Fig. 1. *A*, Cut surfaces of white male rat kidneys photographed in artificial light. From top to bottom: kidneys from 1-year-old, 190-day-old, 100-day-old, and 30-day-old rats. *B*, Same kidneys showing phosphorescence.

and pinned, with the cut surfaces facing upward, to a piece of the black cardboard ordinarily used for photographic mounting. The cardboard and tissue were then submerged in liquid nitrogen for approximately 3 to 5 minutes beyond the period of vigorous fuming that accompanies the initial heat transfer. The mounting board was then removed from the liquid nitrogen and immediately illuminated at a distance of 6 inches for 25 seconds with an ultraviolet lamp having a light output area of 16.5 cm by 8.1 cm and producing 110 mw/ ft^2 of short ultraviolet (2537 A) at 2 feet from the light source. A camera with a polaroid back loaded with Polaroid 3000 ASA speed film was focused at the specimen surface. The camera shutter remained closed during the illumination period. After 25 seconds of illumination, the ultraviolet light was turned off and the shutter of the camera was opened. The room was totally dark except for the phosphorescence of the tissue. The camera shutter was left open until all visible afterglow disappeared.

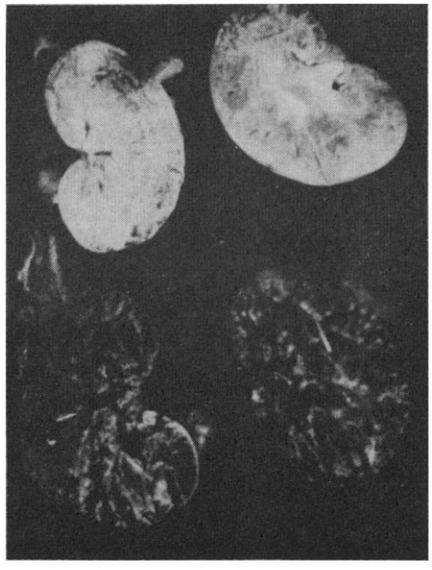

Fig. 2. Longitudinal sections of kidneys from a white male rat. Top, the nonligated kidney shows an intense phosphorescence. Bottom, the kidney which had the renal artery ligated for a period of 6 hours.

Fine structural details were visible in the phosphorescence photographs of a cut kidney surface. Of the many organs studied, only the tooth (if associated with certain kinds of pathology) gave such a specific detailed pattern of phosphorescence activity (*1*).

Figure 1*A* is a photograph, taken in artificial light, of the cut surfaces of four kidneys from white male rats of different ages. In Figure 1*B* are the same kidneys photographed in a totally dark room by the light of their phosphorescence.

To study the effect of renal circulation on kidney phosphorescence, we ligated the renal artery in one kidney of a male rat and left the other kidney intact. After 6 hours the rat was killed with ether and the kidneys were removed. Each kidney was then cut into halves. The halves were pinned to the mounting board with the two pieces of the nonligated kidney on top and the two pieces of the ligated kidney on the bottom. Figure 2 is a photograph of the phosphorescence of these kidney sections. It shows a considerable loss in the intensity of phosphorescence of the kidney which was ligated for the 6-hour period.

In another experiment to explore the effects of 24-hour alteration of blood and urine flow on kidney phosphorescence, four white male Wistar rats were subjected to surgical procedures which resulted in the ligation of the renal artery in the kidney of one rat, the renal vein in another, the renal vein and renal artery in a third and the ureter in the fourth. One kidney in each animal was left intact as a control.

The kidneys showed no phosphorescence when renal veins or arteries were ligated for 24 hours. Phosphorescence was not lost when the ureter was ligated.

One factor to consider in this loss of phosphorescence caused by renal-vessel ligation, is the possible extravasation of blood into kidney tissue resulting from the abnormal pressure and the anoxia caused by the vascular ligations. That such an occurrence might affect kidney phosphorescence is particularly reasonable since perfused blood causes a 41 percent diminution in the intensity of the fluorescence exhibited by kidney slices. This diminution was attributed to an absorption of the exciting wavelength (366 mμ) by erythrocytes (*2; 3*).

