ENDOCRINE-IMMUNE SURVEILLANCE

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For a number of years, Veterinary pathologists have noted varying degrees of adrenal disease histopathologically often not correlated or diagnosed clinically. With the availability of more recent and more direct immunological methods and procedures; we have found good evidence that adrenal disorders exist clinically in veterinary practice, and differ from the classic Addison and Cushing syndromes.

The etiology of these disorders in man are often listed as idiopathic. There is some evidence in man, that suggests that these kind of adrenal lesions may be a sequence to certain bacterial infections, prolonged steriod therapy, or an immunological mechanism directed against the adrenal cortex or adrenal medulla.

Certain idiopathic hypo-adrenal cortical diseases in man are thought to be autoimmune. This syndrome tends to be familial and is thought to be due to an autosomal recessive gene.

In man and dog, the most common lesion is atrophy of all three layers of the adrenal cortex. Varying degrees of atrophy may be present. Apparently, there is no associated pituitary malfunction. In man there is a tendency to form auto antibodies to the thyroid gland concurrently with auto antibodies to the adrenal cortex.

The disease in dogs often clinically causes lethargy, anorrhexia, vomiting, diarrhea, muscle weakness (including cardiac disease), with Na and K imbalance. Typically the disease occurs from two to five years of age. Many of these dogs may have frequent partial remissions. There tends to be no breed predilection.

The adrenal disorders that we are investigating clinically, have occurred mainly in Doberman, German Shephard, Basset Hounds and mixed breeds, with one of these breeds as one of the parents.

The disorder occurs both in the male and the female dogs and has been observed as early as three weeks of age and late as ten years. From past clinical investigations, there are indications that this problem may be a predictable genetic disorder. Sodium and potassium levels are usually normal.

Early in the disease the syndrome manifests itself as a disease of the integument. There is localized alopecia with or without pruritus. The distribution of lesions may be localized at first and then become generalized. As the syndrome progresses, the skin lesions become more severe. The lesions tend to become thickened and

pustular. Generalized demodecosis often accompanies this disease. Resident fungi may complicate the skin lesions. Localized or generalized lymphodenopathy is often apparent in this phase. As the severity of the disorder increases, more typical types and distribution of lesions occur. The skin lesions at this time may reflect pruritus, hyperthermia, indurated areas, with enough damage to the integument that serum begins to appear on the surface of the skin. The areas of involvement may now be generalized with a greater degree of damage periorbitally, ears, ventral chin and chest, and abdomen, and all legs and paws. Much enema may occur in all four paws. Interestingly enough, the most severe lesions tend to occur in areas of the greatest concentration of mast cells, therefore, the greatest histamine release.

These dogs demonstrate an overall hypersensitivity. Standard diets, flea bites, fly bites and hot weather all worsen the condition. Standard methods of therapy include organic phosphates topically and antibiotics systemically. This treatment may slow the problem temporarily, but the condition will continue to deteriorate. To date we have not observed any permanent spontaneous remissions with this disorder.

Many cases have been treated and observed with no steroid therapy with other cases steroids have been used. Our clinical impression at this point in time, is that steroids may be of value in treating this disorder.

CASE REPORTS:

Case One:

Six month old male Irish setter with a generalized demodicosis and dermatitis was referred to the hospital for treatment. The more serious areas of skin involvement were periorbitally, ears, ventral mandibular area, thorax, abdomen, and legs. The following tests were performed with the following results:

LABORATORY RESULTS:

