SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188

1a REPORT SECURITY CLASSIFICATION (U)	1b RESTRICTIVE MARKINGS NA
2 SECURITY CLASSIFICATION AUTHORITY NA	3 DISTRIBUTION/AVAILABILITY OF REPORT Distribution Unlimited
2b DECLASSIFICATION/DOWNGRADING SCHEDULE NA	
4 PERFORMING ORGANIZATION REPORT NUMBER(S) Uniformed Services University of the Health Sciences	5 MONITORING ORGANIZATION REPORT NUMBER(S) NA
6a NAME OF PERFORMING ORGANIZATION Uniformed Services Univ. of the Health Sciences 6b OFFICE SYMBOL (If applicable) NA	7a NAME OF MONITORING ORGANIZATION Office of Naval Research
6c ADDRESS (City, State, and ZIP Code) Department of Surgery 4301 Jones Bridge Road Bethesda, Maryland 20814-4799	7b ADDRESS (City, State, and ZIP Code) 800 North Quincy Street Arlington, VA 22217-5000
8a NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research 8b OFFICE SYMBOL (If applicable) ONR	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N0001488WM24020
8c ADDRESS (City, State, and ZIP Code) 800 North Quincy Street Arlington, VA 22217-5000	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 61153N PROJECT NO: RR04108 TASK NO: 441f83301 WORK UNIT ACCESSION NO:

11 TITLE (Include Security Classification)

Cachectin/Tumor Necrosis Factor and the Pituitary-Adrenal Axis

12 PERSONAL AUTHOR(S)
Diana S. Malcolm, Ph.D.

13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Final	FROM 4/88 TO 12/89	90/2/28	19

16 SUPPLEMENTARY NOTATION

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
08			Tumor Necrosis Factor, Cachectin, ACTH/Corticosterone thyroglobulin/macrophage/LPS/dexamethasone/cAMP

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Results from our studies investigating immune-neuroendocrine interactions have yielded interesting and novel findings. In unanesthetized rats, TNF (10ug/kg) was a potent stimulus for ACTH release without affecting hemodynamics. In vitro, TNF was without effect on the basal secretion of corticosterone (CS) but inhibited ACTH-stimulated CS release. Likewise, TNF inhibited TSH-stimulated thyroglobulin release from cultured human thyroid cells. In vitro, ACTH tended to inhibit LPS-induced TNF release from cultured macrophages, however, in the presence of serum-free media, ACTH appeared to potentiate TNF release. Also, the dose-response curves for CS and dexamethasone (inhibitors of TNF release) were shifted to the right. Together these findings suggest that factors present in sera and absent in serum-free media may interfere with LPS binding and/or uptake into macrophages. Results from these studies have provided additional knowledge and insight into the bidirectional communication between the neuroendocrine and immune systems.

20 DISTRIBUTION/AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	(U)
22a NAME OF RESPONSIBLE INDIVIDUAL Dr. Jeannine A. Majde	22b TELEPHONE (Include Area Code) (202) 696-4055 22c OFFICE SYMBOL ONR

DD Form 1473, JUN 86 Previous editions are obsolete SECURITY CLASSIFICATION OF THIS PAGE

S/N 0102-LF-014-6603

FINAL REPORT

Cachectin/Tumor Necrosis Factor and the Pituitary-Adrenal Axis

ONR Contract No. N00014-88-WM-24020

Brief Statement of Objectives:

Several lines of evidence indicate that the immune system by way of macrophage hormones can influence neuroendocrine function and vice versa suggesting that these two systems are involved in a complete regulatory feedback loop. The purpose of our research studies was to determine whether the monokine TNF can alter pituitary ACTH and adrenal corticosterone release in vivo and in vitro and further to determine whether these neuroendocrine hormones can alter TNF release from macrophages in culture.

Specific Research Objectives:

1. Determine what effect Tumor Necrosis Factor (TNF) has on ACTH and corticosterone secretion in vivo in unanesthetized rats.

2. Determine what effect TNF has on the basal and stimulated release of ACTH and corticosterone from cultured pituitary and adrenal cells, respectively.

3. Determine whether ACTH and/or glucocorticoids (corticosterone, dexamethasone) can alter endotoxin-induced TNF release from cultured macrophages.

Research Results:

The first year of this project was devoted to the in vivo studies (Research Objective #1), as well as, a part of the in vitro studies involving cultured adrenal cells (Research Objective #2). Based on our observations of TNF's actions on adrenal cells, we also explored the effects of TNF on cultured human thyroid cells, specifically, the effects of TNF on TSH-induced thyroglobulin and cAMP production.

The second year of this project was largely devoted to the in vitro studies involving cultured macrophages (Research Objective #3). In addition to studying the effects of ACTH, corticosterone and dexamethasone on LPS-induced TNF release, we sought to determine whether these effects were altered when the macrophages were cultured with media (HL-1, Ventrex Corp) which was devoid of fetal calf serum.

We report here the results of our studies which are both interesting and new. In addtion, results from these studies have provided possible insights into how the immune system by way of macrophage-derived peptides may regulate endocrine function, and conversely, how pituitary-adrenal hormones ativated during infection or stress may act to regulate the release of TNF and thus modulate immune function.

1. Effects of recombinant human TNF-alpha on plasma levels of ACTH and corticosterone in the unanesthetized rat. (Figure 1 and 2)

TNF at doses of 0.01, 0.03 and 0.10 mg/kg was injected as a bolus into unanesthetized rats. Within 15 minutes of TNF injection, plasma ACTH levels were

maximal and not statistically different between the various doses of TNF administered. Thus, it appears that TNF induces a maximal ACTH response at 0.01 mg/kg which is not further elevated even with a ten-fold greater TNF dose (0.10 mg/kg). Although this dose of TNF did not produce significant changes in hemodynamics (data not shown) and was not associated with mortality, it appears to be a potent dose for neuroendocrine stimulation.

As expected, plasma corticosterone levels were elevated following TNF injection. This rise in plasma corticosterone is most likely ACTH-mediated, however, it is unknown from these in vivo studies alone whether TNF has direct effects on the adrenal gland to stimulate corticosterone release apart from or in addition to ACTH. Our in vitro experiments have attempted to answer some of these questions.

2. Effects of recombinant human TNF-alpha on basal and stimulated corticosterone release from cultured adrenal cells. (Table 1)

TNF alone at all doses tested (100, 300, 1000 ug/ml) had no effect on baseline corticosterone release from cultured adrenal cells. However, TNF clearly inhibited ACTH stimulated corticosterone secretion. This inhibition was reproducible and consistent and is seen at concentrations of TNF similar to that reported in patients with sepsis and with AIDS. There was no difference in cell number or viability following TNF application with or without ACTH present, indicating that the inhibitory effects of TNF are not due to cytotoxicity. This finding represents a significant and new interaction between the immune and endocrine systems.

3. Effects of recombinant human TNF-alpha on basal and stimulated thyroglobulin and cyclic AMP release from cultured thyroid cells (Tables 2 and 3).

Based on our observation of TNF's inhibitory actions on adrenal cells, we explored the effects of recombinant human TNF on human thyroid cells. TNF inhibited TSH stimulated thyroglobulin secretion from cultured thyroid cells in a dose-dependent manner. In all experiments, TSH exposure resulted in a brisk increase in cAMP production. However, even at the highest concentration, TNF had no effect on TSH stimulated cAMP production. This suggests that TNF's inhibition of TSH-stimulated thyroglobulin secretion is not mediated through cAMP.

4. Effects of ACTH on LPS-induced TNF release from cultured macrophages (Table 4 and Figure 3 & 4).

LPS (E. coli K235) at doses of 1,10 and 100 ug x 10^{-4} induced a biphasic dose-response effect on TNF release from macrophages cultured in the presence of Fetal Calf Serum (FCS) (Table 4). Maximal TNF release was elicited by the 10 ug x 10^{-4} LPS dose, and this LPS dose was subsequently used to assess the effects of ACTH on stimulated TNF release from macrophages.

