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⑥
USE OF COMPUTERS IN THE ANALYSIS OF PLASMA HORMONES. RESULTS WITH INSULIN.

(Apport de l'informatique a l'analyse des hormones dan le plasma. Resultats concernant l'insuline.)

⑩ A. J. VALLERON ~~and~~ G. E. ROSSELIN

Ann. Biol. Clin. 29:145-152, 1971

(Translated by Phebe W. Summers)

// 26 Jun 1972

The radioimmunologic method of analysis of protein hormones in complex media (1) presents an interesting advance in diabetology and endocrinology. Several central laboratories have resolved the problems with regard to levels of protein hormones in plasma during analysis annually of several thousand samples. We present here results obtained by information analysis of plasma hormones work, carried out during a study of epidemiologic order in a large group of healthy subjects (2-4). ^{are presented.} The informational study was applied to determinations of insulin using separation on adsorbant powder. It can be applied equally well to automation of determinations of other hormones such as somatotropic hormone (5) and luteinizing hormone. ^{We} will not discuss here the problems posed by automation of the manipulations, we will discuss automation of the necessary calculations for control of quality and management of the results.

Material and Methods

I. Technique of Sampling

The blood samples are drawn and centrifuged using conditions previously described (6).

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II. Method of Determination Used

The radioimmunologic assay of hormone is carried out using separation on silicate following the general principles previously presented (7). The technique described for insulin (6), somatotropic hormone (8, 9) and luteinizing hormone (10, 11) have been presented by others. Only the general principles in the informational problem applicable to this method will be mentioned.

Free hormone is absorbed by silicate and after centrifugation, its radioactivity is measured in an automatic counter. Control tubes are measured at the time for a reference or standard curve and for the plasma. They are given as the quantity of iodized hormone adsorbed by the silicate in the absence of immune serum. In the usual experimental conditions, the controls (T) represent 90% of the total radioactivity. It has been verified that this percentage decreases under some conditions thus distorting the results: alteration of the hormone used as tracer at the time of iodination or denaturation of the iodinated hormone at the time of incubation. When the iodinated hormone is bound to an excess of antibody, the percentage of radioactivity is of the order of 10%.

The tubes of the normal or standard curve are obtained by cross-quantities of unlabeled hormone or cold in the presence of the same system of iodinated hormone-antihormone antibody. Under these conditions, the quantity of iodinated hormone adsorbed on the silicate (F) increases and the activity present in the supernatant in the form of a antigen-antibody complex decreases. The ratio of F/T of the quantity of iodinated hormone to the activity of the controls increases as a function of the quantity of

cold hormone. Based on the technique used for separation, it is necessary that the mean of the standard curve should be identical to the mean of plasmas determined. To attain this condition, the curve can be incubated in plasma without hormone, or even a quantity of plasma known to contain small quantities of hormones is added just before the separation replacing an equivalent amount of the incubation mixture. These two techniques, with the immune serum that we used, give similar results. Also, it is easiest to obtain plasma containing a little insulin than plasma not containing all the insulin, the procedure regularly used is that which consists of adding plasma low in hormone before the separation.

III. Calculation Techniques

The programs for treatment of the data have been written in Fortran IV with the exception of the program for decoding the perforated tape coming from the scintillation counter. Classical statistical methods (12) have been used, adjustment of the polynomeals of least squares on observations equally spaced which is utilized for several repetitions in the program was described in particular by Bennet and Franklin (13).

Results

The various informational steps necessary for automation of the determinations are shown schematically in Figure 1. If one follows it the plasma sample from the moment of its being taken to the moment where it results in determination, one can distinguish the following steps: identification of the sample tube of the series, placing in the memory the number of counts, calculation of the quantity of hormone contained in each tube and storage of the results for later use.

I. Identification Step

At the Health Center where the sample is taken, one writes for each plasma sample, on the left side of the card shown in Figure 2, the dates of sampling, name, first name and number of the subject file, the nature of the assay for which the sample was taken, the eventual result and finally the number which is fixed on the tube. This number is used to mark the tubes; at the time when they are introduced into the counter, the right part of the card is filled with the corresponding number at the place in the belt. This procedure eliminates confusing tubes; it allows the laboratory liberty to organize the order of determinations. The second identification problem is the identification of the series of determinations in which the preceding sample is included: date of test, date of incubation, date and specific activity of the iodinated hormone used, date and specificity of the immune serum, date and number of the albumin lot, date and lot of the hormone which was used to establish the standard curve, are noted on a separate card. The group of cards is then transformed to perforated cards for handling by the computer.