CASE I

Cat
Dog
Other

Patient AP 121 Doctor PLECHNER
Hospital C.A.H. Date 2/16/77

	CBC	Results	Canine Normal	Feline Normal
1	WBC x 10^3	11	6.0-17.0	5.0-19.0
2	RBC x 10^3	6.1	5.5-8.5	5.0-10.0
3	HGB	14.1	12-18	8-15
4	HCT	43	37-55	24-45
5	SEGS	67	60-77	35-75
6	NON-SEG		0-3	0-3
7	META		0	0
8	LYMPHS	29	12-30	20-55
9	MONOS	1	3-10	1-4
10	EOS	3	2-8	2-12
11	BASO		Rare	Rare
12	NUC. RBC		0	0
13	MCV	70.5	60-77	39-55
14	MCHC	32.8	32-36	30-36
15	PLATELETS x 10^3	Normal	150-700	250-700
16	RETIC		0-1	0.4-6.4
17	RBC MORPH	Normal		
18	POLYCHROMISIA			
19	ANISOCYTOSIS			
20	SPHEROCYTES			
21	HYPOCHROMIA			
22	FELV			
23	FIA			
24	ALK, PHOS	130	20-150	10-44 IU/L
25	AMYLASE	700	300-1200	300-1200 S.U.
26	T. BILIRUBIN		0.03-0.45	0-0.69 mg%
27	BUN	18	9-25	16-30 mg%
28	CALCIUM		8.1-11.3	8.3-10.5 mg%
29	CHOLESTEROL	248	151-337	44-192 mg%
30	CREATININE	1.2	1-2	0.8-1.8 mg%
31	SODIUM	144	141-155	143-157 mEq/l
32	POTASSIUM	5.3	3.9-5.7	3.3-4.7 mEq/l
33	CHLORIDE		98-113	105-121 mEq/l
34	CO_2		18-24	17-21 mEq/l
35	GLUCOSE	95	60-110	70-110 mg%
36	LDH		56-502	50-514 IU/L
37	PHOSPHOROUS		2.6-6.0	3.6-6.4 mg%
38	SGOT	27	20-80	16-36 IU/L
39	SGPT	38	17-45	13-39 IU/L
40	T-3	48	37-54	37-50 %
41	T-4	1.8	1.6-5.6	1.2-5.2 µgm%
42	T. PROTEIN	7.0	5.3-7.4	5.5-7.9gm%
43	GLOBULIN	3.0	2.7-4.4	2.6-5.1gm%
44	ALBUMIN	4.0	2.5-3.8	2.4-4.0gm%
45	URIC ACID	1.5	0.2-1.9	0.5-1.1mg%
46	A/G	1.33		

URINALYSIS

Character	Normal	Color	Light Yellow
S.G. 1.0	1.038	pH	5.0
Albumin	1	WBC	0 /hpf
Sugar	0	RBC	0 /hpf
Acetone	0	EPC	Few /hpf
Bacteria	Very Few	Crystals	Tri-phosphate
Other		Casts	Few Hyaline /lpf

COMMENTS ACTH 110 (17-98 pmg/ml normal) TESTOSTERONE 329 ng % (400-800 ng % normal); T-Lymphocytes-Normal; CORTISOL-resting-1.0, stimulation-14.7; LE PREP (–); ANA (–); COOMBS (–); IgG-2800 mg%; ADRENAL GLAND-Histopathology, Immunopathology.

Case Two:

A two year old male mixed shepard was referred to the hospital for treatment. The dog was suffering from generalized demodicosis. The dog had been treated for one and one-half years with Ronnel topically and oral Keflex. No steroids had been used. Skin lesions were present periorbitally, on the ventral mandibular area, ventral cervical area, thorax and legs. Localized lymphodenopathy was present with edema of all four feet. Lesions were pruritic. The following tests were performed with the following results:

LABORATORY RESULTS:

