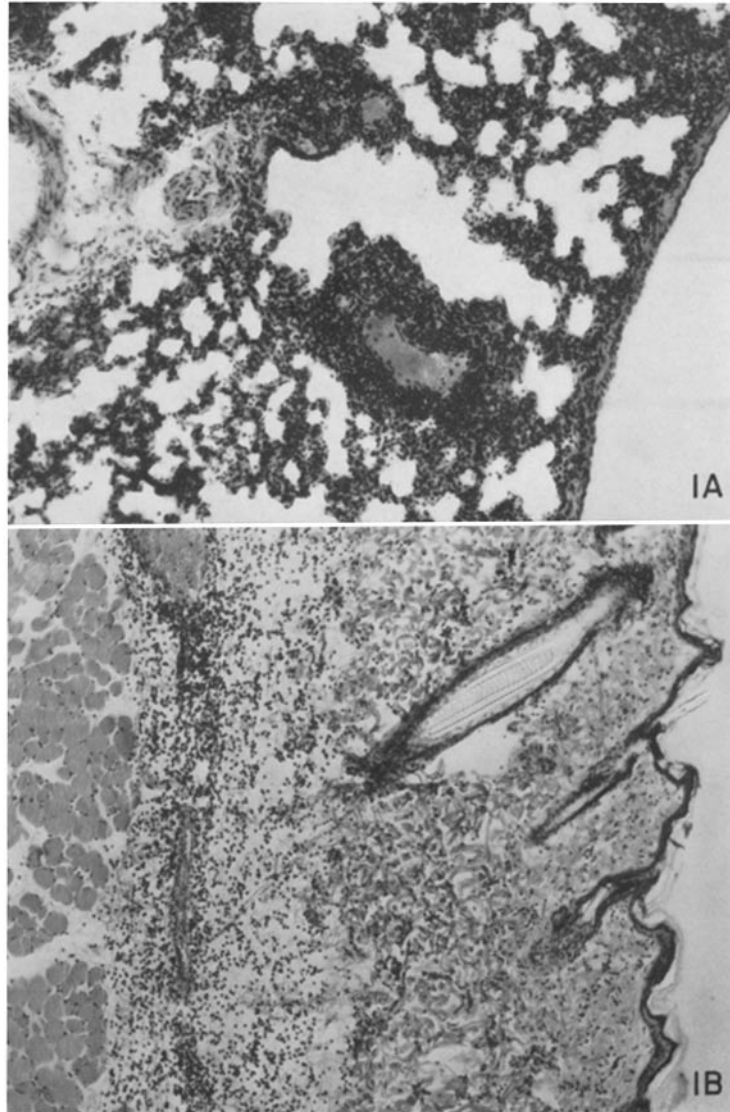
EXPLANATION OF PLATES

Plate 98

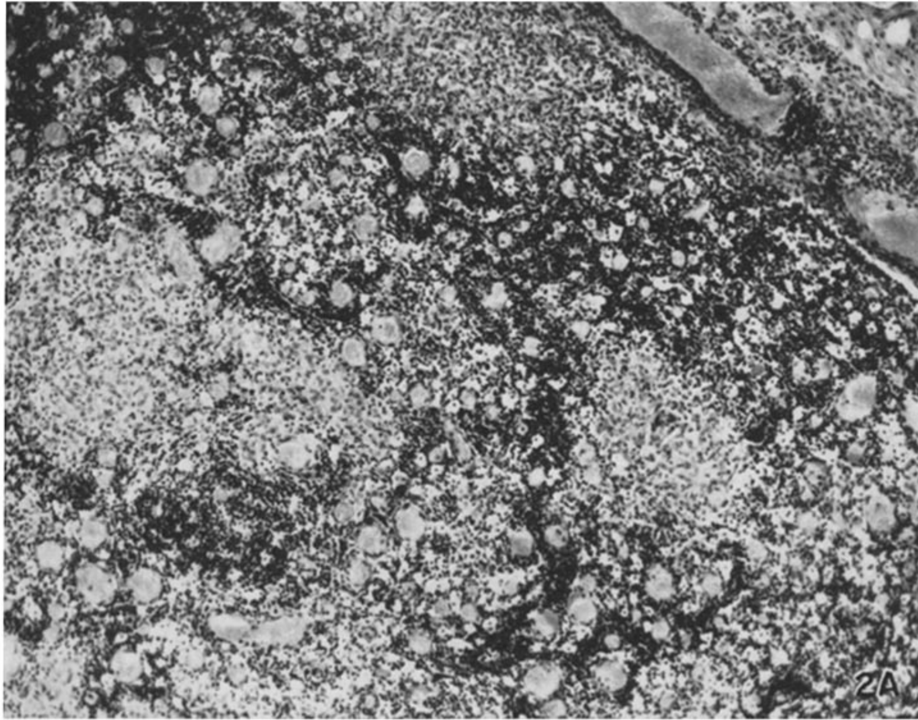
Fig. 1. 1A. Perivenous collections of lymphocytes in lung of contact-sensitive guinea pig 24 hours after second dose of ALAS. 1B. Body wall of normal guinea pig 24 hours after single subcutaneous dose of ALAS. Hematoxylin and eosin. A $\times$ 80. B $\times$ 60.



