amounts of tissue or to a failure of the rudiment to differentiate cytologically and to produce corticotropin remains to be elucidated.

Summary. Hypophyseal rudiments from tail bud stage embryos and hypophyses from tadpoles of various stages were homografted under the median eminence of adult *Bufo bufo* males with extirpated pars distalis. The rudiment had no effect on moulting or survival of the recipient, but the tadpole hypophysis increased survival time in 23 of 30 recipients. Most of the toads surviving longer than the controls showed periods with normal moulting. The best results were obtained with transplants from the advanced tadpole stages. Corticotropic activity of the transplant was probably responsible both for normal moulting and increased survival time. Homograft reactions resulted in destruction of the transplants after several weeks or months.

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Formation of Collagen. I. Antigenicity of Collagen Fractions. (28288)

M. A. PAZ,* O. W. DAVIDSON, C. J. GOMEZ AND R. E. MANCINI
(Introduced by J. Seifter)
Instituto de Anatomia General y Embriologia, Facultad de Medicina, and Catedra de Quimica Biologica Patologica, Facultad de Farmacia y bioquimica, Universidad de Buenos Aires, Argentina

Extracts of rat and rabbit collagen made with acetic acid or neutral salts have proved antigenic insofar as they induce complement fixing antibodies(1,2). Since these studies were performed with crude and heterogeneous preparations of connective tissue, we have now attempted to determine the antigenicity and immunological specificity of purified soluble and insoluble collagen preparations.

Materials and methods. Leg tendons from old (6-8 months) and young (20-30 days) Leghorn chickens were used. Acetic acid extract of tendon collagen was prepared as previously reported by others(1). The fractionation procedure of Jackson(3,4) was applied to tissues of young chickens in order to obtain larger quantities of the water-soluble protein fraction, neutral salt-soluble fraction and acid-soluble fraction(3,4,5); the insoluble fraction was obtained from the tissues of older chickens. With exception of the portion extracted with acetic acid, collagen fractions were purified by successive washings and reprecipitations(3,4), and finally lyophilized. Proline and hydroxyproline contents were determined by established methods(6, 7).

Two lots (6 animals each) of adult male rabbits, each 6 months old and weighing 3 kg, were immunized, one group with acetic acid extract and the other with insoluble fraction. In each case an homogenate of the antigen in 0.01 N acetic acid (1 mg protein/ml) was administered intraperitoneally, a schedule of 4 injections per week with regularly spaced rest periods being followed. Blood samples were obtained at 4, 5 and 6 months after start of immunization. Antibodies against the soluble fractions were induced by injection of 3 lots (6 animals each)

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TABLE I. Proline and Hydroxyproline Contents of Different Collagen Fractions.

	Proline (mg/100 mg)	Hydroxy-proline (mg/100 mg)	Ratio (pro : hypro)
Neutral salt-soluble	10.62	9.87	1.0
Citrate-soluble	10.68	9.73	1.1
Insoluble	12.23	10.63	1.1
Water-soluble proteins	10.48	1.14	9.2

of adult guinea pigs; in these instances, antigens were dissolved in 0.01 N acetic acid (1 mg protein/ml) and homogenized with 1 ml of complete Freund's adjuvant. Animals were injected intracutaneously at multiple sites twice a week for 4 weeks with aliquots of 1 ml of this mixture. As controls, 5 guinea pigs received the adjuvant alone and another 6 were injected with chicken serum; 4 rabbits also received chicken serum.

Complement fixation(8), test tube precipitin reaction(9) and passive cutaneous anaphylaxis by the method of Ovary(10) were applied. All fractions used as antigens in these immunological tests were homogenized in 0.01 N acetic acid, using buffer to adjust the pH to 7.0 before use in the tests. Specificity of antibodies was checked by absorbing the antisera with the purified preparations of collagen and comparing results obtained in the complement fixation test with those obtained using unabsorbed solutions of antisera.

Results. As seen in Table I, the determination of proline and hydroxyproline contents of soluble and insoluble collagen fractions used in this study yielded ratios between these components which agree with established values for similar collagen fractions.

