± 0.32 mm (36 observations; P = >.1) compared with 2.46 ± 0.27 mm (36 observations) for controls. Cortisone administration resulted in a reduction of body weight to 14% below that of untreated controls by the end of 6 weeks which was quickly regained following cessation of treatment. There was no significant difference at autopsy, 6 weeks following cessation of treatment, between weights of the adrenals of treated and control rats.

Discussion. It has been reported(1,3) that hypophysectomy in the rat produces a severe retardation in rate of eruption of the incisors and that growth hormone is ineffective in restoring the normal rate. The reduction in rate of eruption observed following thyroidectomy in the rat is not so severe as that reported for hypophysectomy(3). This would indicate that factors other than absence of thyroid hormone may be involved. The depression observed in eruption rate following adrenalectomy and the stimulation in rate noted following cortisone administration indicates that the adrenal must be considered in any theory involving a hormonal control of incisor eruption rate. Whether the cortisone-induced acceleration in eruption is due to a direct stimulation of the incisor, as has been reported for thyroxin(3), or whether it is due to some other factor or factors remains to be determined.

Summary. Adrenalectomy in young female rats resulted in a significant reduction in rate of eruption of incisors which averaged 28% below that of unoperated controls during 6 weeks of measurement. Daily administration of cortisone produced a significant acceleration in rates of adrenalectomized rats to levels above that of nontreated intact controls. It is suggested that the severe reduction in rate of eruption of the continuously erupting incisor observed by others following hypophysectomy is due to loss of effect of both thyroid and adrenal hormones.

1. Schour, I., Van Dyke, H. B., PROC. SOC. EXP. BIOL. AND MED., 1932, v29, 378.

2. Baume, L. J., Becks, H., Evans, H. M., *J. Dent. Res.*, 1954, v33, 80.

3. ———, *ibid.*, 1954, v33, 104.

4. Schour, I., Rogoff, J. M., *Am. J. Physiol.*, 1936, v115, 334.

5. Leroy, P., Domm, L. V., *Ann. Endocrinol.*, 1955, v16, 773.

6. Domm, L. V., Marzano, R., *Anat. Rec.*, 1954, v118, 383.

Received May 31, 1960. P.S.E.B.M., 1960, v104.

Specificity of Allergic Reactions. II. Azoproteins in the Anamnestic Response. (25916)

S. B. SALVIN AND R. F. SMITH (Introduced by Carl L. Larson)
Dept. of H.E.W., Rocky Mountain Lab., Hamilton, Mont.

Delayed and Arthus hypersensitivities differ in their immunologic specificity(1,2). Guinea pigs sensitized with 5-15 μg of a conjugate of picryl-hen egg albumin in Freund's adjuvant (without mycobacteria) develop delayed hypersensitivity and ultimately circulating antibody to the whole conjugate or the protein moiety, but only circulating antibody to the hapten. Delayed hypersensitivity can differentiate widely different antigens, such as hen egg albumin from bovine gamma globulin, but cannot readily distinguish a conjugated protein from the same unconjugated protein. Antibodies can differentiate between minor chemical differences in antigens(3). For example, an antigen composed of a protein diazotized to one acid radical, when injected into animals, formed antibodies which did not cross-react with the same protein diazotized to a different acid radical. An immune serum to para-aminobenzoic acid-horse serum formed precipitates with the homologous antigen, but not with horse serum conjugated to the heterologous haptens such as

para-sulfanilic acid, para-arsanilic acid, or aniline radicals(4). Antibody even differentiated between ortho, meta, or para position of a carboxyl radical on a benzene ring. In previous experiments(2,5), an anamnestic effect was produced in guinea pigs after a primary injection of a homologous protein in quantities sufficient to produce delayed hypersensitivity but not circulating antibody and after a subsequent injection of hapten-homologous protein conjugate. In animals given a primary injection of a homologous hapten-heterologous protein, an anamnestic response was not detected on subsequent introduction of conjugate. The haptens studied were picryl chloride and 1, fluoro, 2-4 dinitrobenzene; the proteins, purified hen egg albumin and bovine gamma globulin. Results obtained with various combinations of proteins and haptens indicated that the basic protein might be modified (denatured) to varying extents depending upon the hapten. Therefore, to obtain information on relative specificities of delayed and Arthus reactions with more uniformly conjugated proteins, further experiments were conducted with antigens composed of specific diazotized radicals coupled to purified crystalline proteins. The present paper is concerned primarily with anamnestic responses wherein induction of delayed hypersensitivity to a homologous protein or azoprotein prior to a secondary injection of azoprotein plus a hapten caused enhancement of antibody formation to hapten.