In the presence of FCS, ACTH appeared to inhibit LPS-stimulated TNF release (Table 4, Figure 3). However, judging from the 95% confidence interval associated with each mean value, there was no statistical difference between treatment groups. Since each value represents the mean of just 4-6 experiments, data from more experiments may show statistical significance between treatment groups.

Interestingly, macrophages cultured in HL-1 media (100x Concentrate; Ventrex Labs, Inc., Portland, ME) instead of FCS and challenged with LPS, exhibited a more linear dose-response in TNF release (Table 4). The biphasic response to LPS was not seen in macrophages cultured with HL-1.

In contrast to the observed effects of ACTH on macrophages cultured with FCS, in the presence of HL-1, ACTH appeared to potentiate LPS-stimulated TNF release (Figure 4). Because of the small sample size, statistical significance was not achieved, however, the trends in both treatment groups appear to be quite different suggesting that factors present in FCS may influence LPS-induced TNF release and its modulation by peptides.

5. Effects of Corticosterone on LPS-induced TNF release from cultured macrophages (Table 5)

As expected, corticosterone inhibited LPS-induced TNF release in a dose-related manner. Corticosterone's inhibitory effect appeared to be also present in the absence of FCS, however, owing to the small sample size this inhibitory effect was not statistically proven. The dose-response curve for corticosterone appeared to be shifted to the right in HL-1 cultured macrophages as compared to those cultured with 2% FCS.

6. Effects of Dexamethasone on LPS-induced TNF release from cultured macrophages (Table 6 and Figures 5 & 6)

Dexamethasone also inhibited LPS-stimulated TNF release from cultured macrophages, however, dexamethasone appeared to be a more potent TNF inhibitor than corticosterone when compared at the same molar concentrations (Tables 5 & 6). Dexamethasone's inhibitory effects were also present in the HL-1 cultured macrophages, however, the dose-response curve like that of corticosterone appeared to be shifted to the right in the presence of HL-1.

Summary and Conclusions

Results from our studies investigating immune-neuroendocrine interactions have yielded several interesting and novel findings. In unanesthetized rats, TNF at a dose of 0.01 mg/kg was a potent stimulus for ACTH release. A dose of 0.1 mg/kg TNF did not further elevate this maximal ACTH response. Following TNF injection, plasma corticosterone was also elevated and most likely this release was mediated through ACTH since TNF was without effect on the release of corticotserone from cultured adrenal cells. At all doses tested, TNF was without significant effect on mean arterial pressure, heart rate or pulse pressure suggesting that TNF's effect on pituitary ACTH release was not secondary to a cardiovascular effect. Also our results indicate that TNF exerts a more potent effect on the pituitary-adrenal axis than on the cardiovascular system.

Interestingly, in vitro TNF inhibited ACTH-stimulated corticosterone release. It is unknown whether this inhibition also occurs in vivo, however, it is interesting to speculate that TNF may modulate the actions of ACTH in addition to influencing ACTH release. Glucocorticoids are potent inhibitors of TNF release both in vitro and in vivo and it is possible that TNF may influence corticosterone release (via ACTH) as part of a regulatory feedback loop.

TNF was also found to be a potent inhibitor of TSH-mediated thyroglobulin release from cultured human thyroid cells. The effects of TNF on the pituitary-thyroid axis are largely unknown. Clinically it has been observed that TNF is

elevated during medical conditions that are associated with the "sick euthyroid syndrome". One of the hallmarks of the "sick euthyroid syndrome" is the apparent suppression of thyroid hormone release in the face of adequate TSH stimulation. In other words, the thyroid gland's response to TSH is suppressed. It is interesting to speculate based on our observations that TNF may contribute to the thyroid gland suppression present in the "sick euthyroid syndrome" and warrants further study.

Data from our cultured macrophage studies suggest that ACTH may act to inhibit LPS-stimulated TNF release. If ACTH does influence macrophage secretion of TNF, this would imply that both the pituitary gland (ACTH) and adrenal gland (corticosterone) participate in the regulation of macrophage TNF release. This does not exclude the possibility that extra-pituitary sources of ACTH may likewise be involved. As expected, both corticosterone and dexamethasone potently inhibited TNF release in response to LPS.

Our studies using HL-1, a serum-free media, provided curious and new insights into the possible effects of serum factor(s) on LPS-induced TNF release. The LPS dose-response curve in macrophages cultured with serum-free media was linear and plateaued at the highest concentrations rather than being biphasic. The dose-response curves for both corticosterone and dexamethasone were shifted to the right compared to studies run using fetal calf serum in the tissue culture media. Most interesting was the observation that ACTH no longer appeared to inhibit but rather potentiated LPS-induced TNF release from macrophages cultured with serum-free media. These findings suggest that factor(s) present in sera and absent in HL-1 media may act to inhibit LPS-induced TNF release. It is unknown whether these factor(s) interfere with LPS binding and/or uptake into macrophages or whether they bind or restrict the activity of other modulators of TNF release (e.g. other peptides, hormones, calcium).

Publications

Poth, M., Tseng, Y.L., Wartofsky, L. Does Cachectin mediate altered thyroid function in systemic illness? A cell culture model. Presented at the 1989 Endocrine Society meeting.

Brennan, M.J., Betz, J.A., Poth, M. Tumor Necrosis Factor inhibits ACTH stimulated corticosterone secretion by rat adrenal cortical cells. Presented at the 1989 Endocrine Society meeting.

Malcolm, D.S., Poth, M. Tumor Necrosis Factor (TNF) and the pituitary-adrenal axis: In vivo and in vitro studies. Circulatory Shock (accepted).

