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Atrial natriuretic factor in Meriones unguiculatus, the Mongolian gerbil

Kyriakos Tarasidis

ATRIAL NATRIURETIC FACTOR IN
MERIONES UNGUICULATUS, THE MONGOLIAN GERBIL

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KYRIAKOS TARASIDIS

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ATRIAL NATRIURETIC FACTOR IN

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BY

KYRIAKOS TARASIDIS

F. B. Leftwich
COMMITTEE CHAIRMAN

W. John Hayden
COMMITTEE MEMBER

COMMITTEE MEMBER

EXAMINING FACULTY

W. S. Woolcott
William Shanabruch
Gary Radice
Mary Smith
R. Dean Decker

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PREFACE

Meriones unguiculatus, the Mongolian gerbil, is a small mammal indigenous to the dry, sandy areas of eastern Mongolia, northern China, and western Manchuria (Tanimoto, 1943). This arid environment is characterized by soil with high salt concentrations. In this area there are about forty species of halophytic plants (Printz, 1921 [cited by Donaldson and Edwards, 1981]) upon which the Mongolian gerbil is known to feed, consuming, primarily, electrolyte-rich leafy tissues in summer and dry seeds in winter (Tanimoto, 1943). Well suited for its natural environment and diet, the Mongolian gerbil displays a pronounced ability to conserve water, drink concentrated saline solutions and excrete sodium by producing only drops of highly concentrated urine (Winkelmann and Getz, 1962; McManus, 1972; Edwards et al., 1983). Hagood (1982) found that the gerbil can drink a 6% sodium chloride solution (as opposed to the white rat which can drink no greater than a 3% sodium chloride solution) and excrete a significantly higher than normal concentration of sodium in its urine. He also found that they could drink a sodium-free solution (also in contrast to the white rat) and excrete a significantly lower than normal concentration of sodium in the urine. These unique abilities are in part believed to be due to the adaptations in renal anatomy and also to certain endocrine mechanisms.

The renal anatomy of the Mongolian gerbil is comparable to other desert rodents in that it displays extended and highly vascularized renal papillae, differentiation of glomeruli by size, and great bundles of the vasa recta in the outer medulla (Getmanova, 1975). These characteristics function to create a greater surface area within the kidney for osmoregulation, which partly helps in explaining the Mongolian gerbil's great osmoregulatory abilities.

Previous investigations of the osmoregulatory ability in the Mongolian gerbil placed particular interest on adrenal function. The adrenal gland hormones are important under stress conditions and serve in adaptive capacities. For instance, when food and water intake is not possible, cortisol stimulates gluconeogenesis, aldosterone stimulates sodium retention, and epinephrine stimulates both mobilization of energy-providing substances and cardiovascular and neuromuscular adaptation. The Mongolian gerbil has been characterized as extremely adrenal dependent. Following adrenalectomy, the animal survives only 4-5 days despite the presence of high sodium chloride levels in drinking water to compensate for loss of sodium retention ability (Cullen and Scarborough, 1970). In contrast, the white rat survives adrenalectomy for several weeks if sodium chloride is offered in the drinking water (Gaunt et al., 1935). In addition,the white rat lives indefinitely following adrenalectomy if supplemented with adrenocortical hormones (aldosterone and cortisol) (Bia et al., 1982). There are, however, conflicting results in the Mongolian gerbil concerning aldosterone and cortisol treatment. Varied doses and combinations of these hormones have been shown to extend life an additional 5 days following adrenalectomy (Cullen and Scarborough, 1970), but in a similar study have been shown to have life extended for only 2 days (Baggia, 1983). Regardless, life was extended only a short time when compared with the white rat and the mineralocorticoids mentioned do not seem to be the major osmoregulating hormones in the Mongolian gerbil. Epinephrine replacement in adrenalectomized gerbils has also been shown not to extend life significantly (Ussery, 1983).

