TABLE II. Response to the *First* Incubated Sample Injected into Each of the 11 Assay Dogs, And the Effect of Injecting Renin that had been Incubated with Control Plasma into Assay Dogs that had Received Renin plus Immune Plasma 60-120 Minutes Before.

	No. of experiments	Mean change (± standard error) in Aldosterone secretion (mμg/min)	17-hydroxycorticoid secretion (μg/min)	Systolic blood pressure (mm Hg)
Hog renin & control plasma (1st inj)	2	14.5	.4	63
Hog renin & immune plasma (1st inj)	5	1.0 ± 1.0*	−.5 ± .2	6 ± 4 *
Hog renin & control plasma after immune plasma	6	6.8 ± 2.8	1.5 ± .5	48 ± 9.5
Dog renin & control plasma (1st inj)	2	25.0	4.1	45
Dog renin & immune plasma (1st inj)	2	1.0†	.3	3†
Dog renin & control plasma after immune plasma	2	14.5	−.1	35

* Significantly different from hog renin and control plasma, $p < .01$.
† Significantly different from dog renin and control plasma, $p < .01$.

The adrenals of the assay dogs remained responsive to ACTH at the end of the experiment in the 10 dogs tested. The mean aldosterone output in these dogs after ACTH was 32.3 ± 4.3 mμg/min, and the mean 17-hydroxycorticoid output was 8.2 ± 0.8 μg/min.

Summary. The aldosterone-stimulating and 17-hydroxycorticoid-stimulating activity, as well as the pressor activity, of both hog and dog renin were inhibited when prior to injection into nephrectomized hypophysectomized assay dogs, the renin preparations were incubated with the plasma of dogs immunized with hog renin. Incubation with normal dog plasma had no effect on their activity.

ACTH was generously supplied by Dr. J. Stucki, Upjohn Co., Kalamazoo, Mich. The authors wish to thank Miss Angela Boryczka, Miss Margaret Saari, and Mr. Roy Shackelford for technical assistance.

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Received February 15, 1963. P.S.E.B.M., 1963, v112.

Auto-Antigenicity of Connective Tissue Extracts.* (28252)

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We have demonstrated(1,2) that injection of saline extracts of rabbit tendons into young guinea pigs elicits the production of antibodies to these extracts and to similarly prepared extracts of guinea pig tendons. The antigenic component of these connective tissue extracts (CTE) could not be isolated; therefore these antibodies were designated with the general term "connective tissue antibodies" (CTA). The growth of the injected animals was stunted, but following cessation

* We are grateful to Dr. John Peters, Boston, Mass., for valuable advice in the initial phases of these connective tissue studies and to the Medical Illustration Service, V.A. West Side Hospital for indispensable assistance in microphotography.

of the injections the animals started to grow in a normal pattern. Circulating complement fixing antibodies to rabbit and guinea pig CTE were present. In addition, by fluorescent technics, tissue-fixed globulin was demonstrated in the interstitial spaces and along the basement membranes of several parenchymal organs of the injected guinea pigs. Thus the injection of heterologous antigen had elicited antibodies which reacted with the recipient's own tissue, suggesting lack of species specificity of the CTA.

The present report deals with the demonstration of isologous and autologous antibodies to tendon extracts in rabbits.

Methods. Saline extracts from tendons of the hind legs of several sacrificed adult albino rabbits weighing 3-3½ kg were prepared as previously described(1,2). These pooled extracts were used in experiments designed for production of isologous antibodies. Such saline extracts were also prepared from one hind leg, amputated from each of 12 young albino rabbits (1½-2 kg) below the hip joint under sterile conditions with wound closure by carefully prepared skin flaps. These 12 extracts were prepared separately without pooling for reinjection into the donor animals in an attempt to induce autologous antibodies.

