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9831A Desktop Computer

9815A Desktop Computer

AN ECONOMICAL APPROACH TO RAPID RIA DATA REDUCTION

Radioimmunoassay is one of the most powerful tools in modern laboratory medicine, and it is not surprising that the majority of clinical laboratories are now doing RIAs. Radioimmunoassay is also one of the researcher's primary investigatory methods into endocrine, metabolic and reproductive functions.

But only part of any RIA takes place chemically in a test tube. The other part is mathematical and consists of generating a standard curve, interpolating unknowns from the curve, doing error analysis and keeping quality control records. For most routine assays, this mathematical portion of RIA is tedious, repetitive and prone to mistakes when done manually with pencil, ruler and graph paper.

In fact, for a typical 100-tube assay, mathematics can consume over one hour of valuable technologist time. Any kind of exhaustive error analysis or quality control record keeping may be ignored because it simply takes too much time.

Now, with a desktop computer system and HP's latest and most comprehensive software, complete data reduction of a 100-tube assay takes less than 5 minutes and provides the following:

- Data entry with punched paper tape, eliminating transcription errors;
- Unweighted and iterative weighted least squares regression of % bound versus concentration, for an accurate standard curve;
- Interpolation of patient unknowns with correction for nonspecific binding, aliquot factors, % recovery due to extractions and quench correction from CPM to DPM for liquid scintillation counters;
- Exhaustive error analysis of standard curve and patient unknowns showing all observed data with associated coefficient of variations (C.V. %), standard deviations (S.D.), confidence limits and figures of merit (NSB, BØ/T, ED 50, slope, intercept, correlation, etc.) for each assay;
- Storage of quality control data for each and every assay for listing and plotting upon command at any time.

Either the 9815 or 9831 system and software will perform all of these calculations for each of your assays. They will also output neat, formal reports at a single keystroke in less time and with greater accuracy than any manual or graphic method and at substantially less cost per assay than computer or time share methods.

Radioimmunoassay Data Reduction on the HP 9815A and the HP 9831A

HEWLETT hp PACKARD

SETTING UP A DESKTOP COMPUTER FOR RIA DATA REDUCTION IN YOUR LAB

You perform the same assay (Digoxin, HTSH, B12, etc.) many times using the same kit protocol and the same gamma (or liquid scintillation) counter. It is natural, then, that a desktop computer permits you to store the common protocols for each of your assays, as well as the data output format for each of your counters. The magnetic tape cartridge will store up to 15 assay protocols and up to 5 different counter punched paper tape formats, for automatic and repetitive access for any assay/counter combination used in your lab.

For each assay, you assign a code number from 1 to 15 and sequentially answer questions to store the assay name, date of entry, number of total activity tubes used, number of nonspecific binding (blank) tubes used, number of zero concentration standard tubes used and the value and number of replicates for all other standard concentration tubes used (see Figure 1). Then you answer whether the assay requires quench correction, aliquot correction, % recovery correction and how many tube replicates are used for patient unknowns. Where appropriate, you store these constants. You do this only once for each assay routinely performed in your lab, and the computer's cartridge remembers.

Similarly, for each gamma or liquid scintillation counter you use (up to 5), you sequentially enter those numbers which relate to position and character length of tube number, time of counting, gross counts and AES (or channels ratio) number, where appropriate (see Figure 2). These stored values then permit you to access the correct punched paper tape routine for whatever counter you use at any future date.

