NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

PROGRESS REPORT

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Title: The Effect of Changing Gravity and Weightlessness on Vasopressin Control Systems

A. LAP6/WVU SYSTEM

The LAP6/WVU system was developed by Clyde G. Roby, Jr. in order to simplify the computer programming effort associated with this project. LAP6/WVU is a modified version of the LAP6 system described by M.A. Wilkes in "LAP6 HANDBOOK", Technical Report No. 2, Computer Research Laboratory, Washington University, St. Louis, Missouri (May 1, 1967). LAP6/WVU is designed to take advantage of the additional 1024 words of memory available on the basic LINC-8 or PDP-12 manufactured by the Digital Equipment Corporation. The extra memory has been used to add (1) character by character deletion during the editor mode which facilitates the preparation of input data as well as program manuscripts, (2) eight character symbolic names of which there can be a maximum of 192, (3) tape identification, and (4) a date which is available for listings. A high speed version of LAP6/WVU has been developed in which the LAP6/WVU system resides on a disk. This system requires an 8K LINC-8 with at least 64K of disk storage.

This manual was designed to function as a supplement ot the "LAP6 HANDBOOK" and assumes that the user has a copy of it available. All conventions of LAP6, not specifically contradicted, are identical in the two systems. Minor changes in PROGOFOP have also been made.

LAP6/WVU became operational in October 1968 and has remained in its present form since January 1969. We have been in contact with M. A. Clark at Washington University concerning an acceptable name for the system. The system will be released once a final agreement has been reached. The five copies submitted with this report are NASA's preprint copies

B. COMPARISON OF THE IMMUNIOASSAY AND THE ANTIDIURETIC BIOASSAY OF VASOPRESSIN

In May, 1969, a comparative study of the immunochemical and the biological assays of vasopressin was begun in collaboration with Dr. Myron Miller of the V. A. Hospital in Syracuse, New York. This study consists of five steps of which four have been completed.

1. CALIBRATION OF REFERENCE STANDARDS

A master reference standard was prepared from U.S.P. Posterior Pituitary Reference Standard powder. This master reference standard solution contained 100 mU of vasopressin/ml. as well as 0.3723 gm. of disodium ethylenediamine tetra acetate, 2.0 gm. of sodium chloride, and 0.285 ml. of glacial

acetic acid per liter. 250 ml. of the master reference standard was frozen and shipped to Dr. Miller. At the same time, 10 ml. of this standard was frozen and subsequently thawed at the same time that Dr. Miller thawed the standard that had arrived in Syracuse.

Five aliquots of the master reference standard (never frozen) were assayed in the rat using our current reference standard. Five doses of each aliquot were used. The same procedure was carried out on the master reference standard that had been frozen and then thawed.

Biological (antidiuretic) Assay of
Master Reference Standard - 100mU/ml.

Non-frozen	Frozen
104.168	111.552
103.677	113.900
98.634	118.835
98.2412	79.7644
97.098	99.7647
100.363 ± 0.33 (S.D.) mU/ml.	104.763 ± 1.56 (S.D.) mU/ml.

Immunoassay of Master Reference Standard - 100mU/ml.

101.0 ± 6.8 (S.D.) mU/ml.

The accuracy of the master reference standard was established as being approximately 100mU/ml. and was used by both laboratories as a common reference point in the following steps.

2. RECOVERY AND LINEARITY OF METHODS

In the second step, calculated amounts of master reference standard were added to fresh plasma prior to extraction. The amounts of added vasopressin were calculated to fall within a range that could easily be detected by both methods. The four plasma samples were extracted with TCA and them chromatographed on CG-50 resin columns. The acid-ethanol eluates from the columns were split and then evaporated to dryness. 1/3 of each eluate was redissolved in rat injection solution for bioassay. The other 2/3 was redissolved in phosphate buffer (pH 6), frozen and then shipped to Syracuse for immunoassay.

SAMPLES

ØX	60 ml. of plasma + No vasopressin
1X	30 ml. of plasma + 1500 μU of vasopressin
2X	30 ml. of plasma + 3000 μU of vasopressin
3X	30 ml. of plasma + 4500 μU of vasopressin

The following results were obtained:

	Sample No.	ADH Added μU/ml.	ADH Bioassay μU ± (S.E.)/ml.	ADH Immunoassay μU ± (S.E.)/ml.
ØX	2851	0	5.20	24.38
	2852	0	5.41	4.68
	2853	0	4.56	8.12
	2854	0	4.81	11.26
	2855	0	7.97	15.94
			5.59 ± 0.613	12.88 ± 3.42
1X	2856	50	47.2	55.72
	2857	50	42.3	60.0
	2858	50	41.4	65.6
	2859	50	41.5	71.88
	2860	50	32.9	32.6
			41.06 ± 2.30	57.16 ± 6.71
2X	2862	100	95.2	87.6
	2863	100	94.8	123.0
	2864	100	90.9	78.2
	2865	100	91.5	84.4
	2866	100	95.7	78.8
			93.62 ± 1.00	90.4 ± 8.33
3X	2867	150	153.	137.6
	2868	150	142.	115.6
	2869	150	148.	109.4
	2870	150	154.	104.4
	2871	150	155.	100.0
			150.4 ± 2.42	113.4 ± 6.58

The biological assay was linear and closely approximated the theoretical concentration of vasopressin (if the losses during extraction of plasma taken into account). The immunoassay was working at the lower limit of its sensitivity with the ØX sample as evidenced by the coefficient of variation of 59%. The 3X value fell far below the linear extension of ØX, 1X, and 2X values. The two liner regressions were not statistically different.