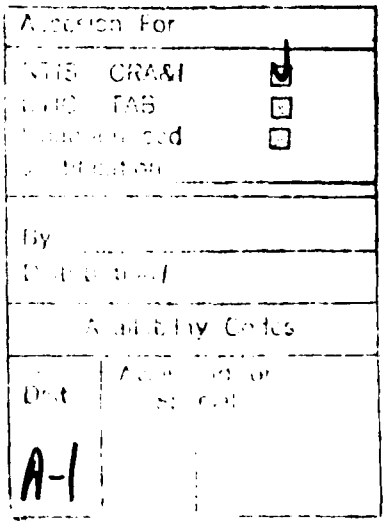


Figure 1. Effects of TNF on plasma levels of ACTH

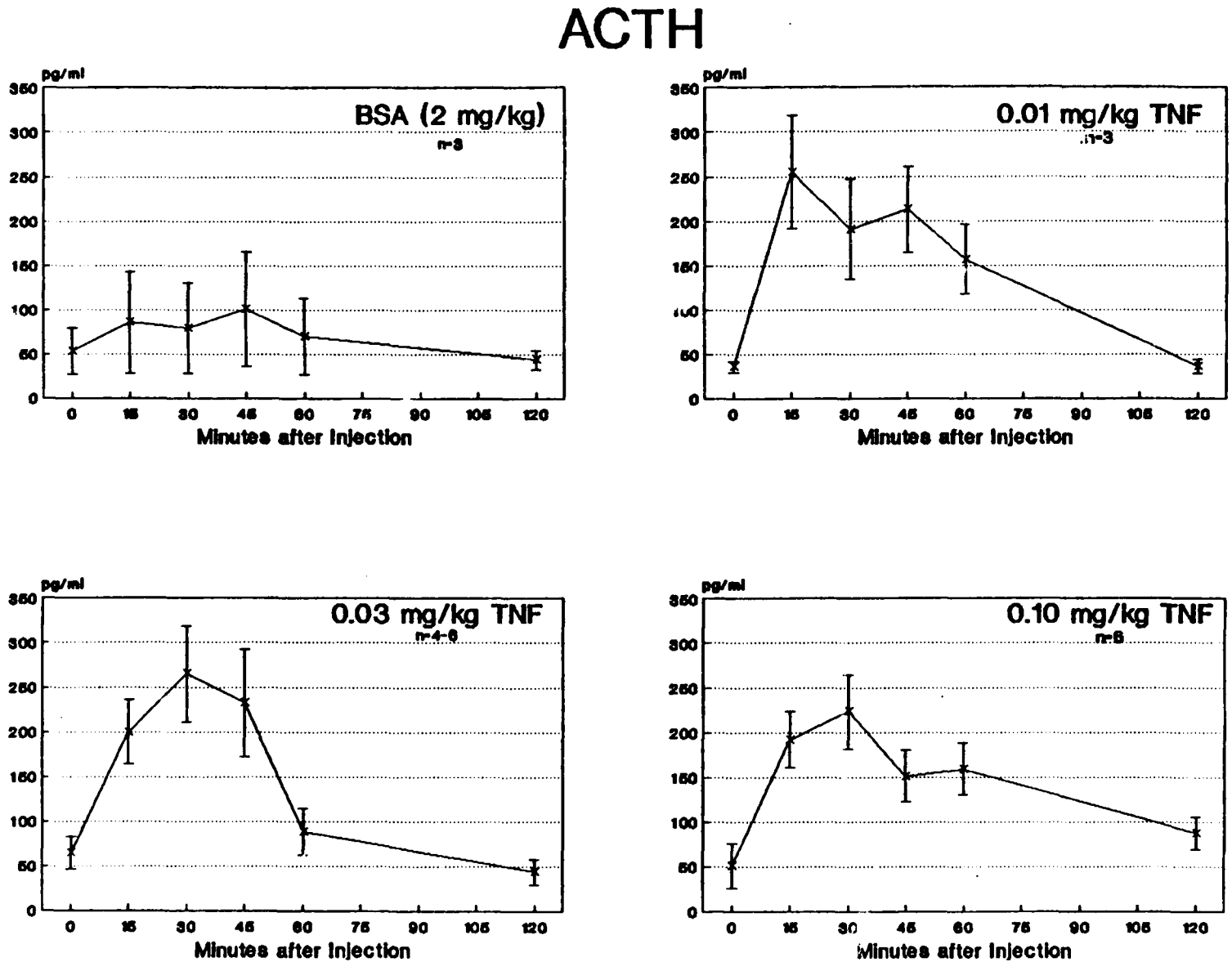


Figure 2. Effects of TNF on plasma levels of corticosterone

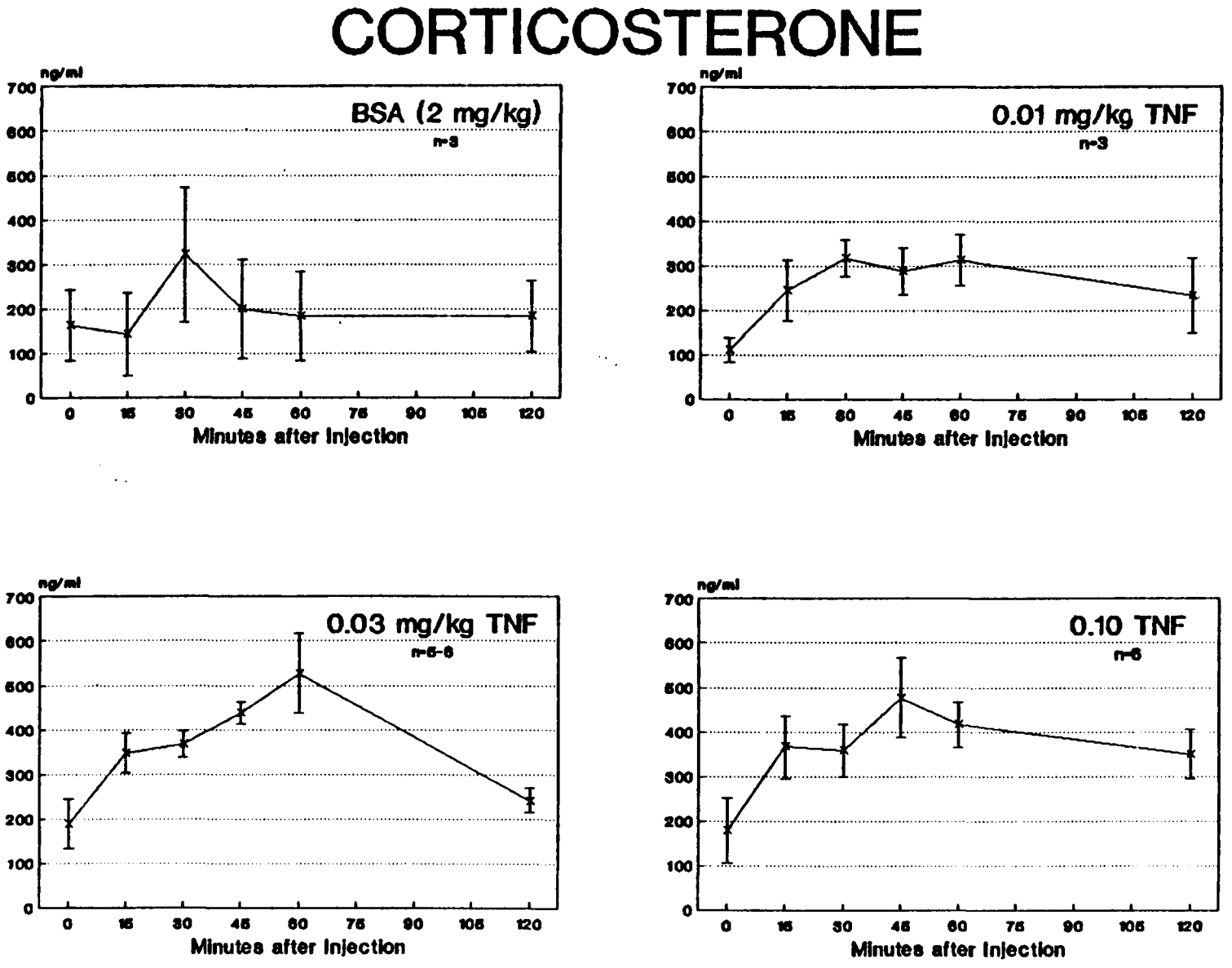


TABLE 1. Effects of TNF on stimulated corticosterone release

		Corticosterone secreted (ng/well)	
ACTH	TNF (ug/ml)	Experiment I	Experiment II
0	0	14 ± 2	6.4 ± 1.2
30	0	41.7 ± 7.8	16.9 ± 5.3
100	0	77.3 ± 6.2	63.0 ± 2.0
30	100	2.6 ± .20	8.1 ± 5.3
30	300	.28 ± .03	
30	1000	.31 ± .02	3.6 ± .20
100	100	1.21 ± .17	10.9 ± 1.7
100	300	3.98 ± 3.0	
100	1000	3.70 ± 2.15	3.5 ± 0

Numbers are means of 3 wells

TABLE 2. Effects of TNF on basal and stimulated thyroglobulin release

Experiment I Thyroglobulin (ng/well) at 0-24 hours

	TNF (pg/ml)			
	0	100	300	1000
Without TSH	70 ± 6	79 ± 3	73 ± 1	65 ± 5
With TSH	340 ± 35	212 ± 25*	171 ± 17*	65 ± 21*

Experiment I Thyroglobulin (ng/well) at 24-48 hours

	TNF (pg/ml)			
	0	100	300	1000
Without TSH	114 ± 33	94 ± 10	79 ± 8	56 ± 12
With TSH	630 ± 126	251 ± 25*	160 ± 23*	63 ± 10*

Data is mean ± S.D. using data from 3 wells
*Different from control (TNF=0) with $p<0.01$

Experiment II Thyroglobulin (ng/well) at 24-48 hours

	TNF (pg/ml)			
	0	100	300	1000
Without TSH	369 ± 19	334 ± 5	258 ± 16*	223 ± 36*
With TSH	1025 ± 15	704 ± 44*	285 ± 51*	105 ± 11*

*Data significantly different from control (TNF=0) according to ANOVA t-test ($p<0.05$).

TABLE 3. Effects of TNF on TSH-induced cAMP release

	cAMP (picomoles/well/2 hours)			
	TNF (pg/ml)			
	0	**100**	**300**	**1000**
Without TSH	<0.5	<0.5	<0.5	<0.5
With TSH	5.6	5.0	4.7	5.1

Data are Mean of 3 wells.