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Angiotensinase with a High Degree of Specificity in Plasma and Red Cells

Abstract. *A peptidase with a high degree of specificity for angiotensin II occurs in normal human plasma and red cells. Preparations from both sources have the same* p*H optimum, require calcium ions, and hydrolyze valyl*^{5}*- or isoleucyl*^{5}*-angiotensin II, but do not hydrolyze* β*-aspartyl*^{1}*-angiotensin II, arginyl*^{1}*-angiotensin II or deaminoangiotensin II. This enzyme, given the name angiotensinase A, requires* α-L*-aspartic acid or* α-L*-asparagine as the* N*-terminal amino acid in its angiotensin substrate, and thus differs from kidney leucine aminopeptidase. Other peptidases known to hydrolyze angiotensin also hydrolyze at least one of the other angiotensin analogs with substitution in the one position.*

The pressor activity of the octapeptide angiotensin II is short lasting, and this may be due, in part, to its rapid destruction by plasma or tissue enzymes called "angiotensinases." By using tritiated angiotensin (*1*) we have shown that within half an hour, peptide fragments are recoverable from the organs of rats infused with it.

Experiments with crude angiotensin showed rapid inactivation of the pressor action of the peptide by blood in vitro. Sapirstein, Reed, and Page (*2*) found that hemolyzed red blood cells had several hundred times the pressor activity of plasma, while intact red cells had none. Most of the early work on the destruction of angiotensin has been reviewed by Braun-Menendez *et al.* (*3*). Since the synthetic peptide became available in 1958, several studies concerning destruction of angiotensin have been conducted. Wood (*4*) reported that whole blood from hypertensive patients and their relatives destroyed angiotensin at a slower rate than did blood from normal controls. In contrast, Wolf *et al.* (*5*) reported that serum from hypertensive human beings destroyed it more rapidly than normals. But Klaus (*6*) found no difference in angiotensinase activity of sera of normal persons and hypertensive patients. Recently, increased levels of plasma angiotensinase were reported in renal hypertensive patients by Hickler, Lauler, and Thorn (*7*).

We have studied the destruction of angiotensin II and some of its analogs by fresh normal human plasma and washed hemolyzed red blood cells. We have not studied angiotensin degradation by serum because it contains various peptidases that are released into it during clotting.

Fresh human heparinized plasma (0.5 ml) was incubated wih 2.5 μg of asparaginyl^1-valyl^5-angiotensin II at 37°C for varying periods of time. The reaction was stopped by adding 0.4 ml of 0.1*N* acetic acid and 2.5 ml of 0.9 percent saline, and then heating the mixture for 10 minutes in a boiling water bath. Angiotensin analogs were similarly incubated in the following concentrations: β-aspartyl^1-isoleucyl^5-angiotensin, 2.5 μg/0.5 ml; arginyl^1-isoleucyl^5-angiotensin, 4.0 μg/0.5 ml; succinyl^1-iosleucyl^5-angiotensin (deaminoangiotensin), 4.0 μg/0.5 ml, and *N*-(poly-O-acetylseryl)-isoleucyl^5-angiotensin, 3.0 μg/0.5 ml (Fig. 1). The β-aspartyl^1-isoleucyl^5-angiotensin was isolated by chromatography on a carboxymethylcellulose column (*1*) from preparations of isoleucyl^5-angiotensin after removal of the protecting groups by hydrogenolysis under acid conditions. This peptide is not split by leucine aminopeptidase (*8*). The α- and β-aspartyl peptides can also be separated by paper electrophoresis at *p*H 2.1 (*9*).

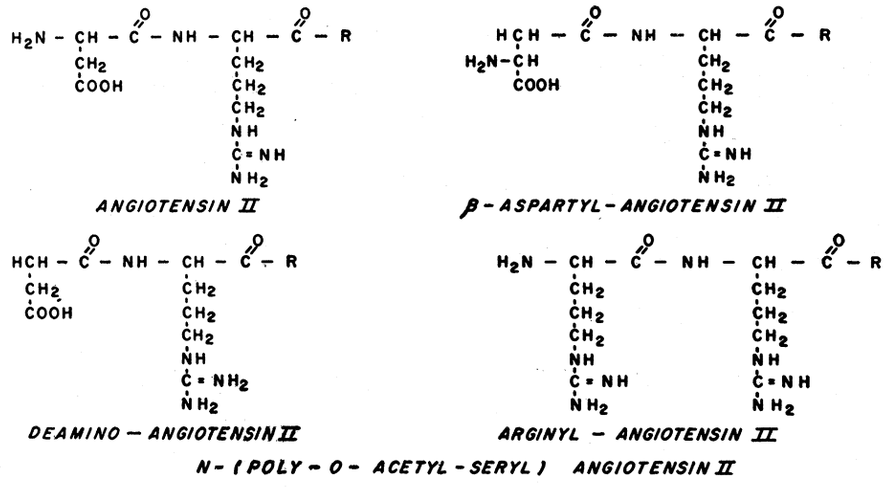

Fig. 1. *N*-Terminus structures of angiotensin analogs used as substrates.

