PREPARATION OF HIGH-QUALITY IODINE-125-LABELED PITUITARY LUTEINIZING HORMONE FOR RADIOIMMUNOASSAY

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ABSTRACT

High quality pituitary luteinizing hormone labeled with ^{125}I was obtained after separating out the more heavily iodinated fractions, through starch gel electrophoresis, using the cathodal component (fraction 1) which was further purified on Sephadex G 100, with the obtention of an almost pure ^{125}I-LH preparation, presenting excellent immunoreactivity and low levels of damage on incubation in plasma. The quality control of the steps of the technique was done with plasma-coated talc (200 mg) which compared favorably, as far indicating undamaged labeled LH with the more time-consuming chromatoelectrophoresis.

INTRODUCTION

For radioimmunoassay it is needed a labeled antigen (hormonal or not) and its specific antibody as well as some technique for separating antibody-bound and free labeled hormone. The use of isotopically labeled antigen ("tracer") with the advantage of the high precision for the measurement of radioactivity associated with the great sensitivity of the immunological reactions, makes the radioimmunoassay techniques quite useful. The general principle that the quantity of an isotopically labeled "tracer" substance introduced in the system should be small compared to the quantity of unlabeled substance present in the reaction requires particular emphasis in application to radioimmunoassay of peptide hormones (Berson and Yalow, 1968). This implicit requirement for isotopically labeled proteins of high specific activity demands greater attention when it is necessary to detect small quantitites of hormonal antigen, since it is the concentration of tracer that so strongly influences the sensitivity and precision of the assay (Yalow and Berson, 1968).

The essential minimal criteria for suitability of labeled hormone in an immunoassay is the absence of damaged components and the complete bindability to antibody. It is still possible for a labeled hormone preparation to fulfill these criteria and yet not be completely satisfactory for one of two reasons (Berson and Yalow, 1968): 1) Although free of damaged fractions initially, the labeled hormone may be highly susceptible to subsequent damage with time as manifested by production of damaged components on incubation with plasma (incubation damage) or by excessive deiodination, which may be due partly to incubation damage and partly to chemical instability. Heavily labeling of the peptide hormone with radioiodine is frequently associated with high susceptibility to later damage; 2) Labeled hormone may still have impaired immunoreactivity.

In this paper, we report the techniques employed in our Laboratory for purification of labeled luteinizing hormone, for radioimmunoassay, with the obtention of high quality ^{125}I-LH, separating out more heavily iodinated fractions from the iodination mixture (through starch gel electrophoresis) and further Sephadex gel filtration of the best component, which

presents good immunoreactivity and lower levels of damage on subsequent incubation in plasma.

MATERIAL AND METHODS

a) Iodination procedure:

The human luteinizing hormone (LH) employed for iodination was obtained from pituitary glands containing 943 IU LH and 1.9 IU FSH per mg(LER 960) and kindly supplied by the National Pituitary Agency, Baltimore, Md., U.S.A.

The hormone was labeled with ^{125}I (provided by Union Carbide and with a specific activity of at least 200 mCi/ml) by the method of Hunter and Greenwood (1962) with several modifications as suggested by Yalow and Berson (1969). To 20 ul phosphate buffer 0.5M, pH 7.5, in a small test tube, are added in rapid sucession 1-10 ul ^{125}I containing appropriate amounts of radioactivity (to a final specific activity of 60 to 90 uCi/ug of LH), 20 ul of LH (2 ug/20 ul in phosphate buffer 0.01M, containing sodium chloride 0.14M, pH 7.5)-LER907, 10 ul chloramine T (2mg/ml 0.5M phosphate buffer), 50 ul sodium metabisulfite (2.5 mg/ml 0.5M phosphate buffer) and 100 ul "blue" plasma (normal human plasma stained with blue bromophenol). Part of the mixture was removed for assessment of the radioiodinated hormone and the remaining immediately applied to a starch gel electrophoresis for purification.

b) Assessment of the radioiodinated hormone

The preparation mixture in the iodination tube was tested for the absence of radioiodide and damaged components by chromatoelectrophoresis and/or by the talc test.

1. Chromatoelectrophoresis: In this techique 20 ul of the iodination mixture was applied to a strip of Whatman 3 MC or Toyo 514 paper. Electrophoresis was performed on phosphate buffer 0.1 M, pH 7.5,2 mA per strip at a voltage of 300 V, for 1 hour. At this time, the serum proteins have moved about 3 inches towards the anode with only slight resolution of the individual proteins. A scan of the radioactivity along the strip identified 3 peaks (Figure 1): (1) a peak at the origin composed of undamaged hormone that has adsorbed to the paper at the site of application; (2) a peak with the serum proteins representing damaged components bound to serum proteins or migrating with them because of loss of ability to adsorb to the cellulose; (3) a peak significantly beyond the serum proteins corresponding to radioactive iodide. The efficiency of iodination was expressed as the percentage of the total radioactivity under the peak at the origin.

2. Talc test. Both plasma-coated charcoal (Palmieri, Yalow and Berson 1971) -100 mg/ml 0.05 M phosphate buffer to which 100 ul of blood bank plasma is added - and talc,(Rosselin, Assan, Yalow and Berson 1996), from 50 to 350 mg/test tube*, were tested to separate intact ^{125}I-hormone from damaged ^{125}I-hormone, ^{125}I and other reactants, the former being adsobed to the precipitate and the remaining radioactivity staying in the supernatant. The radioactivity in the precipitate, as percentage of the total radioactivity put in the test tube, indicated the efficiency of the labeling.

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* Talcum powder in 50 or 100 mg Tablets (Gold Leaf Pharmaceutical Co., Englewood, N. J., U.S.A.) + 100ul blood bank in 0.05 M phosphate buffer to a total volume of 2.1 ml.

c) Purification of the labeled hormone:

To purify the labeled hormone preparation freeing it of unreacted iodine and of damaged components, starch gel electrophoresis was employed followed by further purification in a Gel Sephadex column.