Table 4 Effects of ACTH on LPS-induced TNF release in vitro

Treatment	TNF Bioactivity (U/ml) Fetal Calf Serum*	HL-1 Media*
LPS (1 ug x 10^{-4})	637 (265-1533)	160 (97-264)
LPS (10 ug x 10^{-4})	11790 (6641-20931)	6741 (1947-23342)
LPS (100 ug x 10^{-4})	6789 (4487-10270)	36098 (7421-175606)
LPS (500 ug x 10^{-4})	--	81961 (34064-197205)
LPS (1000 ug x 10^{-4})	--	96279 (46583-198988)
LPS (1 ug x 10^{-4}) +		
--ACTH (10^{-12}M)	683 (183-2548)	--
--ACTH (10^{-10}M)	415 (175-982)	--
--ACTH (10^{-8}M)	537 (150-1923)	--
LPS (10 ug x 10^{-4}) +		
--ACTH (10^{-12}M)	9082 (4836-17052)	6946 (1081-44623)
--ACTH (10^{-10}M)	3866 (925-16155)	10945 (3572-33557)
--ACTH (10^{-8}M)	11521 (6823-19458)	16059 (3874-66570)
LPS (100 ug x 10^{-4}) +		
--ACTH (10^{-12}M)	--	59874 (8656-414157)
--ACTH (10^{-10}M)	--	129185 (47810-349060)
--ACTH (10^{-8}M)	--	144930 (66436-316159)
LPS (1000 ug x 10^{-4}) +		
--ACTH (10^{-12}M)	--	42574 (2873-630961)
--ACTH (10^{-10}M)	--	>163898
--ACTH (10^{-8}M)	--	>163898

* mean ± 95% confidence interval
each value represents mean of 4-6 experiments

Figure 3. Effects of ACTH on LPS-induced TNF release from macrophages cultured in the presence of fetal calf serum. (Representative experiment)

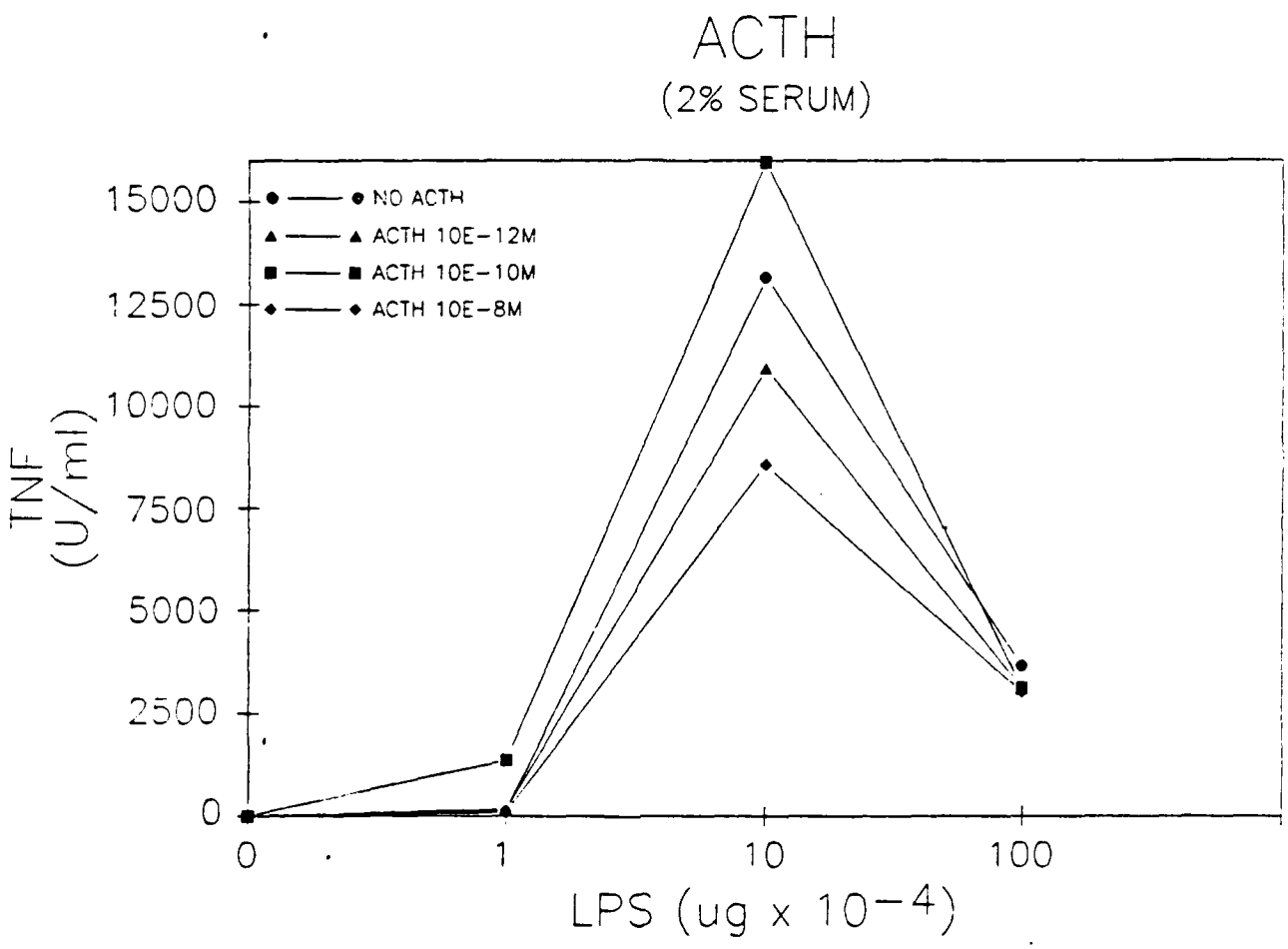


Figure 4. Effects of ACTH on LPS-induced TNF release from macrophages cultured in the presence of HL-1 culture media. (Representative experiment)