CASE II

Cat
Dog
Other

Patient AP 127 Doctor PLECHNER
Hospital C.A.H. Date 4/16/77

	CBC	Results	Canine Normal	Feline Normal
1	WBC x 10^3	8.5	6.0-17.0	5.0-19.0
2	RBC x 10^3	7.1	5.5-8.5	5.0-10.0
3	HGB	16.3	12-18	8-15
4	HCT	49	37-55	24-45
5	SEGS	62	60-77	35-75
6	NON-SEG		0-3	0-3
7	META		0	0
8	LYMPHS	28	12-30	20-55
9	MONOS	5	3-10	1-4
10	EOS	5	2-8	2-12
11	BASO		Rare	Rare
12	NUC. RBC		0	0
13	MCV	69	60-77	39-55
14	MCHC	33	32-36	30-36
15	PLATELETS x 10^3	Normal	150-700	250-700
16	RETIC		0-1	0.4-6.4
17	RBC MORPH	Normal		
18	POLYCHROMISIA			
19	ANISOCYTOSIS			
20	SPHEROCYTES			
21	HYPOCHROMIA			
22	FELV			
23	FIA			
24	ALK. PHOS	150	20-150	10-44 IU/L
25	AMYLASE	400	300-1200	300-1200 S.U.
26	T. BILIRUBIN		0.03-0.45	0-0.69mg%
27	BUN	23	9-25	16-30 mg%
28	CALCIUM		8.1-11.3	8.3-10.5mg%
29	CHOLESTEROL	300	151-337	44-192mg%
30	CREATININE	1.0	1-2	0.8-1.8mg%
31	SODIUM	150	141-155	143-157mEq/l
32	POTASSIUM	5.1	3.9-5.7	3.3-4.7mEq/l
33	CHLORIDE		98-113	105-121mEq/l
34	CO_2		18-24	17-21mEq/l
35	GLUCOSE	97	60-110	70-110mg%
36	LDH		56-502	50-514 IU/L
37	PHOSPHOROUS		2.6-6.0	3.6-6.4mg%
38	SGOT	60	20-80	16-36 IU/L
39	SGPT	40	17-45	13-39 IU/L
40	T-3	51	37-54	37-50%
41	T-4	1.5	1.6-5.6	1.25.2µgm%
42	T. PROTEIN	7.2	5.3-7.4	5.5-7.9gm%
43	GLOBULIN	4.0	2.7-4.4	2.6-5.1gm%
44	ALBUMIN	3.2	2.5-3.8	2.4-4.0gm%
45	URIC ACID		0.2-1.9	0.5-1.1mg%
46	A/G	0.80		

URINALYSIS

Character	Normal	Color	Straw Yellow
S.G. 1.0	1.030	pH	6.0
Albumin	2	WBC	0 /hpf
Sugar	0	RBC	0 /hpf
Acetone	0	EPC	Few /hpf
Bacteria	0	Crystals	0
Other		Casts	0 /lpf

COMMENTS ACTH-90 (17-98 pgm/ml Normal); CORTISOL-resting-1.4,stimulation-20.2; LE PREP (–); ANA (–); COOMBS (–); IgG-1600 mg%

Case Three:

A ten month old male basset hound was referred to the hospital for an allergic dermatitis. A standard allergy workup was performed. Food allergies were ruled out and intradermal tests done. The dog reacted to a large number of antigens and desensitization began with an alum precipitate vaccine. Within four weeks a dramatic improvement was observed. After

six months, the dog's skin appeared normal. A maintenance dose of monthly vaccine was established. Soon after the dog reacted badly to a standard pet shampoo. Signs of flea allergy dermatitis developed. Certain foods began to cause severe reaction. The alum precipitate vaccine was stopped and the resting and stimulation cortisol performed. **Resting Cortisol 1.2 and Stimulation 10.8.** Based upon past clinical observations, we suspicioned a depressed zona fasciculata.

The typical pruritic pustular skin lesions developed with generalized lymphodenopathy and marked edema of the ears and feet. Steroid therapy was instigated with good results. Over a one year period the dog's condition deteriorated. The following tests were performed with the following results:

LABORATORY RESULTS:

CASE III

Cat
Dog
Other
Patient AP 133 Doctor PLECHNER
Hospital C.A.H. Date 10/2/77

	CBC	Results	Canine Normal	Feline Normal
1	WBC x 10^3	9.8	6.0-17.0	5.0-19.0
2	RBC x 10^3	8.7	5.5-8.5	5.0-10.0
3	HGB	18.3	12-18	8-15
4	HCT	54	37-55	24-45
5	SEGS	64	60-77	35-75
6	NON-SEG		0-3	0-3
7	META		0	0
8	LYMPHS	32	12-30	20-55
9	MONOS	1	3-10	1-4
10	EOS	3	2-8	2-12
11	BASO		Rare	Rare
12	NUC. RBC		0	0
13	MCV	62	60-77	39-55
14	MCHC	34	32-36	30-36
15	PLATELETS x 10^3	Normal	150-700	250-700
16	RETIC		0-1	0.4-6.4
17	RBC MORPH	Normal		
18	POLYCHROMISIA			
19	ANISOCYTOSIS			
20	SPHEROCYTES			
21	HYPOCHROMIA			
22	FELV			
23	FIA			
24	ALK, PHOS	230	20-150	10-44 IU/L
25	AMYLASE	800	300-1200	300-1200S.U.
26	T. BILIRUBIN		0.03-0.6	0-0.4 mg%
27	BUN	18	9-25	16-30mg%
28	CALCIUM		8.1-11.3	8.3-10.5mg%
29	CHOLESTEROL	330	151-337	44-192mg%
30	CREATININE	1.8	1-2	0.8-1.8mg%
31	SODIUM	154	141-155	143-157mEq/l
32	POTASSIUM	4.8	3.9-5.7	3.3-4.7mEq/l
33	CHLORIDE		98-113	105-121mEq/l
34	CO_2		18-24	17-21mEq/l
35	GLUCOSE	107	60-110	70-110mg%
36	LDH	64	56-502	50-514 IU/L
37	PHOSPHOROUS	3.5	2.6-6.0	3.6-6.4mg%
38	SGOT	33	20-80	20-70 IU/L
39	SGPT	42	20-80	20-70 IU/L
40	T-3		37-54	37-50%
41	T-4		1.6-5.6	1.2-5.2µgm%
42	T. PROTEIN	6.8	5.3-7.4	5.5-7.9gm%
43	GLOBULIN	2.7	2.7-4.4	2.6-5.1gm%
44	ALBUMIN	4.1	2.5-3.8	2.4-4.0gm%
45	URIC ACID		0.2-1.9	0.5-1.1mg%
46	A/G	1.52	0.1-1.11	0.45-1.19