1. Starch gel electrophoresis (Ferguson and Wallace, 1961; Rosselin and Dolais, 1967) This method serves as an analytic as well as a preparative procedure and permits not only the elucidation of certain characteristics of the labeled hormone to which the other purification methods are blind but also the selection of Iodo-LH components of different iodine numbers since the molecules with diiodotyrosyl residues migrate more rapidly towards the anode than molecules with only monosubstituted tyrosyl groups. At pH 8-8 6, each diiodinated tyrosyl residue confers an ext a negetive charge on the molecule so thet the more highly iodinated molecules are found closer to the anode according to the number of didiodotyrosyls present.

The starch gel was prepared by the method of Smithies (1955, 1959), using Connaught hydrolyzed starch. It was allowed to set at 4 C for at least 3 hours. 40 ul of the iodination mixture, containing bromphenol blue-stained human plasma, was applied to each of two slits in the starch gel. The blue stain marked the zone of migration of the serum albumin. Electrophoresis was carried out in a borate buffer 0 3 M, pH 8.0-8.2, being performed in 6 hours or 12 hours at 200 or 100V, respectively. The time of electrophoresis was controled by the migration of the blue plasma which should move about the half of the total distance from the slots to the apposite edge of the glass plate. LH migrates behind of the albumin but heavily iodinated preparation of the hormone moves closer to the anode (Figure 2). After completion of electrophoresis, both surfaces of the gel were placed in contact with Royal Blue Kodak film through intervening layers of parafilm Exposures were taken for about 20 minutes and the films developed immediately.

Using the autoradiographs as a guide, several narrow sections were cut from the gel, frozen for at least 3-4 hours and, after thawing, were then eluted with phosphate buffer 0.01 M, NaCL 0.14 M, pH 7.0 containing 1% bovine serum albumin (BSA: PBS 1%) and squeezing gently. The eluates were decanted and centrifuged to remove gel particles As with all purified fractions the supernatant eluates were tested for damaged and undamaged fractions with talc (200 mg/test tube) test. As indicated in figure 2, the autoradiograph of starch gel of LH - ^{125}I, showed a main cathodal component (Fraction 1) and a fraction which migrated behind the region of albumin stain (fraction 2).

2. Purification by Sephadex gel filtration - The labeled hormones eluted from the two fractions of the starch gel were further purified in Sephadex G 100 column (50 x 1 cm) which had been previously coated with phosphate buffer 0.05 M containing 1% bovine serum albumin. The several radioactive components were eluted with phosphate buffer 0.05 M pH 7.5, collecting separate 1 ml eluates. Purified labeled hormone emerged significantly later than plasma proteins and damaged components but before free iodine. However, starch gel electrophoresis seperating out damaged components and free iodine, the radioactive eluate was generally free hormone only (figure 3).

The eluted fractions corresponding to the peak of activity were submited to the quality control (talc test). Thus, eluates corresponding to the undamaged labeled hormone were

identified and among them, those with the highest proportion of free hormone were saved to be used in the radioimmunoassay

Components 1 and 2 from starch gel electrophoresis purified on gel filtration were tested for their immunoreactivity and then used that one with the better immunoreactivity and more stable on subsequent incubation in plasma

Before using Sephadex G 100 it was compared with G 75 as far yield of free hormone, since some investigators have used Sephadex G 75 columns (Odell, Ross and Rayford, 1967; Levine, Donabedian and Sobrinho, 1971).

d) Immunoassay

LH was measured by the double-antibody radioimmunoassay technique of Midgley (1967) modified by Mahesh (1973). The first antibody was supplied by the National Pituitary Agency and represents rabbit antisera to human LH extracted from pituitaries. Separation of antibody-bound from free hormone was accomplished by addition by sheep anti-rabbit gama globulin serum, prepared by Dr. V.B. Mahesh, in sufficient quantity to achieve maximal precipitation. LER 907 reference preparation (provided by the National Pituitary Agency) served as the LH standard (1 ng LER 907 = 0.048 mIU) and our results are reported as ng (LER 907)/ml. The lower limit of sensitivity for our LH assay is 10 ng/ml. The intra-assay coefficient of variation is ± 4.7% and between assays ± 12%. The accuracy evaluated through recovery of known quantities of purified hormone indicated that the percentage recovery varied between 72 and 102%, the correlation coefficient r between theoretical and found LH values being 0.910 (Pinto 1973).

To test their immunoreactivity, fractions 1 and 2 from starch gel electrophoresis (figure 2) further purified in Sephadex G 100, were evaluated simultaneously, at the same level of radioactivity (counts/min.) in setting up two standard curves and using the same reagents. A standard curve for LH with the use of the 2nd. antibody, as done routinely in our laboratory, was compared with another one, performed simultaneously, using talc to separate antibody-bound and free hormone, as suggested by Levine et al.(1971, with the difference that 200 mg of talc rather than 300 mg per test tube was used, based in the observation that 200 mg talc compares better than the larger amount with chromatoelectrophoresis as far as adsorbing intact ^{125}I-LH.

RESULTS

Assessment of the radioiodinated hormone:

The efficiency of 6 labeling procedures in almost 1 year averaged 42% (ranging from 38 to 47%) and 47.5% (ranging from 41 to 50%)as evaluated by chromatoelectrophoresis and talc, respectively (table 1). It can be seen that plasma-coated talc, in the amount used in our experiments, compares favorably with the standard chromatoelectrophoresis as far as an indication of undamaged ^{125}I-LH, however giving slightly higher results.