Heparinized plasma was fractionated with ammonium sulfate; the fraction between 35 and 50 percent ammonium-sulfate saturation was collected. After dialysis against saline, this fraction was lyophilized and 5.0 mg of the dry powder was used for incubations with peptide substrates.

Human red cells, washed three times with saline, were disrupted by ultrasonic vibration and lyophilized. This preparation was used as such, or fractionated with ammonium sulfate between 35 and 50 percent saturation, and lyophilized. Again 5 mg of lyophilized powder was used for incubation.

Both plasma and red cells contain an enzyme that inactivated angiotensin. Incubation for 1 hour at 37°C destroyed about 70 percent of its pressor activity and at 23°C about 40 percent. Heating plasma or red cells at 70°C for 10 minutes inactivated the enzyme. The fraction between 35 and 50 percent saturation with ammonium sulfate from both plasma and red cells destroyed angiotensin. Dialysis of plasma or hemolyzed red cells against 9.0 percent NaCl solution or distilled water did not inhibit activity; however, dialysis against saline containing $3 \times 10^{-3}M$ disodiumethylenediaminetetraacetic acid, or against $3 \times 10^{-3}M$ sodium pyrophosphate, completely inactivated the enzyme. Activity could be fully restored by addition of $10^{-3}M$ Ca^{++}. Addition of Mn^{++} and Co^{++} partially restored activity, while Mg^{++} had no effect. The *p*H optimum for both plasma and red-cell enzymes is between 7.5 and 8.0.

Neither red cells nor plasma destroyed β-aspartyl^1-angiotensin II, arginyl^1-angiotensin II, deaminoangiotensin II, or poly-O-acetylseryl-angiotensin II (Fig. 2). On the other hand, partially purified hog-kidney leucine aminopeptidase (*10*) destroyed both angiotensin II and arginyl^1-angiotensin II, but not β-aspartyl^1-angiotensin II and deaminoangiotensin II. Also, leucine aminopeptidase requires Mg^{++} for activity (*11*) while the plasma and red cell angiotensinase requires Ca^{++}. Brunner and Regoli (*12*) reported that plasma did not destroy the pressor activity of β-aspartyl angiotensin.

Because of the conflicting reports concerning angiotensinase levels in normal and hypertensive patients, better characterization of normal plasma angiotensinase seemed necessary. Angiotensinase of plasma and red cells are similar, or probably identical. Both exhibit the same *p*H optimum, ionic requirements, and substrate specificity. This angiotensinase seems to be specific for L-aspartyl peptides in contrast to leucine aminopeptidase which will hydrolyze arginyl^1-angiotensin II and differentiates it from other angiotensin-destroying enzymes referred to as "an-

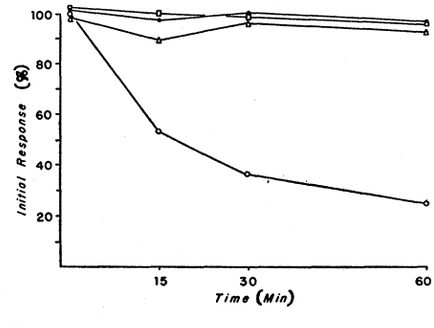

Fig. 2. Incubation of normal plasma with different substrates. Open circles, angiotensin II; closed circles, deaminoangiotensin II; triangles, arginyl angiotensin II; squares, aspartyl angiotensin II.

giotensinases." We, therefore, suggest this plasma and red-cell enzyme from normal human beings be called angiotensinase A.

With respect to substrate specificity, angiotensinase A resembles aminopeptidase A, an enzyme found in a particulate fraction from rat kidney that splits α-aspartyl- but not β-aspartyl-β-naphthylamide, and which is also activated by calcium ions (*13*). Identity between these two peptidases has not been established.