Materials and methods. Animals. Guinea pigs of the Hartley strain weighing 400 to 450 g were used for studies on hypersensitivity. White or albino guinea pigs not necessarily of the Hartley strain weighing 300 to 400 g were employed in the passive cutaneous anaphylaxis (PCA) tests for determination of antibody in test sera. Once guinea pigs had been skin tested, they were not used again. *Antigens.* Proteins were prepared as described previously(2). Diazotization and coupling of proteins were carried out with modifications of basic methods previously described(4,6). As an example, the method used for coupling of para-aminobenzoic acid (PABA) with hen egg albumin (HEA) is given. Other simple chemicals used for coupling were employed in quantities equivalent to that of the PABA. 1.37 g (0.01 Mole) of PABA were dissolved in 150 ml of distilled water and 26 ml (0.026 Mole) of N HCl. Diazotization was carried out with 0.011 M sodium nitrite at a temperature of 0°C. Diazotized protein was then precipitated by acidification to litmus paper with 10% trichloroacetic acid. The precipitate was centrifuged, resuspended in 19 ml saline, and dissolved with 0.1N NaOH. Seven additional reprecipitations were carried out. Finally, the precipitate was dissolved in 20 ml saline adjusted to pH 7.0 with 0.1N NaOH. Other preparations were treated in a similar manner. Thus, aniline (An), p-aminosulfonic acid (PASA), p-aminoarsonic acid (PAAA) or m-aminobenzoic acid (MABA) were diazotized and coupled to HEA, bovine gamma globulin (BGG), human gamma globulin (HuGG), or bovine plasma albumin (BPA). *Sensitization.* Antigens were dissolved either in physiologic saline plus 1% guinea pig serum or in Freund's adjuvant (Difco) without mycobacteria, and a total volume of 0.5 ml injected intracutaneously into the digits of the feet. *Skin tests.* Guinea pigs were tested on the flanks by intracutaneous injection of 0.1 ml of a solution containing 50 μg protein/ml saline. Reactions were observed and diameters of areas of induration measured at intervals up to 4 hours after sensitization and between 18 and 24 hours. *Antibody determination.* Guinea pigs were bled prior to being skin tested, and the resulting sera assayed for antibody by the passive cutaneous anaphylaxis (PCA) reaction(7). By this technic, as little as 0.002-0.003 μg AbN can be detected. In these tests, 0.1 ml antiserum was injected intradermally in the back or sides. About 3 hours later, 350 μg protein in 0.5 ml physiologic saline and 0.5 ml 1% Evans blue in physiologic saline were administered intravenously. Fifteen to 30 minutes later, areas of blueing pigmentation were measured and recorded.

Results. Hypersensitivity to proteins coupled to diazotized acid radicals. Guinea pigs were sensitized in the footpads with 15 μg of hen egg albumin (HEA), hen egg albumin

TABLE I. Cross-Reactions in Guinea Pigs Sensitized with 15.0 μg PABA · HEA in Adjuvant.*

No. of guinea pigs	Day tested	Skin tested with 5 μg PABA · HEA				PAAA · HEA		PASA · HEA		An · HEA		HEA			BGG			An · BGG			PABA · BGG			PABA · BPA	PAAA · BGG
		A	Ab	D	A	Ab	D	Ab	A	Ab	D	A	Ab	D	A	Ab	D	A	D	A	Ab	D	Ab	Ab
2	5		N	++						N	0	N	+	+			0		0			0		
3	7		N	"						N	±	N	+	"			0		0			0		
5	8		N	"	0	N	+			N	+	N	+		0	N	0	0	0			0		
5	9		N	+	0	N	+	N	0	N	±	0	N	+	0	N	0	0	0	0		0			N
3	10	++	½	+	0	N	+					0	N	+	0	N	0			++	½	0	½	P	N
3	11	++	P		0	N						0	N	+	0	N	0			++	P	+	P	P	N
5	12	"	P		½			N	0	N	+	0	N	+	0	N	0	0	0	"	P	+	P	P	N
2	15	"	P		N			N	0	N	"	0	N	±	0	N	0	0	0	"	P	+	P	P	N
2	20	"	P		½			N	0	N	±	0	N	+	0	N	0	0	0	"	P	+	P	P	N

* Similar results obtained after sensitization with 5 μg PABA · HEA in adjuvant. † Reaction not determinable.