Comparison between adsorbents (plasma-coated charcoal and talc) and chromatoelectrophoresis as a method to separate intact, undamaged ^{125}I-LH from damaged

fractions and free iodine (table 2). As it can be seen in table 2, of the 3 techniques used to detect the percentage of intact, undamaged, iodine-labeled LH, of the fractions eluted from Sephadex G 100 colums, after previous purification in starch gel electrophoresis, talc in the amount of 200 mg gave the best results in comparison with chromatoelectrophoresis.

Purification of the labeled hormone: Starch gel electrophoresis

Fractions 1 and 2 (figure 2) were tested in relation to the percentage of purity (undamaged ^{125}I-LH) of the correspondent eluates table 3. It is evident that fraction 2, with increased anodal electrophoretic mobility, has a lower proportion of undamaged hormone than component 1, probably related to its greater proportion of more heavily iodinated components. It has been found that overiodinated components from the more anodal spots are less stable - preparation damage - besides being frequently associated with high susceptibility to later damage on incubation damage (Berson and Yalow, 1968). Therefore, the eluates corresponding to the highest proportion of intact hormone - fraction 1 - were further purified on Sephadex columns. The slowest migrating fraction 1 despite including unlabeled molecules, because of its proximity to origin, resulting in a lower specific activity of the ^{125}I-LH preparation than that desired, but yet free of overiodinated molecules, is preferable to fraction 2 in the assay not only for its greater proportion of undamaged hormone (table 3) but also for its greater immunoreactivity (figure 4).

Sephadex gel filtration:

As the proportion of intact labeled hormone from the gel sections was not sufficiently high, after starch gel electrophoresis alone, to be used in the assay without further purification and considering the added incubation damage, Sephadex gel filtration was used further.

The comparison between the efficiency of sephadex G 75 and G 100 columns (table 4), indicated that the yield of pure labeled LH is sligtly greater with G - 100 column in the eluates known to present the greatest proportion of intact, undamaged, hormone.

Quality control (talc test) of the eluated fractions from Sephadex G-100 column, after previous purification of labeled LH in starch gel electrophoresis - table 5 - indicated that the fractions with the highest proportion of undamaged hormone correspond the effluents 19 to 22, on the descending limb of the radioactivity peak (figure 3).

In the published papers the labeled hormone is usually purified only through a G-75 or G-100 Sephadex column, before being used in the radioimmunoassay, then not allowing the selection of components of different iodine numbers which this method of purification cannot accomplish, since either fraction 1 or 2 (figure 2) have the same elution pattern as shown in figure 3.

As indicated in table 6, the labeled hormone purified in two systems, starch gel and Sephadex G-100, presented a smaller degree of degradation (incubation damage), during the 7 days of incubation with plasma, in the immunoassay, then after purification only in the Sephadex column.

Because plasma coated talc was shown to adsorb intact ^{125}I LH, being comparable to the

standard chrometoelectrophoresis technique (table 2) it was decided to test it for separating free and antibody boud hormone, in the radioimmunoessay, in the place of the 2nd, antibody, which would make the assay simpler, faster and economical. When two standard curves were performed simultaneously, one using the sheep ant rabbit gamma globulin serum as the 2nd. antibody and the other using plasma coated talc (200mg) to separate free from antibody-bound LH (figure 5) it could be clearly seen that talc did not separate bound from free hormone.

Finally, to test the immunoreactivity of fractions 1 and 2, from starch gel electrophoresis further purified in Sephadex G 100 column, two sets of standard curves were done, using the same number of counts of each fraction and the same standards, as indicated in table 7 and figure 4. As it could be seen, fraction 2 exhibit diminished immunoreactivity in comparison to fraction 1.

DISCUSSION

As its well known, the essential needs for radioimmunoassay include the availability of the individual reactants - namely, the labeled antigen and the specific antibody - and some technique for separating the antibody-bound and free labeled hormone, since the antibody antigen complexes do not precipitate spontaneously at the low concentrations of reactants employed. In this paper we will consider only the preparation of the labeled hormone and the factors that determine its suitability for radioimmunoassay.

As most workers we use the method of Hunter and Greenwood (1962) as modified by Yalow and Berson (1968) to attach the radioactive iodine to ortho positions of tyrosyl residues of the hormon, adding human plasma to minimise damage to hormone and adsorptive losses, the reagents being put in a sequence as quick as possible. However, the labeled hormone must satisfy certain criteria if the assays are to be valid and precise.

An important aspect in radioimmunoassay is the labeling of peptide hormones with radiactivity levels which would permite statistically suitable counting rates while maintaining, at the same time, a low concentration of the tracer hormone. Indeed, it is advisable that the concentration of the labeled hormone be not much greater than and preferably smaller than the lowest concentration of unlabeled hormone to be measured. Therefore, it is required a high specific activity (uCi labeled Iodine/ug hormone).

By increasing the number of substitutions of radioidine atoms in the hormone molecule we could increase the specific activity of the labeled hormone. However, overiodination tends to decrease the immunoreactivity of the preparation, as it can be seen in table 7 and figure 4. Thus, the hormone must be labeled at a specific activity to be consistent with the maintenance of the immunochemical integrity and stability of the labeled hormone.

The specific activity depends on a number of other factors, including the sensitivity of the couting system, the volume of incubation mixture to be counted and, as indicated, the concentration of hormone to be assayed (Berson and Yalow, 1968); the more efficient the counting system and the larger the volume counted, the lower the concentration of labeled hormone with a given specific activity required for the same total counting rate.