De Cova (1984) proposed that the rapid and irreversible cause of death in the Mongolian gerbil following adrenalectomy was due to increased plasma levels of potassium. She found that adrenalectomized Mongolian gerbils do not reabsorb as much sodium as intact ones, resulting in decreased plasma levels of sodium. The increased excretion of sodium causes a concommitant loss of water in the urine, which de Cova suggested leads to hemoconcentration as well as a drop in blood volume and blood pressure. In addition, low plasma levels of sodium cause a net movement of potassium out of the cells into the blood. The resulting hyperkalemia may diminish the potential difference across cell membranes and so inhibit critical life processes such as cardiac muscle contraction and nerve transmission.

Although the above research clearly shows that the adrenal is very important to the Mongolian gerbil, the organ does not appear to play a major role in osmoregulation. It is possible that a natriuretic factor from the heart is more important as the present research investigates.

ABSTRACT

The basis for the great osmoregulatory ability in *Meriones unguiculatus*, the Mongolian gerbil, has been studied previously, but as yet no clear mechanism has been determined. The present study investigated the level of cardiac atrial natriuretic factor (ANF) under high and low sodium stress. In addition, cardiac ANF values for the Mongolian gerbil were compared with another desert mammal (*Mesocricetus auratus*, the Syrian golden hamster) and a non-desert mammal, (*Rattus norvegicus*, the white rat). ANF was found to exist in the heart atria of the Mongolian gerbil at a level of 388 $\pm$ 66 μg ANF/g tissue. High and low sodium stress did not have a significant effect on the cardiac level of ANF in the Mongolian gerbil. Finally, the Mongolian gerbil had cardiac ANF levels similar to the Syrian golden hamster but 3 to 4 times less than the white rat.

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INTRODUCTION

The Mongolian gerbil has the ability to osmoregulate under extreme environmental conditions. The traditional mineralocorticoids do not appear to be the major osmoregulatory hormones in the Mongolian gerbil (Cullen and Scarborough, 1970; Baggia, 1983; Ussery, 1983; De Cova, 1984). It is possible that the atrial natriuretic factor (ANF), characterized by de Bold et al. (1981), has an involvement in this animal's unique sodium regulatory ability. De Bold's group noted a rapid and short-lived diuresis and natriuresis in white rats as a result of atrial homogenate injections. A small peptide was subsequently isolated from atrial homogenates and found to be the causative agent. This substance (ANF) is a 126 amino acid peptide (Asn-1 to Tyr-126) in the prohormone form (Thibault et al., 1984), found primarily in granules in the atria of mammals (Garcia et al., 1982). Although in mammals ANF is primarily located in the atria, it has been isolated in small quantities in the ventricles (Cantin et al., 1987). De Bold (1982) also identified the hormone in the ventricles of non-mammalian vertebrates as well. ANF is released from the granules in the atria (Garcia et al., 1982) in an active 28 amino acid form (Thibault et al., 1984). Activation of stretch receptors after increased blood volume (Lang et al., 1985) causes the release of ANF. Sodium loading increases blood volume by drawing in water causing an increase in venous return and a subsequent increase in atrial pressure. In the white rat, high sodium in the diet lasting several weeks has been shown to significantly increase plasma ANF levels by approximately 25% and significantly decrease the atrial ANF content by approximately 33% (Takayanagi et al., 1985; Schwartz et al., 1986). Blood ANF acts on the kidney to increase glomerular filtration rate and to produce a diuresis and natriuresis (Atlas et al., 1984; Camargo et al. 1984).

ANF has been identified in the atria of various mammals, including the white rat, the hamster, and others, displaying great homology in peptide sequence among them (Vlasuk et al., 1986, Forssmann et al., 1984, Oikawa et al., 1985). However, ANF has not been investigated in the Mongolian gerbil. The present research investigates the presence and level of ANF in cardiac tissue of the Mongolian gerbil and attempts to determine if this level is greater than the white rat. If the Mongolian gerbil has significantly higher levels of the hormone in cardiac tissue under no sodium stress and displays a significantly lower level in cardiac tissue under high sodium stress (depletion in tissue due to release into the blood stream), then it can be proposed that ANF has a greater importance in osmoregulation in the Mongolian gerbil than in the rat. The research also attempted to identify the location (atria versus ventricle) of the hormone in the cardiac tissue and to quantify it under high and low sodium chloride regimens. In addition, atial ANF values were determined for the Syrian golden hamster and the white rat, to compare both with the literature values and with the Mongolian gerbil. The Syrian golden hamster was chosen because it is a desert mammal for which there are ANF values available. The white rat was selected because of the extensive information in the literature concerning ANF in this animal. Importantly, the white rat is also the only animal for which there is a sodium stress model for ANF.