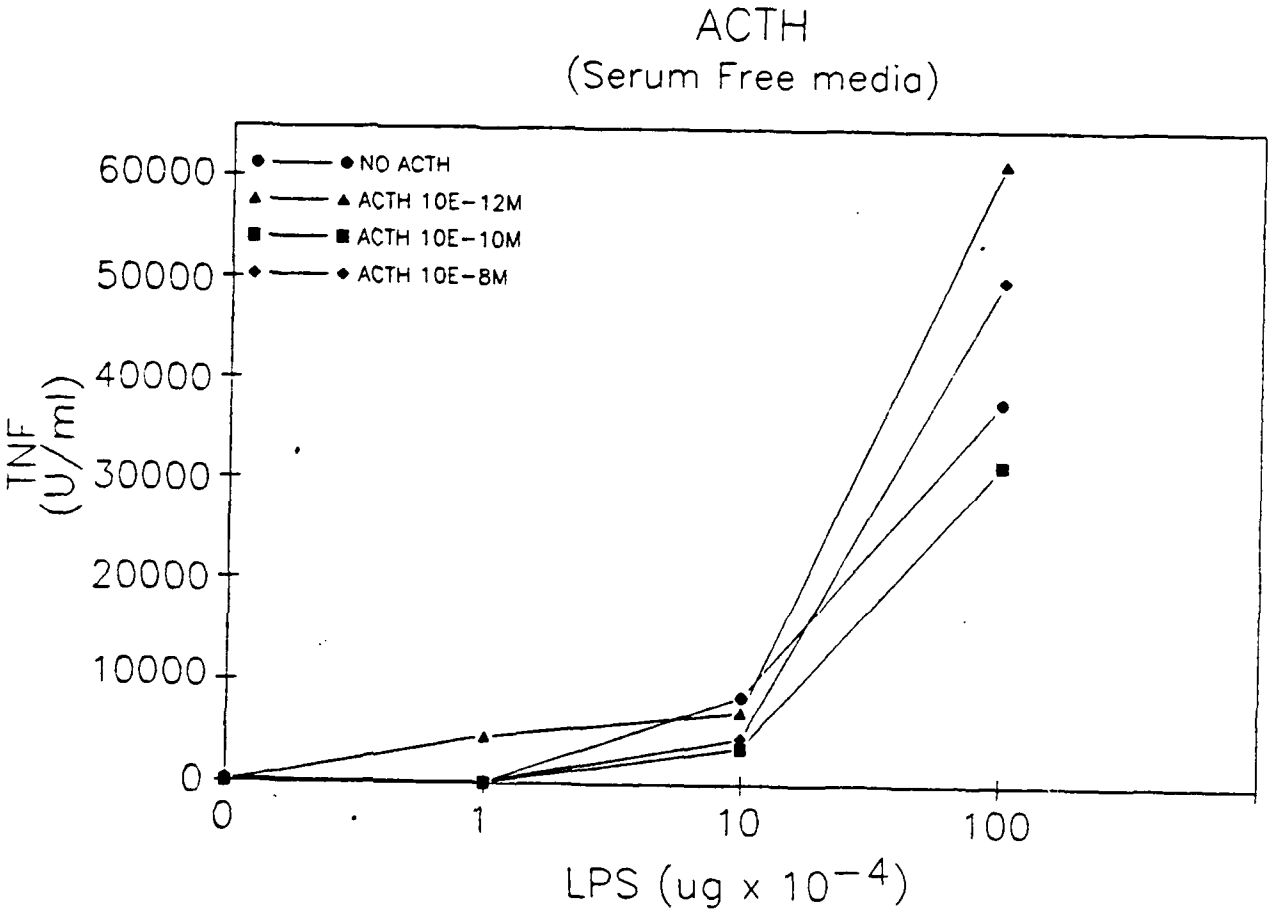


Table 5 Effects of Corticosterone (CS) on LPS-induced TNF release in vitro

Treatment	TNF Bioactivity (U/ml) Fetal Calf Serum*	HL-1 Media*
LPS (1 ug x 10^{-4})	637 (265-1533)	160 (97-264)
LPS (10 ug x 10^{-4})	11790 (6641-20931)	6741 (1947-23342)
LPS (100 ug x 10^{-4})	6789 (4487-10270)	36098 (7421-175606)
LPS (500 ug x 10^{-4})	--	81961 (34064-197205)
LPS (1000 ug x 10^{-4})	--	96279 (46583-198988)
LPS (1 ug x 10^{-4}) +		
--CS (10^{-10}M)	149 (99-225)	--
--CS (10^{-8}M)	117 (80-171)	--
--CS (10^{-6}M)	81 (79-82)	--
LPS (10 ug x 10^{-4}) +		
--CS (10^{-10}M)	9009 (5409-10000)	14750 (1470-148004)
--CS (10^{-8}M)	2705 (1389-5265)	8417 (564-125492)
--CS (10^{-6}M)	356 (250-507)	637 (102-3987)
LPS (100 ug x 10^{-4}) +		
--CS (10^{-10}M)	--	62944 (15741-251702)
--CS (10^{-8}M)	--	58395 (8242-413742)
--CS (10^{-6}M)	--	13440 (1498-120571)
LPS (1000 ug x 10^{-4}) +		
--CS (10^{-10}M)	--	70404 (12991-381551)
--CS (10^{-8}M)	--	33962 (1458-790958)
--CS (10^{-6}M)	--	31225 (1133-860269)

*mean $\pm$ 95% confidence interval
each value represents the mean of 4-6 experiments

Table 6 Effects of Dexamethasone (DEX) on LPS-induced TNF release in vitro

Treatment	TNF Bioactivity (U/ml) Fetal Calf Serum*	HL-1 Media*
LPS (1 ug x 10^{-4})	637 (26501533)	160 (97-264)
LPS (10 ug x 10^{-4})	11790 (6641-20931)	6741 (1947-23342)
LPS (100 ug x 10^{-4})	6789 (4487-10270)	36098 (7421-175606)
LPS (500 ug x 10^{-4})	--	81961 (34064-197205)
LPS (1000 ug x 10^{-4})	--	96279 (46583-198988)
LPS (1 ug x 10^{-4}) +		
--DEX (10^{-10}M)	573 (143-2280)	--
--DEX (10^{-8}M)	98 (74-129)	--
--DEX (10^{-6}M)	<80	--
LPS (10 ug x 10^{-4}) +		
--DEX (10^{-10}M)	9009 (5239-15490)	1194 (9-163570)
--DEX (10^{-8}M)	441 (169-1150)	226 (28-1806)
--DEX (10^{-6}M)	213 (116-389)	99 (65-153)
LPS (100 ug x 10^{-4}) +		
--DEX (10^{-10}M)	--	7237 (904-57931)
--DEX (10^{-8}M)	--	1428 (217-9414)
--DEX (10^{-6}M)	--	371 (55-2500)

*represents mean $\pm$ 95% confidence interval
each value represents mean of 4-6 experiments

Figure 5. Effects of dexamethasone on LPS-induced TNF release from macrophages cultured in the presence of fetal calf serum (Representative experiment)