Numerator of fraction indicates No. of guinea pigs showing antibody by PCA test. Difference between denominator and numerator indicates animals possibly showing delayed hypersensitivity. P, Circulating antibody present; N, circulating antibody not detected. ±, Mean diameter of induration about 10 mm. +, " " " 11 to 14 mm. ++, " " " 15 to 19 " . +++, " " " 20 to 24 " .

A, Arthus-type hypersensitivity.

Ab, Circulating antibody (by PCA test(4)).

D, Delayed hypersensitivity.

0, Mean diameter of induration <10 mm (in animals showing particular reaction).

coupled to diazotized p-aminobenzoic acid (PABA · HEA), to p-aminoarsonic acid (PAAA · HEA), or to p-aminosulfonic acid (PASA · HEA), bovine gamma globulin (BGG), or bovine gamma globulin coupled to diazotized p-aminobenzoic acid (PABA · BGG) in Freund's adjuvant without mycobacteria. In animals sensitized with 15 μg HEA in Freund's adjuvant, delayed hypersensitivity could be detected not only to HEA itself but also to any of the HEA conjugates, such as HEA coupled to diazotized (a) aniline (An · HEA), (b) p-aminosulfonic acid (PASA · HEA), (c) p-aminoarsonic acid (PAAA · HEA) or (d) p-aminobenzoic acid (PABA · HEA). Delayed hypersensitivity could not be discerned in any of the guinea pigs skin-tested with the foregoing diazotized radicals coupled to heterologous proteins such as BGG, human gamma globulin (HuGG), or bovine plasma albumin (BPA). However, from the 9th to 20th day, when Arthus type hypersensitivity and circulating antibody could be detected, reactions occurred only with the homologous HEA, and not with any of its conjugates. Antibody to a closely related conjugate such as An · HEA could not be detected.

Sensitization of guinea pigs with PABA · HEA resulted in delayed hypersensitivity from the 5th to at least the 10th day (Table I). This hypersensitivity was directed only against HEA and conjugates containing HEA. On the 10th day, following appearance of circulating antibody and Arthus reactions, specificity was no longer directed against the protein but against the hapten PABA conjugated to any of the proteins (HEA, BGG, BPA). Two of the 30 sera from these animals did cross-react with PAAA · HEA in the PCA test. Since, however, they did not react with PAAA · BGG, their specificity was presumably directed toward the HEA portion of the conjugate.

Similar results were obtained in 38 guinea pigs sensitized with PASA · HEA, 23 sensitized with PAAA · HEA, and 33 sensitized with PABA · BGG. In all cases, the animals first showed delayed hypersensitivity to any conjugate containing the homologous protein

TABLE II. Anamnestic Response to PABA · HEA in Guinea Pigs Administered a Primary Dose of 1 μg PABA · HEA in Saline and a Secondary of 5 μg PABA · HEA in Adjuvant. (10 days between inj.)

		Detectable antibody by PCA test						
		Antigen						
No. of guinea pigs	Day tested	PABA · HEA	PABA · BGG	PABA · BPA	MABA · HEA	MABA · BGG	PAAA · HEA	PAAA · BGG
4	4	N	N	N	N	N	N	N
4	5	¼	N	N	N		N	N
4	6	P	¾	P	⅔	⅔	N	N
4	7	¾	¾	¾	¾	¾	N	N
4	8	P	P	¾	"	¾	N	N
4	9	P	¾	¾	"	"	N	N
4	10	P	P	P	P	¾	N	N
4	11	P	P	P	P	"	¾	N
4	12	P	P	P	P	"	N	N
3	14	P	P	P	P	P	⅔	N
3	16	P	P	P	P	⅔	N	N
3	18	P	P	P	P	P	⅔	N

See Table I for legend.

and then Arthus hypersensitivity to any conjugate containing the homologous hapten. Sensitization with these conjugates further indicated (a) that PASA · HEA was a weaker antigen than the others in that Arthus reactions did not appear until the 12th to 13th day after sensitization and areas of induration and erythema were smaller; and (b) that PAAA · HEA induced delayed hypersensitivity to HEA and to its conjugates PABA · HEA and PASA · HEA up to the last skin tests on the 16th day, although Arthus reactions appeared on the 10th day to PAAA coupled with HEA, BGG, or HuGG.