3. SEMIQUALITATIVE COMPARISON

The third step was more complicated. It consisted of three parts. For the first and second parts, 30,000 μU of master reference standard was added to 50 ml. of whole blood. 20 ml. of this blood sample was centrifuged and the plasma was extracted with TCA and chromatographed on CG-50 resin. The acid-ethanol eluate was then collected in 2 ml. fractions numbered 2900-2909. These were split and assayed in the same manner as in step 2. The values below represent the recovery of 12,000 uU of ADH from 20 ml. of whole blood.

Sample No.	ADH Bioassay μU	ADH Immunoassay μU
2900	68.2	N.D.
2901	3470	2500
2902	3170	2219
2903	278	N.D.
2904	53.3	N.D.
2905	45.2	N.D.
2906	31.9	N.D.
2907	25.0	N.D.
2908	N.D.	N.D.
2909	N.D.	N.D.

The values from the immunoassay indicate that the biological and immunochemical activities of vasopressin have the same coefficient of distribution during adsorption chromatography on CG-50 resin columns. The lack of adequate sensitivity prevented the immunochemical method from demonstrating the entire curve.

Another 20 ml. of the whole blood sample to which 12,000 μU of vasopressin had been added was processed. This time each step was saved and processed as a separate sample. The red cells were separated, washed three times with saline and then laked with distilled water. Each TCA extract of the plasma sample was saved separately. Three additional 5% TCA extractions were carried out.

	Sample No.	ADH Bioassay μU	ADH Immunoassay μU
TCA-1 - 10%	2910	6040	5469
TCA-2 - 8%	2911	1770	688
TCA-3 - 5%	2912	160	24
TCA-4 - 5%	2913	90.2	N.D.
TCA-5 - 5%	2914	+	N.D.
TCA-6 - 5%	2915	N.D.	N.D.
TCA-7 - 5%	2916	+	N.D.
LAKED RBC'S	2917	40.6	N.D.
NACL - 1	2918	N.D.	N.D.
NACL - 2	2919	47.7	N.D.
NACL - 3	2920	691	3000

Again the immunochemical activity matched the biological activity of vasopressin with the limitation of sensitivity of the immunoassay still evident.

The third series 2922-2927 consisted of artifical plasma with vasopressin added to equal vasopressin concentrations that would be encountered in normal plasma. This part did not work out too well as the bioassay was forced to work at 1/3 of its usual sensitivity.

Sample No.	ADH Added μU/ml.	ADH Bioassay μU/ml.	ADH Immunoassay μU/ml.
2922	0.6	N.D.	N.D.
2923	0.0	N.D.	N.D.
2924	1.6	1.18	N.D.
2925	0.2	0.46	N.D.
2926	0.0	+	N.D.
2927	2.5	+	N.D.

These normal plasma levels were beyond the reach of the immunoassay even with the CG-50 resin column concentrating procedure.

4. VASOPRESSIN IN RAT URINE

Because of the lack of an adequate sensitivity for the assay of plasma vasopressin levels, Dr. Miller turned to the use of the immunochemical assay for the estimation of the vasopressin content of urine. In this experiment, five samples of urine collected from normal rats and five samples of urine collected from rats with heritary diabetes insipidus were assayed in the manner described in step 2. No vasopressin was found in the DI urine by either method. The values from the normal rats are listed below.

	Sample No.	ADH Bioassay μU	ADH Immunoassay μU
NL - 1	2954	645.5 ± 20.6 (n=4)	480
NL - 2	2955	382.5 ± 18.5 (n=4)	420
NL - 3	2956	944.7 ± 53.4 (n=4)	950
NL - 4	2957	1298. ± 71.9 (n=4)	1250
NL - 5	2958	919.0 ± 58.9 (n=4)	910

Again, a fairly good correlation was obtained.

I think that the results obtained so far indicate that the immunochemical assay of vasopressin as performed in Dr. Miller's laboratory accurately determines the vasopressin content of urine. His present materials are not sensitive enough to be used in determining ADH in human plasma.

The final step will involve a sophisticated chromatographic test of specificity and is in the process of being performed.