II. Calculation of Results Step

To make the calculations possible, a strict plan must be followed, the data coming from the scintillation counter have to be interpreted and, finally, a general program must be written.

A. Protocol of Determination

Preparation of an automation program necessitates definition of a protocol (Table I). A series of determination has been defined as 200 tubes

or more; this quantity is adjustable to the equipment available, but it has been preferable to have a standard curve of this order of numbers of tubes. When more than 200 tubes must be determined, the same protocol is repeated. To establish the standard curve, 8 determinations are used for each in duplicate to allow assessment of the precision of the curve. The chosen determinations are in geometric doubling progression. For insulin, they go from $3\mu\text{U/ml}$ to $400\mu\text{U/ml}$ of plasma, or 0.3 to $40\mu\text{U/ml}$ of incubat? The control tubes are equal spaced every 10th on the belt and the test plasma tubes are placed between them..

B. Interpretation of the Data Coming from the Counter

At the end of the counting, numbers of counts corresponding to each tube are punched on a paper tape and become a part of the program, other information is punched at the same time. The perforated tape is decoded and transcribed by a special program onto a magnetic tape for later treatment. The final program uses this magnetic tape for selecting the useful information (number of counts on the channel concerned, etc.); at this transcription step from the perforated tape to the magnetic tape, a list comes to the lab which can briefly verify each item for errors and damage to the perforated tape.

C. Calculation Step

1) Evaluation of the standard curve.

Table II gives the results obtained in an exaple. From the difference observed d_1 between the 2 percentages corresponding to the same dose, one can estimate (12) the variance of the percentage of activity or silicate by the quantity

$s^2 = \frac{1}{16} \sum_{i=1}^8 d_i^2$. In the example treated here one finds $s^2 = 0.70$
 $\times 10^{-4}$ with 8 degrees of freedom.

This supposes that the percentage activity on silicate has a constant variance whatever the dose, and is a reasonable approximation.

The problem of linearization of the standard curve of a radioimmunoassay is often proposed (14). The usual transformations proposed (probit, logit...) do not suffice to linearize the standard curve in our experience (5). Finally, the automatization makes it possible to have confidence in the quality of the method of linearization chosen so as to correct the program for each occurrence. We do not linearize the standard curve but approach it either by polygonal adjustment or by the polygon of least squares of the 4th degree; the log doses being equally space, the use of orthogonal polygons allows easy calculation (13), the polynomial of which can be verified in Figure 3 and fits the facts perfectly. This is the expression of the standard curve which is used by the rest of the program for later calculations. In Figure 3 are shown by dashed lines, the upper and lower limits that can be assigned to the standard curve Y , Y_1 sup. and Y_1 inf. are calculated by the relationship Y_1 sup. = $\hat{Y}_1 + t_8 s$ and Y_1 inf. = $\hat{Y}_1 - t_8 s$ in which \hat{Y}_1 is the value estimate of the standard curve, and t_1 is the Student t for 8 degrees of freedom corresponding to the chosen probability (for a confidence interval having 95 changes in 100 of containing the true value, one has $t_8 = 2.306$). These limits of the standard curve permit giving for each dosage a CL of 95%.

2) Taking account of the variation of the control tubes

The decrease of the radioactivity of iodine in the course of determination, the fluctuations of the measure of the radioactivity and diverse events make the number of total counts of the control tubes vary from one end to the other of the run. One must take into account the systematic variations of these controls, to eliminate the variations of these controls, to eliminate the variations due to imprecision in the number of counts and to develop a procedure capable of measuring the very large variations of these controls which can be interpreted as the smallest number of incidents able to affect the quality of the determinations. Figure 4 shows the variations of the controls during the course of one run. The line trace is the polynomial of least squares. Each sample of plasma will be reported as a theoretical control which is found at the same place and whose value will be calculated by the preceding polynomial. Finally, when the 2 experimental controls on either side of a tube differ one from the other by 75% of the calculated value of the control, a signal appears in the results, asking for verification of the origin of this control variation.

