(15). Issekutz and Miller(16) observed that in dogs NEFA levels rapidly decreased during exercise. These findings suggest that expected effects of stress-released catecholamines and corticoids were not observed because of the increased caloric expenditure of the dogs in restraint.

Summary. A meal containing 1 g of lard per pound body weight in a meat patty was given to male mongrel dogs after a 16 hour fast. Lipemia clearance was observed in dogs of 4 age groups as follows:

Group 1, less than 1 year, Group 2, 1 to 3 years, Group 3, 4 to 8 years and Group 4, over 9 years. Peak lipemia was apparent after 4 hours in Groups 1-3 and was greater in Group 2 than in Group 1 and in Group 3 than in Group 2. In Group 4 dogs peak lipemia occurred later than in the others but was not as great as that observed in Group 3. During restraint in a modified Pavlov rig for 6 hours urinary catecholamine and corticoid excretion increased approximately 4-fold without regard to age. Contrary to expectation, lipemia clearance was accelerated and serum NEFA did not rise. Increased clearance seemed to be correlated with activity in the rig.

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Angiotensin II Degradation by Human Cohn Plasma Fractions.* (27994)

ROBERT L. WOLF, MILTON MENDLOWITZ, STANLEY E. GITLOW, JULIA ROBOZ, AND NOSRAT NAFTCHI

Department of Medicine and Andre Meyer Department of Physics, Mount Sinai Hospital, New York City

Plasma or serum from patients with untreated primary benign hypertension(1) degrades more angiotensin II-I^{131} *in vitro* than plasma or serum from normotensive or secondary renal hypertensive subjects(2-7). Similarly, the *in vitro* biological half-life of angiotensin II is shorter in patients with primary hypertension than in normotensive subjects(8). Previous heat inactivation of the serum or plasma, before reaction with the angiotensin II-I^{131} *in vitro*, increases the amount of angiotensin II-I^{131} degraded(2,7, 9). These observations prompted investigation of the identity of the plasma fractions which enhance and inhibit angiotensin II-I^{131} degradation.

Methods. Angiotensin II-I^{131} was prepared by the Newerly(10) modification of the Pressman and Eisen method. Twelve different Cohn fractions prepared from different batches of pooled human plasma were obtained. Various concentrations of each of the

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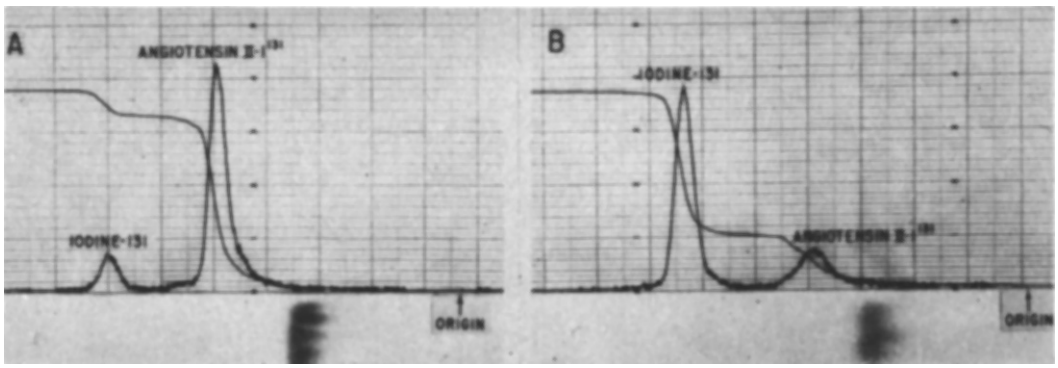

FIG. 1. Typical paper radiochromato-electrophoretograms of mixtures of angiotensin II-I^{131} and Cohn fraction V after refrigeration for 24 hr at 4°C: (A) Without prior heat inactivation; 12% of angiotensin II-I^{131} is degraded; (B) After prior heat inactivation; 76% of angiotensin II-I^{131} is degraded.

