Serum Transamidinase in Renal Disease.* (29757)

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It is well known that disease of a tissue or organ frequently results in release of some intracellular enzymes into the blood stream. In the case of enzymes which are found principally in high concentration in certain tissues or organs, measurement of their concentrations in body fluids in disease frequently provides valuable diagnostic information as to both the location and nature of the lesion(s). Transamidinase, an enzyme which catalyzes amidine transfer, has been found in significant concentration only in the kidney of mammals(1,2), and in dog pancreas(3). This selective distribution in mammals of transamidinase suggested that detection of concentration changes of this enzyme in serum in renal disease might be of diagnostic importance. This paper reports a) the profound elevation of transamidinase found in sera of rats with various types of experimental nephropathy, b) finding of the enzyme in significant concentration in human kidney and, c) some preliminary observations on serum transamidinase activities in humans with renal disease.

Materials and methods. Unilateral renal trauma was produced in male Sprague-Dawley rats by partial burning or crushing of the kidney; hydronephrosis was created by ureter ligation. A form of nephrotic syndrome was produced by administration of 6-dimethyl-amino-purine, 3-amino-d-ribose (puromycin aminonucleoside, PAN)† (4,5). Blood was obtained from rats by cardiac or vena caval puncture.

The enzyme assay developed employs canavanine and ornithine as substrates(6). Serum activity is expressed in units. Each unit represents the synthesis of one millimicromole of product (arginine) in one hour per ml of serum. Arginine was measured by the Sakaguchi procedure of Satake and Luck(7). All determinations were performed in duplicate.

The assay was as follows: 0.7 ml of a buffered substrate solution (1 part 1 M phosphate buffer pH 7.5 plus 3 parts each of 2.4 mM solutions of L-canavanine sulfate and L-ornithine hydrochloride adjusted to pH 7.5) was incubated in a 3 ml centrifuge tube for 15 minutes at 37°C then 0.2 ml of serum added. One hour following addition of serum, 0.4 ml of 30% trichloroacetic acid was added to the incubation tube and the mixture centrifuged. Color development was carried out by adding 1.5 ml water, 2.0 ml of 0.02% 8-hydroxyquinoline in 3 N NaOH and 1.0 ml of 0.1% N-bromosuccinimide to a 0.5 ml aliquot of supernatant liquid from the centrifugation. After 10 minutes absorbance was read (Bausch and Lomb Spectronic 20) at 500 mμ employing 13 mm diameter tubes; the color is stable for at least one-half hour.

Because Sakaguchi positive chromophores are normally present in serum, it was necessary to prepare a serum blank. This was done by adding serum to a buffer-substrate-TCA mixture without prior incubation and proceeding directly with color development.

To measure transamidinase activity in homogenates, the amount of all of the constituents of the incubation mixture was increased 5-fold and the incubation conducted in a Dubnoff incubator-shaker apparatus.

Ascending paper chromatography was employed to identify the reaction products in rat serum. Concentrates of ether extracted TCA supernatant fluids from both the serum blank and incubated mixtures were chromatographed on Whatman No. 1 paper using an isopropanol, formic acid (90%), water sol-

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TABLE I. Effect of Renal Trauma on Rat Serum Transamidinase Activity.

Post oper. time (hr)	Serum transamidinase*					
	Burn injury Unilateral		Crush injury Unilateral		Hydronephrosis	
					Unilateral	Bilateral
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6
0	0	0	0	0	0	0
12	293	80	590	1338	170	397
24	273	159	420	1188	273	478
36	273	80	357	1100	400	318
48	<50	—	210	1025	380	159
60	—	—	107	875	297	159

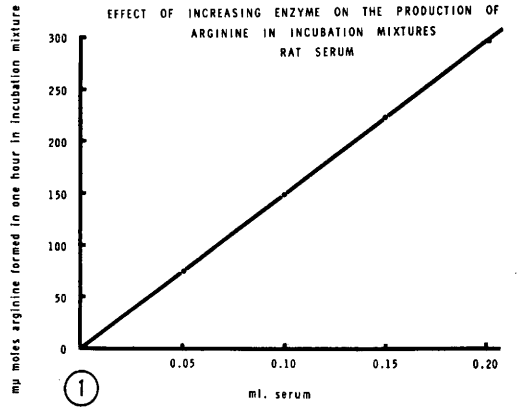
* Sham-operated rats showed no serum transamidinase activity.

vent system (120:30:50). Guanidines were identified with diacetyl spray.