C. PUBLICATIONS

1. Moran, W.H., Jr.: The endocrine response to injury, Journal of St. Barnabas Medical Center, 7(1):2-9 (1970).

EXPERIMENTS SUPPORTED IN PART BY NGR-001-019
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E 19	ADH STABILITY I, EFFECT OF TEMPERATURE
E 20	EFFECT OF BLEED ON ADH AND CRF
E 21	ADH STABILITY II, REPEATED FREEZE-THAW PLASMA
E 22	ADH STABILITY III, REPEATED FREEZE -THAW STANDARD SOLUTION
E 23	ADH STABILITY IV, EFFECT OF TEMPERATURE
E 31	ANTIDIURETIC ACTIVITY OF ANGIOTENSIN
E 32	ANGIOTENSIN RECOVERY ON CG-50 RESIN COLUMNS
E 33	ADH STABILITY V, NA OXALATE AND EDTA AS INHIBITORS
E 34	ADH RECOVERY I, NO ETHER WASH
E 39	ADH STABILITY VI, NA OXALATE AND EDTA AS INHIBITORS
E 40	CRF ISOLATION I, TO AMES
E 41	CRF ISOLATION II, TO AMES
E 42	ADH RECOVERY II, NO ETHER WASH
E 43	ADH RECOVERY III, PH 4.0 RESIN COLUMN
E 44	ADH RECOVERY IV, EE VS NEE
E 45	ADH RECOVERY V, EE VS NEE
E 46	ADH RECOVERY VI, EE VS NEE
E 47	CRF ISOLATION III, TO AMES
E 49	VEITNAM SAMPLES, CRF TO AMES
E 50	ADH ISOLATION I, DIURETIC PEAK
E 53	ADH ISOLATION II, NAOX AND EDTA ON DIURETIC PEAK
E 54	ADH RECOVERY VII, PLASMA
E 55	ADH ISOLATION III, NA ION IN EXTRACT
E 57	ADH ISOLATION IV, THIOGLYCOLATE CORD BLOOD
E 58	ADH ISOLATION V, THIOGLYCOLATE CORD BLOOD
E 61	ADH ISLOATION VI, PITRESSIN VS. USP POST PIT REF IN RAT
E 63	ADH STABILITY VII, STORAGE OF DILUTED EXTRACT INJECT SOL
E 64	ADH CHROMATOGRAPHY I, PITRESSIN
E 65	ADH CHROMATOGRAPHY II, ELUTION FROM PAPER
E 66	ADH CHROMATOGRAPHY III, USP PIT REF
E 67	ADH CHROMATOGRAPHY IV, RESIN + PAPER WITH USP REF
E 68	ANGIOTENSIN CHROMATOGRAPHY I,
E 69	OXYTOCIN CHROMATOGRAPHY I,
E 70	ADH CHROMATOGRAPHY VIII, PLASMA-LUNG CA POOL: GM
E 71	CRF ISOLATION IV, TO AMES
E 72	ADH CHROMATOGRAPHY V, CORD BLOOD
E 73	ADH RECOVERY VIII, RESIN PLUS PAPER CHROMATOGRAM
E 74	ADH CHROMATOGRAPHY VI, USP REF STD - DIST COEF
E 75	ADH CHROMATOGRAPHY VII, CG-50 ION EXCHANGE COL
D 7000	BIOLOGICAL HALF-LIFE OF ADH I, DOG
D 7001	BIOLOGICAL HALF-LIFE OF ADH II, DOG
D 8000	EFFECT OF TILT ON ADH SECRETION
E 76	ADH CHROMATOGRAPHY IX, PLASMA-LUNG CA POOL: CN
E 77	ADH CHROMATOGRAPHY X, PLASMA-LUNG CA POOL: WC
E 78	ADH CHROMATOGRAPHY XI, TISSUE-NORMAL LUNG: CN

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E 79 ADH CHROMATOGRAPHY XII, TISSUE-LUNG CA: CN
E 80 ADH CHROMATOGRAPHY XIII, TISSUE-LIVER METAST.: CN
H 191933 ADH CHROMATOGRAPHY XIV, PLASMA-HEAD TRAUMA: FJ
E 81 ADH RESPONSE I, URATE, CHOH, COSM VS. DOSE
E 82 APOLLO 9 PLASMA SAMPLES
E 83 ADH IMMUNIOASSAY-BIOASSAY COMPARISON I
E 84 ADH IMMUNIOASSAY-BIOASSAY COMPARISON II
D 7002 BIOLOGICAL HALF-LIFE OF ADH III, DOG
E 85 ADH IMMUNIOASSAY-BIOASSAY COMPARISON III
E 86 SHOCK PLASMA ULTRAFILTRATE I
D 7003 BIOLOGICAL HALF-LIFE OF ADH IV, DOG
E87 ADH PLASMA, DIABETES INSIPIDUS
E88 ADH IMMUNIOASSAY-BIOASSAY COMPARISON IV
E89 ADH PLASMA, INAPPROPRIATE SECRETION SYNDROME
E90 ADH PLASMA, HEAD TRAUMA I