Summary. Adult male and female iguanas, *Ctenosaura pectinata*, were auto- and allografted. Skin allografts and autografts behaved like those of other vertebrates; they healed in initially but allografts later showed signs of rejection recognized mainly by pigment cell destruction. At 25°C chronic rejection of first-set allografts in the iguana, as indicated by the survival times, is more like the rejection pattern of urodeles and apodans than that of anuran amphibians or fishes. The data support a view that allograft rejection is by an immune process since accelerated rejection of some second-set grafts occurred while the rest showed enhanced survival. In addition, an inflammatory response, characterized by lymphocytic infiltration, was always associated with destroyed grafts.

The authors wish to extend appreciation to Miss Maria de la Paz Rodriguez, Mrs. Gloria Aponte, Mrs. Margarita Moncada and Mrs. Alicia Bifano for technical assistance. We also wish to thank Dr. Francisco Garcia-Herrera who provided the laboratory facilities.

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Received Dec. 22, 1967. P.S.E.B.M., 1968, Vol. 128.

Studies on the Nature of the Synalbumin Insulin Antagonist* (32967)

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Excess plasma synalbumin insulin antagonism has been proposed to explain coexistence of normal or above normal plasma insulin levels and abnormal glucose tolerance in some maturity-onset diabetics (1). Although it is generally agreed that something associated with plasma albumin antagonizes the effect of insulin on glucose uptake by the rat hemidiaphragm *in vitro* (1–4) and in other *in vitro* systems (5–7), there are conflicting reports regarding the ability of synalbumin to function in whole animals (8,9). Consequently, the actual role of this antagonist in the pathogenesis of diabetes is not clear.

Recently published data indicates that at least part of the antagonism exhibited by Debro "albumin" is due to an artifactual antagonist derived from the Visking tubing used to dialyze the "albumin" (10). The present studies were carried out to gain information on the nature of synalbumin antagonist extracted by the Debro procedure.

Materials and Methods. The rat hemidiaphragm assay of Vallance-Owen (1) was used to measure insulin antagonism. Rats used as donors of hemidiaphragms were Upjohn Wistars or Upjohn pathogen free rats. Insulin concentration was 1000 μU/ml with hemidiaphragms from Wistars and 500 μU/ml with tissue from pathogen free animals. Boiled dialysis membranes were prepared by boiling 50-cm lengths of Visking tubing, size 27/32, (Union Carbide Company) in 4 liters of distilled water for 4 hours with the water changed at 1-hour intervals. Antagonistic "albumin" was extracted from outdated human

* This investigation was supported in part by a U. S. Public Health Service Research Grant (AM-10997-01) from the National Institute of Arthritis and Metabolic Diseases.

plasma by the TCA–ethanol method of Debro (11) as modified by Vallance-Owen (12). The "albumin" was dialyzed through boiled membrane for 7 days against running distilled water to remove the TCA and ethanol and then lyophilized. Ten gm of nonantagonistic "albumin" were dissolved in 200 ml of 0.15 *M* sodium chloride and passed through a 3.8 × 80-cm Dowex 50W X-8 (200–400 mesh) column (Na^+ phase) (13,14). The eluate was dialyzed through boiled membranes against running distilled water for 3 days and lyophilized. Redialyzed "albumin" was prepared by dissolving 5 gm of nonantagonistic "albumin" in 100 ml of distilled water and dialyzing through Visking tubing (boiled or unboiled) against running distilled water for 3 days prior to lyophilizing. Reextracted "albumin" was prepared by dissolving 5 gm of nonantagonistic "albumin" in 100 ml of distilled water, extracting by the Debro procedure and dialyzing the resulting solutions through boiled or unboiled membranes against running distilled water for 3 days. The solutions were then lyophilized.

Dialysis membrane extract was obtained by immersing five 50-cm lengths of Visking tubing, size 27/32, in 1 liter of distilled water and boiling for 4 hours. The tubing was removed and the resulting solution lyophilized.

Statistical analysis of responses to insulin were done by analysis of variance (15).