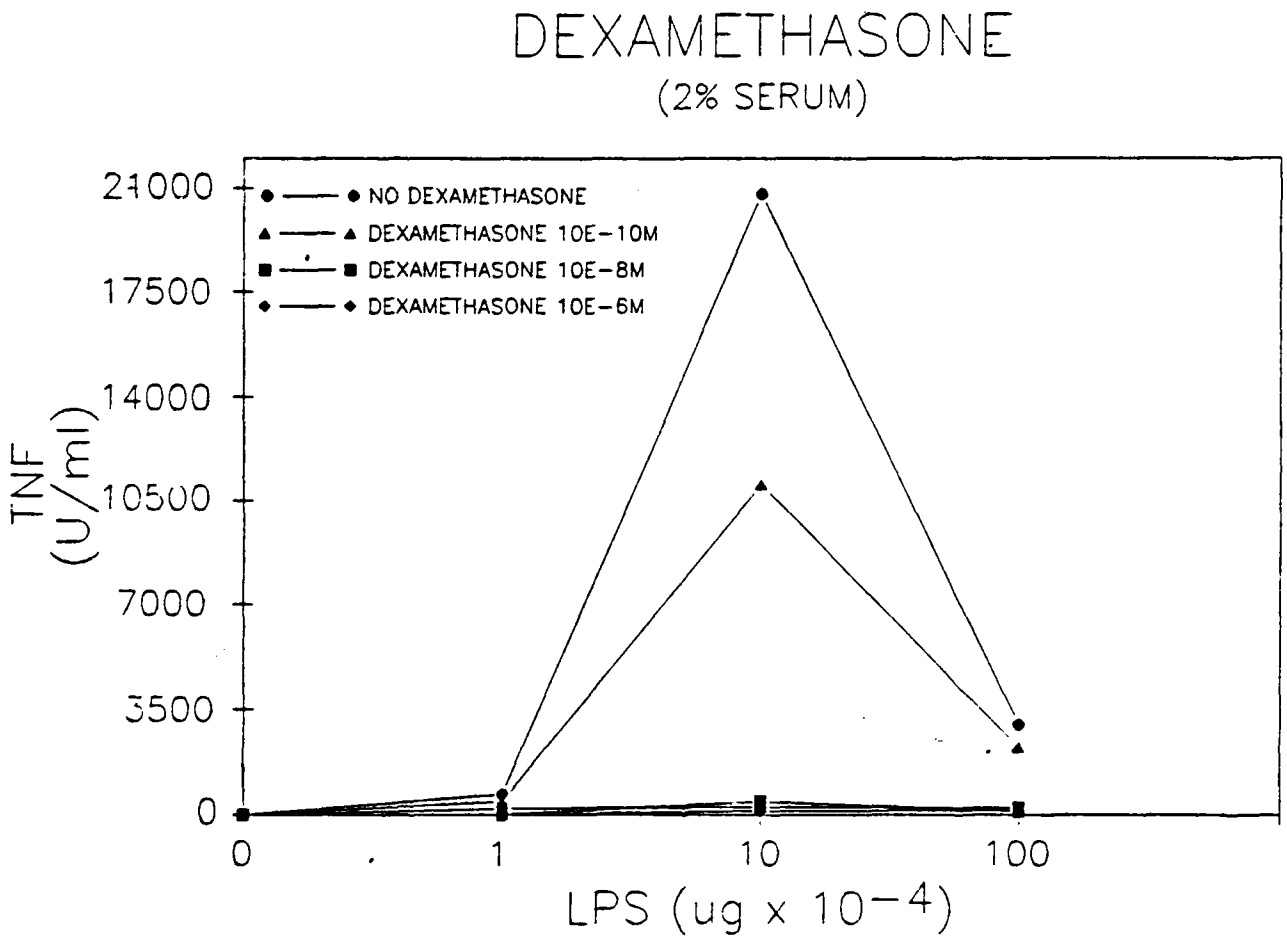
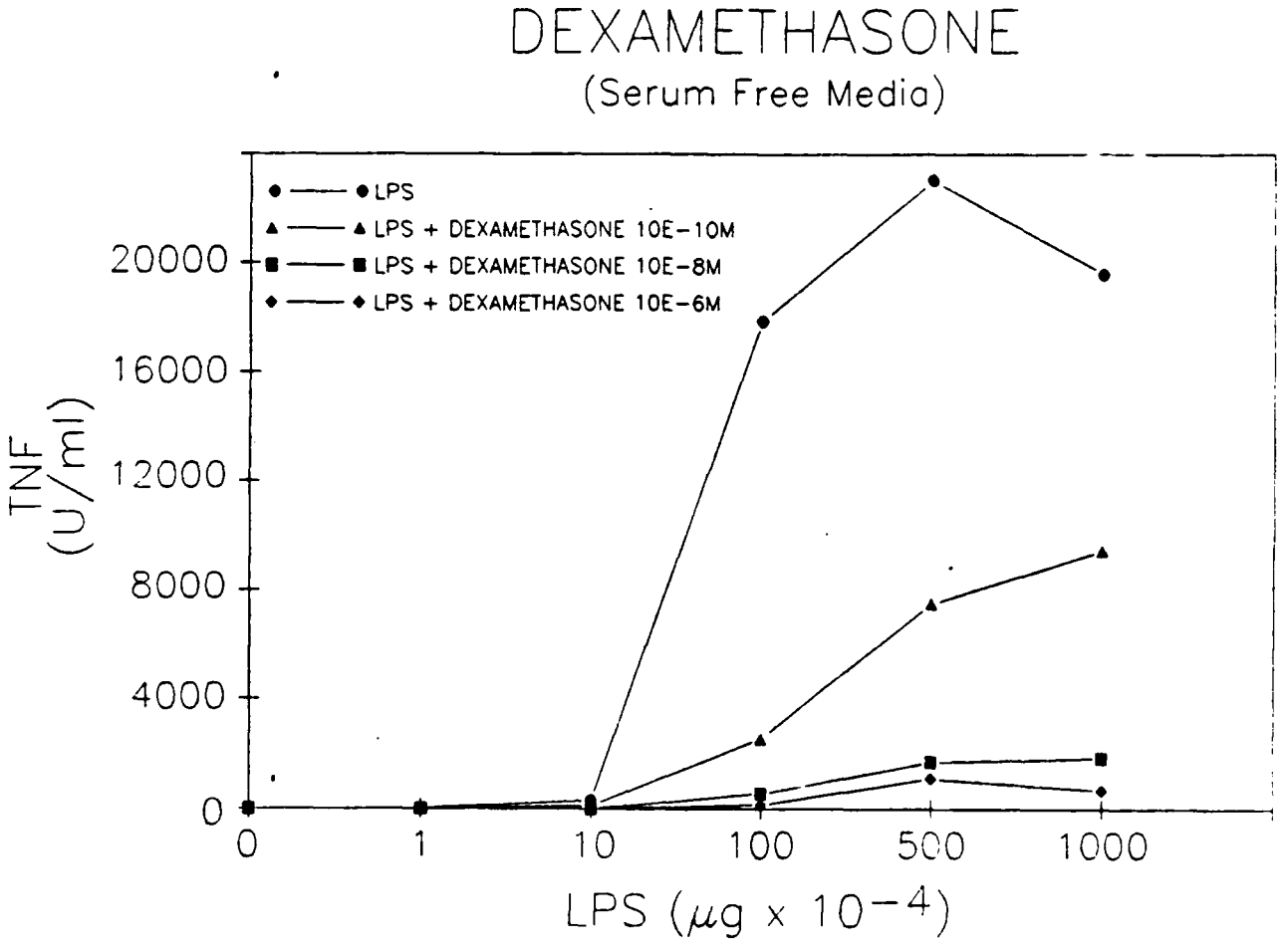


Figure 6. Effects of dexamethasone on LPS-induced TNF release from macrophages cultured with HL-1 media (serum free) (Representative Experiment)



12/21/89 JM

DO NOT FOLD OR BEND THIS FORM. ABSTRACTS WILL BE PUBLISHED AS TYPED.

The Endocrine Society

1989 ABSTRACT FORM

IMPORTANT

Read all instructions before you type abstract. Also see sample abstracts and typing instructions on reverse side.

ALL ABSTRACTS MUST BE POSTMARKED

Outside USA — January 2, 1989
USA only — January 9, 1989
ONLY ACCEPTED ABSTRACTS WILL BE PUBLISHED.

MAIL TO:

The Endocrine Society
9650 Rockville Pike
Bethesda, MD 20814
Tel. (301) 571-1802

PRESENTATION PREFERENCE

Do you strongly prefer a poster?
☐ Yes ☒ No

Preferences will not be binding on the Program Committee.

Indicate appropriate categories for your abstract (See Topic Category List):
1st Choice: #. 2 . Title. Adrenal Cortex
2nd Choice: #. . . .Title.....

Would you be willing to have your abstract taken out of its category and placed in a clinical session?
☒ Yes ☐ No

Do you think that your abstract is of interest to the lay public and, if asked, are you willing to write an abstract for the lay press?
☐ Yes ☒ No

1. The original typed copy of this abstract form (for reproduction by photo-offset in Program/Abstracts book) must be submitted together with 7 photocopies.

2. Your entire abstract, including a concise, descriptive title, author(s), sponsor if any, location where work was done, text and acknowledgments, must be within the rectangle outlined at the right. See reverse side for sample abstracts and full instructions.

3. Each Abstract Form submitted MUST BE SIGNED below by the member of THE ENDOCRINE SOCIETY.

Name and Telephone no. of presenting author

Michael J. Brennan

(202) 576-0055

TUMOR NECROSIS FACTOR INHIBITS ACTH STIMULATED CORTICOSTERONE SECRETION BY RAT ADRENAL CORTICAL CELLS. M.J. Brennan*, J.A. Betz*, and M. Poth. (SPON: D. Bunner) Walter Reed Army Medical Center, Washington, D.C. 20307 and The Uniformed Services University of the Health Sciences, Bethesda, M.D. 20814.

Tumor Necrosis factor (TNF) is a biologically active peptide secreted by macrophages and monocytes. TNF secretion is stimulated by endotoxin and TNF has been implicated in the pathogenesis of septic shock. To determine if TNF has specific actions on the adrenal gland, we studied the effects of ACTH and TNF on the in vitro secretion of corticosterone by rat adrenal cells. Adrenal glands from adult Sprague-Dawley rats were harvested, digested with collagenase, and cell suspensions were prepared. Cell cultures were incubated for 90 minutes in media with various concentrations of ACTH, TNF, or ACTH and TNF in combination. The cells were then centrifuged and the supernatants were assayed by RIA for corticosterone.