The saline extracts were mixed with an equal or somewhat larger volume of complete Freund's adjuvant to result in mixtures containing approximately 1 mg of extract protein per ml(3). Each injection of 1 ml was given into 2-3 subcutaneous and intramuscular sites. This was repeated weekly for 10 weeks, then 0.5 ml was given in monthly intervals. The injection regimen was the same for the intact animals injected with isologous pooled extract as for the amputees which received extract from their own tendons after the operative wound had completely healed. Control animals in either group were injected with saline and adjuvant, others with rabbit serum and adjuvant or received no injections. All animals were weighed weekly and their behavior, food intake, fur, and body surface were carefully observed. Blood was obtained from the ear artery prior to the experiment and at monthly intervals for serologic and hematologic tests. Complement fixing antibodies were determined according to the standard technic of Kolmer and precipitating antibodies by a micro-agar gel double diffusion technic(4). The test antigens were the described saline tendon extracts and an acetic acid extract from these tendons containing collagen(5). The protein concentration(3) was 1 mg/ml.

The animals were sacrificed periodically, beginning in the 12th week following start of the injection regimen, for histopathological examination and demonstration of fixed antibodies by fluorescent technics. For the latter purpose parts of the tissue to be examined were quickly frozen in absolute ethanol precooled in a bath of acetone-Dry Ice and embedded in paraffin(6). The 3-5μ thick sections were then processed according to the classical technic of Coons(7) with minor modifications. The antirabbit globulin sera were conjugated with fluorescein isothiocyanate according to the technic of Rinderknecht (8). All slides were prepared in pairs, one slide being incubated with the fluorescent antiserum, the other for the purpose of blocking being first treated with non-fluorescent antiserum. Tissue slides from non-injected animals were first overlaid with the CTA-serum, then placed in a moist sealed container which was mechanically shaken for one hour at 4°C. Following 3 washings in buffered saline these slides were incubated with fluorescent antirabbit globulin. The washed and air dried preparations were overlaid with a semipermanent mounting medium(9) and cover slips applied. For microphotography a Zeiss fluorescent microscope was used with Osram HBO 200 as light source and BG 12 as exciter filter and OG5 as barrier filter. Magnification was 200 X. Exposure time was 90-120 seconds and the used Anscochrome Daylight films were developed by forced processing to achieve ASA 200.

Skin sensitivity of each animal was determined a few days prior to sacrifice by intracutaneous injection of 100 γ antigen protein (0.1 ml). Rabbit serum (100 γ protein/0.1 ml) was used as control.

Results. The rabbits injected with isologous tendon extracts gained less weight than the control animals but the difference be-

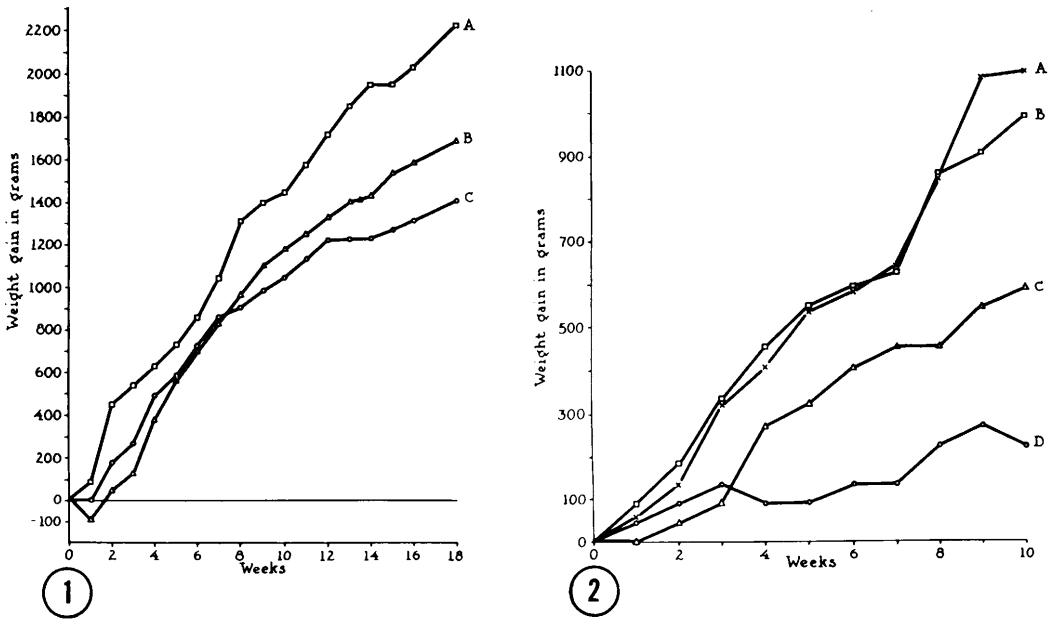

FIG. 1. Weight gain of rabbits injected with isologous CTE (mean values of each group). A. Controls (no injection). B. Controls (serum and adjuvant). C. Experimental animals (CTE and adjuvant).