Azoproteins in an anamnestic response. A primary injection of 1.0 μg PABA · HEA in serum-saline was administered in the footpads of guinea pigs. This dosage was used since typical delayed hypersensitivity develops, but circulating antibody can not be detected by PCA tests. Ten days later, the animals were injected with 5.0 μg PABA · HEA in Freund's adjuvant (Table II). On the 5th to 6th day after secondary sensitization, antibody to the homologous PABA · HEA was detected. In contrast, animals given a single injection of 5.0 to 15.0 μg PABA · HEA in adjuvant usually develop antibodies on the 10th or 11th day (Table II). The antibody formed to PABA · HEA in animals given a secondary stimulation also reacted with PABA · BGG and PABA · BPA, but not with An · HEA, PASA · HEA or HEA. Six sera reacted with PAAA · HEA, but not with heterologous protein conjugate PAAA · BGG. The cross-reaction was apparently associated with the protein portion of the conjugate or with the conjugate as a unit.

Guinea pigs sensitized with a primary dose of An · HEA, HEA, or PAAA · HEA, sufficient to develop delayed reactions but not detectable antibody, produced an anamnestic response to a subsequent injection of PABA · HEA (Table III). Delayed hypersensitivity to HEA after primary injection must have served as a basis for the anamnestic response, since the protein was the only common antigenic factor in 2 injections. The capacity of homologous protein to induce an anamnestic effect was particularly significant in that enhancement occurred in rate of antibody production against a hapten (PABA) which was not contained in the primary injection.

When secondary stimulation with PABA · HEA was preceded by injection of a homologous hapten-heterologous protein (PABA · BGG), an anamnestic response did not follow (Table III). Antibody to PABA · HEA appeared at the same interval after the secondary injection as did antibody in control animals that received only a single injection of antigen. Reintroduction of hapten on a heterologous protein therefore did not accelerate antibody response to the hapten.

Discussion. In previous work(1,2), haptens such as picryl chloride were connected

TABLE III. Anamnestic Response to PABA•HEA and Variants. (10 days between inj.)

Antigen		No. of guinea pigs	Day tested	Detectable antibody Antigen					
Primary	Secondary			PABA•HEA	PABA•BGG	PAAA•HEA	PAAA•BGG	An•HEA	HEA
1 μg An•HEA in saline	5 μg PABA•HEA in adjuvant	3	6	N	N	N	N	N	N
		3	7	2/3	1/3	N	N	N	N
		3	8	P	P	N	N	N	N
		3	9	P	P	N	N	N	N
		3	17	P	P	2/3	N	N	N
		3	20	P	P	N	N	N	N
1 μg HEA in saline	*Idem*	3	6	N	N	N	N	N	N
		3	7	1/3	N	N	N	N	N
		3	8	P	1/3	N	N	N	N
		3	9	P	P	N	N	N	N
		3	10	P	P	N	N	N	N
		3	20	P	P	2/3	N	N	N
		3	22	P	P	N	N	N	N
1 μg PAAA•HEA in saline	"	3	7	N	N	N	N		N
		3	8	P	P	N	N		N
		3	9	P	P	N	N		N
		3	11	P	P	1/3	1/3		N
		3	12	P	P	"	"		N
		3	17	P	P	2/3	2/3		N
		3	25	P	P	1/3	N		N
		3	27	P	P	2/3	1/3		N
1 μg PABA•BGG in saline	"	4	7	0	0				
		4	8	0	0				
		4	9	1/4	0				
		4	10	"	1/4				
		4	11	"	"				
		4	12	P	P				
		4	14	P	P				

See Table I for legend.

directly to proteins. Each hapten is probably different in its chemical reactivity and therefore, in the process of conjugation, could have altered the basic nature of the protein in a different way. To minimize the differences produced in the protein molecule by conjugation, haptens were first diazotized and the protein conjugated under similar chemical environments. Comparisons in immunologic activity of haptens could then be made with the knowledge that what differences existed were probably due to the hapten and not to modifications in the basic protein molecule.