Results:

Corticosterone Secretion (pg/400,000 cells)

	TNF (pg/ml)		
ACTH (pg/ml)	0	100	1000
0	6.4 ± 1.2	5.8 ± 1.2	4.4 ± .20
30	16.9 ± 5.3	8.1 ± 5.3	3.6 ± .20
100	63.0 ± 2.0	10.9 ± 1.7	3.5 ± .00

Incubation with ACTH at concentrations of 10, 30 and 100 pg/ml produced a dose-response related stimulation of corticosterone secretion. Incubation with TNF alone at concentrations of 100, 300, and 1000 pg/ml had no effect on corticosterone secretion. However, when adrenal cells were incubated with ACTH and TNF in combination, corticosterone secretion was significantly inhibited. TNF at 100 pg/ml inhibited ACTH stimulated corticosterone secretion by 75-100% ($p < .001$), while TNF at 1000 pg/ml produced 100% inhibition ($p < .001$). Statistical significance was determined by multiple regression analysis. Conclusion: TNF inhibits ACTH stimulation of corticosterone secretion by rat adrenocortical cells. This is a new, potentially clinically important interaction between the immune and endocrine systems. Speculation: TNF may potentiate septic shock by inhibiting the body's ability to mount an appropriate glucocorticoid response to the stress of sepsis.

Member Signature Required Below as Sponsor of Abstract: NO MEMBER MAY SIGN MORE THAN ONE ABSTRACT.

The signing member certifies that any work with human or animal subjects related in this abstract complies with the guiding principles for experimental procedures as set forth in the *Declaration of Helsinki*, in the *Statement of The Endocrine Society Concerning the Care and Use of Animals in Research*, and in the *NIH Guide for the Care and Use of Laboratory Animals*, 1985. The signature also certifies that the scientific material in this abstract will not have been published or presented at any national meeting prior to The Endocrine Society Annual Meeting. Failure to adhere to this rule will result in deletion of the paper from the Program.

David L. Bunner — David L. Bunner — (301) 663-7181

Member signature — *Printed or typed name* — *Telephone number*

DO NOT FOLD OR BEND THIS FORM. ABSTRACTS WILL BE PUBLISHED AS TYPED.

The Endocrine Society

1989 ABSTRACT FORM

IMPORTANT

Read all instructions before you type abstract. Also see sample abstracts and typing instructions on reverse side.

ALL ABSTRACTS MUST BE POSTMARKED

Outside USA — January 2, 1989
USA only — January 9, 1989
ONLY ACCEPTED ABSTRACTS WILL BE PUBLISHED.

MAIL TO:

The Endocrine Society
9650 Rockville Pike
Bethesda, MD 20814
Tel. (301) 571-1802

PRESENTATION PREFERENCE

Do you strongly prefer a poster?
☐ Yes ☒ No

Preferences will not be binding on the Program Committee.

Indicate appropriate categories for your abstract (See Topic Category List):
1st Choice: #. 5 Title. Thyroid
2nd Choice: #. . . Title.....

Would you be willing to have your abstract taken out of its category and placed in a clinical session?
☒ Yes ☐ No

Do you think that your abstract is of interest to the lay public and, if asked, are you willing to write an abstract for the lay press?
☐ Yes ☒ No

1. The original typed copy of this abstract form (for reproduction by photo-offset in Program/Abstracts book) must be submitted together with 7 photocopies.

2. Your entire abstract, including a concise, descriptive title, author(s), sponsor if any, location where work was done, text and acknowledgments, must be within the rectangle outlined at the right. See reverse side for sample abstracts and full instructions.

3. Each Abstract Form submitted MUST BE SIGNED below by the member of THE ENDOCRINE SOCIETY.

Name and Telephone no. of presenting author

Dr. Merrily Poth

(202) 576-0055

DOES CACHECTIN MEDIATE ALTERED THYROID FUNCTION IN SYSTEMIC ILLNESS? A CELL CULTURE MODEL.

M. Poth, Y.L. Tseng*, and L. Wartofsky. Walter Reed Army Medical Center, Washington, DC 20307 and Uniformed Services University of the Health Sciences, Bethesda, MD 20814

Thyroidal economy in systemic non-thyroidal illness (SNTI) is marked by reductions in both central thyroid function and peripheral T_4 to T_3 conversion presumed to reflect a homeostatic mechanism to conserve energy. TSH levels tend to be normal in SNTI, and the mechanism underlying reduced thyroidal secretion is unknown. Recently, Ozawa (Endocrinol 123:1461, 1988) treated mice with tumor necrosis factor (TNF), as an animal model for SNTI, and reported diminished T_3 and T_4 responses to TSH administration. We have employed a primary thyroid cell culture system derived from surgical specimens to assess the effects of TNF on thyroidal responses to TSH. Cells at a density of 100,000/well were incubated with various concentrations (0-1000 pg/ml) of recombinant alpha-TNF (Genentech) and bTSH (1 mU/ml). Media were analyzed for cyclic AMP by RIA, and for thyroglobulin (Tg) by ELISA. TNF had no effect on either basal or TSH-stimulated cAMP generation.

Tg (ng/well) secreted into media by thyroid cells (100,000 cells/well) in the presence of TNF and bTSH (Mean ± SEM)

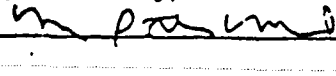
TNF	0-24 hr		24-48 hr	
(pg/ml)	(-) TSH	(+) TSH	(-) TSH	(+) TSH
0	212 ± 7	365 ± 56	369 ± 19	1025 ± 15
100	186 ± 23	266 ± 57	334 ± 5	704 ± 44*
300	266 ± 73	144 ± 23*	258 ± 16*	285 ± 51*
1000	240 ± 41	89 ± 12*	223 ± 36*	105 ± 11*

*Data significantly different from control (TNF=0) according to ANOVA t-test ($p < 0.05$).

While TNF alone had no effect on Tg release at 24 hrs, TNF blunted TSH-stimulated Tg release by 27-76%. At 48 hrs, TNF blunted Tg release by 9-39% and TSH-stimulated Tg release by 31-90%. These results are consistent with the in vivo observations of Ozawa et al. and demonstrate a cytostatic effect on human thyrocytes by TNF in concentrations comparable to blood levels in man during SNTI. Thus, increases in circulating TNF in SNTI may be responsible for reduced thyroid function in these patients.

Member Signature Required Below as Sponsor of Abstract: NO MEMBER MAY SIGN MORE THAN ONE ABSTRACT.

The signing member certifies that any work with human or animal subjects related in this abstract complies with the guiding principles for experimental procedures as set forth in the *Declaration of Helsinki*, in the *Statement of The Endocrine Society Concerning the Care and Use of Animals in Research*, and in the *NIH Guide for the Care and Use of Laboratory Animals*, 1985. The signature also certifies that the scientific material in this abstract will not have been published or presented at any national meeting prior to The Endocrine Society Annual Meeting. Failure to adhere to this rule will result in deletion of the paper from the Program.



Dr. Merrily Poth — Printed or typed name

(202) 576-0055 — Telephone number

THIRTEENTH ANNUAL CONFERENCE ON SHOCK

Tamarron
Durango, Colorado
June 8-11, 1990
H. Richard Adams, Scientific Program Chair

ABSTRACT FORM

DEADLINE: Abstracts must be received by January 26, 1990

PLEASE READ CAREFULLY AND FOLLOW DETAILED INSTRUCTIONS PROVIDED ON REVERSE SIDE.

NAME & MAILING ADDRESS OF FIRST AUTHOR

Dr. Diana S. Malcolm
Department of Surgery
Uniformed Services University
of the Health Sciences
4301 Jones Bridge Road
Bethesda, Maryland 20814-4799

Telephone: (202) 295-3707

IMPORTANT: Please check if appropriate:

☐ Please send registration materials.

PRESENTATION PREFERENCE (check one).
NOTE: *See reverse side for details.*

☒ Poster session ☐ Minisymposium ☐ Indifferent

SELECT CATEGORY *(See Topic Category List)*

1st TNF/Cytokines

2nd Hormones

Prepare abstract carefully on this form only. Return the original typed copy of this abstract plus 8 photocopies by January 26, 1990 to address on reverse side. Please enclose a non-refundable abstract handling fee of $15.00 made payable to the Shock Society.
Abstracts will be reviewed by the Scientific Program Committee, H. Richard Adams, Chair.