Tissue damage in the injured kidneys was assessed by making routine H & E stained sections of the injured kidneys.

Results and discussion. Enzyme assay. Direct proportionality between enzyme activity and amount of enzyme (serum, or kidney homogenate) was achieved throughout a wide range of enzyme activity. Typical results are given in Figs. 1 and 2. Recovery of arginine added in various amounts to enzyme-substrate incubation mixtures was found to range from 90 to 108%. The amount of arginine synthesized approached a maximum within the one-hour incubation period. Maximal synthesis of arginine was achieved using equimolar substrate concentrations; a similar result has been reported(2) for arginine and glycine substrates. All assays were performed in duplicate. Chromatography of concentrates of the supernatant fluid from the incubation mixture revealed a diacetyl positive spot with an Rf (0.55) identical to that of authentic arginine. A similar spot was not detected when the serum blank was chromatographed.

Rat serum. The results obtained for rat sera at various time intervals following crushing, burning and ureter ligation are given in Table I. As would be anticipated, rapid release from damaged cells and consequently high serum enzyme levels were observed following partial crushing of a kidney. Much less change in the serum enzyme concentration was observed with the burn injury probably indicating the lack of release of active enzyme into the blood due to the marked destruction of the renal enzyme within the burned area. Microscopically, the kidneys subjected to crush injury revealed a marked disruption of the renal architecture.



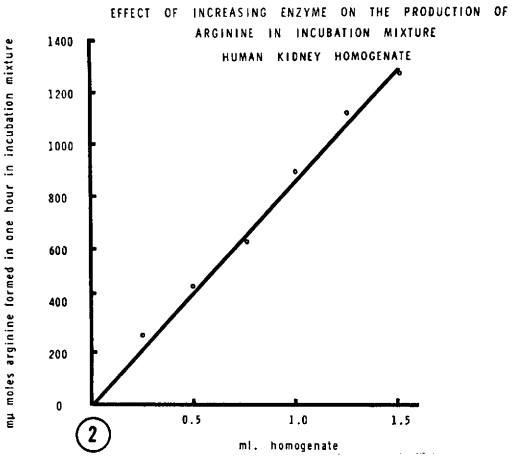


FIG. 1. Pooled serum from rats with unilateral crush injury was used. For details of the composition of the incubation mixture see text.

FIG. 2. Activity was determined on a homogenate of normal human kidney prepared by homogenizing one gram (wet weight) of kidney with 10 ml of 0.1 M phosphate buffer, pH 7.5. For details of the composition of the incubation mixture see text.

TABLE II. Changes in Serum Transamidinase* in Rats Given Puromycin Aminonucleoside (PAN).

Group 1†				Group 2‡			
Animal No.	PAN administration, days	Serum transamidinase, units	Blood urea nitrogen, mg/100 ml	Animal No.	PAN administration, days	Serum transamidinase, units	Blood urea nitrogen, mg/100 ml
1	5	150	24	1	5	185	20
2	11	375	38	2	11	185	32
3	13	375	64	3	14	375	133
4	15	595	56	4	17	935	180
5	19	935	40	5	19	745	—
				6	22	635	42

* No significant transamidinase activity could be detected in serum from normal rats.
† .3 ml of 0.017M PAN administered daily; rat weights at sacrifice ranged from 135 to 180 g.
‡ .3 ml of 0.058M PAN administered daily; rat weights at sacrifice ranged from 440 to 475 g.