The observation to be emphasized concerns accelerated production of antibody to the hapten of a conjugated protein when immunization is preceded by injection of the protein alone. This phenomenon can best be explained by the assumption that an early step in antibody production is a state of delayed hypersensitivity to the protein as a whole, and that this step is superseded by production of circulating antibody if enough antigen is present or if a booster dose is administered. The specificity of the circulating antibody is, however, directed toward small chemical configurations which may be present only in the booster antigen.

Earlier observations suggested the possibility that delayed hypersensitivity might represent an early stage in production of circulating antibody(8). Administration of egg white or horse serum to tuberculous guinea pigs produced a state of hypersensitivity characterized by delayed and prolonged skin reactions, absence of demonstrable antibodies in the blood stream, failure of passive transfer with serum, and insusceptibility to anaphylactic shock after intravenous injection of the specific antigen. Others(9-12) subsequently noted that repeated skin-testing of man or animals caused the subjects to develop a 24-hour inflammatory reaction of the delayed type, which, when the series of intradermal

injections were continued, was replaced by a hypersensitivity of the early type. Histological evidence has also indicated(13,14) that early in the course of immunization a delayed reaction develops characterized by mononuclear cells and absence of serum antibody, but that maturation of mononuclear cells to plasma cells occurs simultaneously with detection of circulating antibody.

Delayed allergy was further implicated as a possible phase in the immune process when a single intradermal injection of a minute quantity of highly purified soluble protein (*e.g.*, 1 Lf diphtheria toxoid) in Freund's adjuvant was found to induce typical delayed reactions. Antitoxin could not be detected by the rabbit intracutaneous test, which can detect 0.0024 μg AbN, and hypersensitivity could be transferred passively by washed lymph node cells(15). Duration of delayed hypersensitivity varied with amount and type of antigen, but was eventually masked by the appearance of circulating antibody and the concomitant Arthus reaction. However, if the amount of antigen injected is lowered sufficiently, delayed hypersensitivity may be produced which is not masked by circulating antibody(5). Evidence that the delayed reaction may be the basis for the anamnestic response has also been presented((15,16,5).

Studies of immune reactions in guinea pigs sensitized with conjugated proteins revealed the specificity of delayed allergy to be associated with the protein portion of the molecule, while specificity of the circulating antibody was directed primarily toward the hapten(1,2). If delayed hypersensitivity is an early stage in antibody production, then delayed hypersensitivity should precede detection of antibody in the circulation. The failure to observe delayed hypersensitivity to haptens prior to formation of antibody appears at first to indicate that delayed and Arthus type hypersensitivities are independent reactions, since antibody is formed to a chemical group (the hapten) without previous induction of a delayed hypersensitive state. If delayed hypersensitivity to the protein and antibody production to the hapten are completely unrelated, the 2 phenomena should have no effect on one another. The foregoing data, however, indicate that there is an intimate relation between delayed hypersensitivity to the protein portion and Arthus hypersensitivity to the haptenic portion of a conjugated protein. When an animal is in a state of delayed hypersensitivity to a protein, the immunological mechanism is primed to produce antibody to antigenically specific groups on that protein or to haptens attached to it. The primed mechanism responds to the second stimulus of the protein, but the resulting antibody is apparently specific for certain particular loci on the protein surface. When, therefore, HEA, An • HEA, or PAAA • HEA is used as the primary sensitizing antigen, the primed cell responds anamnestically to reintroduction of the homologous protein. If a heterologous hapten such as PABA is attached to HEA, response to the hapten is simultaneously accelerated.

In experiments involving anamnestic response, in which a protein was used as a primary dose and a homologous protein conjugate as the secondary dose to cause a typical anamnestic response to the hapten, occasional sera reacted with a heterologous hapten-homologous protein conjugate (Tables II and III). When a conjugate with the same heterologous hapten but a heterologous protein was assayed with these sera, no reaction occurred. Also, the homologous protein by itself failed to react with the antiserum. Hence, the antibody is probably being oriented toward a modified protein, such as may exist in the conjugate, or more likely toward the whole conjugate.

