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The stability of norepinephrine (NE) and epinephrine (EPI) during long-term storage at -70 C was analyzed in plasma from humans, canines, and rats. In human plasma, heparin and EDTA as anticoagulants, in combination with reduced glutathione and EGTA (GSH/EGTA) as a preservative, were compared. Canine plasma was heparinized and contained GSH/EGTA as a preservative. Rat plasma was heparinized without the addition of preservative. Norepinephrine and EPI levels were measured by high pressure liquid chromatography (HPLC) with electrochemical detection. Optimal storage conditions for NE and EPI in human plasma require heparin as an anticoagulant without the addition of GSH/EGTA as a preservative. Norepinephrine and EPI in human plasma were stable for approximately 18 months (550 days). In heparinized canine plasma and heparinized rat				
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19. plasma, NE and EPI were stable for 6 months. We conclude that simple heparinization of human, canine, and rat plasma provides optimal storage conditions for quantitation of the in vivo concentration of NE and EPI. In addition, canine plasma cannot be stored for the extended periods of time demonstrated with human plasma.

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TABLE OF CONTENTS

	<u>Page No.</u>
Acknowledgements	iv
Introduction	1
Materials and Methods	2
Results	4
Discussion	6
References	8
Figure 1	10
Figure 2	11
Figure 3	12
Figure 4	13
Figure Legends	14

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INTRODUCTION

Plasma catecholamine measurements are important in the diagnosis and management of nervous system related disorders (1, 2), hypertension (3, 4), pheochromocytoma (5, 6), shock and/or trauma (7, 8). Since much research in critical care medicine is directed toward patient resuscitation following shock and/or trauma, the relevance of changes in plasma catecholamine content is evident. Catecholamine stability has been of particular concern when determining quantitative levels (7). The stability of catecholamines in plasma and whole blood must be maintained since the chemical structure of the catechol ring is susceptible to spontaneous oxidation.

We have previously reported that the addition of heparin to human plasma provided for optimal conditions for quantitation of in vivo concentration of NE and EPI for up to 24 hours at room temperature (9). In addition, NE and EPI were stable at -70 C in heparin or EDTA plasma for up to eight months. Canine plasma catecholamines with heparin as an anticoagulant were relatively stable after 24 hours at room temperature and after long-term storage (approximately 4 months) at -70 C (10).

We have extended these initial long-term degradation studies because it was apparent, owing to logistical and technical difficulties, that sample extraction and quantitation of catecholamines could be delayed longer than anticipated. We have also measured catecholamine degradation in heparinized rat plasma during long-term storage at -70 C.

MATERIALS AND METHODS

Animals:

Twelve purpose-bred healthy male Beagles (1 to 2 years old, 10 to 12 kg) were phlebotomized as part of an ongoing study at the Armed Forces Radiobiology Research Institute (AFRRI). Dogs were housed in an AAALAC accredited facility and provided commercial dog chow and tap water ad libitum. Male Crl:CDBR rats (250-300 gm), also part of an ongoing study at AFRRI, were used. Rats were housed in an AAALAC accredited facility in microisolator cages and provided with commercial food pellets and acidified tap water (pH 2.5) ad libitum. Animal holding rooms were maintained at 20 C with 50%±10% relative humidity, using at least 10 air changes per hour of 100% conditioned fresh air. The animals were maintained on a 12-hour lighting cycle with no twilight.

Sample collection:

Human: Blood samples were collected from non-fasted healthy women and men, ages 25-55 years. Venous blood (60 ml) was drawn into tubes containing either sodium EDTA (4 mmol/L) or heparin (14.3 USP units/ml) as anticoagulant. All blood samples were centrifuged and aliquoted within one hour of collection.

Dog: Blood was collected by venipuncture from the lateral saphenous vein into syringes containing EDTA (4 mmol/L) or heparin (14.3 USP Units/ml). All blood samples were centrifuged within one hour of collection.

Rat: Animals were anesthetized with halothane (to effect) and exsanguinated by means of cardiac puncture. Blood was heparinized (14.3 USP Units/ml) and centrifuged within one hour of collection.

Blood samples were centrifuged at 3000 rpm (1000 x g) for 10 minutes at 4 C to pellet cellular elements and platelets. The plasma supernatant was aliquoted (1 ml) into 1.5 ml microcentrifuge tubes with or without EGTA (8 mmol/L final concentration) and glutathione (GSH, reduced form, 6.5 mmol/L final concentration) as a preservative. Samples were frozen immediately at -70 C for analysis after long-term storage. Plasma pools were different for the various experiments. The inherent individual variability of NE and EPI values we observed accounted for differences in the initial baseline values of pooled samples. All plasma collected for both human and animal studies were samples provided from ongoing protocols.