Burned kidneys showed discrete areas of total destruction and an inflammatory reaction surrounding this area. Unilateral ligation of the ureter was followed by a slow rise in serum enzyme activity reflecting the gradual destruction of renal cells by pressure. Bilateral ligation, as expected, resulted in more rapid changes. At autopsy, a marked thinning of the renal parenchyma, the result of dilatation of the pelvic lumen, was observed.

Five young and six older rats were injected daily with puromycin aminonucleoside to produce a form of nephrotic syndrome in the animals. The rats were sacrificed at various time intervals and serum transamidinase determined. The results are given in Table II. Sera from both the young and old rats sacrificed at 5 days showed significant elevations of transamidinase activity. A maximum was achieved from the 15-19th day. Alterations in blood urea nitrogen and serum protein electrophoretic patterns were noted at the 11th day. Signs of fluid retention appeared at the 15th day. Although only single rats were used for each time interval, the renal lesions produced in all 11 animals were all associated with significantly elevated transamidinase activity. In addition, the extent of increase of the transamidinase activity correlates with the severity of the disease as indicated by serum protein changes, BUN elevations and evidence of fluid retention.

TABLE III. Transamidinase Activity of Human Kidney.*

Tissue source	Transamidinase specific activity
	mμmoles arginine synthesized/hr/g/× 10^{-3}
1 Cortex	5.8
Medulla	1.2
2 Cortex	7.6
Medulla	3.3
3 Cortex	9.8
4 "	6.5

* Normal human kidneys obtained 4 to 6 hours post-mortem.
Enzyme activity was determined on homogenates of human kidney prepared by homogenizing one gram (wet weight) of kidney with 10 ml of 0.1M phosphate buffer, pH 7.5.

Human kidney and serum. Table III shows values obtained for transamidinase in the normal human kidney. These values can be considered as approximations only since the earliest such specimens could be obtained was 4 hours post-mortem. It can be seen, however, that the human kidney contains the enzyme in significant concentration (a finding not reported previously), particularly in the cortex. These findings suggested that transamidinase activity might be found in the sera of humans with renal disease and preliminary observations on humans with renal disease confirmed this point.

Serum from a number of normal humans was assayed for transamidinase; no transamidinase activity was detected. In a preliminary study, serum was obtained from 10 patients‡ with acute and chronic renal disease. No transamidinase was detected in the serum of the 6 patients with chronic disease. Serum obtained from the 4 patients with acute renal disease during the very active

‡ The authors wish to thank Drs. George E. Schreiner and John F. Maher, Dept. of Medicine, for making available the above patients.

TABLE IV. Serum Transamidinase Activity in Patients with Acute Renal Disease.*

Patient	Age	Sex	Diagnosis	Transamidinase units
J.W.	13	♀	Acute glomerulonephritis	
			May 17th admitted	—
			May 28th	670
			June 9th	74
			June 11th	0
			June 12th discharged	—
W.McI.	63	♂	Massive necrotizing papillitis, chronic pyelonephritis, and nephrosclerosis, pancreatitis with fat necrosis; (autopsy)	
			May 17th admitted	—
			May 22nd	300
			May 23rd	485
			May 24th	634
			May 28th	560
			June 9th	+
T.E.	34	♀	Acute glomerulonephritis, chronic pyelonephritis	485
R.C.	38	♂	Acute renal failure, secondary to surgery	112

* Enzyme activity of normal human sera is essentially zero.
Values reported here are from patients of Georgetown Univ. Hospital, Washington, D. C.

phases of their disease showed significant enzyme activity (Table IV). It is interesting that the serum transamidinase activity of patient J. W. paralleled the clinical course of the disease process. Classical signs and symptoms of acute glomerulonephritis were found upon admission and these persisted until early in June. Serum obtained during this period showed significant transamidinase activity. During the subsequent recovery phase, the transamidinase activity decreased markedly. In contrast, patient W. McI. whose acute disease and its complications eventually resulted in death continually displayed markedly elevated serum enzyme levels. Since the intercellular distribution of transamidinase is unknown, the significance of these findings at a specific tissue level cannot now be deduced. However, the determination of transamidinase in serum of patients with renal disease might prove to be of diagnostic significance.