Neighboring controls are very close to each other in value; the principle of calculation of a theoretical control for each standard tube is maintained; still, it is a straight line of least squares which is used for each calculation.

III. Expression of the Results

Results are preserved as a printed document and on magnetic tape for later statistical treatment. The printed results are divided into 3 parts:

one concerns the calculations related to the standard curve and its precision, and the estimations of the standard and plasma controls for each tube; the second concerns the detailed results (fig. 5) where are shown all the identification in the middle of the previous determination, the estimated quantity of insulin and its 95% confidence limits relative to the imprecision on the standard curve, a recall of the number of counts observed in its tube, the neighboring experimental controls and the calculated controls. The third part is a summary of the results (fig. 6) and is printed in several copies. Where the controls are too variable or where an incident has been signaled at the time of manipulation, the results are marked with "à verifier." (to verify)

Comparison between the manual estimation of 96 determinations and the estimation using our program was made: there was very high correlation ($r = 0.99$, $p < 0.001$).

More interesting is the highly significant difference ($p < 0.001$) between the 2 estimates. This difference can be interpreted by the fact that manual estimation does not allow close approximation of the standard curve and the variation in the controls.

Discussion and Conclusions

The method of expressing the standard curve which we propose avoids all the limitations imposed by linearization methods (15).

Another factor for our procedure permits controls throughout the determinations: identification controls of the tubes, control of quality of the information recorded from the counter, control of the stability of

the measure of radioactivity in the counter; the confidence interval calculated for each determination permits comparison of several series of determinations against the same standard curve. The use made of this confidence interval permits ignoring for the first time on the one hand the error of the hypothesis of equality of the variance as a function of dose (14), on the other, the fact that it is only a reflection of the imprecision of the standard curve. It will still be necessary to bring into the calculation of the confidence interval the imprecision on the number of counts obtained at each determination; this last operation loses nevertheless its importance from the moment where one compares it to the previous calculation.

Finally, it should be noted that the informational application to radioimmunologic determination (15) has the same advantages as those implicated in other methods of analysis. These advantages are of particular interest in radioimmunologic determinations because determinations can be carried out in a large series when plasma specimens have been stored for several months at -20°C . We note in particular the increase in quality of the measurements, the gain in time for the laboratory in taking the counts and the grouping of the calculations.

INFORMATIQUE ET DOSAGE D

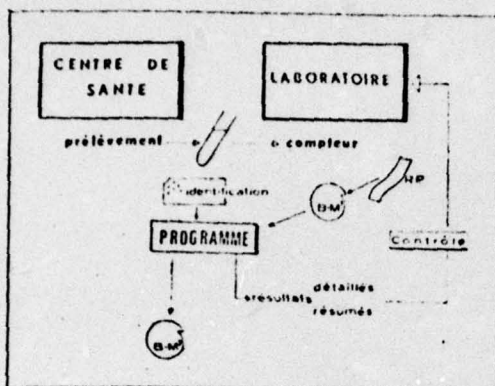


Fig. 1. -- Etapes informatiques de l'automatisation des dosages. R.P. : ruban perforé. (perforated tape)
 B.M. : bande magnétique sur laquelle sont enregistrées les données sortant du compteur.
 B.M.F. : bande magnétique contenant les dossiers des sujets correspondant aux prélèvements donnés.

AUTOMATISATION DU DOSAGE : Feuille 3

IDENTIFICATION DES PLASMAS

Partie à remplir au moment des prélèvements						Partie à remplir au moment du dosage		
Date du prélèvement						date (rappel)		
<input type="text"/>						<input type="text"/>		
Nom	Prénom	N° enquête ou dossier	N° Labo U 55	Temps prélevé	Remarques	N° programme ou 2	N° trou à 100	date tube plasma contrôle 3
25	367	4243	4219	5		67	70	80

Fig. 2. -- Fiche d'identification des tubes.
 La partie gauche est remplie au lieu du prélèvement.
 La partie droite est remplie au moment du comptage.