URINALYSIS

Character	Normal	Color	Clear Yellow
S.G. 1.0	1.028	pH	6.5
Albumin	1	WBC	0 /hpf
Sugar	0	RBC	0 /hpf
Acetone	0	EPC	Few /hpf
Bacteria	0	Crystals	Tri-phosphate
Other	0	Casts	0 /lpf

COMMENTS LE PREP (–); ANA (–); COOMBS (–); CORTISOL - 10/18/77 resting-1.2, stimulation 10.8; CORTISOL - 10/2/77 resting-2.1, stimulation 8.6; IgG-2300 mg%

All three dogs' conditions with standard therapy deteriorated. All the owners were considering euthanasia. Based upon our past experimental, clinical research, we recommended bilateral adrenalectomy. (We had performed twelve such adrenalectomies by this time.) The owners were given information as to the severity of the procedure and certainly the experimental nature of the surgery.

MATERIALS AND METHODS:

Adrenal gland tissue was taken at the time of surgery from all three dogs. Part was fixed in a 10% neutral buffered formalin solution for routine histopathology and part was quick frozen for the preparation of frozen sections to

be used for immunopathology.

The formalin-fixed tissue was processed in the routine manner and mounted sections stained with H & E microscopic examination. The frozen tissue was sectioned and was mounted on slides. They were then flooded with fluorescene conjugated anti-canine IgG, incubated at 37°C for twenty minutes and then washed three times with phosphate buffered saline solution at ph. 7.2. The sections were then examined under a fluorescent microscope.

Blood serum was collected from all three cases and assayed for levels of IgG by double diffusion in agar gel plates against anti-canine IgG, along with the control serum from Miles Laboratories. Blood was also assayed for resting and ACTH. stimulated cortisol. Resting blood samples were taken from all three dogs at 9:00 a.m. and then 1 mg./lb. of body weight of ACTH gel given intramuscularly. Two hours later another blood sample was taken from all three dogs. The cortisol levels were again checked a week later and all corresponded. RIA was used for Cortisol determination. Blood serum was taken for ACTH determination with Cases I and II. Ria was used as the method of determination.

Blood serum was taken for testosterone - with Case I - RIA was used for method of determination.

Adrenalectomies were preferred at this time in our clinical research to OP-DDD. (1) There is still much controversy over the specificity of layers of the adrenal cortex directly affected by this drug, (2) due to the severity of the diseases we did not want to risk the toxic effects of the drug (3). We also were interested in the histopathology and immuno-pathology of the glands.

Blood serum was taken from Case One defibrinated by stirring with the syringe, and the lymphocytes were purified and cultered with mitogens added to the culture media to determine the responsiveness of the T - lymphocyte population in the peripheral blood. The lymphocytes were without mitogen as a control, and with the added pokeweed mitogen, Concanvalin A, phytohemagglutinin, sodium metaperiodate, and a water soluble extract of Nocardia Opaca. The media contained tridiated-thymidine for incorporation during DNA synthesis. Following culters for 72 hours, the samples were analyzed in a scintillation counter to assay for tridiated-thymidine uptake by the stimulated lymphocytes.