(Waksman *et al.*: Inhibition of hypersensitive reactions)

Plate 99

Fig. 2. Lymph nodes draining inoculation sites in guinea pigs receiving nervous tissue plus adjuvant. Nodes removed at time of sacrifice at 14 days. 2A. Guinea pig treated with ALAS and failing to develop encephalomyelitis. Node shows epithelioid cell masses but is markedly depleted of small lymphocytes; follicular architecture is lost. 2B. Control guinea pig, which developed ++ encephalomyelitis. Normal follicles are seen in addition to large area of epithelioid cells. Vacuoles probably represent droplets of adjuvant mixture. Hematoxylin and eosin. × 80.



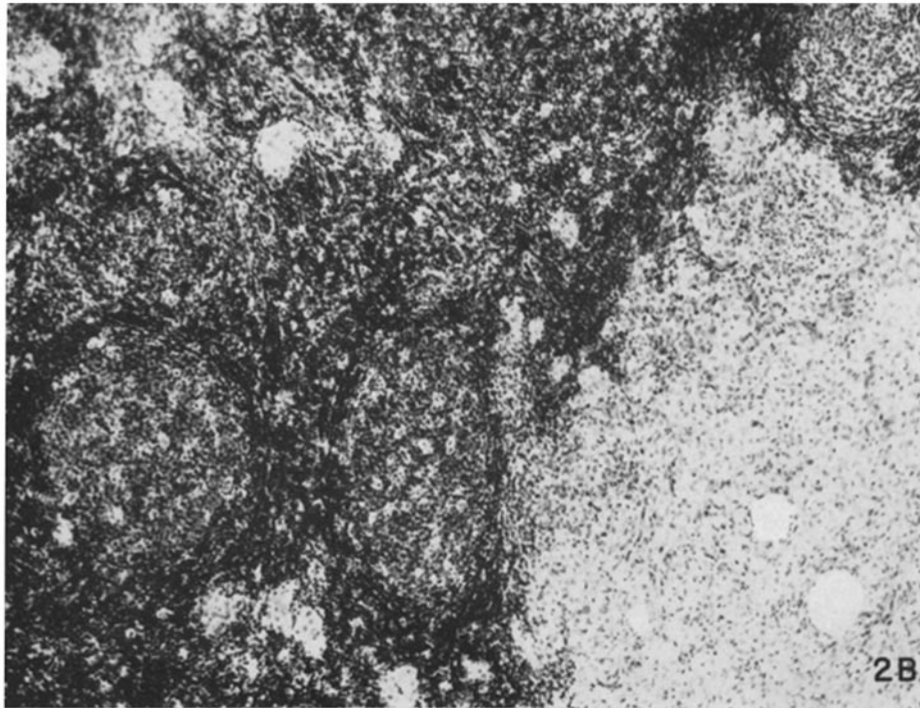


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PLATE 100

FIG. 3. 3A. 24 hour reactions to old tuberculin 1:20, 1:100, and 1:500 in guinea pigs 1, 2 and 3 shown in Table III. 3B. PCA reactions elicited in the same three animals at sites sensitized 24 hours earlier with 1.0, 0.2, and 0.04 μg antibody N and photographed 20 minutes after injection of the eliciting dose of antigen and dye. In both 3A and 3B the animal treated with ALAS is on the left, that treated with APAS in the center, and that given control serum on the right. Photomicrographs of the tuberculin reactions are shown in Fig. 4.