FIG. 2. Weight gain of rabbits injected with autologous CTE (mean values of each group). A. Controls (not amputated, no injections). B. Controls (amputated, no injections). C. Controls (amputated, serum and adjuvant). D. Experimental (amputated, CTE and adjuvant).

tween the 2 groups was not statistically significant (Fig. 1). On the other hand this difference was highly significant for the animals injected with autologous antigen ($P<.001$) (Fig. 2). The animals injected with CTE exhibited a positive skin test to this antigen which was of the delayed type. Normal rabbit serum did not elicit any reaction on the skin of the CTE injected animals and the CTE-skin reaction was negative in control animals.

Complement fixing antibodies to the isologous and autologous antigen were demonstrated in gradually increasing titer (Table I). The rabbits which were injected with autologous CTE developed antibodies sooner than the animals injected with isologous CTE. Collagen as test antigen reacted only weakly (Table I) with 4 isologous sera in the complement fixation test. Circulating precipitating antibodies were demonstrated in none of the animals throughout the experiment.

The tissue slices from the animals which had been injected with CTE showed specific fluorescence following incubation with fluorescein conjugated antirabbit globulin. The fluorescence was limited to the interstitial tissue and the basement membranes. The examined organs included liver, kidney (Fig. 3),

TABLE I. Titer of Complement Fixing CTA of Rabbits Injected with Isologous or Autologous CTE.

Animal No.	4 wk	8 wk	12 wk	20 wk
		Isologous		
1*	—	—	128	64
2*	—	—	64	32
3*	—	—	64	128
4	—	—	32	128
5*	—	—	64	64
6	—	±	16	8
7	—	16	16	16
8	—	8	32	8
9	—	8	32	32
10	—	±	16	16
		Autologous		
1	16	8	16	
2	8	8	16	
3	32	32	64	
4	32	32	64	
5	8	16	64	
6	16	32	64	

* The sera of these rabbits were also tested with rabbit tendon collagen as test antigen, the maximal titer was 1:8 in the 20th wk.