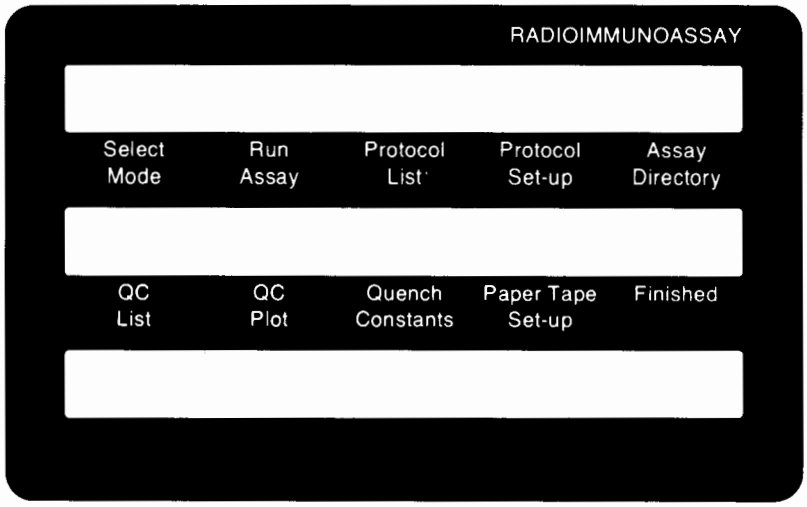
The assay code number and counter code number are all that are required to specify any assay/counter combination used in your lab. All these numbers and values can be updated at any time to adapt to changing circumstances in the lab (e.g., if you adopt new assays and/or replace counters).

```
================
SELECT KEY B-J.
================

 PROTOCOL LIST

WHICH ASSAY #?
                1*

HTSH
ASSAY # 1
DATE: 8.18.75
---------------
# TOTALS=       2
# NSB'S=        3
# B0'S=         3
RESPONSE=B/B0
# CONC.>0=      6
  CONC. 1=   0.60
  CONC. 2=   1.20
  CONC. 3=   2.50
  CONC. 4=   5.00
  CONC. 5=  10.00
  CONC. 6=  20.00
STD. REPL.=     3
UNKNOWN REPL.= 2
ALIQ. FTR.=1.000

USE BOUND COUNTS
NO QUENCH CURVE
RECOVERY=100%

================
```

Figure 1. Typical assay protocol setup — up to 15 protocols can be stored.

```
    PAPER TAPE
      SET-UP

PROGRAM?
  1=CALL
  2=STORE
                1*

WHICH COUNTER #?
  (1-5)
                1*

  PROGRAM FOR
COUNTER #1 IN
MEMORY.
================

DATA ENTRY?
  1=MANUAL
  2=P-TAPE
                2*
```

Figure 2. Program option to accept/select appropriate paper tape format from gamma counter — up to 5 different formats can be stored.



Overlay supplied with software for Special Function keys on the computer keyboard — identifies specific repetitive operations, routines, or reports that can be generated by pressing a single key.

RAPID AND ACCURATE INTERPOLATION OF PATIENT UNKNOWNS

Only at this point will the computer proceed to interpolate patient unknowns from the standard curve selected as the best fit. In a fraction of a second, each set of patient unknown CPM is interpolated from the standard curve, corrected for aliquot reduction, adjusted for % recovery and printed, along with the tube numbers, C.V. %, and ± 95% confidence limits (see Figure 9). Any number of patient unknowns can be computed in this manner.

As indicated earlier, the whole process from standard curve generation to patient unknown interpolations for a typical 100-tube assay usually takes less than 5 minutes...or roughly 10% of the time required to do the job by hand.

UNKNOWNS: Weighted Regression

Aliquot Factor= 1.000

PATIENT #	TUBE #	CPM	B/B0	LOGIT	CALCULATED DOSE	+OR- 95% CONFIDENCE	COEFF OF VAR
1	326	10230					
	327	10170					
	B=	9831	0.950	2.938	0.30	0.041	6.7%
2	1	9780					
	2	9970					
	B=	9506	0.918	2.425	0.45	0.124	13.6%
3	3	3270					
	4	3400					
	B=	2966	0.287	-0.913	6.23	0.426	3.4%
4	5	11090-OFF CURVE					
	6	10980-OFF CURVE					
5	7	11460-OFF CURVE					
	8	11360-OFF CURVE					
6	9	8200					
	10	7970					
	B=	7716	0.745	1.075	1.31	0.170	6.5%

**=HIGH VARIATION

Figure 9. Fully labeled patient unknowns interpolated automatically from user-selected curve.