TYPE ABSTRACT IN SPACE BELOW — DO NOT FOLD

TUMOR NECROSIS FACTOR (TNF) AND THE PITUITARY-ADRENAL AXIS: IN VIVO AND IN VITRO STUDIES. Diana Malcolm and Merrily Poth*. Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Several lines of evidence indicate that the immune system by way of macrophage products can influence neuroendocrine function and vice versa suggesting that these two systems are involved in a complete regulatory feedback loop. The purpose of our studies was to determine what effect TNF has on ACTH and corticosterone (CS) release both in vitro and in vivo, and conversely, what effect ACTH and CS have on endotoxin-induced TNF release from cultured macrophages. Intravenous injections of low doses of TNF (0.01-0.10 mg/kg) in unanesthetized Sprague-Dawley rats (250-300 g) resulted in dose-related elevations in plasma ACTH and CS. In vitro, TNF (0, 0.1, 1.0 ng/ml) inhibited ACTH-induced CS release from cultured adrenal cells by 83% and 94%, respectively ($p \leq 0.01$). TNF alone at these doses had no effect on the basal secretion of CS. Conversely, both ACTH (10^{-8}M-10^{-12}M) and CS (10^{-6}M-10^{-10}M) suppressed TNF release from endotoxin (LPS)-stimulated cultured macrophages in a dose-dependent manner. Furthermore, when macrophages were cultured in serum-free media (HL-1, Ventrex) instead of 2% fetal calf serum, the LPS dose-response curve was shifted to the right suggesting that factor(s) present in serum may facilitate LPS-induced TNF release. In summary, (1) plasma ACTH and CS levels are elevated following TNF injection, (2) TNF inhibits ACTH-stimulated CS release in vitro without affecting basal CS secretion and (3) both ACTH and CS inhibit TNF release from cultured macrophages. These findings represent new and significant interactions between TNF and the pituitary-adrenal axis and further support the existence of a regulatory feedback loop between the neuroendocrine and immune systems.

* Paper to be judged for the Young Investigators Awards. My signature attests that this work will not be presented in any form at any other meeting prior to the Shock Society meeting.

NAME OF AUTHOR

Diana S. Malcolm, Ph.D.

Diana S. Malcolm

DISTRIBUTION LIST

Behavioral Immunology Program

Annual, Final and Technical Reports (1 copy each except as noted)

INVESTIGATORS

Dr. Itamar B. Abrass
Department of Medicine
University of Washington
Harborview Medical Center
Seattle, WA 98104

Dr. Prince K. Arora
NIDDK, Bldg. 8, Room 111
National Institutes of Health
Bethesda, MD 20892

* Dr. Andrew S. Baum
Dept. of Medical Psychology
Uniformed Services University
of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814-4799

Dr. Charles A. Bowles
Merrifield Research Lab, Inc.
P.O. Box 2362
Merrifield, VA 22116-2362

Dr. Karen Bulloch
Dept. of Pediatrics M009D
Univ. of California,
San Diego, School of Medicine
La Jolla, CA 92093

Dr. Michael D. Cahalan
Dept. of Physiology and
Biophysics
Univ. of California, Irvine
Irvine, CA 92717

Dr. Donald A. Chambers
801 S. Paulina - Room J30E
Univ. of Illinois at Chicago
P.O. Box 6998
Chicago, IL 60680

* Dr. Christopher L. Coe
Department of Psychology
Harlow Primate Laboratory
University of Wisconsin
Madison, WI 53715

* Dr. Sheldon Cohen
Department of Psychology
Carnegie-Mellon Univ.
Pittsburgh, PA 15213

Dr. Walla L. Dempsey
Dept. of Microbiology and
Immunology
Medical College of
Pennsylvania
3300 Henry Avenue
Philadelphia, PA 19129

Dr. Robert L. Hunter
Dept. of Pathology
Emory Univ. School of
Medicine
WMB 760
Atlanta, GA 30322

Dr. Terry C. Johnson
Division of Biology
Ackert Hall
Kansas State University
Manhattan, KS 66506

* Dr. Jerome Kagan
Dept. of Psychology
Harvard University
Cambridge, MA 02138

* Dr. Keith W. Kelley
Lab. of Immunophysiology
University of Illinois
809 S. Wright Street
Champaign, IL 61820-6219

Dr. James M. Krueger
Dept. of Physiology
University of Tennessee
894 Union Street
Memphis, TN 38163

* Dr. Sandra Levy
WPIC
Univ. of Pittsburgh
School of Medicine
3811 O'Hara Street
Pittsburgh, PA 15213

Dr. Roger M. Loria
Virginia Commonwealth Univ.
Dept. of Microbiology &
Immun. Box 678, MCV Station
Richmond, VA 23298-0001

* Dr. Lester Luborsky
Dept. of Psychiatry
308 Piersol Bldg. G1
Hospital of the University
of Pennsylvania
Philadelphia, PA 19104

* Dr. Steven F. Maier
Dept. of Psychology
University of Colorado
Campus Box 345
Boulder, CO 80309

Dr. Diana S. Malcolm
Dept. of Surgery,
Uniformed Services Univ.
of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814-4799

Dr. Michael H. Melner
Dept. of Reproductive Biology
Oregon Regional Primate Center
505 N.W. 185th Avenue
Beaverton, OR 97006

Dr. Vera B. Morhenn
Department of Dermatology
Martinez VA
Martinez, CA 94553

Dr. Jose R. Perez-Polo
Dept. of Biochemistry
Gail Borden Bldg., Room 436
Univ. of Texas Medical Branch
Galveston, TX 77550-2777

Dr. Merrily P.M. Poth
Dept. of Pediatrics, A3027
Uniformed Services Univ.
of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814-4799

Dr. Eric M. Smith
Dept. of Psychiatry
Univ. of Texas Medical
Branch
Galveston, TX 77550

Dr. G. John Stanton
Dept. of Microbiology
Univ. of Texas Medical
Branch
Galveston, TX 77550

** Dr. Ross R. Vickers, Jr.
Naval Health Research Center
Building 346
P.O. Box 85122
San Diego, CA 92138

* Supported by ONR Code 1142BI
** Supported by NMRDC

Annual, Final and Technical Reports (one copy each except as noted)

ADMINISTRATORS

Dr. Jeannine A. Majde, Code 1141SB (2 copies)
Program Manager, Systems Biology
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

Program Manager
Biotechnology Program
Office of Naval Research
Code 1213
800 N. Quincy Street
Arlington, VA 22217-5000

Administrator (2 copies) (Enclose DTIC Fm 50)
Defense Technical Information Center
Building 5, Cameron Station
Alexandria, VA 22314

Program Manager
Support Technology Directorate
Office of Naval Technology
Code 223
800 N. Quincy Street
Arlington, VA 22217-5000

Administrative Contracting Officer
ONR Resident Representative
(address varies - obtain from business office)

Mr. Michael McCracken
218 23rd Ave NW
[illegible] DC 20006

DoD ACTIVITIES

Commanding Officer
Naval Medical Center
Washington, DC 20372

Commander
USAMRIID
Fort Detrick
Frederick, MD 21701

Commanding Officer
Naval Medical Research & Development Command
National Naval Medical Center
Bethesda, MD 20814

Directorate of Life Sciences
Air Force Ofc of Scient. Res.
Bolling Air Force Base
Washington, DC 20332

Director, Infectious Diseases Program Center
Naval Medical Research Institute
National Naval Medical Center
Bethesda, MD 20814

Library
Armed Forces Radiation
Research Institute
Bethesda, MD 20814-5145

Commander
Chemical & Biological Sciences Division
Army Research Office, P.O. Box 12211
Research Triangle Park, NC 27709

Commander
U.S. Army Research and Development Command
Attn: SGRD-PLA
Fort Detrick
Frederick, MD 21701

Final and Technical Reports Only

Director, Naval Research Laboratory (6 copies)
Attn: Technical Information Division, Code 2627
Washington, DC 20375