Complement fixation tests demonstrated that some of the animals in each group developed antibodies against proteins found in the acetic acid extract and in the soluble and insoluble collagen fractions. Titers in general were low, and ranged between dilutions of 1:16 to 1:128; they were highest for antisera obtained with the neutral salt-soluble fraction and lowest for those obtained with the citrate-soluble fraction. Variable and negligible titers were obtained by the precipitin test on overnight incubation. Antisera produced by injection of acetic acid extract not only reacted with this fraction, but also with the neutral salt-soluble and citrate-soluble fractions; however, they reacted only to an insignificant extent with the insoluble fraction and not at all with the water-soluble protein fraction. Antisera to the citrate-soluble and insoluble fractions reacted only with corresponding antigens; whereas cross reactions were observed between antisera to the water-soluble protein fraction and the neutral salt-soluble fraction *per se* as well as between antisera to the neutral fraction and the water-soluble proteins. Table II shows typical results obtained with the complement fixation and tube precipitin tests. The Ovary test gave variable and non-specific results not only with antigens in direct reactions, but also in cross reactions and, in some instances, even with normal rabbit serum. No antibodies to the soluble or insoluble fractions or to the acetic acid extract could be found in sera of animals injected with Freund's adjuvant alone or with chicken serum. After absorption with corresponding purified collagen fractions, antisera gave negative results in complement fixation tests.

Discussion. The antigenic potencies of purified collagen fractions used in this study were revealed by induction of complement fixing and possibly precipitating antibodies; titers obtained were often low. This is in accord with results of others as concerns the acetic acid extract(1) and collagen-containing saline extracts of connective tissue from tendons of young or adult rabbits(2). Of special interest is the high titers obtained with antisera to the neutral salt-soluble fraction. That these antigens were not contaminated with chicken serum proteins was demonstrated by negative serologic results obtained with their respective antisera. Evidence of specificity of the antigens used in the direct reaction was shown by negative complement fixation tests obtained after absorption procedures.

From our results alone one cannot conclude whether the several fractions of collagen are different or similar. More data com-

TABLE II. Specificity of Anticollagen Antibodies. Direct and cross reactions.

Antiserum obtained by injection of:	Fraction used as antigen in test	Complement fixation test	Oudin precipitin test
		Maximum dilution of antiserum yielding positive test*	
Rabbits with acetic acid extract	Acetic acid extract	1:64	1:32
	Insoluble fraction	1:8	neg.
	Water-soluble proteins	neg.	"
	Neutral salt-soluble fraction	1:32	1:16
	Citrate-soluble fraction	1:16	neg.
Rabbits with insoluble fraction	Insoluble fraction	1:64	1:8
	Water-soluble proteins	neg.	neg.
	Neutral salt-soluble fraction	"	"
	Citrate-soluble fraction	"	"
Guinea pigs with water-soluble proteins	Water-soluble proteins	1:32	1:16
	Insoluble fraction	neg.	neg.
	Neutral salt-soluble fraction	1:16	1:8
	Citrate-soluble fraction	neg.	neg.
Guinea pigs with neutral salt-soluble fraction	Neutral salt-soluble fraction	1:128	1:64
	Insoluble fraction	neg.	neg.
	Citrate-soluble fraction	"	"
	Water-soluble proteins	1:32	1:16
Guinea pigs with citrate-soluble fraction	Citrate-soluble fraction	1:16	neg.
	Insoluble fraction	neg.	"
	Neutral salt-soluble fraction	"	"
	Water-soluble fraction	"	"

* Negative.

paring amino acid compositions, physicochemical properties and additional serological behavior are needed to support the significance of our immunological findings. Recently we have used fluorescent antibody techniques similar to those employed in other collagen studies(11,12,13,14) to obtain information concerning the location in tissues of the various types of collagen. Results of these studies support the concept that several collagen fractions may be precursors of others during the development of collagen fibers in embryos and wound healing tissue.

Summary. These studies appear to demonstrate existence of a common immunogenic determinant in the fraction of water-soluble proteins of connective tissue and the neutral salt-soluble fraction; again, the acid-extractable collagen fraction, being heterogeneous, shares a common determinant with other extractable fractions. The so-called insoluble fraction of collagen, while being immunogenic, did not yield antibodies capable of cross reaction with the soluble collagens.

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