MATERIALS AND METHODS

Twenty-one, 13-18 week old, 70-80 g male Mongolian gerbils were obtained from Tumblebrook Farms, MA. The animals were acclimated to a standard temperature range of 20-24 C and photoperiod of 12h light-12h dark for five days prior to experimentation.

Three groups of seven animals each were established with all groups fed Purina Lab Chow 5001 (0.40% Na^+) *ad libitum.* Different degrees of sodium stress were induced through manipulation of sodium chloride levels in drinking water. The control group received tap water, whereas the low sodium group received deionized water (Barnstead D1794) and the high sodium group received 6% sodium chloride water, all *ad libitum.* The animals were held under these regimens for 21 days and then euthanized by cervical dislocation. No anesthesia was administered since Gutkowska (1987) reports that anesthetic agents may alter ANF levels. In addition, the American Veterinary Medical Association (AVMA) recommendations (Smith et al., 1986) do not require anesthesia for mammals weighing less than 200 g. The twenty-one day period was chosen because Hagood (1982) found peak sodium levels in the urine of the Mongolian gerbil under the regimen of 6% sodium chloride drinking water. Following the procedure of Gutkowska (1987) to liberate ANF from the heart muscle, 12 mg of tissue were removed using both atria of each animal and rinsed in ice cold 0.1 M phosphate buffer pH 7.4, 0.9% NaCl. The tissue was then homogenized for 60 sec in 0.1 M acetic acid containing the protease inhibitors EDTA (1 mg/ml), phenylmethylsulfonyl fluoride (10^{-5} M), and pepstatin A (5 µM). The homogenates were then centrifuged for 20 min at 3000 rpm at 4 C and the supernatants frozen at -70 C until use. At the time of use, the frozen supernatants were thawed at 4 C and once again centrifuged at 3000 rpm before the ANF assay was conducted.

The presence and concentration of ANF in the supernatants were investigated with a radioimmunoassay for rat ANF (Ser-99 to Tyr-126) from Peninsula Laboratories, Inc., Belmont, CA, Catalog No. RIK9103. The dilutions of the supernatants for assay were made at 1:2000. To make the comparison with other mammals, an additional group of seven, 13-18 week old, 70-80 g male Mongolian gerbils were obtained from Tumblebrook Farms, MA. In addition, five, 12-15 week old, 175-200 g male white rats (Dominion Laboratories, VA) and two, 6-8 week old, 100-125 g male Syrian golden hamsters (Dominion Laboratories, VA) were also used for literature comparison and direct comparison with the Mongolian gerbil. For seven days prior to experimentation, all groups were fed Purina Lab Chow 5001 and given tap water, both provided *ad libitum*. All the tissue from both atria was homogenized to compare with the literature value, where values are often expressed as µg ANF/2 atria.

Mean ANF values of the control, low, and high sodium groups were compared statistically using the unpaired t-test performed at a 95% confidence level. In addition, the mean ANF value of the Mongolian gerbil was statistically compared to the means of both the white rat and the Syrian golden hamster using the unpaired t-test also performed at a 95 % confidence level.

RESULTS

ANF was found in low, but easily measured quantities in the heart of the Mongolian gerbil. In the control group (tap water), it was found in the atria at a level of 388 ± 66 µg ANF/g tissue and in the ventricle at levels less than 0.01 µg ANF/g tissue (Table 1). There was no statistical difference in ANF levels when the control, low, and high sodium groups were compared (Table 2). The low sodium group had a tissue ANF level of 350 ± 75 µg ANF/g tissue, and the high sodium group 434 ± 69 µg ANF/g tissue. Although there were no significant difference between these groups, there was a trend of increased atrial ANF concentration with increased sodium in drinking water (Figure 1).

The Mongolian gerbil (293 ± 43 µg ANF/g tissue) and the Syrian golden hamster (334 ± 20 µg ANF/g tissue) had statistically similar atrial levels of ANF whereas the level in the white rat (632 ± 160 µg ANF/g tissue) was significantly higher than in both the Mongolian gerbil and the Syrian golden hamster (Table 3).