The fact that β-aspartyl-angiotensin is not destroyed by angiotensinase A may lead to erroneous results since the α-aspartyl compound is easily converted to the β-aspartyl linked compound. For example, when angiotensin II which had been dissolved in 0.1*N* HCl for several months was used undiluted plasma did not destroy angiotensin II (*1*). Subsequently, we found that this treatment of angiotensin yielded a peptide with electrophoretic mobility the same as that of β-aspartyl-angiotensin. We would again recommend that paper electrophoresis at *p*H 2.1 be used on all angiotensin II samples (*9*) since this can separate the α- and β-linked aspartyl peptides. The β-linked peptide obtained in this way retains full pressor activity, but is not split by plasma or red-cell enzyme. The possibility of a mixture may account for some of the disparity in results reported.

When measuring angiotensinase concentrations, several angiotensin analogs should be used to differentiate between angiotensinase A and other enzymes which may destroy angiotensin. For example, the plasma of a hypertensive patient in uremia who had been subjected to biweekly hemodialysis showed increased destruction of angiotensin activity. But since it destroyed deaminoangiotensin (Fig. 3), an angiotensin-destroying enzyme or enzymes other than, or in addition to, angiotensinase A were present in this patient's plasma.

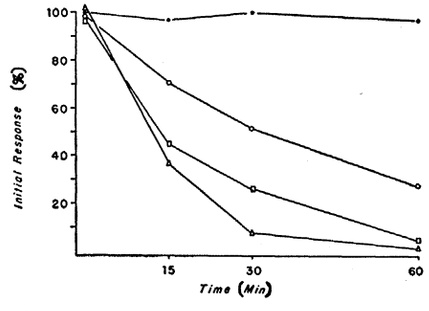

Fig. 3. Normal and abnormal plasma angiotensinase. Open circles, normal plasma plus angiotensin II; closed circles, normal plasma plus deaminoangiotensin II; triangles, abnormal plasma plus angiotensin II; squares, abnormal plasma plus deaminoangiotensin II.

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Lemming Cycle at Baker Lake, Canada, during 1959–62

Abstract. *Brown and varying lemmings of the central Canadian arctic showed changes in reproduction, mortality, and the properties of the individuals over a 4-year period of fluctuating population. These changes were not due to starvation or malnutrition, nor were there any obvious symptoms of stress. They may have been associated with changes in the behavior of the animals.*

Periodic fluctuations in small mammal populations have not yet been explained. The lemming cycle of the tundra is a classic example of the 3- to 4-year cycles found in many rodent populations (subfamily Microtinae) of temperate and arctic regions. Elton (*1*) has described the early work on periodic fluctuations.

Since 1949 the brown-lemming (*Lemmus trimucronatus*) cycles of northern Alaska have been studied in detail (*2*). Populations of the Norwegian lemming (*L. lemmus*) and the Siberian lemmings (*L. sibiricus* and *Dicrostonyx torquatus*) have been studied only sporadically and not in detail.

The purpose of this paper is to summarize a 4-year study on brown- and varying-lemming (*D. groenlandicus*) populations of the central Canadian arctic, and to relate these findings to three currently held hypotheses. The main area studied was at Baker Lake, 425 miles north of Churchill, Manitoba. Data were obtained from about 4000 animals (*3*).

Beginning in the summer of 1959 and continuing throughout the winter of 1959–60, both species increased in number. The crude estimates of this winter increase were 25- to 50-fold in *Lemmus* and 5- to 10-fold in *Dicrostonyx*. Little further increase occurred during the summer of 1960, when the population was highest. The population decreased over the winter of 1960–61, so that only 5 to 10 percent of the *Lemmus* population and 20 to 30 percent of the *Dicrostonyx* survived from August 1960 to June 1961. In the main area this decline continued and accelerated during the summer of 1961. The population changed little during the winter of 1961–62 but began to increase again in the summer of 1962. This cycle was synchronous in both species over a very wide zone of the central Canadian arctic.

But not all areas showed this sharp decline in 1961. At Aberdeen Lake, 115 miles west of Baker Lake, the decline was very gradual, as it was at several islands in Baker Lake within a few miles of the main study area.

Two components of reproduction varied with the cycle—length of breeding season and weight at sexual maturity. Winter breeding occurred in the period of increase but not in the period of decline; summer breeding ended in early August in the peak year and in the decline, rather than continuing into September. There was rapid sexual maturation in the period of increase. In particular, young *Lemmus* males born in the summer matured in the summers of increase at 25 to 35 g but did not mature during the peak summer or decline even though they reached weights of 40 to 50 g. Females matured in the summers of increase and