Cohn fractions were mixed with 0.003 to 0.030 μg of angiotensin II-I^{131} (specific activity of 100 to 250 μc per mg) and refrigerated at 4°C for 24 hours. The mixtures were then analyzed by paper radiochromato-electrophoresis for 1.5 hours on Whatman 3 MM filter paper in barbital buffer, ionic strength 0.1, pH 8.6 in order to determine quantitatively the per cent angiotensin II-I^{131} degraded. The strips were assayed for radioactivity in an automatic strip scanner employing a thin window gas flow counter with a sensitivity of 0.6×10^6 counts per minute per μc I-131 above a background of 16 counts per minute. The detector was connected to a linear rate-meter with an integrating count circuit and a dual channel recorder. By this technic, separate angiotensin II-I^{131} and iodine-131 peaks of radioactivity could be delineated on the radiochromato-electrophoretograms and the areas beneath the peaks were automatically integrated. The per cent angiotensin II-I^{131} degraded could, therefore, easily be calculated (Fig. 1).

Results. When 50 μl aliquots of the various Cohn fractions ranging in concentration from 1 to 20 mg per ml were mixed with the angiotensin II-I^{131} in borate buffer at pH 7.36, it was found that there was no significant degradation of the angiotensin II-I^{131} when the concentration of the Cohn fractions was less than 1 mg per ml. In 11 separate experiments, when concentration of the various Cohn fractions was between 5 and 10 mg per ml, the per cent angiotensin II-I^{131} degraded was greater than 10% for fractions I, IV-1, IV-4, IV-5,6, IV-7 and IV-8, and always greater than 15% for fractions IV-4 and IV-5,6 (Table I). Heating the various Cohn fractions at 57°C for 30 minutes before the procedure produced a greater than 4-fold increase in the per cent angiotensin II-I^{131} degraded by fractions I, IV-8 and V (Table I). An 8-fold increase in the per cent degradation of angiotensin II-I^{131} was accomplished only by fraction V (Table I). The results were identical when the radiochromato-electrophoreses were performed in borate buffer at pH 7.40.

When the Cohn fractions were diluted in the borate buffer at pH 7.36, so that the final concentrations were equal to the number of grams of the basic fraction per 100 g of plasma proteins, fractions I, IV-1, IV-4 and V degraded more than 10% of the angiotensin II-I^{131} and only fraction V degraded more than 30%.

Discussion. It is apparent from these studies that prior heat inactivation of the Cohn fractions enhances the degradation of angiotensin II-I^{131} by fractions I and IV-8 and that the greatest increase occurs with fraction V. Fraction I supposedly has an electrophoretic composition of approximately 7% albumin and 60% fibrinogen(11) but the fraction I preparation which we employed in these experiments was composed of 98% albumin when measured by paper electrophoresis in our laboratory. The composition of the remainder of the Cohn fractions was

TABLE I. Per Cent Angiotensin II-I^{131} Degraded by Various Cohn Fractions With and Without Prior Heat Inactivation.

Cohn fraction		Exp No. 1	2	3	4	5	6	7	8	9	10	11
I	A	12	11	14	14	10	14	11	12	11	13	10
	B	53	52	70	75	48	64	72	68	71	67	56
II	A	2	5	2	2	4	2	3	3	4	3	5
	B	5	6	2	2	7	5	4	5	2	4	7
III-0	A	1	7	4	2	8	2	6	7	6	5	4
	B	3	6	3	3	9	3	2	9	6	5	5
III-1	A	1	5	1	1	3	1	2	3	1	1	3
	B	3	2	1	1	4	3	2	3	2	1	4
III-2	A	5	6	5	8	6	5	6	4	6	5	4
	B	18	6	7	10	8	9	7	6	9	7	2
III-3	A	3	7	6	9	5	8	7	6	4	7	8
	B	11	8	8	12	11	13	9	9	7	7	11
IV-1	A	10	12	11	14	14	12	11	13	12	14	10
	B	15	12	21	25	19	21	17	16	14	17	13
IV-4	A	18	16	17	23	26	19	21	28	19	22	21
	B	24	15	23	43	32	24	26	27	24	41	21
IV-5, 6	A	18	15	21	24	18	22	17	25	24	20	17
	B	20	17	20	30	22	28	24	23	30	24	21
IV-7	A	11	10	11	13	14	13	10	14	11	14	10
	B	16	14	12	28	21	19	16	22	19	17	16
IV-8	A	10	10	14	12	14	13	14	10	12	11	14
	B	62	48	68	83	78	72	84	62	84	49	61
V	A	8	11	9	3	9	6	4	7	7	9	7
	B	82	83	74	79	78	84	81	79	82	80	79