For literature comparison, these data were mathematically converted to µg ANF/2 atria. When this was done the Mongolian gerbil had an atrial level of 7.4 ± 1.5 µg ANF/2 atria, the Syrian golden hamster, 8.4 ± 0.5 µg ANF/2 atria, and the white rat, 28.5 ± 7.4 µg ANF/2 atria (Table 3).

DISCUSSION

The primary goal of this study was to investigate the existence and location of ANF in cardiac tissue of the Mongolian gerbil and to determine whether it changes during salt stress. It was no surprise to find the hormone in the Mongolian gerbil as it has been identified in other mammals (humans, flying squirrel, rat, mouse, guinea pig, cat, rabbit, dog, hamster, pig, ox) and in other vertebrates - birds (chicken), reptiles (turtle), amphibians (frog, toad), and fish (shark, lamprey, hagfish, goldfish). In the earliest studies in the white rat, ANF was identified indirectly by observing natriuretic activity from cardiac extracts (de Bold, 1982) while in later studies, it was identified directly with immunocytochemical techniques (Cantin et al., 1984). De Bold (1982) intravenously injected the supernatants of atrial or ventricular homogenates into the various vertebrates and observed increases in sodium excretion and urine volume. Cantin et al. (1984) used gold-labeled antibody against ANF to label fine sections of atrial tissue. This technique indicated the presence of ANF in this tissue and more specifically in the granules found within the tissue (Marie et al., 1976 [cited by Cantin et al., 1984]).

In the present study, ANF was found mainly in atrial tissue with only a trace found in the ventricles. De Bold (1982) found natriuretic activity only in atrial tissue in mammals and in both atrial and ventricular tissue in the nonmammalian tissues. Gutkowska et al., (1984) established a radioimmunoassay (RIA) which confirmed the mammalian results in the white rat. RIA has a high specificity and sensitivity for ANF as it uses an antibody against the peptide. Cantin et al. (1987) showed the rat heart to contain ANF almost exclusively in the atria with little to none present in the ventricles. The atrial ANF concentration increased with age, whereas the ventricular ANF concentration was found to decrease with age to a plateau ratio of 152:1 (atria to ventricle ANF concentration). In addition, by means of morphometric studies in the white rat, Marie et al. (1976 [as cited by Cantin et al., 1984]) showed a greater number of granules present in the right atrium than in the left. RIA quantitatively confirmed this observation showing a 2-3 times greater concentration of ANF in the right atrium than in the left (Gutkowska, 1987). Immunocytochemical and RIA techniques were used to investigate ANF in other tissues such as the anterior pituitary, lung, aortic arch (Gardner et al 1986), brain (Tanaka et al., 1984), eye (Stone and Glembotski, 1986), and kidney (Sakamato et al., 1985). In the white rat, the levels in these tissues were quite low (in the lower ng ANF/g tissue range) compared with cardiac levels (in the μg ANF/g tissue range), therefore the cardiac tissue levels of ANF are considered to be the major source of this hormone. For this reason, only cardiac levels of ANF were measured in the Mongolian gerbil.

Sequencing of the ANF peptide revealed a high degree of homology among the various species which have been analyzed (Vlasuk et al 1986; Forssmann et al., 1984; Oikawa et al., 1985). In fact, human and rat ANF gene sequences showed a 90% homology among coding sequences (Nemer et al., 1984) with only a single amino acid difference in the active 28 amino acid form of the hormone (Kangawa and Matsuo, 1984; Thibault et al., 1984). However, there is an unusual difference between the human and rat sequences in the N-terminal portion. The human sequence lacks the arg-arg dipeptide which is a common site of cleavage in many prohormones. The reason for this difference is unknown (Oikawa et al., 1984; Maack et al., 1985). This high degree of homology in ANF sequence among species leads to the assumption of homology existing between the Mongolian gerbil and the white rat. Commercially, there are two ANF RIA's available - rat and human. The white rat and the Mongolian gerbil are more closely related than the human and the Mongolian gerbil, therefore the rat ANF RIA was selected for the present investigation.