The specific activity attainable depends on the available isotopic abundance of the Iodine

isotopes because ^{125}I is carrier free, not available for ^{131}I, the hormones labeled with isotope, can have lower specific activity the number of tyrosine residues per molecule of hormone 4 tyrosyl residues per molecule for insuli, 8 for human growth hormone and 5 for human pituitary LH the molecular weight of the hormone 6000 for insulin, 20000 for human growth hormone and 30000 for LH and the tolerance of the hormone for labeling with radioiodide

A compromise between a counting time required to obtain statistical accuracy and a maximal specific activity without significant alterations (other the iodine substitution) of the preparation, can be attained by labeling LH at a specific activity between 60 and 90 uCi/ug (Midgley 1967) (table 1) where as from 100 to 300 uCi for insulin and 50 to 100 uCi/ug for growth hormone, respectively (Higa et al , 1973)

The problem of preparation of a suitable labeled LH does not end with consideration of specific activity, as even when labeled at relatively low specific activity, it tends to undergo partial damage, as it can be seen in table 1, where it is clearly shown that less than 50 percent of the labeled LH is undamaged hormone after iodination, as evaluated by the classical technique of chromatoelectrophoresis The damaged components must be removed together with unreacted radioactive iodide before the labeled hormone can be used in radioimmunoassay.

A rapid direct assessment of the iodinated preparation could be done adding a small aliquot of the iodination mixture to plasma coated talc, which compares favorably with the more time consuming chromatoelectrophoresis (tables 1 and 2)

Then, purification of the labeled hormone is always necessary for the removal of unreacted radioiodide, reactants and damaged iodinated proteins

Sephadex gel filtration, used by the majority of investigators as the only process of purification of the labeled LH, despite resulting in the obtention of eluates with very high proportion of purified labeled hormone (usually greater than 90%), free from damaged components and iodide, however it cannot exclude componentes more heavily iodinated, which exhibit reduced immunoreactivity (figure 4) and are less stable in that they undergo greater damage on incubation in plasma, during the assay (table 6).

On the other hand, starch gel electrophoresis, providing a discriminating separative system whith can be used to fractionate iodinated proteins, showing heterogeneity in ^{125}I-LH preparations (figure 2), can be used to select the fraction having the highest immunoreactivity (figure 4). However, as the only method of purification, the yield of undamaged labeled hormone, eluted from the cathodal component (fraction 1) is not greater than 81% (table 3).

The procedures used in our laboratory, starch gel electrophoresis followed by Sephadex chrometography of fraction 1 is an assurance that we start the assay with almost pure labeled peptide preparation without overiodination and practically devoid of damaged components and of free iodine (table 5). In effect, the final yield from Sephadex columns has always been above 85% (figure 5) being used in the assay those effluents with above 90% of undamaged hormone wich also undergo lower level of incubation damage (table 6).

The same sequence of procedures has been used by Berson and Yalow (1968) and has

yielded the best preparations of labeled insulin, growth hormone and gastrin. Therefore, our data indicate that can also be used in the preparation of high quality ^{125}I-labeled LH for radioimmunoassay

Finally, because plasma-coated talc has worked quite well in our hands to separate undamaged labeled hormone from damaged components and free iodide, being comparable to the standard chromatoelectrophoresis technique (table 2) and considering its effectiveness in separating antibody bound from free hormone in radioimmunoassay of several peptide hormones (Palmieri, Yalow and Berson, 1971) it was used in the assay for the measurement of LH being compared with the technique employing a 2nd antibody. However, in contraction to the findings of Levine et al (1971) it did not work satisfactorily at least in our hands.

Acknowledgments

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TABLE 1

Efficiency of Labeling of LH With ^{125}I

Date	Specific activity (uCi/ug)	Efficiency of labeling (percent of total radioactivity)	
		Chromatoelectrophoresis	talc (200mg)
8/25/72	60	42	50
10/10/72	74	39	48
11/24/72	80	40	45
2/27/73	60	38	41
4/24/73	75	46	51
5/15/73	60	47	50

TABELA 2

Quality Control of ^{125}I-LH Fractions Eluted From Sephadex G-100 (After Previous Starch-Gel Electrophoresis) as Evaluated With Charcoal, Talc and Chromatoelectrophoresis.

^{125}I-LH fraction (effluent nº)	CHARCOAL		TALC-TABLETS		CHROMATOELECTROPHORESIS*
	ml	% intact hormone	mg	%intact hormone	% intact hormone
14	0.25	67	50	36	55%
	0.50	70	100	44	
	0.75	76	150	48	
	1.00	79	200	58	
	1.25	79	250	61	
	1.50	84	300	64	
	2.00	90	350	67	
17	0.25	71	50	41	65%
	0.50	74	100	47	
	0.75	78	150	51	
	1.00	82	200	64	
	1.50	90	250	67	
	2.00	93	300	71	

* Chromatoelectrophoresis used as the standard technic.

TABLE 3.

Percentage of Undamaged ^{125}I-LH, Extracted From Fractions 1 And 2 (Starch Gel Electrophoresis)

DATE OF IODINATION	PERCENTAGE OF UNDAMAGED ^{125}I-LH*	
	Fraction 1	Fraction 2
8/25/72	72%	50%
10/10/72	67%	45%
11/24/72	71%	48%
1/ 9/73	81%	56%
2/27/72	64%	40%
8/16/73	72%	49%
1/14/74	70%	50%

* Percentage of total radioactivity in gel section correspondent to undamaged ^{125}I-LH adsorbed to plasma-coated talc.

TABLE 4.

Comparison Between Purification of Labeled LH, After Previous Starch Gel Electrophoresis, on Sephadex G-75 And G-100 Columns

ELUATE nº	PERCENTAGE OF INTACT ^{125}I-LH (TALC-200 mg)	
	G-75	G-100
20	90%	93%
21	86%	94%

TABLE 5.