RESULTS:

Routine histopathologic examination of the adrenal glands from all three dogs revealed in case I and II, a very marked amount of hemorrhagic necrosis of the adrenal cortex, involving all three layers but most extensively in the layers found toward the cortico-medullary junction. The cortex of the glands were quite thinned due to the atrophy of the necrotic tissues. There were some viable cells in the zona glomerulosa and in the more hemorrhagic and necrotic foci were scattered mononuclear inflammatory cells. There was a disruption of the stromal structure of the cortex of the gland as well as loss of epithelial cell structure of the cortex. The medulla of these glands appeared relatively normal, with only hyperemia present. There was no evidence of infectious etiology in the hemorrhagic and necrotic adrenal cortices. Case III revealed a relatively normal adrenal cortex with mild hyperemia.

In Case III, the majority of the adrenal cortex was histologically intact. The inner most layer of the adrenal cortex showed a pressure necrosis due to the hematomma developed in the medullary portion.

The adrenal medulla was almost entirely destroyed. Medullary cells were almost entirely gone due to necrosis and replaced with blood. A few small clumps of medullary cells were found in several areas of the medulla right adjacent to the cortex. There was some organization of the hematoma with formation of fibrin strands. No inflammatory cells were present. Examination of the serum from Case I by electrophoretic analysis showed a heterogenous elevation of the gamma-globulin component. By double diffusion assay, it was found that this serum contained 2800 mg. % IgG, while Case II serum contained 1600 mg. % and Case III serum contained 2300 mg. % IgG.

The assay of the peripheral T-lymphocyte function showed a good response to PWM with 19000 cpm., to Con A with 14000 cpm. and to PHA with 10000 cpm., while the control was 150 cpm.

PATHOLOGY DISCUSSION:

The findings that were described indicate a primary adrenal disease, which consisted of adrenal cortical and medullary hemorrhage, necrosis and atrophy.

The results of Case I show an elevated IgG

level in the serum and that there was specific deposition of this immunoglobulin in the adrenal cortex tissue, suggesting an immunological destruction of the adrenal cortical tissue. Dogs with this kind of lesion may die suddenly and unexpectedly and the principal lesion found is an acute hemorrhagic necrosis of the adrenal cortex. If the lesion develops more slowly, the dog may evidence clinical signs of hypoadreno corticism.

The results of Case II show an acute necrosis of the adrenal medulla with the filling of the defect by blood. This would be considered an acute stress related type lesion.

In Case I with the presence of the chronic generalized demodecosis, a thought of possible immunological insufficiency was considered, and the T-lymphocytes (cell mediated immunity) system evaluated by mitogen stimulation. The results of the test showed that the T-lymphocyte population responded adequately and normally to the in vitro mitogens, when compared to the comparable values obtained from several clinically normal dogs.

ENDOCRINE DISCUSSION:

The adrenal cortex is composed basically of three layers. The outermost layer is the zona glomerulosa which produces aldosterone which is used for reabsorption of sodium and excretion of potassium. This layer is under the primary control of the renin-angiotensin system. The zona glomerulosa is only slightly responsive to ACTH. It is suspicioned in man that ACTH has a supportive effect on the zona glomerulosa but chronic ACTH deficiency does not alter zona glomerulosa cell responsiveness. Zona fasciculata produces cortisol for glucocorticoid action and we feel is involved clinically in endocrine-immune surveillance. This layer is the target area for ACTH and cortisol acts as the substance for negative feed back for ACTH. Cortisol is found in two forms. Cortisol may be bound to albumen which is biologically active and necessary for the negative feed back system to the anterior pituitary to function. Cortisol may be bound to an alpha globulin as transcortin which basically is biologically inactive. Of the cortisol produced 95% is bound and 5% is in a biologically active form. Resting blood cortisols and ACTH stimulated blood cortisols are generally measured by RIA, which measures total cortisol (free and bound). Theoretically speaking, then when normal resting and stimulation values occur like Case I and Case II, are they really normal? If the ratio between free and bound cortisol is incorrect, then adrenal disease may be prevalent even with normal test levels. With the incorrect ratio between bound and free cortisol, the negative feed back mechanism to the anterior pituitary is also less functional. Naturally occurring steroids must have a hydroxyl group at the 11th position to inhibit ACTH release.

Zona reticularis is under the regulation of ACTH and is responsible for the production of estrogens, androgens and prostiglandins.