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TABLEAU I
Protocole pour une série de 200 dosages

Courbe standard	TUBES CONTRÔLE	AUTRES TUBES	
		Traceur	1, 3, 5
Tubes 1 à 25	2, 4, 10, 15, 20	Excès d'anticorps anti-insuline	25
		Standard	Autres tubes
Plasma Tubes 26 à 200	30, 40, 50, ..., 190, 200	Plasmas à doser	Autres tubes (157 tubes en tout)

Les nombres indiquent les ordres de passage des tubes dans le compteur au cours du dosage. Les traceurs sont les tubes contenant l'hormone radioactive et l'immunosérum à la dilution utilisée pour le dosage. Les « standard » contiennent en plus l'hormone en quantité variable.

TABLEAU II
Résultats pour l'établissement de la courbe standard concernant l'exemple traité

DOSE D'INSULINE $\mu\text{U/ml}$ de plasma	0	3,125	6,25	12,5	25	50	100	200	400
Pourcentage d'activité adsorbée sur le silicate (F/T)	40,1	42,8	43,5	47,5	56,3	69,5	82,6	92,7	98,4
	39,7	41,2	43,2	48,4	57,3	71,3	82,8	93,7	99,8
	41,1								

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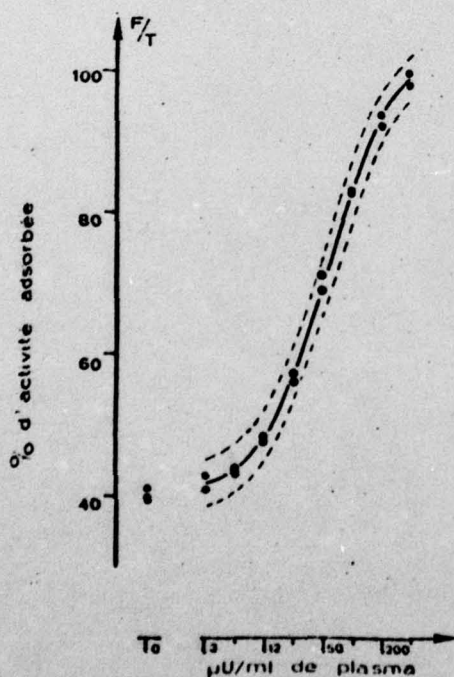


Fig. 3. — Courbe standard. Les points sont les points expérimentaux. La courbe en trait plein est la courbe calculée. Les courbes en pointillés sont les limites de précision (95 p. cent) de la courbe standard.

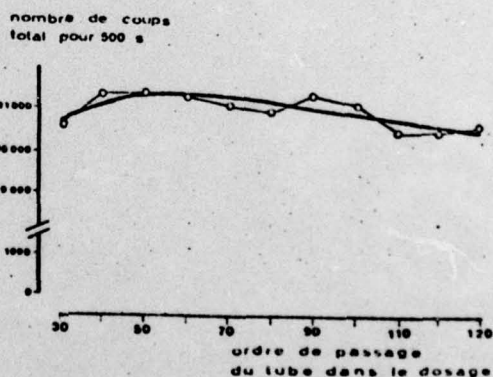


Fig. 4. — Variation de la mesure de la radioactivité des tubes de contrôle. La courbe en trait plein est la courbe calculée servant à calculer la valeur de référence (T) pour chaque tube à doser.