QUALITY CONTROL DONE AUTOMATICALLY

For each assay, every time it is performed, you have the option of storing the following assay parameters and figures of merit:

- N = Nonspecific binding (NSB or blank) counts,
- BØ = Bound counts at zero standard concentration,
- (B/T)ø = Initial percent bound,
- ED 50 = Estimated dose at 50% binding (weighted or unweighted),
- Date of each assay,
- Code number of technologist performing assay.

At a single keystroke, you can output a list of these values for the previous 20 assays and/or print a Levy-Jennings quality control plot with ± 2 standard deviation limits for each parameter (see Figure 10).This permits comparison of quality from assay to assay and is a very powerful reason for performing a linearized regression on RIA standard curves.

This kind of quality control record keeping is of inestimable value in clinical labs where kit shelf-life and variable technologist bench techniques can make or break the precision of an assay on a daily basis. The desktop computer permits this comprehensive QC automatically with a single keystroke.

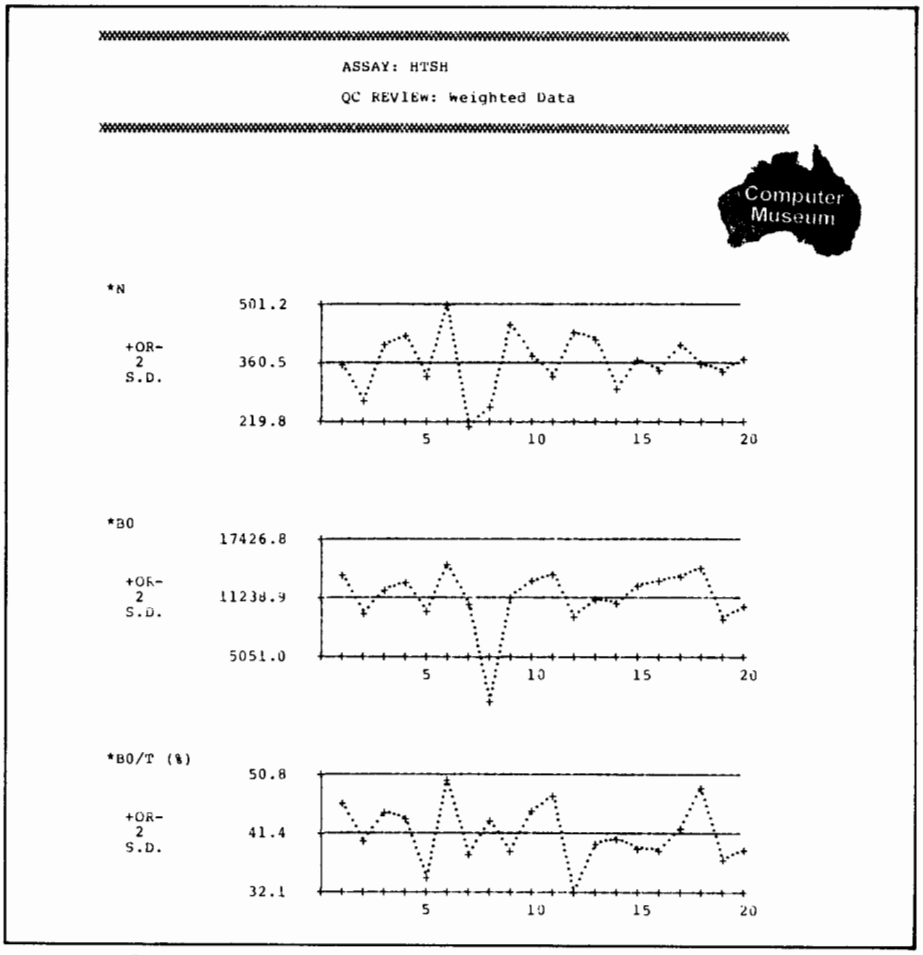


Figure 10. Three of 7 possible QC plots for preceding 20 assays

Once all the standard curve data has been approved and verified, the computer then calculates an unweighted least squares regression of logit (% bound) vs. $\log_{10}$ (concentration) and outputs the equation, correlation coefficient, ED 50, and standard concentration comparison table (see Figure 6).