Quality Control of the ^{125}I-LH Fractions Eluted From Sephadex G-100 Columns (After Previous Starch Gel Electrophoresis)

Eluate No.	Study 1 (jan'73)		Study 2 (feb'73)	
	% damage + free iodine	% undamaged ^{125}I LH	% damage + free iodine	% undamaged ^{125}I LH
11	48	52	49	41
12	44	56	47	43
13	45	55	48	42
14	44	56	43	47
15	43	57	49	51
16	41	59	44	56
17	31	69	29	71
18	27	73	28	72
19	15	85*	20	80*
20	13	87*	12	89*
21	6	94*	8	92*
22	8	92*	15	85*
23	24	76	19	81

* Eluates containing the highest proportion of undamaged ^{125}I LH as tested with talc (200 mg)

TABLE 6.

Levels of Degradation of ^{125}I-LH During 7 Days of Incubation With Plasma Studies in Two Assays

Days of incubation	Percentage of degradation*	
	^{125}I LH purified through starch gel and Sephadex	^{125}I-LH purified through Sephadex G-100
1	9	10
2	9	11
3	11	13
4	13	15
5	14	17
6	14	18
7	14	19
1	7	13
2	7	15
3	8	17
4	9	18
5	10	20
6	11	20
7	11	22

* Total radioactivity in each test tube minus radioactivity precipitated with the 2nd. antibody, as percentage of the total counts.

TABLE 7

Summary of Assay Procedure With Fractions 1 and 2 From Starch Gel Electrophoresis of ^{125}I-LH, After Sephadex G-100 Purification.

TUBES	STANDARD LH (LER 907) VOLUME ml	STANDARD LH (LER 907) CONCENTRATION ng/ml	BUFFER (BSA-PBS 1%) ml	ANTI LH (1:10 000) ml		^{125}I-LH (0.1ml 12000 cpm)		ANTI RABBIT GAMMA GLOBULIN ml		RADIOACTIVITY PRECIPITATED (ANTIBODY BOUND) Fraction 1 (cpm)	Fraction 2 (cpm)
Trace	-	-	0.2 ml			0.1 ml		0.1		12000	12000
Control	-	-	0.2	0.1		0.1		0.1		8637	5126
Standard nº 1	0.1	50	0.1	0.1		0.1		0.1		8126	4837
Standard nº 2	0.1	100	0.1	0.1	4C 24 hrs	0.1	4C 24 hrs	0.1	4C 24 hrs	7436	4518
Standard nº 3	0.1	500	0.1	0.1		0.1		0.1		5187	3519
Standard nº 4	0.1	1000	0.1	0.1		0.1		0.1		4082	3226

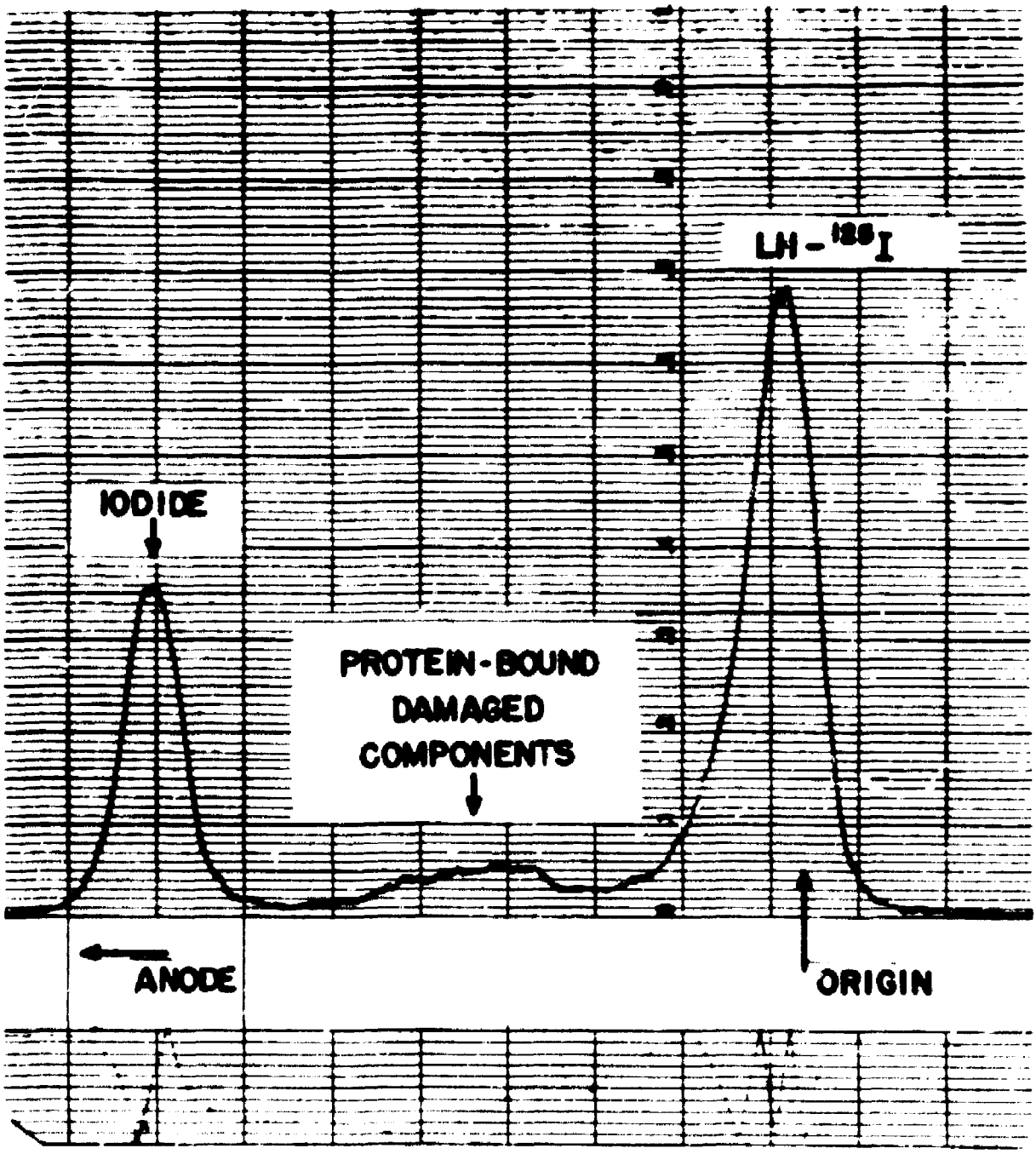


Figure 1

Scan of radioactivity on paper chromatoelectrophoretogram of ^{125}I - LH preparation immediately after iodination.