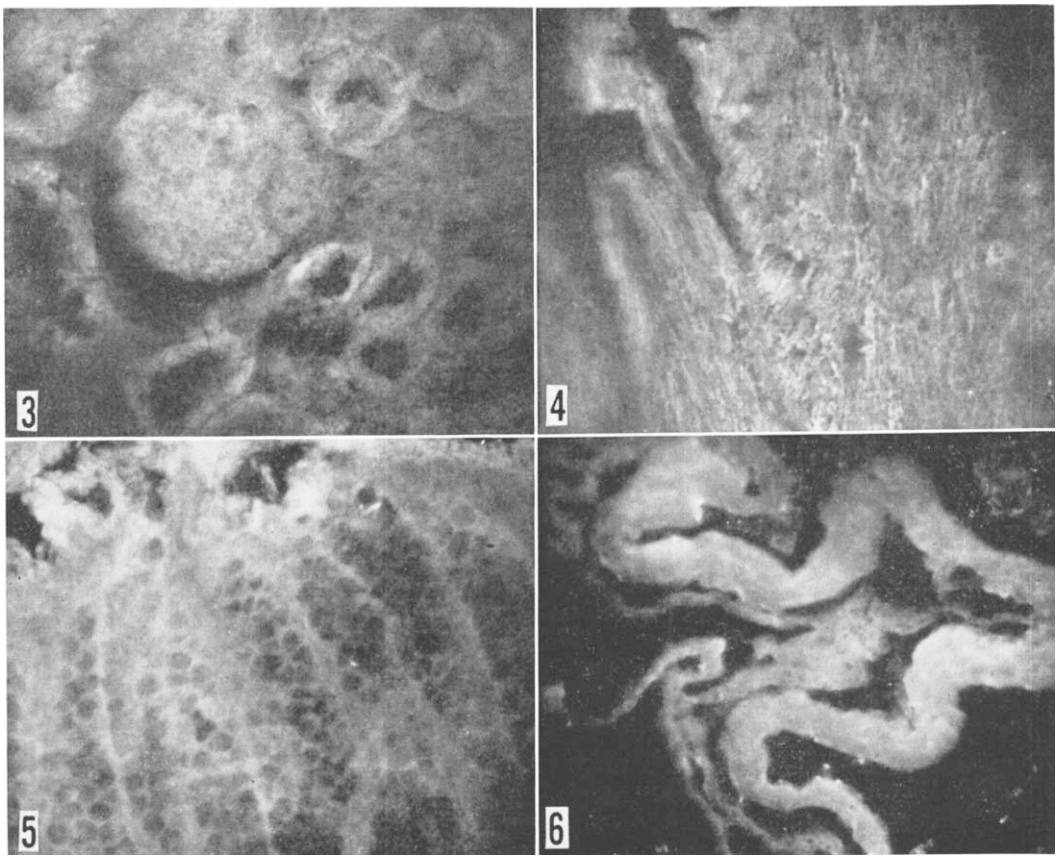

FIG. 3-6. Immunofluorescence of tissues from animals injected with autologous CTE. Reproductions of original color microphotographs. Fig. 3, kidney; Fig. 4, heart; Fig. 5, small bowel; Fig. 6, synovia.

heart (Fig. 4), spleen, lungs, adrenal, small bowel (Fig. 5), synovia (Fig. 6), and subcutaneous tissue. Fluorescence was inhibited in each case by prior incubation with non-fluorescent antirabbit globulin serum. The tissue from the control animals did not show any specific fluorescence, but when incubated with CTA serum first and then with fluorescein conjugated antirabbit globulin the tissues showed the same specific fluorescence as the tissue from the immunized animals.

No definite histologic abnormality could be demonstrated, except for slight thickening of the glomerular basement membranes and of the synovia. These abnormalities are the subject of a separate study. Most animals had a mild normochromic, normocytic anemia, but the direct and indirect Coombs test remained negative.

Discussion. There is a large body of experimental evidence for the existence of autoantibodies(10,11). In the present study connective tissue extract was chosen in an attempt to determine its potential as stimulator of auto-immune phenomena which have long been recognized to be of significance in several connective tissue diseases. Tendon appeared to be suitable because of its richness in connective tissue proteins and its low content of plasma proteins(12).

The presence of gamma-globulin in the connective tissue spaces of various organs, as demonstrated in the experimental animals of this study does not necessarily indicate the presence of antibody in this tissue. The possibility exists that the fluorescein labelled antiglobulin would react with non-antibody globulin present in tissue which is especially true in experiments designed for the demonstration of the antigenicity of autologous tissue by

immunofluorescence. In such a case, however, one would expect that the tissue of the non-injected animals including that of the animals which had been injected with normal serum and Freund's adjuvant would show specific fluorescence. Since this did not occur, the conclusion appears to be justified that the demonstrated globulin represented tissue fixed antibody. This possibility is strengthened by the results of the experiment in which normal tissue was incubated with the antisera to CTE. Moreover, circulating and skin sensitizing antibodies were demonstrable. It cannot be stated with certainty that the production of the "auto-antibodies" was responsible for the diminished growth. It is interesting that the animals injected with autologous material were more severely affected and complement fixing antibodies developed earlier in these animals, suggesting greater antigenicity of the autologous tissue. This unexpected finding requires further study.

Previous experiments(2) have shown that antibodies to rabbit CTE crossreacted with tissue constituents in the injected guinea pigs suggesting that rabbit and guinea pig connective tissues shared antigenic groups. This type of crossreaction is known to be responsible for experimentally induced "autoimmune" phenomena(10,11). In such a case, the autologous tissue is not responsible for the production of antibodies. The possibility, however, that antibodies could be produced by autologous tissue has been widely considered and the results of the present study also suggest the existence of such a mechanism. It is possible that the preparation of the antigenic material has altered the molecular structure of the responsible antigen, or this alteration could have occurred after injection of the material which thus was "displaced" from its anatomic site. In this way the cell units responsible for recognition of antigen might respond to it as to a foreign antigen. Similar conclusions have recently been reached by Weigle(13) in the interpretation of his studies of rabbits made tolerant to bovine serum albumin. In these animals the tolerance was terminated by reinjection of slightly modified bovine serum albumin. Such subtle molecular alterations should be considered among the explanations for autoimmune phenomena. Altered or displaced antigenic molecules could stimulate antibody producing cells, which had atrophied early in embryonic life, but it might not be necessary to invoke the revival of such "forbidden clones" as the source of the production of "auto-antibodies." Normal "uncommitted" antibody producing cells might perform this function provided the antigen is presented to them in a way to make it unrecognizable as a "forbidden" antigen.