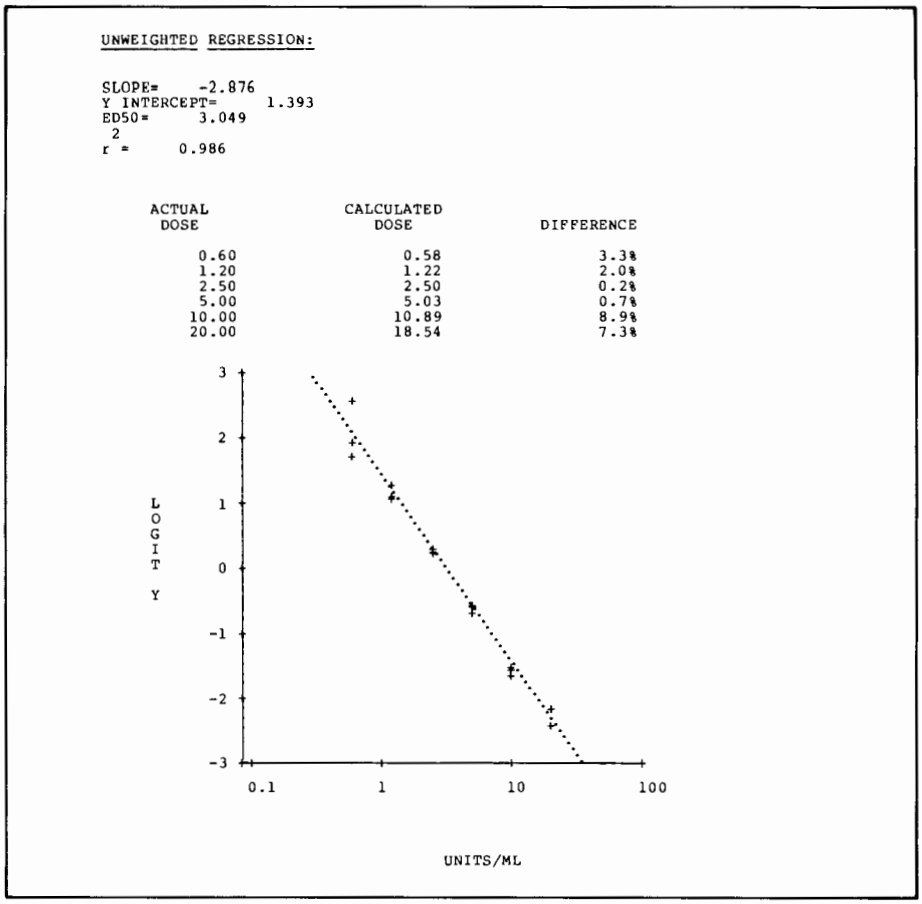


Figure 6. Unweighted least squares regression of transformed (linearized) standard curve data

Then the computer repeats this procedure and calculates the "adjusted", variance-weighted least squares regression, iterating the curve fit based upon each observed count's distance from the computed line (see Figure 7). This gives points farther from the line less contribution to the overall regression than points closer to the line, which is the recommended procedure in nearly 40 years' worth of bioassay methodologies.*

At this point, you are again permitted to delete standard curve data if you discover a previously overlooked broken or mispipetted tube (see Figure 8).

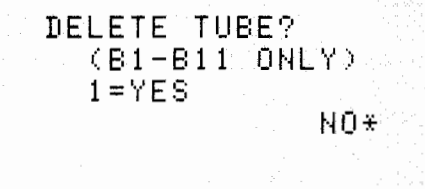


Figure 8. Operator permitted to delete outliers at this point.

At your command, the computer will recalculate all standard curve data in a matter of seconds...giving you complete control over your assay and the most accurate graphical representation of your data in the region(s) of interest.