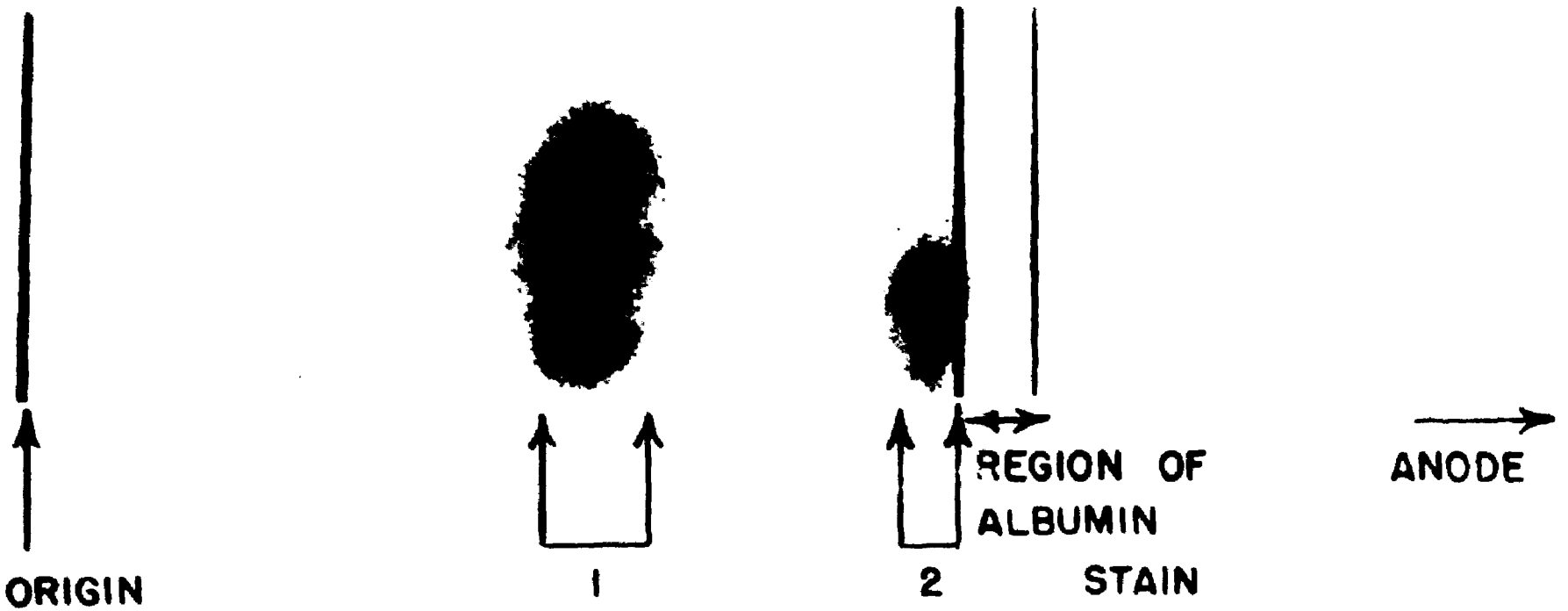


Figure 2

Autoradiograph of starch gel electrophoretogram of ^{125}I - LH immediately after iodination, showing fractions 1 and 2.