In the human male two-thirds of the androgen production occurs in the zona reticularis. In the human female, almost all androgen production occurs in this layer.

The adrenal stimulation cycle may originate in the cerebral cortex due to stress or diurnal factors producing neural secretions. **Figure 1.** These and other secretions act upon the hypothalamus to release CRF (Corticotropic Releasing Factor). CRF acts upon the anterior pituitary and causes release of ACTH. ACTH causes release of cortisol from the zona fasciculata. If cortisol production is normal and enough cortisol is in a free state, then the feed back mechanism functions to stop further ACTH release. **Figure 3.** If the zona fasciculata is malfunctioning, then the negative feed back is damaged and ACTH secretion continues. **Figure 4.** The ACTH then effects further release of estrogens, androgens and prostiglandins from the zona reticularis. Estrogens and prostiglandins cause biologically active cortisol to go into transcortin (the bound state) and therefore cause further disfunction to the negative feed back system with the anterior pituitary. Estrogens and prostiglandins also supply hormones for a positive feed back to the hypothalamus thru their effect on CRF. Estrogens and prostiglandins also cause a shift from biologically active thyroid compounds to a bound state and thereby may cause a change in the metabolic rate. Cortisol is necessary for catecholamine synthesis in the adrenal medulla, brain, spinal cord and other nervous tissue. **Figure 2.** Synthesis of epinephrine at proper levels acts as a negative feed back mechanism involving corticotropic releasing factor and ACTH. Therefore, if the zona fasciculata is impaired, then cortisol levels are decreased. If catecholamine synthesis is reduced or inhibited then ACTH release is increased due to the damaged negative feed back from epinephrine and corti-

sol. **Figure 5**. Resultant increased estrogens and prostiglandins then further the cyclic damage.

SUMMARY:

In summary, it is interesting to speculate about the possibility of various degrees of adrenal mediated disorders. There is good evidence that there may be a number of clinical adrenal diseases that are multi-system disorders. If cortisol is the main secretion for endocrine-immune surveillance, then the possible consequence of a mild adrenal disease might manifest itself as an allergy. A severe adrenal disorder might manifest itself as a more serious endocrine-immune complex with resultant auto-antibodies production. Standard blood cortisols, resting and stimulation, and blood electrolytes if normal, do not rule out adrenal disease. Much more research is needed in this area particularly the endocrine influences of the immune system.

Hopefully, this article will stimulate some interest for continued investigation.