Catecholamine extraction and quantitation:

Plasma samples were extracted as previously described (11). Alumina adsorption of catecholamines was complete after 30 minutes at room temperature. After washing the adsorbed alumina, we released catecholamines by adding 100 μ l acetic acid containing 0.05% EDTA and 0.1% sodium disulfite. To determine extraction efficiency and quantitate catecholamine levels, we used 3,4-dihydroxybenzylamine as an internal standard. Samples were assayed by HPLC with electrochemical detection and computer

analyzed. The extraction and HPLC methods allow for reliable detection of NE and EPI levels greater than 20 pg/ml. The intra-assay coefficient of variation was 5% for NE and 8% for EPI. Assays were run in triplicate. Data are reported as pg/ml $\pm$ standard error. The conversion factor for expressing NE as nmol/L is 0.00591 and for expressing EPI as pmol/L is 5.458. Statistical differences were determined by analysis of variance with the Duncan test between means for repeated measures.

RESULTS

Human: Norepinephrine and EPI plasma values were relatively stable for up to 18 months when stored at -70 C with heparin as the anticoagulant ($p>0.1$) (Fig. 1). There was no apparent requirement for the addition of GSH/EGTA as a preservative (Fig. 1, Table 1). With EDTA as the anticoagulant, NE decayed at a faster rate ($p<0.01$) (Fig. 2). There was negligible degradation of EPI with extended storage when EDTA was used as the anticoagulant ($p>0.2$) (Fig. 2). Decay rates of NE and EPI in plasma during long-term storage were determined by linear regression analysis (Table 1). A negative slope was indicative of catecholamine decay (pg/day $\pm$ SE).

Table 1. Decay rate of norepinephrine (NE) and epinephrine (EPI) from heparinized and EDTA blood following long-term storage at -70 C.

PLASMA	GSH/EGTA*	DECAY RATE, pg/d $\pm$ SE**
Heparin		
NE	-	-0.13 $\pm$ 0.08
	+	0.02 $\pm$ 0.02
EPI	-	-0.004 $\pm$ 0.180
	+	-0.075 $\pm$ 0.098
EDTA		
NE	-	-0.27 $\pm$ 0.06
	+	-0.20 $\pm$ 0.05
EPI	-	0.021 $\pm$ 0.020
	+	0.018 $\pm$ 0.015

*Minus sign indicates plasma without 6.5 mmol/L GSH and 8 mmol/L EGTA; plus sign indicates plasma with GSH and EGTA.

**Decay rates were determined by linear regression analysis.

Dog: Following long-term storage at -70 C for 6 months (200 days), there was no degradation of NE and EPI in heparinized samples containing preservative (Fig. 3). However, increasing the length of storage beyond 200 days resulted in degradation of both NE and EPI. After 397 days' storage, the mean decay rate for NE was 0.22 ± 0.2 pg/day ($p < 0.05$) and 0.17 ± 0.05 pg/day ($p < 0.05$) for EPI (Table 2).

Table 2. Decay rates of plasma norepinephrine (NE) and epinephrine (EPI) from heparinized blood of rats and canines.*

		DECAY RATE, pg/d $\pm$ SE**
Canine*		
	NE	-0.22 $\pm$ 0.08
	EPI	-0.17 $\pm$ 0.06
Rat		
	NE	-0.22 $\pm$ 0.33
	EPI	0.42 $\pm$ 0.44

*Canine blood contained reduced glutathione and EGTA (GSH/EGTA) as preservative. No preservative was added to rat blood.

**Decay rates were determined by linear regression analysis.

Rat: Norepinephrine and EPI in pooled heparinized rat plasma stored without the addition of preservative was stable for 6 months at -70 C (Fig. 4). The NE decay rate was -0.22 ± 0.33 pg/day ($p > 0.05$). There was no apparent change in EPI, as evidenced by a slope of 0.42 ± 0.44 pg/day ($p > 0.05$) (Table 2).