Summary. Injection of 15 μg of a hapten-azoprotein, such as p-aminobenzoic acid diazotized to hen egg albumin, in Freund's adjuvant, into footpads of guinea pigs produces delayed hypersensitivity in 5 days and circulating antibody in 10-11 days after sensitization. Injection of 1 μg of such conjugate in saline produces delayed hypersensitivity but no detectable circulating antibody. Specificity of delayed hypersensitivity is oriented toward the protein; that of circulating antibody, toward the hapten. When a primary dose of 1 μg in saline of protein, azoprotein or

heterologous hapten-homologous protein is followed by a secondary dose of hapten-homologous protein, antibody response to the hapten is accelerated. A primary sensitizing dose of hapten, such as p-aminobenzoic acid (PABA) + protein such as hen egg albumin (HEA), followed by a secondary dose of the same hapten attached to heterologous protein, does not result in accelerated response to the hapten. These phenomena are explained if delayed hypersensitivity is considered an early step in antibody synthesis.

Technical assistance of Jane Nishio, Aspascia Cobure, and Leroy F. Peel is gratefully acknowledged.

1. Benacerraf, B., Gell, P. G. H., *Immunol.,* 1959, v2, 53.

2. Salvin, S. B., Smith, R. F., *J. Exp. Med.,* 1960, v111, 465.

3. Landsteiner, K., *The Specificity of Serological Reactions.* Harvard Univ. Press, Cambridge, 1945.

4. Landsteiner, K., van der Scheer, J., *J. Exp. Med.,* 1927, v45, 1045.

5. Salvin, S. B., Smith, R. F., *J. Immunol.,* 1960, v84, 449.

6. Goebel, W. F., Avery, O. T., *J. Exp. Med.,* 1931, v54, 431.

7. Ovary, Z., *Internat. Arch. Allergy,* 1952, v3, 162.

8. Dienes, L., Mallory, T. B., *Am. J. Path.,* 1932, v8, 689.

9. Jones, T. D., Mote, J. R., *N. England J. Med.,* 1934, v210, 120.

10. Simon, F. A., Rackemann, F. M., *J. Allergy,* 1934, v5, 439.

11. Dienes, L., Simon, F. A., *J. Immunol.,* 1935, v28, 321.

12. Mote, J. R., Jones, T. D., *ibid.,* 1936, v30, 149.

13. Gell, P. G. H., Hinde, I. T., *Internat. Arch. Allergy,* 1954, v5, 23.

14. Kaplan, M. H., Dienes, L., *Henry Ford Hosp. Internat. Symp. Mar.* 1958, pp. 435-449, Little, Brown & Co., 1959, Boston.

15. Salvin, S. B., *J. Exp. Med.,* 1958, v107, 109.

16. Pappenheimer, A. M., Jr., Scharff, M., Uhr, J. W., *Henry Ford Hosp. Internat. Symp. Mar.* 1958, p417, Little, Brown & Co., 1959, Boston.

Received June 1, 1960. P.S.E.B.M., 1960, v104.

Antigenicity of Connective Tissue Extracts II. Stimulation of Auto- and Iso-antibodies by Heterologous Antigen.* (25917)

PAUL HELLER AND VINCENT J. YAKULIS (Introduced by H. J. Zimmerman)
Research Lab. and Medical Service, Vet. Admin. West Side Hospital, and Dept. of Medicine, University of Illinois College of Medicine, Chicago

Previous studies in our laboratory(1) have shown that saline extracts from rabbit tendons combined with Freund's adjuvant are antigenic to guinea pigs. Circulating complement fixing (C.F.) and precipitating antibodies to these connective tissue extracts could be demonstrated(1). Growth of the majority of the guinea pigs injected subcutaneously with extracts of rabbit tendon was markedly diminished as compared to growth of control animals injected only with saline and adjuvant. This effect was not observed following injection of other tissue extracts (liver, leukocytes, kidney) and serum. It was considered possible that the stunted growth was related to development of antibodies against components of connective tissue. The present report deals with demonstration of fixed antibodies in various tissues of the sensitized animals.

Methods and materials. Preparation of extracts from rabbit tendon has been previously described(1). Since the previous study showed connective tissue extracts (C.T.) from old rabbits to be more antigenic than those from young animals, only tissue extracts from rabbits older than one year were used. Extracts from leg and foot tendons of guinea pigs were prepared in the same fashion and used as test antigen only. Guinea pigs of the Hartley Strain weighing approximately 300 g received weekly injections of C.T. suspended in Freund's adjuvant. The injection aliquots contained 150 μg of connective tissue protein

* The authors are grateful to Lemuel Hall for technical assistance and to Medical Illustration Service for preparing the photographs.