DATE DU PRELEVEMENT 7 AVR 70			
CHROMONE NO 1	NOM PETIT	MAX	NO ENQUETE 70-625 (R 24)
TROU NO 39	TEMPS 1PO 2M		
QUANTITE D'INSULINE ESTIMEE 63.5 MIU/ML (56. - 73.)			
Rappel			
		HEURE DE COUPS	8304.8
		CONTROLE ESTIME	11151.5
		CONTROLE PRECEDENT	10668.
		CONTROLE SUIVANT	11405.
ATTENTION : LES CONTROLES ENTOURANT CE TIME DIFFERENT DE PLUS DE 5 P. CENT REVOIR LE RESULTAT			
DATE DU PRELEVEMENT 7 AVR 70			
CHROMONE NO 1	NOM PETIT	MAX	NO ENQUETE 70-625 (R 26)
TROU NO 41	TEMPS 1PO 3M		
QUANTITE D'INSULINE ESTIMEE 37.5 MIU/ML (33. - 42.)			
Rappel			
		HEURE DE COUPS	7.46.8
		CONTROLE ESTIME	11507.4
		CONTROLE PRECEDENT	11 05.
		CONTROLE SUIVANT	11460.
DATE DU PRELEVEMENT 6 AVR 70			
CHROMONE NO 1	NOM MARTINOU	MARCEL	NO ENQUETE 70-626 (R 29)
TROU NO 44	TEMPS 1PO 0M		
T.VIDE QUANTITE D'INSULINE INFERIEURE A 3.0 MIU/ML (**** -****)			
Rappel			
		HEURE DE COUPS	2052.0
		CONTROLE ESTIME	11275.7
		CONTROLE PRECEDENT	11405.
		CONTROLE SUIVANT	11360.

Fig. 5. — Exemple de résultats « détaillés ». Le tube 39 était dans une zone où les contrôles variaient de façon jugée trop importante. Le tube 44 avait été signalé au moment du prélèvement comme « tube vide ».

INFORMATIQUE ET DOSAGE DES HORMONES PROTEIQUES

RESULTATS RESUMES

DATE DU DOSAGE 11 JUIN 70-1

NUMERO TROU DATE 4 JUIN 70 ACTIVITE SPECIFIQUE 200NIC/MIA

IN'INSERUM DATE 2-09-1966 SPECIFIC: AC 41

ALBUMINE DATE - - - NO DU LOT B 356

NUMERO LIURE DATE FEVRIER 70 NO DU LOT 41066

DATE PRELEV	NOM PALADE	PRENOM	NO ENQUETE	NO USS	TEMPS PRELEV	INCIDENT	QTE D'INSULINE	CHROMONE	TROU
6 AVR 70	ALASSIER	J-L	70-624	R 11	1PO 0M		14.5	1	26
6 AVR 70	APASSIER	J-L	70-620	R 12	1PO 3M		39.5	1	27
6 AVR 70	APASSIER	J-L	70-620	R 13	1PO 1M		100.3	1	28
6 AVR 70	APASSIER	J-L	70-620	R 14	1PO 0M		117.0	1	29
6 AVR 70	APASSIER	J-L	70-620	R 16	1PO 2M	A VERIFIER.	94.5	1	31
6 AVR 70	APASSIER	J-L	70-620	R 17	1PO 3M	A VERIFIER.	50.1	1	32
6 AVR 70	APASSIER	J-L	70-620	R 18	1PO 0M		67.5	1	33
6 AVR 70	APASSIER	J-L	70-620	R 19	1PO 5M	A VERIFIER.	64.3	1	34
6 AVR 70	APASSIER	J-L	70-620	R 20	1PO 0M	A VERIFIER.	8.0	1	35
7 AVR 70	PETIT	MAR	70-625	R 21	1PO 3M	A VERIFIER.	50.1	1	36
7 AVR 70	PETIT	MAR	70-625	R 22	1PO 0M	A VERIFIER.	77.3	1	37
7 AVR 70	PETIT	MAR	70-625	R 23	1PO 0M	A VERIFIER.	94.0	1	38
7 AVR 70	PETIT	MAR	70-625	R 24	1PO 2M	A VERIFIER.	64.5	1	39
7 AVR 70	PETIT	MAR	70-625	R 26	1PO 3M		37.3	1	41
7 AVR 70	PETIT	MAR	70-625	R 27	1PO 0M		21.9	1	42
7 AVR 70	PETIT	MAR	70-625	R 28	1PO 3M		14.0	1	43
6 AVR 70	MARTINOU	MARCEL	70-626	R 29	1PO 0M	T.VIDE A VERIFIER.	0000	1	44
6 AVR 70	MARTINOU	MARCEL	70-626	R 30	1PO 3M		54.7	1	45
6 AVR 70	MARTINOU	MARCEL	70-626	R 31	1PO 1M		81.0	1	46
6 AVR 70	MARTINOU	MARCEL	70-626	R 32	1PO 0M		94.0	1	47
6 AVR 70	MARTINOU	MARCEL	70-626	R 33	1PO 2M		27.0	1	48

Fig. 6. — Exemple de résultats « résumés ». Au début, identification de la série de dosages ; les listes de résultats sont accompagnées de messages éventuels relatifs à ces résultats.