Results. Debro isolated "albumin" is antagonistic, even when the dialysis step is carried out with boiled membranes. The antagonist can be separated from the "albumin" by ion-exchange chromatography producing a nonantagonistic "albumin" preparation (Table I). Nonantagonistic "albumin" does not become antagonistic when redialyzed with boiled or unboiled dialysis membranes against running distilled water for 3 days (Table I). Reextraction and redialysis of nonantagonistic "albumin", produced a preparation which was found to be antagonistic and there was no difference whether this preparation was dialyzed through boiled or unboiled membranes (Table I).

An extract of the dialysis membrane failed to antagonize insulin at concentrations of 250 or 1000 μg/ml in buffer alone or at a concentration of 1000 μg/ml in buffer containing 4% nonantagonistic "albumin" (Table II).

Discussion. The insulin antagonism demonstrated by the Debro isolated "albumin" confirms the work of others (1–4). Several observations reported here do not support the conclusion of others (10) that a water soluble extract from the dialysis membrane was responsible for the insulin antagonism. These are: (i) Since "albumin" dialyzed through boiled membranes was antagonistic, the antagonism was not due to the water soluble artifactual antagonist described by Ensinck (10); (ii) nonantagonistic "albumin" does not become antagonistic when redialyzed through either boiled or unboiled membranes; and (iii) the dialysis membrane extract failed to demonstrate significant insulin antagonism.

Antagonism demonstrated by the reextracted "albumin" was not physiological since the starting "albumin" was nonantagonistic. This antagonist is obviously an artifact resulting from the isolation procedure. Several possibilities exist regarding the nature of this artifactual antagonist. The antagonism could be caused by: (i) TCA which was not completely removed from the "albumin" by dialysis; (ii) A material produced by the action of alcoholic TCA on "albumin"; or (iii) A material extracted from the dialysis membrane with alcoholic TCA. If the latter explanation is true it is reasonable to assume that this substance could not have been extracted from the membrane with boiling distilled water since antagonism was present in both reextracted preparations, i.e., those dialyzed through boiled membranes as well as those dialyzed through unboiled membranes.

These results confirm the presence of an artifactual insulin antagonist which occurs in conjunction with Debro isolated "albumin" preparations (10) but this does not preclude the possibility that a physiological insulin antagonist is also present in these "albumin" preparations. Evidence is available that there are physiological insulin antagonists in whole serum or plasma (16). Data presented here emphasizes the need for caution in interpreting the results of experiments carried out with Debro "albumin" and the necessity of over-

coming technical problems before the actual role of the synalbumin insulin antagonist in the pathogenesis of diabetes can be determined.

Summary. "Albumin" extracted from human plasma by the method of Debro and dialyzed through boiled dialysis membranes for 7 days has been shown to antagonize the effect of insulin on the isolated rat hemidiaphragm. The antagonist has been separated

TABLE I. Effect of Various Albumin Preparations on Insulin Stimulation of Glucose Uptake by the Rat Hemidiaphragm.[a]

Albumin preparation	Albumin conc. (%)	No. of hemidiaphragms for testing	Glucose uptake (mg%/10 mg dry wt. tissue) ± SE[b]			
			B	I	A	A + I
Debro	5	3	17.4 ± 2.01	25.7 ± .02[c]	16.1 ±2.03	14.9 ±1.57
	5	3	14.4 ± .84	22.6 ± .87[c]	15.9 ± .69	17.5 ± .39
	5	3	17.1 ± .68	24.6 ± .74[c]	17.0 ± .55	16.9 ± .64
	5	3	16.9 ± .99	25.3 ± .02[c]	15.0 ± .58	13.6 ± .78
Nonantagonistic	5	3	14.0 ± .13	24.7 ± .73[c]	15.0 ± .44	24.1 ± .67
(from Dowex	5	3	13.7 ± 1.13	19.9 ± .09[c]	10.6 ± .47	19.2 ± .55
column)	5	3	13.4 ± .83	20.3 ± .95[c]	11.6 ± .76	20.2 ± .85
Nonantagonistic redialyzed (unboiled membrane)	5	6	14.5 ± 1.39	21.0 ± .39[d]	12.9 ± .70	20.8 ± .41
Nonantagonistic redialyzed (boiled membrane)	5	6	14.5 ± 1.39	21.0 ± .39[d]	12.8 ± .70	19.7 ± .68
Nonantagonistic reextracted by Debro + dialyzed (unboiled membrane)	4	6	11.9 ± .67	22.5 ± .83[d]	11.0 ± .69	13.0 ± .94
Nonantagonistic reextracted by Debro + dialyzed (boiled membrane)	4	6	11.9 ± .67	22.5 ± .83[d]	10.0 ± .69	13.6 ±1.03