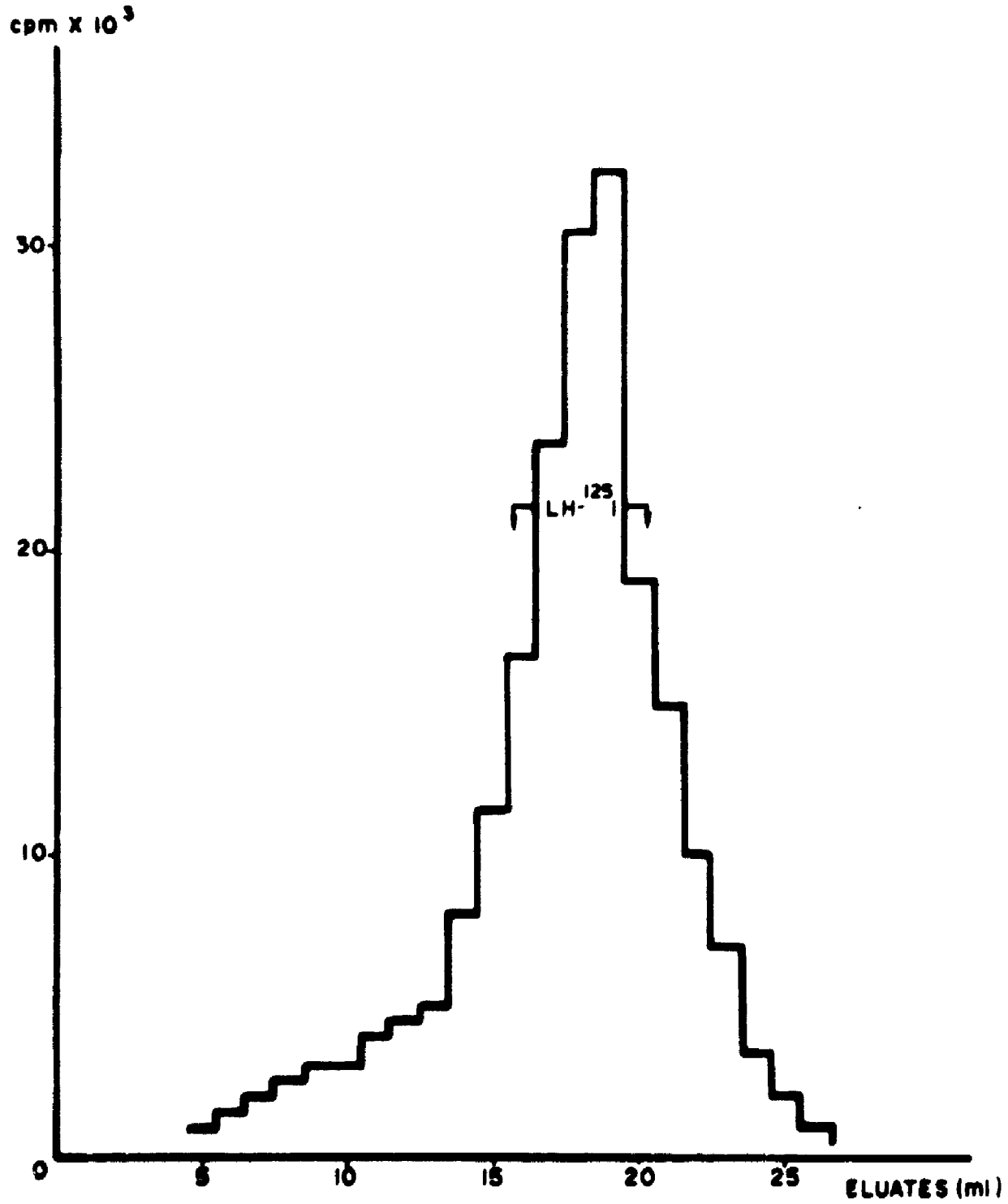


Figure 3

Gel filtration on Sephadex G-100 of ^{125}I - LH after previous purification on starch gel electrophoresis