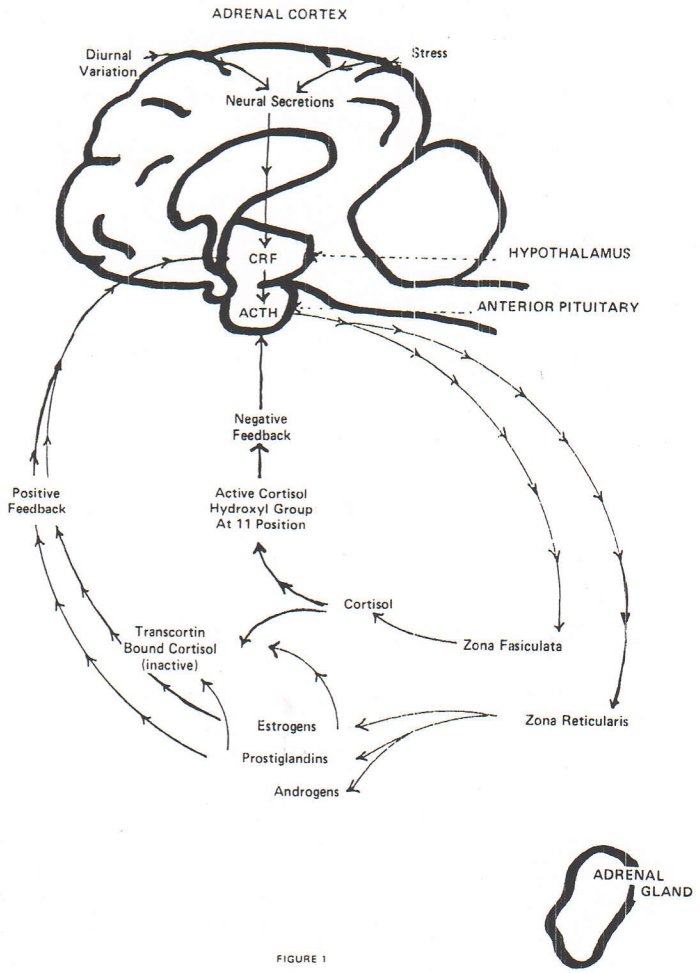

FIGURE 1

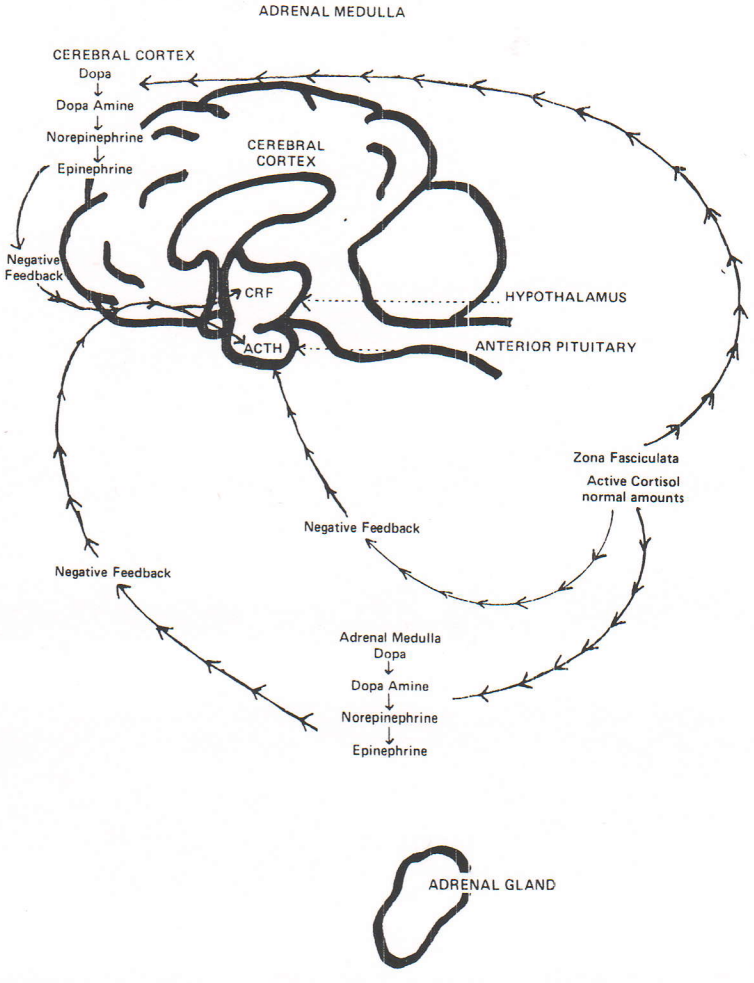

FIGURE 2

ACTH-CORTISOL NEGATIVE FEEDBACK INTACT

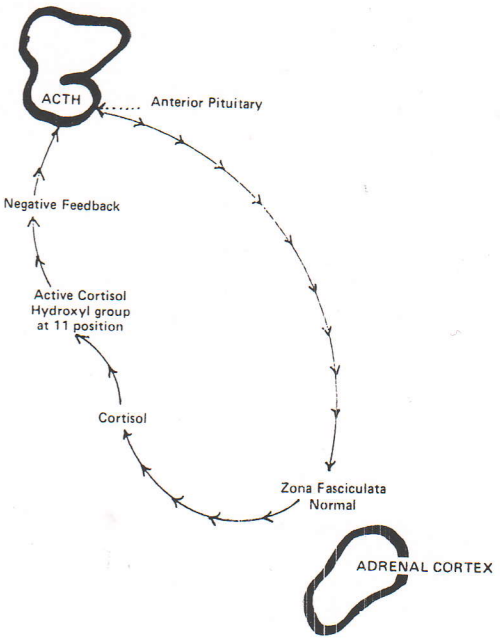

FIGURE 3