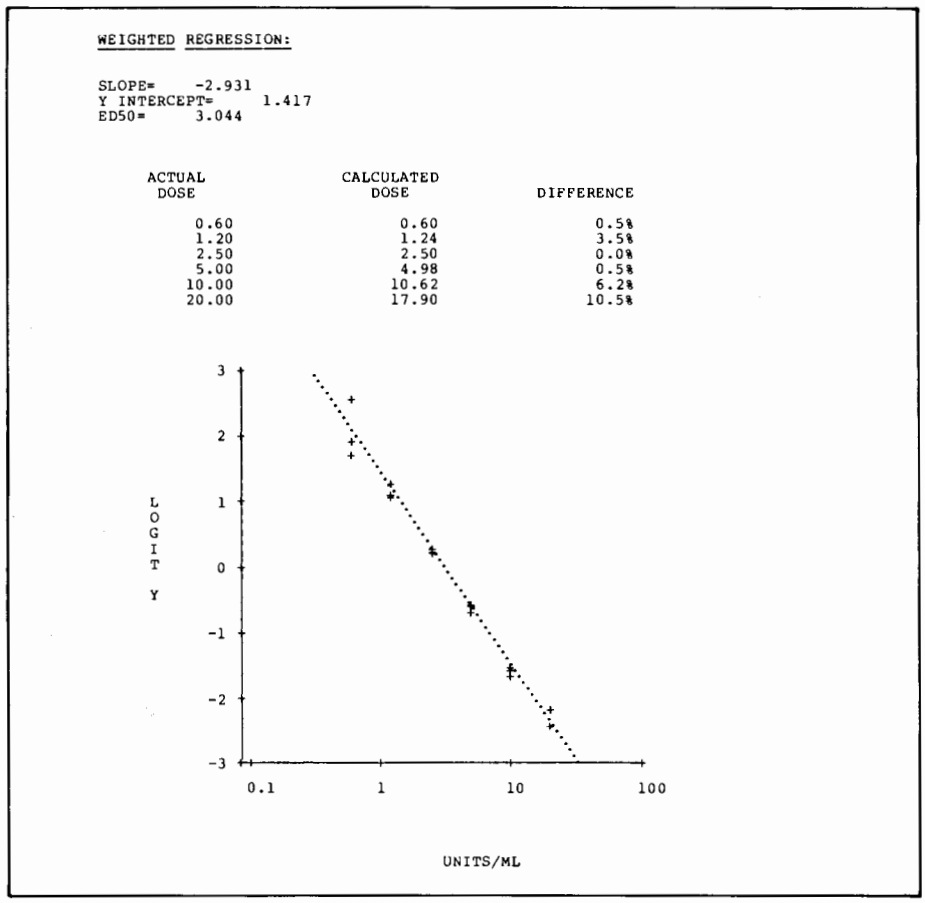


Figure 7. Variance-weighted linear regression of standard curve data, iterated for optimum fit

*See "Radioimmunoassay Theory for Health Care Professionals", HP Part No. 09830-75250 (included in RIA Software Package).

SIMPLIFIED PREPARATION OF YOUR STANDARD CURVES

After performing your assay chemistries and collecting your counter data (CPM for each tube), it is a simple matter to enter the data manually via the keyboard or preferably via punched paper tape. We strongly recommend that your counter be equipped with a teletype punch to facilitate rapid and error-free data entry by punched paper tape.

The computer, already knowing the assay protocol and counter format (previously described), now takes the observed CPM for each tube (either *bound* or *free*) and corrects each for nonspecific binding (and converts to DPM where a liquid scintillation counter is used requiring quench correction). The observed counts for the standard curve are stored in memory. Each count can be manually changed or deleted if it is discovered that erroneous values are present, due to pipetting errors, broken or spilled tubes, failure to add antibody, etc. (see Figure 3). Each set of tube replicates is analyzed for pipetting error, C.V. %, and standard deviation (see Figure 4). Appropriate error messages are printed if variations exceed acceptable limits (e.g., 4% pipetting error, 15% C.V., etc. . . .which can be specified by user). An automatically plotted % bound vs. concentration standard curve on the HP 9871A Printer helps here to detect gross outliers (see Figure 5).

```
WHICH ONE?
  0=NO CHANGES
  1=CORRECT TUBE
  2=DELETE TUBE
              0*
```

Figure 3. Operator permitted to change or delete data at this point.

