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April 25, 1962

OFFICE OF NAVAL RESEARCH

ANNUAL PROGRESS REPORT

AD No. 280202  
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Report Prepared By: Bernard Zimmermann, M. D.

For Period: July 1, 1961 to June 30, 1962

NR: 101-441

CONTRACT: Nonr - 710 (25) current  
Nonr 3256(00) - formerly

ANNUAL RATE: \$9,900.00

CONTRACTOR: West Virginia University

PRINCIPAL INVESTIGATOR: Bernard Zimmermann, M. D.

Co-Investigators: Walter H. Moran, Jr., M. D.  
Ronald G. Severs, M. D.

TITLE OF PROJECT:

Further Investigation of the Role of Endocrines and  
the Regulation of Electrolyte Balance in Trauma  
and Surgery.

ABSTRACT (OR SUMMARY) OF RESULTS

a. See annual reports for 1958 through 1961.

b. During the period of the present report, the project has consisted of two phases. The first has been concerned with the study of the relationship between antidiuretic hormone and surgical stress. This project has been directed by Dr. Moran with the technical assistance of Mr. Ted Browne and Mrs. Janet Gregg. The second phase of the program has been concerned with the factors involved in the formation of peptic stress ulcers following major surgical procedures. This study has been prosecuted by Dr. Ronald Severs with the technical assistance of Mrs. Richard Garretson.

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I. ROLE OF ANTIDIURETIC HORMONE IN SURGICAL FLUID BALANCE

As the first step in the development of a chemical method for antidiuretic hormone, a rat intravenous bioassay was developed. Most bioassays in current use possess a sensitivity of only 10 mU per injected dose and required at least thirty minutes for recovery from the response. Present

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work was directed toward the development of a method possessing a greater sensitivity and a shorter response time. This was achieved (1) by using an intravenous alcohol-water loaded rat instead of the more widely used orally alcohol-water loaded rat, (2) by controlling the rate of water load through a servo mechanism dependent upon the rat's weight, (3) by using a continuously recording conductivity bridge to monitor the change in urine concentration, and (4) by collecting urine within a pressure transducer whose voltage output is fed through a differential operational amplifier which in turn produces a signal representing the rate of urine flow. The signals from the conductivity bridge and the operational amplifier may be amplified to visualize responses that would not otherwise be appreciable. Responses which involved no change in urine output were detected by the conductivity system.

A dose-response curve was linear between 1 mU and 20 mU of pitressin. The difference between the control conductivity and the conductivity at the height of the response was selected as the response metameter. Five-point assays were carried out. The regression lines were not parallel but tended to converge toward the zero point, indicating a linear, non-logarithmic response. This assay satisfied all of the following requirements: (1) A sensitivity sufficient for measurement of antidiuretic hormone in 5-10 ml of human peripheral plasma, (2) An easily-recorded response metameter, (3) and a very rapid recovery from the response permitting many samples to be analyzed within a single working period.

An interesting by-product of this assay has been its ability to follow the very rapid changes that occur subsequent to antidiuretic hormone administration. The phase of water retention followed by salt excretion is clearly demonstrated and can be easily studied. The output voltage from the recording devices can be fed into analog computing circuits to give immediate analysis of data or may be fed back into the rat in servo systems. This biological system may prove to be most helpful in the study of antidiuretic hormone mechanisms.

Preliminary work has been completed on collection, extraction, and crude separation of antidiuretic hormone from plasma samples. Extraction methods of several authors have been tried, but the procedure developed by the group at Western Reserve seems to be the most satisfactory. This

consists of using laked fresh blood, and precipitation with trichloroacetic acid. The precipitate is then washed with ether to remove the TCA. Residue is then passed through a cation exchange column (GC-50), and acid alcohol is used to elute the antidiuretic peptide.

Recoveries and precision are still to be evaluated. Preliminary studies are being undertaken to develop a physico-chemical assay of antidiuretic hormone. Initial trials are being carried out with known microgram quantities of the hormone. This work is in its early stages, but appears promising.

## II. SURGICAL STRESS AND PEPTIC ULCERS

The object of the second group of studies was to determine etiologic factors in stress ulcer and relationship of stress ulcers to types of gastrointestinal ulceration e.g. chronic gastric, duodenal, cortisone ulcers. Studies were done to determine the effect of cortisone on gastric secretion in Heidenhain pouch dogs. Considerable time was spent on the development of appropriate collection apparatus for the pouch dogs.

The samples were collected through a threaded teflon cannula 2" long with a 1/2" inside diameter, which was equipped with one flange to secure it inside the Heidenhain pouch, and another flange on the outside next to the skin to prevent retraction after bringing it through. A large Penrose tube was used to drain the secretions from the cannula to a polyethylene bottle suspended between the dog's forelegs. The tubing was attached proximally to a spindle which screwed on the cannula and distally to a cap which screwed on the bottle. Collections were made by removing the bottle from the cap. Every 4 hours during the day the bottle was emptied and the secretions measured, filtered and the filtrate pooled until a 24 hour collection was accumulated. Collections over night were for a period of 12 hours. Determinations of free and total HCl and pH were done. Titration with a pH meter using 0.1 N Na OH were done to pH 3.5 for free acid and pH 8.5 for total acids.

To date 6 Heidenham pouch preparations in 15-25 kg. castrated female dogs have been studied. Control studies consisted of 10 consecutive 24 hour daily collections. Control runs were followed by collections during a 10 day

period during which cortisone 180 mg./day was administered intramuscularly. One of the 6 dogs was discarded because of bleeding around the cannula; another has been incompletely studied. The remaining 4 dogs showed increases of 14%, 150%, 190% and 200% in free HCl. Two of the dogs had their total acids increased 132% and 210%, and 2 had no change in total acids. The volume increased in two dogs and decreased in two dogs.