A indicates without prior heat inactivation.
B indicates with prior heat inactivation.

normal when analyzed by paper electrophoresis. The major electrophoretic component of Cohn fractions IV-8 and V is albumin, which comprises more than 90% of the protein in these fractions(11). The heat labile factor which inhibits angiotensin II-I^{131} degradation, therefore, appears to have the electrophoretic mobility and cold-ethanol characteristics of albumin whereas the alpha and beta globulins, the major component of Cohn fractions IV-4 and IV-5,6, are richly endowed with the factor or factors which enhance angiotensin II-I^{131} degradation. The fact that the degradation of angiotensin II-I^{131} by human plasma, serum or Cohn fractions is accompanied by a corresponding loss of pressor activity is indicated by Hickler *et al.*(8) who demonstrated diminished biologic activity of angiotensin II in hypertensive plasma as compared to normotensive plasma.

Summary. Reaction of various Cohn fractions *in vitro* with angiotensin II labeled with I-131 has demonstrated that there are quantitative differences in the per cent angiotensin II-I^{131} degraded by the fractions. There is evidence for a heat labile inhibitor of angiotensin II-I^{131} degradation with electrophoretic and cold-ethanol characteristics of albumin. The alpha and beta globulin fractions are enriched with the factor or factors which enhance angiotensin II-I^{131} degradation.

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Modification of Irradiation Effects Through Actively Acquired Tolerance to Homologous Bone Marrow Grafts.* (27995)

DON H. SHAW AND HALVOR VERMUND

Departments of Radiology and Genetics, University of Wisconsin, Madison (Paper No. 898)

Successful transplantation of isologous, homologous and in most instances, heterologous hematopoietic elements prolongs the life of lethally irradiated animals. Increased survival appears to depend upon partial or total repopulation of the host's depleted hematopoietic system by cells proliferating from the graft(1,2). Although 30 day survival was much improved even when marrow grafts were from homologous or heterologous animals, Barnes and Loutit(3) observed a large percentage of deaths between the 30th and 60th days in mice with such grafts. This delayed death syndrome has been referred to as "foreign bone marrow reaction," "secondary disease," "homologous disease," and "heterologous disease"(4,5). Makinodan(6) interpreted "secondary disease" as a host reaction against the proliferating foreign bone marrow antigens, *i.e.*, although radiation injury to the immune mechanism of the host makes successful transplantation of foreign marrow possible, when the host system recovers it develops an *in vivo* antigen-antibody reaction against the grafted tissue. In contrast, Trentin(7), Uphoff(8), and Barnes, *et al.*(1) suggested that the bone marrow bears the immune mechanism of the donor and that death results from an immune reaction of the grafted tissue against the host. Shaw and Vermund(9,10) in studies of homologous and heterologous (dove) bone marrow treatment of lethally irradiated pigeons, demonstrated that a similar or identical phenomenon results.

This report includes data from experiments in which the host and donor pigeons had been made immunologically tolerant in various combinations by skin graft technics of Cannon and Longmire(11).

Materials and methods. Pigeons used were domestic barn pigeons (*Columba livia*) and backcross hybrids which were derived from matings to *C. livia* of hybrids and selected backcross hybrids from crosses between *C. guinea* and *C. livia*. These hybrid birds have been sufficiently backcrossed to be considered homologous with *C. livia,* except for those loci responsible for production of one or 2 of the erythrocytic antigens B^g, C^g, and E^g derived from *C. guinea*(12). Skin grafts were made on 136 newly-hatched pigeon squabs by an adaptation of the technic described for chicks by Cannon and Longmire(11). Details of a successful method for development of actively acquired tolerance in the pigeon will appear elsewhere. Of the 136 squabs, 32 accepted and retained the original homologous skin graft and survived to an age of 8-10 months in proper condition for experimental use. Twenty-five of these 32 accepted challenge grafts of skin from the appropriate primary donor at the age of 6 months and retained them through the 10th month, indicating that actively acquired tolerance had been achieved. These birds were used in the following host-donor combinations: Group 1: The irradiated hosts were presumably tolerant of the marrow donors (9 birds—6 hosts, 3 marrow donors). Group 2: The bone marrow donors were presumably tolerant of tissue from the

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