Sodium stress did not have a significant effect on the atrial ANF levels in the Mongolian gerbil in the present study. This is different from the white rat, in which a high sodium diet not only decreases cardiac levels of ANF (Takayanagi et al., 1985; Schwartz et al., 1986) but also decreases the number of atrial granules (Marie et al., 1976 [cited by Cantin et al., 1984]). If ANF was being released in the Mongolian gerbil for sodium regulation, then a drop in the atrial level of the hormone would be expected for the high sodium regimen, and an increase in atrial level for the low sodium regimen would be predicted. Because there is no change in the cardiac level of ANF under sodium stress in the present study, it appears that the Mongolian gerbil is not releasing higher or lower levels of this hormone. Therefore, a possible conclusion is that this animal does not place a great importance on the ANF system for osmoregulation.

As the results indicate (Table 3), the Mongolian gerbil's atrial level of ANF was similar to that in the Syrian golden hamster, another desert mammal (7.4 ± 1.5 µg ANF/2 atria in the Mongolian gerbil and 8.4 ± 0.5 µg ANF/2 atria in the Syrian golden hamster). The literature value for the Syrian golden hamster was similar at 3.3 µg ANF/2 atria (Cantin et al., 1988). These two desert mammals have comparable abilities in osmoregulation of high sodium chloride in their drinking water (Hagood, 1982). In addition, these two animals have shown similar behavior following adrenalectomy, in that their survival time was minimal despite receiving sodium chloride in the drinking water. However, the Syrian golden hamster, unlike the Mongolian gerbil, has been kept alive indefinitely with adrenocorticoid supplements (Snyder and Wyman, 1951; Gaunt et al., 1971). This suggests that the Mongolian gerbil uses a hormone other than aldosterone or cortisol for osmoregulation (Baggia, 1983) and as one interpretation of this investigation might suggest, other than ANF.

Comparison of the the Mongolian gerbil with the white rat revealed an unexpected result. The present study demonstrated that the white rat had an atrial ANF level three to four times greater (28.5 ± 7.4 µg ANF/2 atria) than the Mongolian gerbil (7.4 ± 1.5 µg ANF/2 atria). Gutkowska (1987) found a level in the white rat (20 µg ANF/2 atria) similar to the level found in the present study. The difference in ANF levels between the Mongolian gerbil and the white rat could be considered unusual as they are not greatly divergent species.

The fact that atrial ANF did not change under sodium stress in the present study suggests that ANF is relatively unimportant in this animal. Another possible interpretation of the results is that the Mongolian gerbil has a very high synthesis of ANF to keep pace with the release of the hormone into the bloodstream. This hypothesis would explain the lack of significant change in atrial ANF concentration observed under sodium stress. Future study of ANF levels in the plasma would help provide the answer to this possibility. In addition, the Mongolian gerbil may have a more efficient release of the hormone into the bloodstream or a more efficient utilization of it at the receptors in the kidney than the white rat. If this were the case, a lower concentration of ANF in the Mongolian gerbil would be necessary to elicit an equal response in the white rat.

In summary, the present investigation identified atrial ANF in the Mongolian gerbil and quantified it at a level of 388 $\pm$ 66 µg ANF/g tissue. Sodium stress was found to have no effect on the atrial level of this hormone. The Mongolian gerbil was found to have an atrial level of ANF similar to that of another desert mammal, the Syrian golden hamster but low compared with a non-desert mammal, the white rat. This research suggests that ANF is not involved in the osmoregulation of the Mongolian gerbil or the possibility of a more efficient ANF system at various possible levels in this animal. Future research, investigating the plasma ANF concentrations under sodium stress, would be a clear next step in understanding the role of ANF in the osmoregulation of the Mongolian gerbil. Additional studies might also monitor the consumption of sodium in drinking water and in the lab chow as well as monitor the amounts of sodium excreted.

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Table 1. A comparison of ANF concentrations in cardiac atrial and ventricular tissue of the Mongolian gerbil.*

N	Body Weight (g)	μg ANF/g tissue	
		atria	ventricle
1	74.5	475	0.00
2	71.0	377	0.03
3	71.5	358	0.01
4	76.5	369	0.00
5	72.5	455	0.00
6	66.5	295	0.01
7	76.0	---**	0.00
		$x = 388 \pm 66$	$x < 0.01$

*animals provided tap water *ad libitum*

**sample lost to denaturation, value not included in calculation of mean

Table 2. The effect of sodium stress on concentrations of atrial ANF in the Mongolian gerbil. Unpaired t-test performed at 95% confidence limits indicate no statistical difference between groups.