DISCUSSION

We initially reported that there was no degradation of human (9) or canine (10) NE and EPI with long-term storage of eight and four months, respectively, in heparinized plasma. Optimal storage conditions for human plasma samples require the addition of heparin as anticoagulant and separation of plasma from blood cellular elements within six hours of collection (9). In the canine, there was no degradation of plasma catecholamines if plasma was separated from heparinized blood within six to eight hours of collection (10). Decay rates were determined during

long-term storage of human and canine plasma catecholamines to better estimate deviations from in vivo values with extended storage. EDTA, when used as an anticoagulant for human plasma, was not as efficacious as heparin in preventing catecholamine degradation. We have extended these long-term studies with human and canine plasma to 18 and 13 months, respectively. Our results indicate that NE and EPI in heparinized human plasma is less susceptible to degradation with long-term storage than NE and EPI in heparinized canine plasma. In addition, NE and EPI in heparinized rat plasma are stable at -70 C for approximately six months.

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FIGURE 1

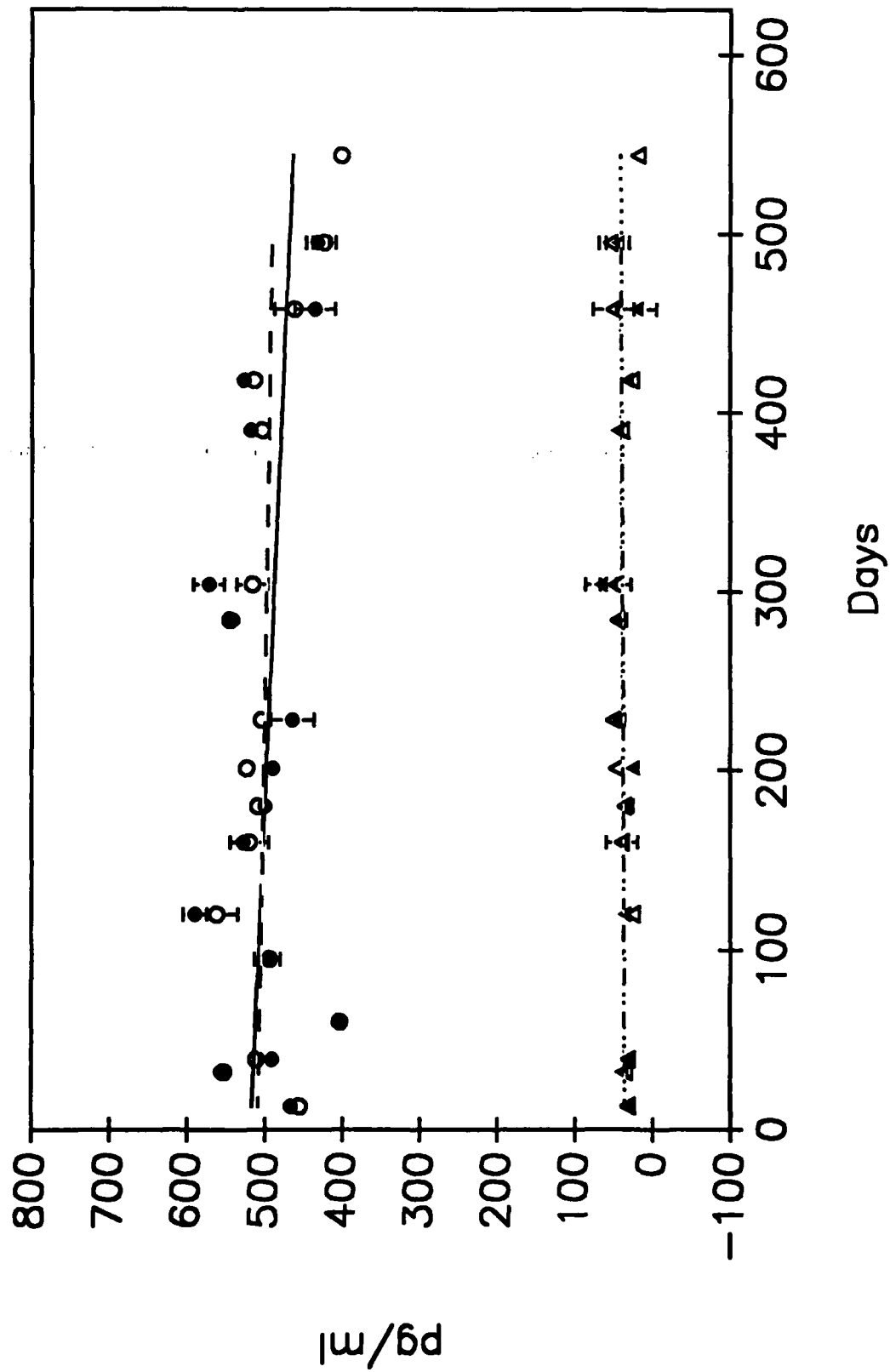


FIGURE 2

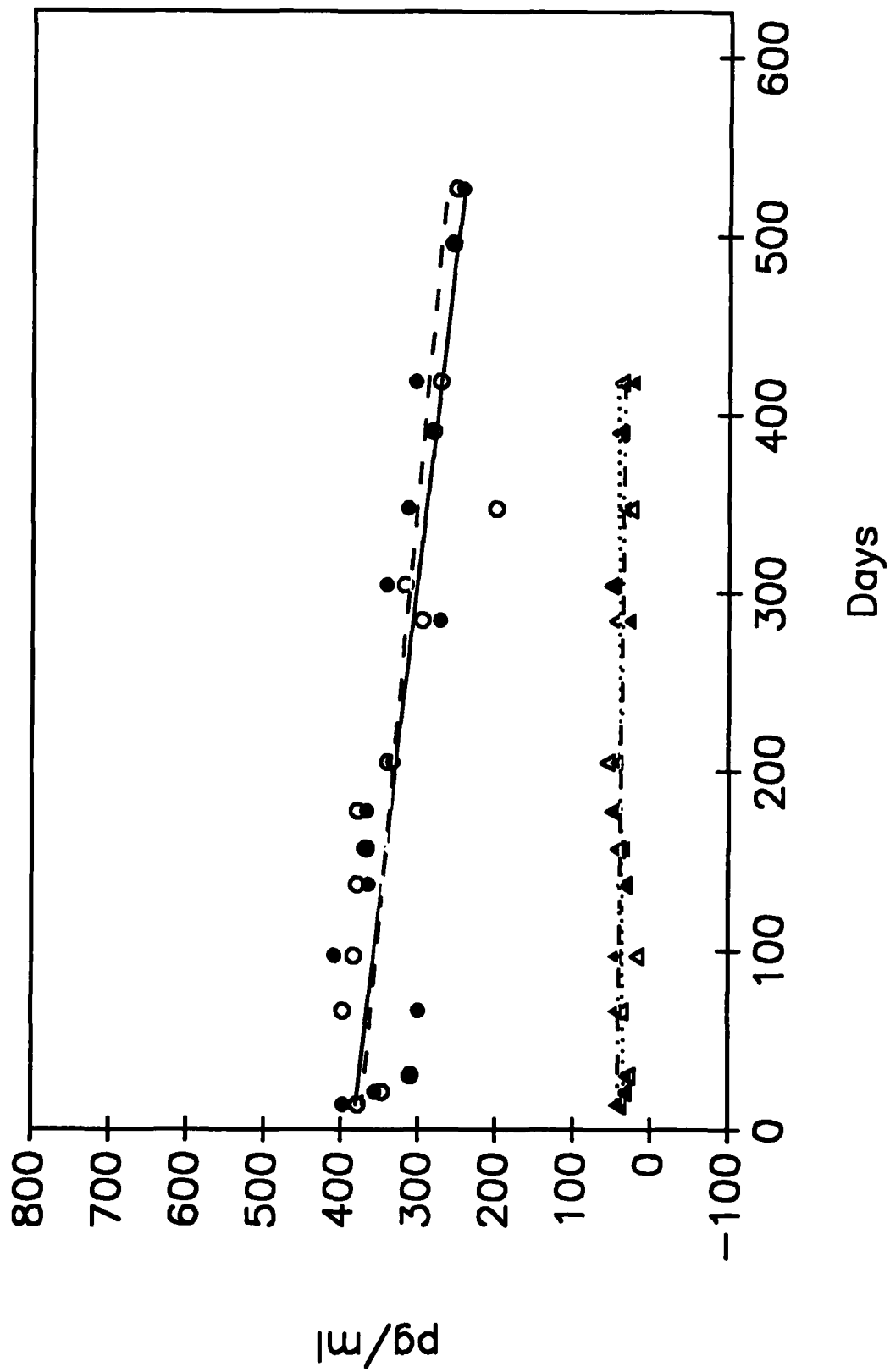