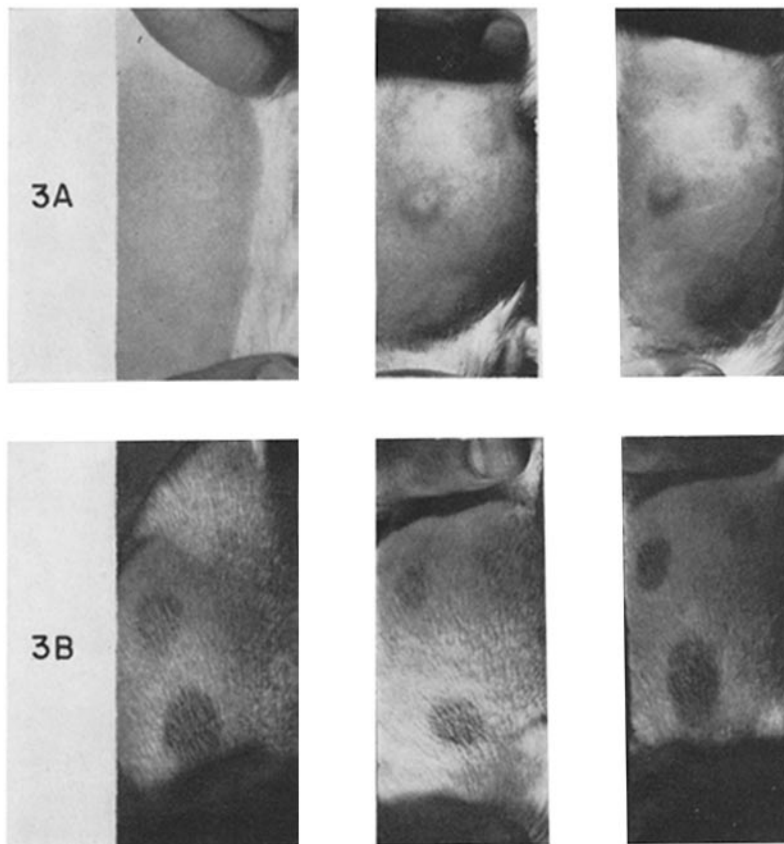

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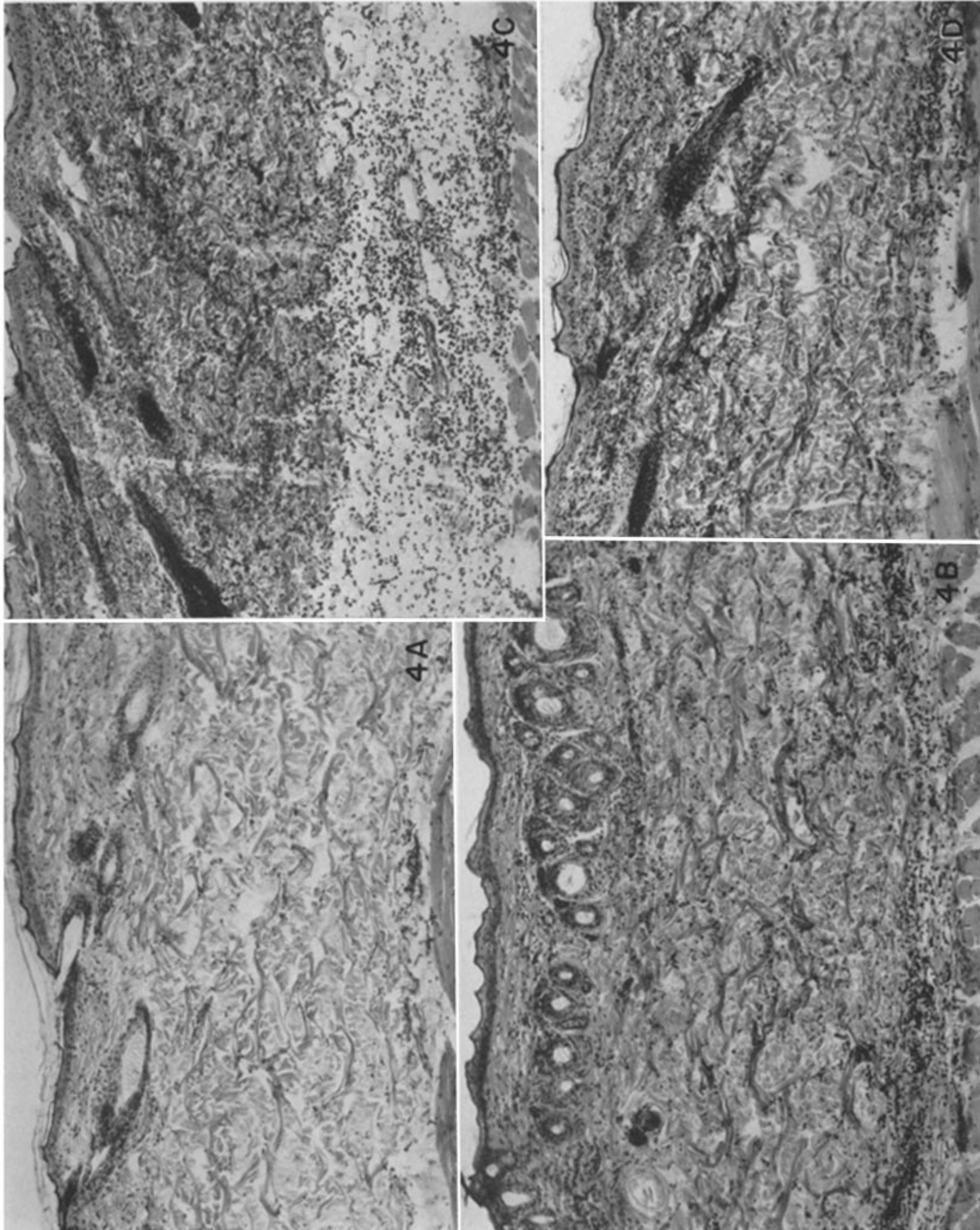
PLATE 101

FIG. 4. Tuberculin reactions at 24 hours in treated and control guinea pigs, described in Table III. Gross photographs of these lesions are shown in Fig. 3. 4A. Reaction to OT 1:20 in animals treated with two doses of ALAS. 4B. Reaction to OT 1:500 in animal similarly treated with APAS. 4C and 4D. Reactions to OT 1:20 and 1:500 in animal given control serum. Reaction is completely suppressed by treatment with ALAS and unaffected by APAS. Hematoxylin and eosin. $\times$ 60.



(Waksman *et al.*: Inhibition of hypersensitive reactions)

PLATE 102

FIG. 5. First set skin grafts. 5A. Autograft, 7 days. There is no sign of inflammation. 5B and 5C. Homografts in ALAS treated animals at 7 and 10 days. These show + and ++ cellular infiltration respectively, and minimal invasion of epithelium. 5D and 5E. Homografts in control animals, at 7 and 10 days. 5D shows infiltration and invasion graded as ++. 5E shows massive cellular infiltration and necrosis of large areas of the graft. Viable epithelium is still visible at the right. 5F. High power view of cellular infiltrate and invasive-destructive lesion of follicle epithelium in control homograft at 10 days. Hematoxylin and eosin. 5A to 5E × 105. 5F × 390.

