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WEST VIRGINIA UNIVERSITY
MEDICAL CENTER
Morgantown

The School of Medicine
Department of Surgery

April 15, 1963

Office of Naval Research
Department of the Navy
Physiology Branch (441)
Washington 25, D. C.

We are submitting herewith application for renewal of
our Navy Grant ONR:101-441 for the period November 1, 1963
to October 31, 1964 in the amount of \$9,900.00.

Name and Title of Official
Authorized to Sign for
Institution

Paul A. Miller, President of
the University and Chief Exec.
Officer of the Board of Governors

Signature of Official
Authorized to Sign for
Institution

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APR 15 1963

WEST VIRGINIA UNIVERSITY
MEDICAL CENTER
Morgantown

The School of Medicine
Department of Surgery

April 30, 1963

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

Dear Doctor Libber:

Enclosed is a progress report on ONR:101-441. I am sending three copies to you as well as to the other offices listed in the distribution list attached to your letter of April 24, 1963.

Now that the laboratories are set up at West Virginia, I believe we are making a good deal of progress. The determinations of antidiuretic hormone in the blood in surgical stress and trauma have turned out to be extremely interesting, and I believe will eventually lay the groundwork for better management of fluid balance in injury. Incidental studies reported here on the effect of stress on gastric secretion and ulcer production are also of considerable practical import.

Attached is a proposed budget for the coming year which I trust you will find satisfactory.

We are extremely grateful to the Office of Naval Research for its continued support, and thank you for your continued interest in this work.

Yours very sincerely,

Bernard Zimmermann, M.D.
Professor and Chairman

BZ/pcj

April 15, 1963

OFFICE OF NAVAL RESEARCH

ANNUAL PROGRESS REPORT

Report Prepared by: Bernard Zimmermann, M.D.
For Period: April 25, 1962 to April 15, 1963
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.
Fred W. Miltenberger, 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 1962.

b. During the period of the present report, the project has consisted of two phases. The study of the relationship between antidiuretic hormone and surgical stress has been directed by Dr. Moran with the assistance of Dr. Miltenberger, Mr. T. Browne and Mrs. C. Romano. The second phase of the program has been concerned with the factors involved in the formation of peptic stress ulcers following major surgical procedures, and has been directed by Dr. Severs with the technical assistance of Mrs. R. Garretson.

1. ROLE OF ANTIDIURETIC HORMONE IN SURGICAL FLUID BALANCE

During the past year, the major portion of research effort has involved the study of the effect of surgical procedures upon the circulating level of antidiuretic hormone. The bioassay of

antidiuretic hormone which was described in the last report has been greatly improved. This procedure utilizes the intravenous route for fluid maintenance and anesthetic administration as well as sample injection in the ethanolized rat. Hydration is maintained through a femoral venous catheter and regulated by photoelectrically coupling the infusion pump to the balance on which the assay preparation rests. Urine from a bladder cannula is collected for 5 minute intervals in small test tubes placed in a fraction collector. The logarithm of the urine conductance at the time of maximum response divided by the urine conductance immediately before sample injection is directly proportional to the dose of vasopressin from 0 to 20 micropressor units. The coefficient of variation is $\pm 12\%$ for replicate standard doses ($n = 5$) over the usable range.

A study of vasopressin stability was undertaken to determine optimum conditions for storage of specimens. Pitressin solutions containing 10^{-3} M EDTA at pH 3.5 were found to be stable for at least four months at room temperature.

The third phase involved the development of an isolation procedure which would render the blood sample acceptable to the rat in terms of both hormone concentration and inorganic salt content. Advantage was taken of an observation by Boardman and Partridge who found the carboxylic cation exchange resins such as IRC-50 avidly adsorbed large polypeptide molecules at low pH's when ionization of the resin was completely suppressed and was, therefore, unable to remove cations from solution. In essence, the procedure for blood concentration and desalting consists of (1) precipitation of proteins of 10 ml of freshly drawn whole blood 12.5% trichloroacetic acid, (2) removal of TCA by diethyl ether extraction, (3) adjustment of pH to 4.5 with ammonium hydroxide, (4) adsorption of vasopressin on cation exchange resin CG-50, (5) elution of vasopressin from CG-50 by a solution of 75% ethanol-hydrochloric acid (pH 2.0), (6) reduction of eluate to dryness in vacuo, and (7) redissolving residue in 1 ml of a solution of 0.2% sodium chloride, 0.0285% acetic acid, and 10^{-3} M EDTA. 200 μ l of this material is used for each sample injection. 60 - 80% recovery of added vasopressin is obtained with this system. All operations are carried out at room temperature.