The antigenic component of the CTE has not been identified. Serum protein has been shown not to elicit the characteristic antibody response and collagen, although not yet tested in active immunization, reacted only very weakly with the antisera.

Recently Coleman, Gendrich and Jeffay (14) isolated a protein from our connective tissue extracts which is similar to but not identical with serum albumin. Its capacity for auto-antigenicity is not known.

Summary. 1. Rabbit tendon extracts, reinjected into the donor animals produced circulating complement fixing antibodies, skin reactivity of the delayed type and fixation of antibody to synovia and interstitial spaces of several organs. 2. The growth of the animals was stunted, but no other abnormalities were demonstrable. 3. Isologous antibodies were produced in a similar way, but the effects were not as marked.

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Received February 18, 1963. P.S.E.B.M., 1963, v112.

The Radiorenogram in Experimental Hypertension. (28253)

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Taplin and co-workers(1) discovered that an intravenous injection of radioactive iodopyracet (Diodrast-I^{131}) produced a characteristic pattern of radioactivity over the renal areas (radioisotope renogram). The value of the radioisotope renogram in detecting hypertension due to unilateral renal disease was soon recognized. Utilization of radioactive sodium iodohippurate (o-iodhippurate-I^{131}) (2) eliminated many of the technical difficulties associated with the use of radioactive iodopyracet and established the renogram in many centers as a clinical test of differential renal function.

However, despite the voluminous literature devoted to the radioisotope renogram in human hypertension, there is no published report of its characteristics in the experimental animal with renal hypertension. The present study was designed to utilize the radioisotope renogram in experimental renal hypertension.

Method. Twenty-one female dogs each had a right nephrectomy; 30 to 119 days later the left renal artery of each animal was constricted. A polyvinyl sponge clamp with an internal diameter approximately 85% of the external diameter of the renal artery was placed on the main trunk of the artery(3).

The blood pressure of each animal was measured by means of a 20-gauge needle placed directly in the femoral artery and was recorded on a Sanborn recorder *via* a 10-lb strain gauge. Multiple blood-pressure readings were recorded after each surgical procedure. The dogs were checked periodically for 9 months after the onset of hypertension.

Blood-urea concentrations were determined at frequent intervals.

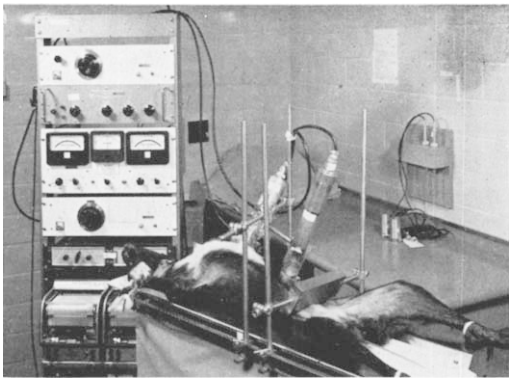

FIG. 1. Method for performing renographic study. Animal is supine on worktable with scintillation counters aimed at heart and left kidney. All dogs in anesthetized group were catheterized at beginning of each experiment.

A minimum of one radioisotope renogram was made on each dog before application of the arterial constriction and after the onset of hypertension. Several dogs had serial renograms taken; 9 animals had renograms taken shortly after application of the arterial constriction and again several weeks later.

The dog was placed supine on a worktable and 2 scintillation counters, one aimed at the heart and the other at the left kidney, were used (Fig. 1). The amount of radioactivity was recorded continuously for about 1 hour after intravenous injection of o-iodohippurate-I^{131} (Hippuran-I^{131})* (Fig. 2). The amount injected per dog was 1 microcurie per kilogram of body weight. Ten of the dogs were studied while they were awake and 11 while under pentobarbital anesthesia. A more reliable tracing could be obtained with the an-

* Supplied by Abbott Laboratories, Oak Ridge, Tenn.