ASSAY: HTSH

TECH. #: 20481
DATE: 9-02-75

STANDARDS:

	TUBE #	CPM				
TOTALS	300	28000				
	301	26000				
	Avg.=	27000	Pipetting Error=	8.5%*		
NSB'S	302	425				
	303	339				
	304	342				
	Avg.=	369				
B0'S	305	10670				
	306	10570				
	307	10925	B0/T	B/B0	S.D.	COEFF OF VAR
	B0=	10353	38.9%	1.000	0.018	1.8%

DOSE	TUBE #	CPM	B/B0	S.D.	COEFF OF VAR	LOGIT
0.60	308	9140				
	309	9410				
	310	9990				
	B=	9145	0.883	0.042	4.7%	2.073
1.20	311	8060				
	312	8460				
	313	8110				
	B=	7841	0.757	0.021	2.8%	1.141
2.50	314	6140				
	315	6150				
	316	6280				
	B=	5821	0.562	0.008	1.3%	0.250
5.00	317	3810				
	318	4090				
	319	4030				
	B=	3608	0.348	0.014	4.1%	-0.626
10.00	320	2140				
	321	2010				
	322	2220				
	B=	1755	0.169	0.010	6.0%	-1.590
20.00	323	1210				
	324	1430				
	325	1430				
	B=	988	0.095	0.012	12.9%	-2.255

*=LARGE ERROR
**=HIGH VARIATION

Figure 4. Standard curve data list and replicate statistics

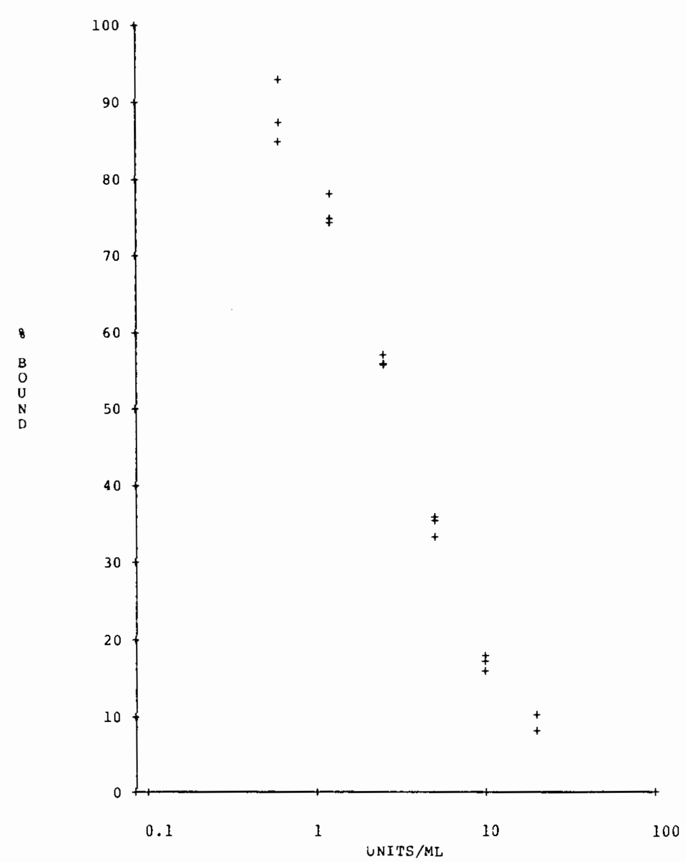


Figure 5. Untransformed dose-response plot

BENEFITS OF USING DESKTOP COMPUTER SYSTEMS AND SOFTWARE

Use of a Hewlett-Packard desktop computer and RIA software dramatically reduces the time required for data reduction of assays (up to 10 to 1), while improving accuracy.

Classical mathematical and statistical methods are used and the program yields easy-to-understand numbers which help you judge the accuracy of each of your assays. You can be more confident that each assay's behavior is within acceptable limits and that patient values are valid. It's very much like having a professional statistician at your side at all times.