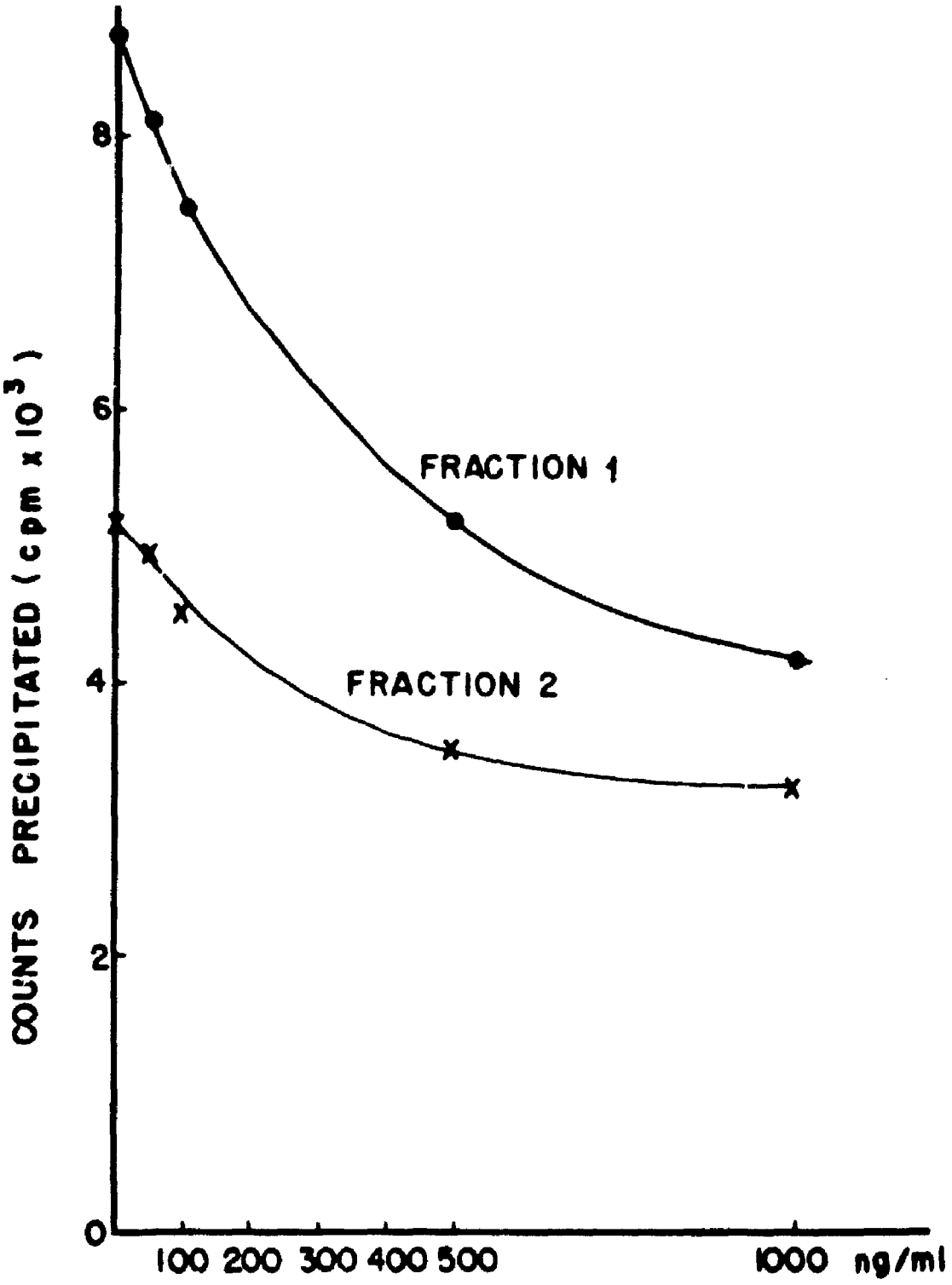
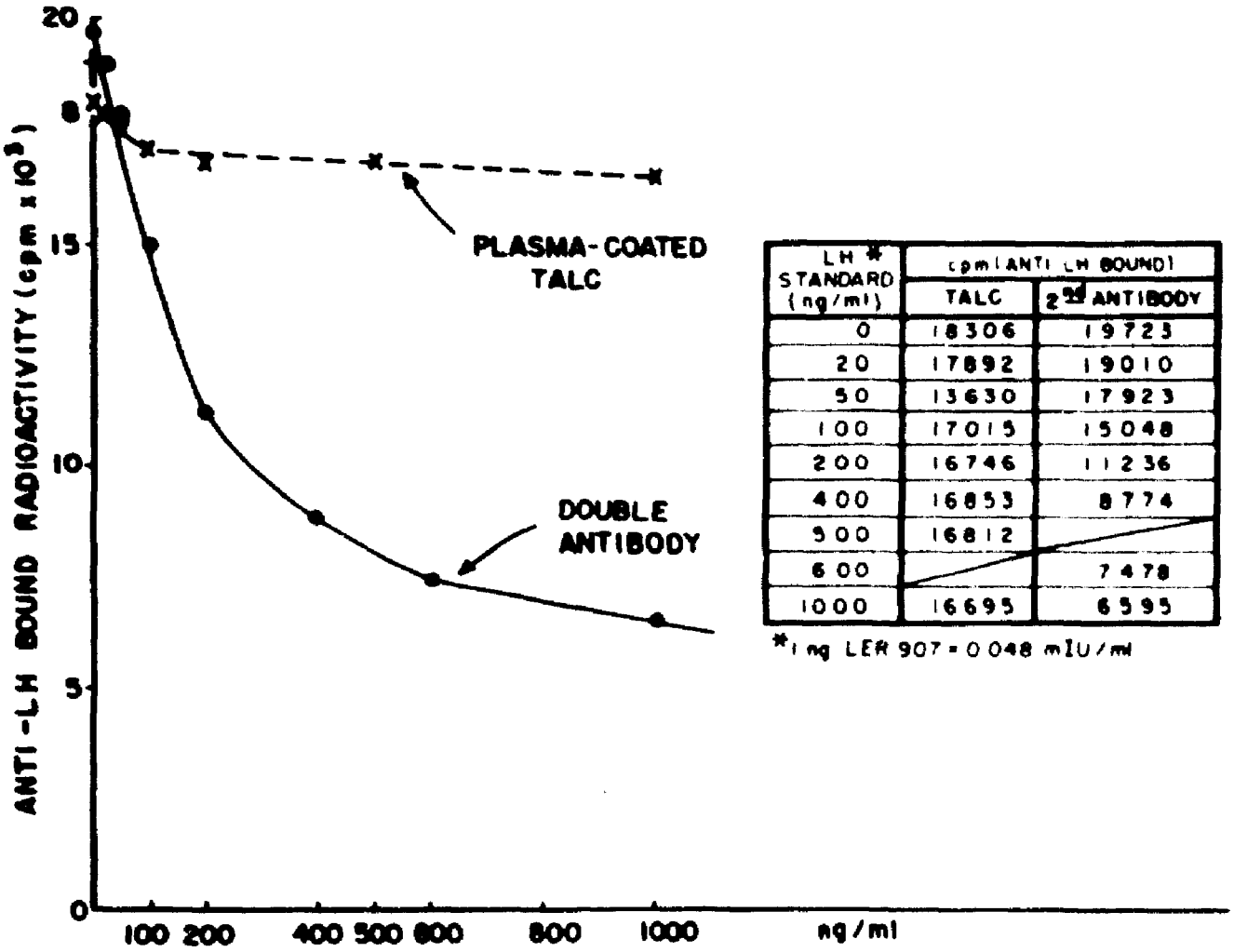


Figure 4

Standard curves for LH assay with the double-antibody technique. Comparison of ^{125}I - LH fractions 1 and 2 (from starch gel electrophoresis and further purified on Sephadex G-100) immunoreactivity.



LH * STANDARD (ng/ml)	cpm (ANTI LH BOUND)	
	TALC	2nd ANTIBODY
0	18306	19723
20	17892	19010
50	13630	17923
100	17015	15048
200	16746	11236
400	16853	8774
500	16812	
600		7478
1000	16695	6595

*1 ng LER 907 = 0.048 mIU/ml

Figure 5

Comparison of LH standard curves obtained by separation of bound and free labeled hormone using the double-antibody technique and plasma-coated talc

RESUMO

A presente comunicação versa sobre a seleção e padronização dos procedimentos técnicos que visam a obtenção do Hormonio Luteinizante hipofisario marcado com ^{125}I de alta qualidade para o radio-imunoensaio.

O LH ^{125}I foi obtido após separação das frações de hormonio marcado de acordo com o grau de iodação através da eletroforese em gel de amido usando-se o componente mais catodico (fração 1). Posteriormente esta fração foi purificada em coluna de gel Sephadex G 100 para separá-lo dos componentes danificados preparando-se assim LH ^{125}I marcado sem excessiva iodação para que a imunoreatividade do hormonio não fique comprometida.

O controle de qualidade foi realizado com talco (200 mg) recoberto com plasma que é comparavel a clássica cromatoeletroforese em papel (Yalow e Berson).

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