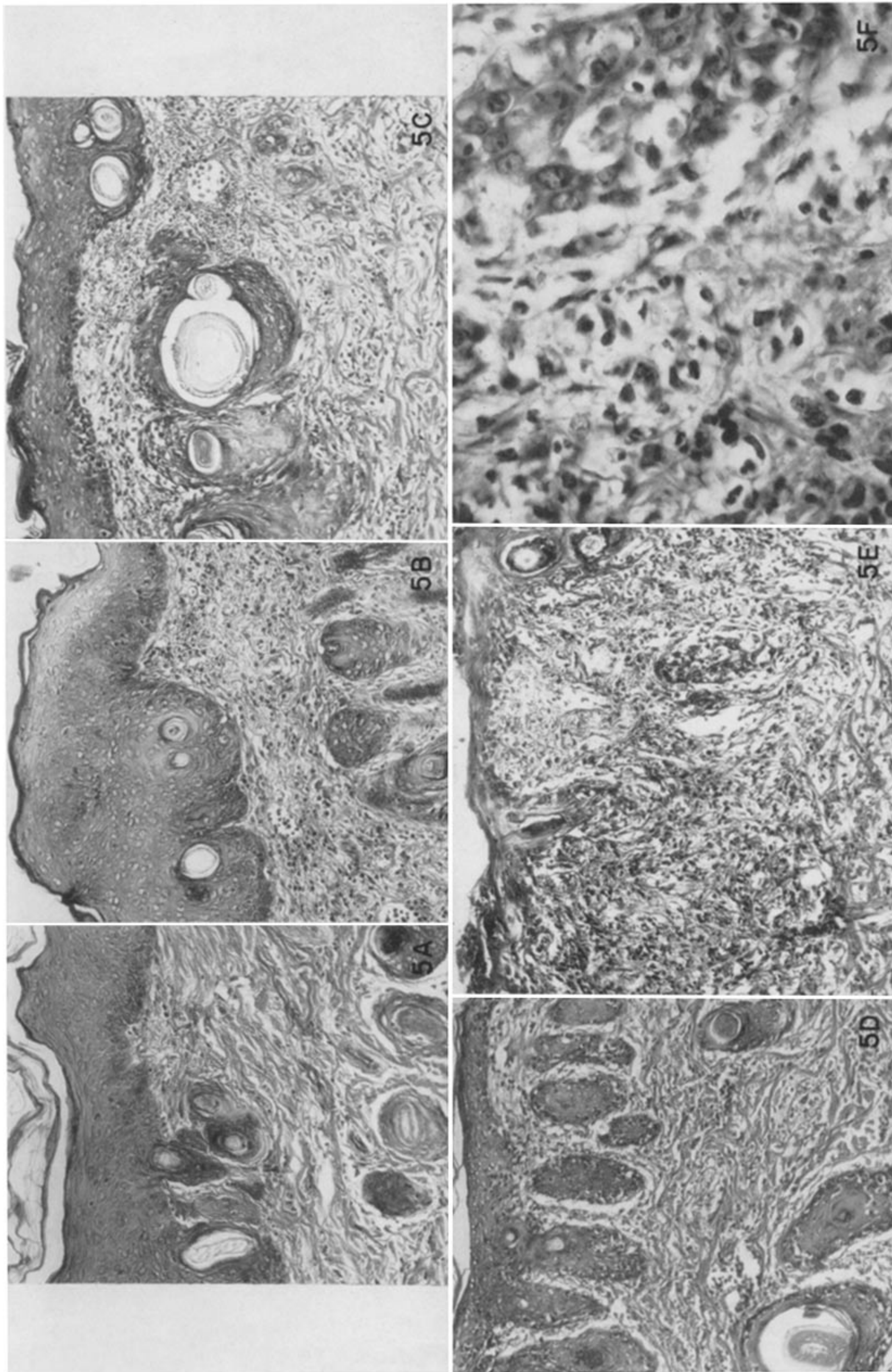


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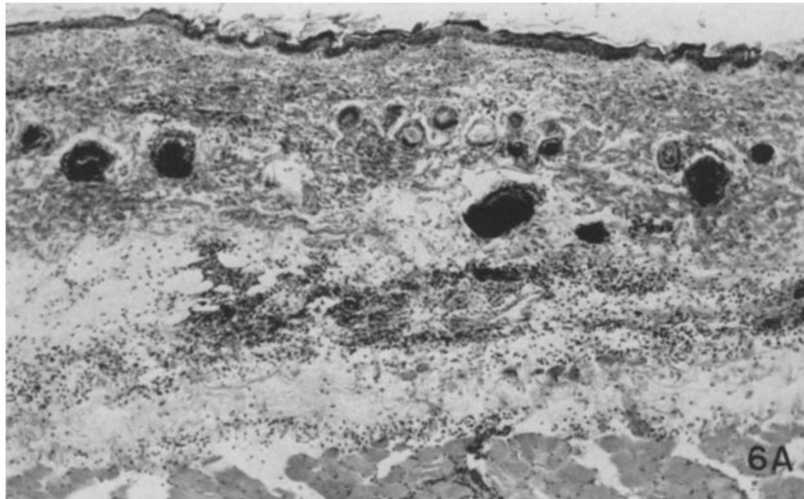
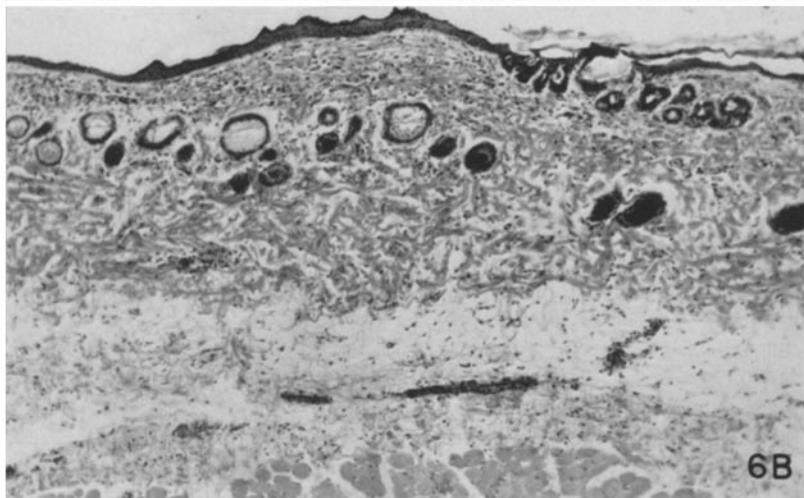
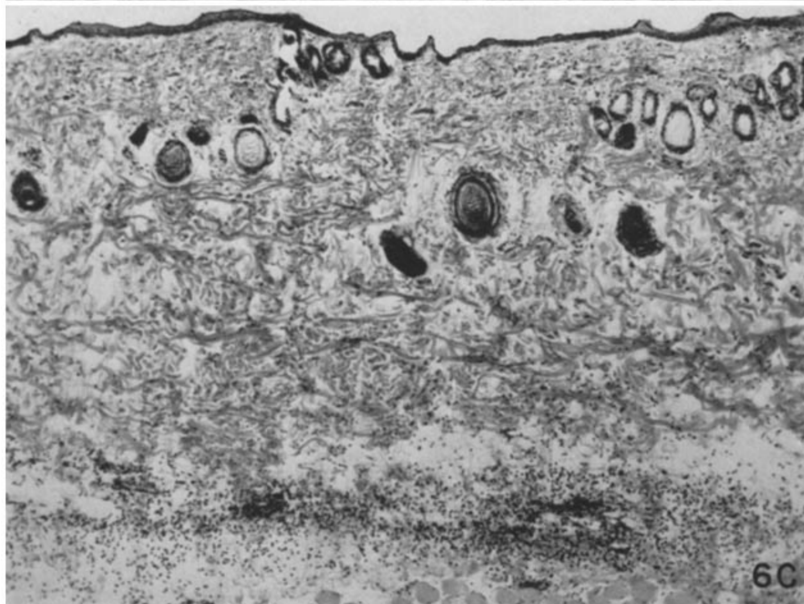
PLATE 103

FIG. 6. 6A, 6B, and 6C. Arthus reactions at 2 hours in guinea pigs treated with two doses of ALAS, APAS, and control serum respectively. Reaction is suppressed by APAS and unaffected by ALAS. Hematoxylin and eosin. × 55.







(Waksman *et al.*: Inhibition of hypersensitive reactions)