ACTH-ADRENAL CORTEX FEEDBACK DAMAGED

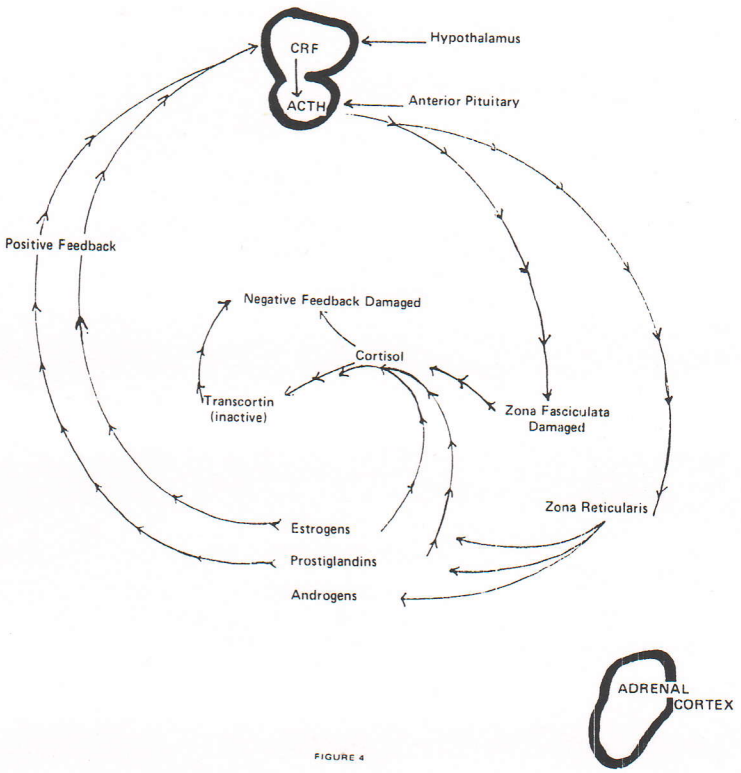

FIGURE 4

EFFECTS OF DAMAGED ZONA FASCICULATA
ON EPINEPHRINE SYNTHESIS AND NEGATIVE FEEDBACK

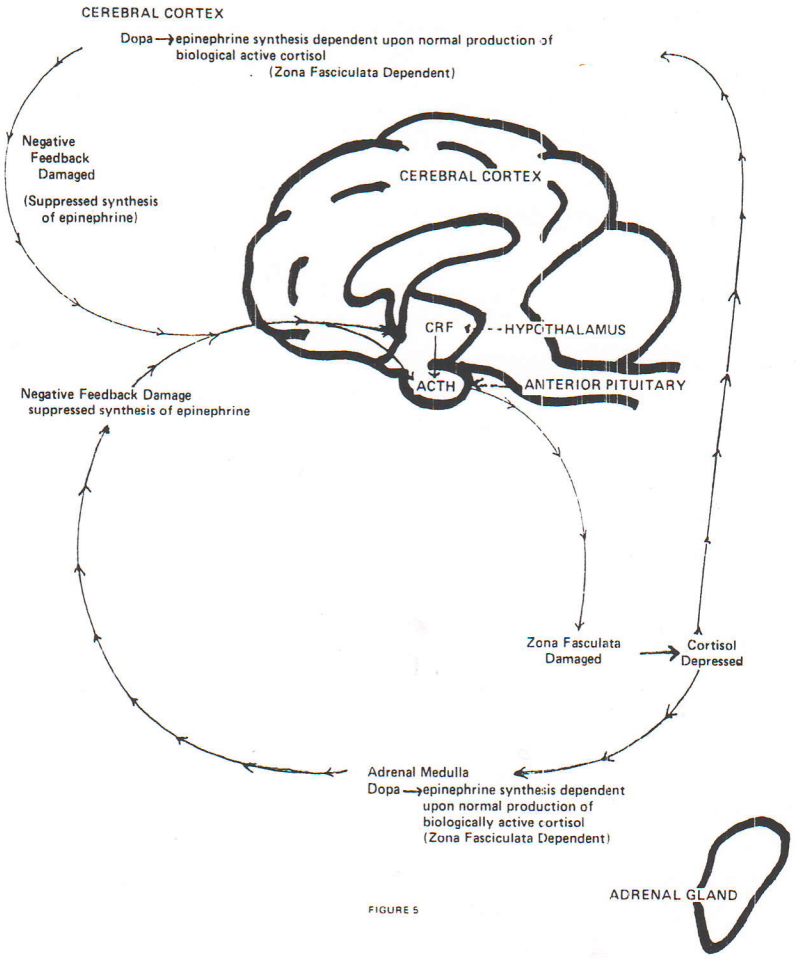

FIGURE 5