Also, with no additional complexity, the computer automatically performs all the QC record keeping that you will need on a daily basis to monitor your bench chemistry procedures. Assay parameters and figures of merit are always at your fingertips for immediate inspection...fully labeled and easy to understand.

The desktop computer is also a versatile tool for non-RIA tasks in your lab. Wherever you have "number crunching" to do or data-logging to perform, these new systems will supply the power and speed to make these jobs a lot simpler.

HP 9815 SYSTEM CONFIGURATION

The 9815 is a fast, small, powerful, yet inexpensive RPN desktop computer. For persons with limited computer knowledge and experience, the 9815 is the ideal laboratory computer and serves as the core of a low-cost Radioimmunoassay Data Reduction system.

The 9815 Desktop Computer system for RIA consists of:

- 9815A Desktop Computer with 2008 program steps (Opt. 001) and I/O capability (Opt. 002),
- Radioimmunoassay Data Reduction software (Part No. 09815-14250) which includes a program cartridge, user's manual, and the RIA Theory Monograph.

For formal reports and plotting or graphic output, you may wish to add the 9871A Character Impact Printer.

HP 9831 SYSTEM CONFIGURATION

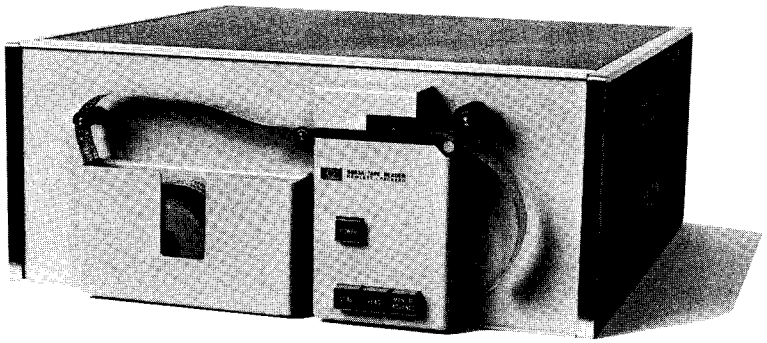
The new 9831 Desktop Computer is an advanced version of the popular HP 9830 Desktop Computer. Programmed in BASIC, a high-level computer language, it is the ideal choice for labs having many different computation tasks to perform.

The 9831 Desktop Computer system for RIA consists of:

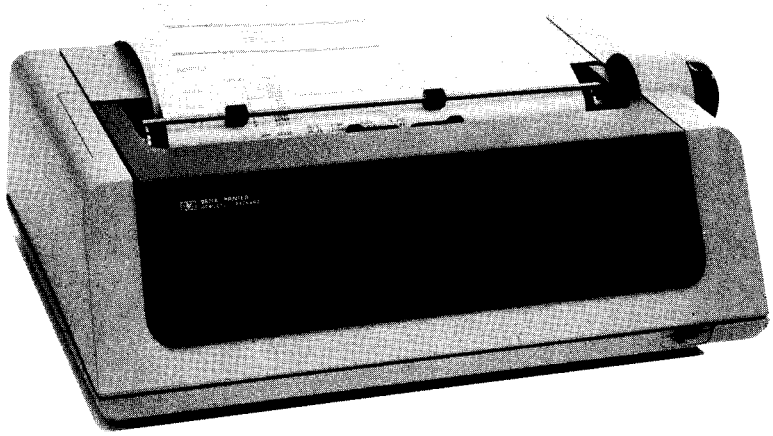
- 9831A Desktop Computer with 16k bytes additional memory (Opt. 001),
- Radioimmunoassay Data Reduction software (Part No. 09831-14250) which includes a program cartridge, user's manual, and the RIA Theory Monograph,
- 9871A Character Impact Printer for plotting and graphic output.

OPTIONAL EQUIPMENT

Complete RIA data reduction can be accomplished on either the 9815 or the 9831 systems. Optional, but recommended, peripherals for use with these systems are either the 9863A or 9883A Tape Reader for automated data input.



9883A Tape Reader



9871A Printer

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