REFERENCES

1. YALOW R.S., BERSON S.A. -- Immunoassay of endogenous plasma insulin in man. *J. clin. Invest., U.S.A.*, 1960, 39, 1157.
2. ROSSELIN G.E., CLAUDE J.R., ESCHWEGE E.P., VARNET J.M., RICHARD J.L. -- Epidemiological study of diabetes and coronary heart disease. - I. The implication of the 75 g 0.2 hour oral glucose tolerance test (0.2h OGTT) in the detection and definition of diabetes. *Excerpta med., internation. Congr. Ser., Pays-Bas*, 1970, 209, 139.
3. ESCHWEGE E., VALLERON A.J., ROSSELIN G.E., CLAUDE J.R., VARNET J.M., RICHARD J.L. -- Diabetes and coronary heart disease epidemiological study. Part. II. Multivariate statistical analysis of the glucose, lipid and hormonal levels systematically measured during 0.2 hours OGTT. *Excerpta med., internation. Congr. Ser., Pays-Bas*, 1970, 209, 127.
4. VALLERON A.J., ROSSELIN G.E., ESCHWEGE E. -- Factor analysis, canonical correlation analysis, and computer techniques used to express the glucose-insulin relationship during the 5 hour OGTT. Experimental data on 310 subjects. *Excerpta med., internation. Congr. Ser., Pays-Bas*, 1970, 209, 119.
5. VALLERON A.J., ROSSELIN G.E. -- Automation of insulin radioimmunoassays using adsorbant as a separation procedure. In : *Radioimmunoassay methods*. European Workshop, Edinburgh, 1970 (Livingstone Press, London, 1971).
6. ROSSELIN G., DOLAIS J. -- Application de la méthode radio-immunologique au dosage de l'insuline humaine et au dosage de l'hormone folliculo-stimulante humaine (H.F.S.H.). In : *Les isotopes. Applications biocliniques*, p. 189 (Expansion Scientifique Française, Paris, 1967).
7. ROSSELIN G., ASSAN R., YALOW R., BERSON S. -- Separation of antibody bound and unbound peptide hormone labelled with iodine 131 by talcum powder and precipitated silica. *Nature, G.B.*, 1966, 212, 355.
8. ROSSELIN G., DOLAIS J., FREYCHET P. -- Adsorbent in hormone assay. In : *7th international. Congress of Clinical Chemistry*, Geneva, 1969.
9. DOLAIS J., FREYCHET P., ROSSELIN G. -- Fuller's earth as separation procedure for HLM HTSH and HGH radioimmunoassay. In : *Radioimmunoassay methods*. European Workshop, Edinburgh, 1970 (Livingstone Press, London, 1971).
10. DOLAIS J., FREYCHET P., ROSSELIN G. -- Dosage plasmatique de l'hormone lutéinisante humaine (HLH) par la méthode radio-immunologique. I. Mise au point de la technique de dosage. *C. R. Acad. Sci., Fr.*, 1970, 267, 1105.
11. DOLAIS J., VALLERON A.J., GRAPIN A.M. -- Etude de l'hormone lutéinisante humaine (HLH) au cours du nyctémère. *C. R. Acad. Sci., Fr.*, 1970, 270, 3123.
12. SCHWARTZ D. -- *Méthodes statistiques à l'usage des médecins et biologistes* (Flammarion, Paris, 1963).
13. BENNET C.A., FRANKLIN N.L. -- *Statistical analysis in chemistry and the chemical industry* (Wiley, New York, 1954).
14. ROBBARD D., BRIDSON W., RAYFORD P.L. -- Rapid calculation of radioimmunoassay results. *J. Lab. clin. Med. U.S.A.*, 1969, 74, 770.
15. MORGAN C.R., HARDIGG J.B., FISHER D.D. -- A computer Program for Immunoassay of Protein Hormones with Special Reference to Insulin and Growth Hormone. *Diabetes, U.S.A.*, 1967, 16, 734.

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