FIGURE 3

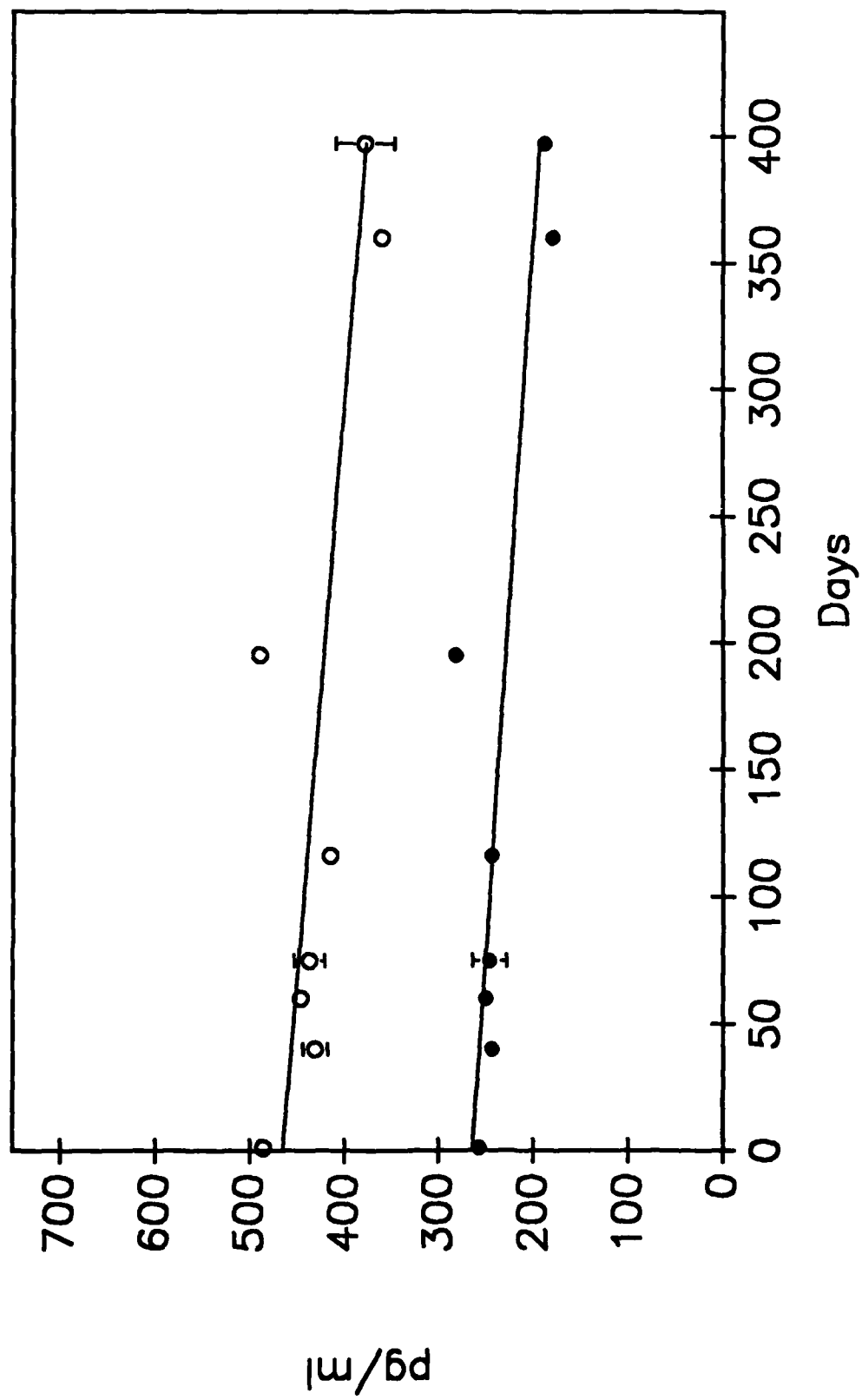


FIGURE 4

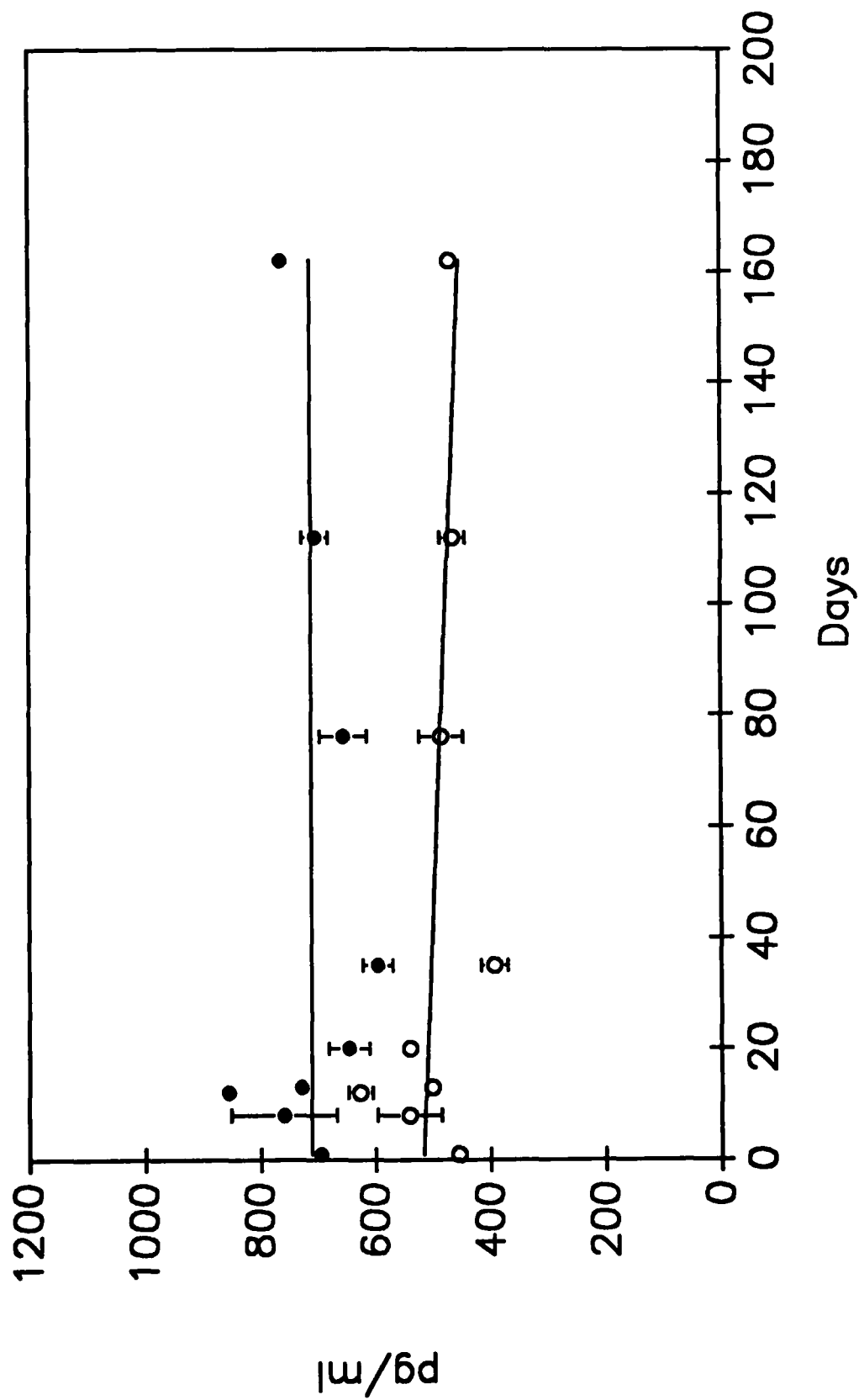


FIGURE LEGENDS

Figure 1. Plasma norepinephrine (NE) and epinephrine (EPI) levels from heparinized human blood following long-term storage at -70 C with and without reduced glutathione and EGTA (GSH/EGTA) as preservative. NE without GSH/EGTA (open circles); NE with GSH/EGTA (closed circles); EPI without GSH/EGTA (open triangles); EPI with GSH/EGTA (closed triangles).

Figure 2. Plasma norepinephrine (NE) and epinephrine (EPI) levels from EDTA-treated human blood following long-term storage at -70 C with and without reduced glutathione and EGTA (GSH/EGTA) as preservative. NE without GSH/EGTA (open circles); NE with GSH/EGTA (closed circles); EPI without GSH/EGTA (open triangles); EPI with GSH/EGTA (closed triangles).

Figure 3. Changes in canine plasma norepinephrine (NE) and epinephrine (EPI) levels during long-term storage at -70 C. Samples were pooled heparinized plasma containing reduced glutathione and EGTA (GSH/EGTA) as preservative. NE (open circles); EPI (closed circles).

Figure 4. Changes in rat plasma norepinephrine (NE) and epinephrine (EPI) levels during long-term storage at -70 C. Pooled heparinized plasma samples contained no preservative during storage. NE (closed circles); EPI (open circles).