[a] Abbrev.: B = buffer; I = insulin; and A = albumin.
[b] Standard error of the mean.
[c] Insulin conc. 1000 μU/ml.
[d] Insulin conc. 500 μU/ml.

TABLE II. Effect of Visking Tubing Extract on Insulin Stimulation of Glucose Uptake by the Rat Hemidiaphragm.[a]

Conc. of nonantag. albumin	Conc. of DME (μg/ml)	No. of hemidiaphragms for testing	Glucose uptake (mg%/10 mg of dry wt. tissue) ± SE[b]			
			B	I	B + DME	B + I + DME
0	250	6	13.8 ± .73	30.7 ± 1.29	12.7 ± .82	29.2 ± 1.75
0	1,000	7	11.9 ± .31	28.3 ± 1.83	11.6 ± 1.12	24.6 ± .86
			B + A	I + A	B + A + DME	B + A + I + DME
4	1,000	7	10.9 ± .56	23.9 ± .71	11.0 ± .81	21.7 ± .36

[a] Abbrev.: DME = dialysis membrane extract; B = buffer; I = insulin 500 μU/ml; and A = albumin.
[b] Standard error of the mean.

from "albumin" by ion-exchange chromatography. This nonantagonistic albumin did not become antagonistic when redialyzed through boiled or unboiled dialysis membranes. Nonantagonistic "albumin" did become antagonistic when reextracted from an aqueous solution by the Debro procedure and dialyzed through either boiled or unboiled dialysis membranes. An aqueous extract from the dialysis membranes did not exhibit significant insulin antagonism at concentrations as high as 1000 μg/ml.

The authors gratefully acknowledge the valuable technical assistance of Mr. G. H. Lund and Mrs. Beatrice Wyse.

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Received Jan. 2, 1968. P.S.E.B.M., 1968, Vol. 128.

Roles of Thyroid and Parathyroid Hormones in Renal Calcification Induced by Magnesium Deficiency in the Rat (32968)

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The mechanism for maintaining plasma magnesium within narrow limits was proposed (1) to be the variation in parathyroid hormone secretion in response to plasma magnesium changes. Other investigators (2–4) have suggested that parathyroid hormone regulates magnesium metabolism either by affecting magnesium absorption from the gut or its mobilization from bone. Heaton and Anderson (5) have theorized that the increased activity of the parathyroid glands during magnesium deficiency is responsible for renal calcification in part through production of hypercalcemia and in part through a direct action of parathyroid hormone (PTH) on the kidney. The known effects of parathyroid hormone on mineral reabsorption from the kidney tubule and on bone resorption seem to support the above theories. It is probable that any condition altering blood-bone mineral balances would tend to alter parathyroid secretion rates which in turn may contribute to soft tissue calcification.

The thyroid gland has also been implicated in the metabolism of minerals. Hyperthyroidism was reported to increase calcium excretion, whereas hypothyroidism decreased calcium excretion (6). Improper function of the thyroid also affects the renal handling of phosphorus (7). Others (8,9) have reported an inhibitory effect by thyroid hormone on renal calcium deposition in magnesium deficient rats.

The present studies were designed to further define the roles of the parathyroid and the thyroid glands in the renal calcification

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