In order to determine the magnitude of peripheral blood vasopressin levels that would be encountered during surgical procedures, a preliminary study was undertaken. Eight blood samples were obtained throughout the surgical procedures from each of five patients. Normal values, obtained preoperatively,

ranged from 1-4 micropressor units/ml of whole blood. Levels rose as high as 130 micropressor units/ml of blood during the procedure. It was apparent from this study that 5 ml blood samples would be adequate during the operation. A more detailed study was completed in one patient in which 30 blood samples were drawn at 5-10 minute intervals in order to determine the temporal relationship between each phase of the procedure and its evoked response. Opening of body cavities and visceral manipulation appeared to be the most potent stimuli of ADH secretion. Observations during nerve blocks and epidural anesthesia pointed to the importance of afferent nerves in mediating the response. Levels remained elevated during the operations and returned toward preoperative values within three hours following skin closure.

2. SURGICAL STRESS AND PEPTIC ULCERS

In order to assess the role of adrenal-pancreatic factors upon gastric hypersecretion, five dogs were prepared with Heidenhain pouches. Following control studies, total pancreatic duct ligations were performed. Two of these animals were subsequently adrenalectomized. Plasma cortisol levels were monitored following these procedures. The adrenalectomized dogs were given 5 mg/Kg of cortisone acetate I.M. each day. At autopsy, adrenals, pituitary, and pancreas were removed, serially sectioned and examined for adenomata or hyperplasia.

Following pancreatic duct ligation gastric secretion increased 120%. Following subsequent adrenalectomy (two dogs), gastric secretions returned to control levels. These findings suggest an extra-pancreatic adrenal factor not involving the adrenal glands. The 5 mg/Kg dose of cortisone maintained gastric secretion at control levels in adrenalectomized but otherwise normal pouch dogs. Plasma cortisol levels were not significantly altered after either pancreatic duct ligation or adrenalectomy.

PLANS FOR FUTURE:

Immediate:

1. Further studies of vasopressin levels will be carried out on patients undergoing surgical procedures with regional blocks, spinal, and general anesthesia. ADH levels will be followed during and after blood donation in normal subjects and during corrective surgery for mitral stenosis.

2. The effect of an adrenal blocking agent, SU-4885, on gastric hypersecretion following stress will be studied.

Long Range:

1. Work will continue on the development of a physioco-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.

2. Changes in gastric mucus following duct ligation and cortisone will be studied. Particular emphasis will be placed on changes in fructose/hexosamine ratios and sialic acid content.

REPORTS AND PUBLICATIONS

See previous reports for accumulated publications.

1. Moran, W.H., Jr. and Miltenberger, F.W.: Use of the Intravenous Route for Maintenance of Water Balance in the Alcoholized Rat Bioassay of Vasopressin. Fed. Proc., Part I, 22:2:386, March-April, 1963. (Abstract).
2. Severs, R.G. and Zimmermann, B.: Cortisone and Gastric Secretion. Fed. Proc. Part I, 22:2:276, March-April, 1963. (Abstract).

BUDGET

Salary:

1. Laboratory Technologist - Mr. W. Browne	\$ 4,800.00
Social Security, compensation, etc.	<u>180.00</u>
	4,980.00

Consumable Supplies:

1. 500 Rats	500.00
2. 100 Dogs	<u>300.00</u>

Animal Care:

1. Rat Care at \$0.02/day/rat	730.00
2. Dog Care at \$0.50/day/dog	<u>1,623.00</u>

Total:	\$ 8,133.00
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Overhead (21.723%)	<u>1,767.00</u>
	\$ 9,900.00