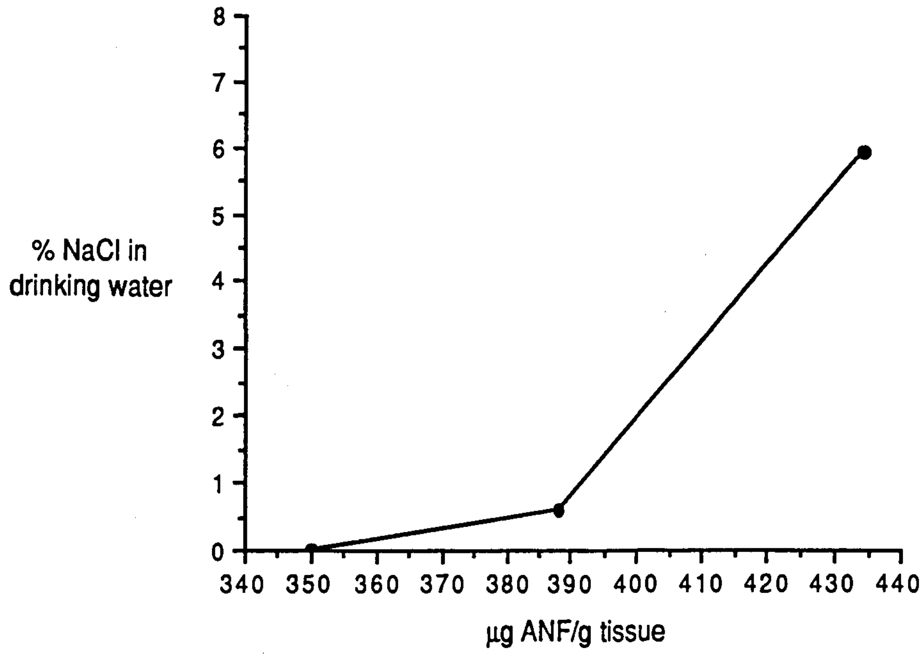
Group	µg ANF/g tissue (atria)	x	SD	t-value
Control	475	388	66	--
(tap water)	377			
	358			
	369			
	455			
	295			
Low Sodium	304	350	75	1.3
(deionized)	259			
	266			
	409			
	442			
	355			
	416			
High Sodium	345	434	69	0.8
(6% NaCl)	496			
	437			
	373			
	525			
	425			

Table 3. A comparison of atrial ANF concentrations in the Mongolian gerbil, the Syrian golden hamster, and the white rat. Unpaired t-test performed at 95% confidence limits indicates that the value for the Mongolian gerbil is significantly lower than the white rat, but is not significantly different from the Syrian golden hamster.*

Animal	μg ANF/ g tissue	μg ANF/ 2 atria	t-value	Lit. value (μg ANF/2 atria)
Mongolian gerbil N=7	293 ± 43	7.4 ± 1.5	--	N/A
Syrian golden hamster N=2	334 ± 20	8.4 ± 0.5	1.0	3.3 (Cantin et al.,1988)
White rat N=5	632 ± 160	28.5 ± 7.4	5.7	20 (Gutkowska, 1987)

*animals provided tap water *ad libitum*

Figure 1. The effect of sodium chloride in drinking water on atrial ANF in the Mongolian gerbil.


% NaCl in drinking water
0
1
2
3
4
5
6
7
8
340 350 360 370 380 390 400 410 420 430 440
µg ANF/g tissue

VITA

Kyriakos Tarasidis was born May 8, 1967, in Danville, Virginia. He attended Wake Forest University, Winston-Salem, North Carolina, where he majored in Biology and received the degree of Bachelor of Science in May, 1989. With a constant interest in biology, he pursued a graduate degree in biology at the University of Richmond. He completed the requirements for the Master of Science Degree in July, 1991.