CHANGES IN GASTRIC ACIDITY AND VOLUME WITH CORTISONE

<u>Dog. No.</u>	<u>Free HCl</u>	<u>Total HCl</u>	<u>Volume</u>
G2	+200%	+210%	+22%
G6	+190%	+132%	+43%
G19	+150%	No change	-43%
G20	+14%	No change	-20%

The consistency of the gastric mucosa appeared to change during the cortisone administration. Hypersecretion of gastric juice did not appear until 3-5 days after onset of cortisone and persisted 7-10 days after discontinuance.

**PLANS FOR FUTURE:**

**Immediate:**

1. As soon as the method for the crude separation for antidiuretic hormone has been standardized, it will be applied to study of peripheral levels of antidiuretic hormone in the blood of dogs undergoing graded surgical stresses.

The first project will consist of peripheral samples drawn at fairly close intervals during the stress situation. The measured levels of antidiuretic hormone will be compared with levels calculated from the dogs own antidiuretic response at that instant.

The dog preparations will be set up in the same electrobiological system as the bioassay rat with both the urine response and plasma levels being monitored at the same time.

After the normal response to this stress has been studied, different means will be employed to block this response, such as alcohol infusion and nerve block. These

studies will provide (1) a convenient model upon which to test validity of the analytical method and (2) information regarding the time sequence of the antidiuretic response due to these various models of surgical stress.

2. The effect of cortisone upon gastric secretion will be determined in additional dogs. Against this background the possible antagonistic or synergistic effects of other steroids will be studied. The effect of cortisone on gastric mucin and mucous will also be investigated.

#### Long Range:

1. Following completion of animal experiments, the crude separation - bioassay method will be applied to the study of the surgical response in humans. Work will continue on the development of a physico-chemical method for assay of antidiuretic hormone. The plan is to achieve a physical separation by several chromatographic systems and to tag the final material with a radioactive compound, either by acetylation or benzoylization. Previous experience in this laboratory with the measurement of aldosterone in plasma would predict that if only one constituent of the vasopressin molecule is labeled, adequate sensitivity to measure peripheral plasma levels in the resting state will be attained.

2. The investigation of cortisone-pancreatic relationships in ulcer formation (e.g. endocrine adenoma, Zollinger-Ellison syndrome) and inter-dependence of adrenals with various other endocrines will also be studied.

#### REPORTS AND PUBLICATIONS

See previous reports for accumulated publications.

Moran, W.H., Martinson, A., and Zimmermann, B.: Estimation of Aldosterone in Small Human Peripheral Venous Samples. Surgical Forum 12:21-22, 1961.

Zimmermann, B.: Electrolyte Disturbances in Trauma for symposium in Journal of Trauma. J. M. Howard, Editor. In preparation.

Ameli, Mohamed, Moran, Walter H., Grage, Theodor B. and Zimmermann, Bernard. The Effect of ACTH and Potassium on Aldosterone Output in Normal Human Subjects: With Reference to Aldosterone Regulation During Surgical Procedures. In preparation.

BOOK

Moran, W.H.: Endocrine Glands - "Surgical Research" Little Brown & Co. - Boston - In preparation. (Edited by W. Ballinger)

BUDGET

SALARY:

Technologist - Ted Browne 4200.00

SUPPLIES:

Animals:	500 rats @ \$1.00	500	
	100 dogs @ \$3.00	300	
Rat care:	.02 a day x 100	730	
Dog care:	.50 a day x 16	<u>2872</u>	4402.00

Subtotal 8602.00

Univ. overhead  
(30.9% of salary) 1298.00

GRAND TOTAL 9900.00

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The School of Medicine  
Department of Surgery

April 25, 1962

Dr. Leonard M. Libber  
Head, Physiology Branch  
Department of the Navy  
Office of Naval Research  
Washington 25, D. C.

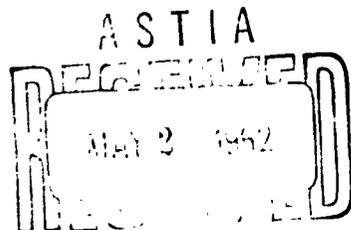
Dear Doctor Libber:

Attached hereto is a progress report on ONR:101-441. Now that we have settled down at West Virginia University, I think that real progress on the project is being made. As you can see here, we are currently working hard on the measurement of antidiuretic hormones and its relationship to fluid balance in trauma, and we are also investigating effects of trauma on the formation of "stress ulcers".

We are still "tooling up" the aldosterone method which we used at Minnesota, and should be determining this on human patients and subjects within the next few weeks. We were fortunate during the past year to receive a grant from the National Institutes of Health for the maintenance of a metabolic unit. This will provide some of the hospital costs for our patients and should greatly facilitate the research being carried out on the ONR grant.

I have attached the proposed budget for the coming year. We are greatly in need of more funds, but I have not applied for a supplementary budget since in recent years I gained the impression that funds for supplementing existing projects have not been available. If, however, you should feel that an additional request would be in order, I would greatly appreciate your advice in this matter.

As in the past, we are extremely grateful to the Office of Naval Research for its support, and thank you for your continued interest in this work on the physiology of trauma.



Yours very sincerely,

Bernard Zimmermann, M. D.  
Professor and Chairman

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