While this investigation was in progress, Levey, Persky and Czarnecki(8) reported their study of serum and kidney transamidinase activity in rats with various types of renal damage. Two types of renal damage studied by these workers are comparable to those employed by us (crust injury and hydronephrosis). In these conditions although the transamidinase activity of the kidney was found to be significantly diminished when the activity was expressed per gram (wet weight) of renal tissue, transamidinase activity was not detected by these investigators in the serum of these animals. Since we have found no evidence for unusual lability of transamidinase in serum, the divergence in findings may be due to the difference in the substrates in the assay system(2) used by these investigators (glycine and arginine) and ours, and/or the dilution factors involved.

Summary. Transamidinase was determined in sera of rats, both normal and with experimental nephropathy. Little or no enzyme activity could be detected in normal sera whereas those from rats with renal lesions showed profound and sustained increases in enzyme activity. Transamidinase was found in human kidney in significant concentration. In a preliminary study of transamidinase activity in the sera of humans with renal disease similar results were obtained indicating the potential value of transamidinase determination in diagnosis and evaluation of renal disease.

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Hypoxia Induced Alteration of the Skin Homograft Reaction.* (29758)

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Among the adaptive responses reported in animals exposed to altitude hypoxia are myeloid hyperplasia(1), increased hemopoiesis (2), increased adrenocortical activity associated with thymic involution(3), and a proliferation of the reticulo-endothelial system (4). Since the immunological potential of an animal is known to depend upon the functional state of both myeloid and lymphatic tissue, it was desirable to determine whether mice exposed to reduced barometric pressure exhibit any change in tolerance to skin transplants. The present study deals with the effects of altitude hypoxia on the median survival time of skin homotransplants between 2 inbred strains of mice.

Materials and methods. Seventy-eight adult male mice of the A/J and the C_{57}BL/6J strains were used in the skin transplant studies. Prior to transplantation half of the mice were maintained for 14 days in a ventilated decompression chamber at 380 mm Hg, which corresponds to simulated altitude of approximately 18,000 ft. Hematocrit measurements showed that after 14 days of continuous exposure at this pressure the mice could be considered as acclimated. The mice maintained at the ambient pressure of University Park, Pa. (1200 ft) are referred to as unacclimated.

Homotransplants were made in the following combinations: (a) unacclimated donor to unacclimated host; (b) acclimated donor to acclimated host; (c) acclimated donor to unacclimated host; (d) unacclimated donor to acclimated host. All the transplants were made using a modification of the technique described by Billingham and Silvers(5), and were of the type commonly referred to as "pinch" grafts. The grafted skin was approximately 1.5 cm in diameter, and was removed from the antero-dorsal surface of the animal. Postoperatively, all mice were maintained in individual cages at ambient pressure. The protective bandages were removed after 5 days and the animals were observed daily to follow the fate of the graft.

Data were obtained on the survival time of the grafts, and the nature and duration of the 3 stages of graft rejection—granulation, retraction, and sloughing (Table I). A separate group of 48 C_{57}BL/6J male mice was used for histological and weight analyses of the spleen and thymus (Table II).

Results. When both the host and donor were unacclimated, transplants had a median survival time of approximately 9 days, while transplants between acclimated mice were retained approximately 15 days. When skin from an acclimated donor was transplanted to an unacclimated host the median survival time was 12 days, and when the donor was unacclimated and the host was acclimated the transplant was retained 13 days. Thus, it was found that the prior acclimation of either or both the donor and host to altitude hypoxia results in an increased tolerance of mice to skin homotransplants.

Graft rejection can be grossly divided into

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