Phytochemistry, 1970, Vol. 9, pp. 73 to 86. Pergamon Press. Printed in England.

PEROXIDASES ASSOCIATED WITH LENTIL ROOT RIBOSOMES

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(*Received* 16 *April* 1969; *in revised form* 25 *June* 1969)

Abstract—Three peroxidases are associated with the ribosomes of lentil roots. During cellular fractionation, the three haemoproteins are only present on these particles and not in the other fractions. The three peroxidases were isolated and obtained in a highly purified form. Their spectral characteristics and their molecular weights were determined. One is slightly acidic at pH 6·8, whereas the other two are slightly basic at this same pH. The incorporation of a ^{14}C-amino acid into these haemoproteins has enabled us to show that the basic peroxidases turn over rapidly whereas the acid peroxidase is very stable. Treatment of the roots with IAA considerably stimulates the *de novo* biosynthesis of the two basic peroxidases but not that of the acid peroxidase.

INTRODUCTION

DURING the last few years, various workers have shown the presence of isoperoxidases[1-6] in homogenates of plant tissues. Some of these haemoproteins are fixed to cellular organelles, in particular to the ribosomes.[7] However, there is no proof that these isoenzymes occur as such in the plant cell. It is not in fact possible to eliminate the hypothesis[8] that these various enzymes are artefacts caused by transformations of a single peroxidase during the extraction or purification procedures. Also, little is known about the hormonal regulation of the biosynthesis of isoperoxidases.[1,9,10] It is well known that the treatment of a plant organ by various hormones leads to RNA synthesis[11-15] and increases in the amount of tissue proteins[16,17] and in various enzymatic activities[18-21] These effects are generally interpreted by

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assuming that the hormones act at the level of gene expression, on the production of messenger RNAs and on *de novo* formation of a series of proteins.[22] However, with the exception of Varner and Galston,[23–26] no worker has been able to show, in the case of plants, the *de novo* biosynthesis of an enzyme, under the action of a phytohormone.

The present paper is concerned with the "ribosomal" isoperoxidases of lentil (*Lens culinaris* Med.) roots and the control of their biosynthesis.

RESULTS

Table 1 shows the distribution of peroxidase activity, of proteins, and of nucleic acids in the various fractions isolated from control roots and those treated with indoleacetic acid. It may be noted that peroxidases (about 67 per cent of the total amount) are associated with the particulate fractions. Treatment of the roots by IAA leads to an increase in their content of peroxidases, proteins, and RNA. The DNA content is slightly diminished; this is not surprising since, at the IAA concentration used, this hormone inhibits cell division.[27]

TABLE 1. DISTRIBUTION OF PEROXIDASE ACTIVITY, PROTEINS AND NUCLEIC ACIDS IN THE VARIOUS FRACTIONS ISOLATED FROM ROOTS TREATED OR UNTREATED WITH IAA $2{\cdot}5 \times 10^{-4}$ M FOR 15 hr

	Control					Treated with IAA				
	Peroxidase (μg/g)	Proteins (μg/g)	RNA (μg/g)	DNA (μg/g)	μg Peroxidase / mg Proteins	Peroxidase (μg/g)	Proteins (μg/g)	RNA (μg/g)	DNA (μg/g)	μg Peroxidase / mg Proteins
Filtrate	55	12,061	1,122	106	4·56	176	15,650	1,417	82	11·25
Supernatant 10,000 *g*	42	11,105	761	41	3·78	166	13,049	1,072	33	12·72
Pellet 10,000 *g*	10	1,342	55	43	7·45	20	1,013	44	22	19·74
Supernatant 30,000 *g*	26	8,656	505	7	3·00	93	10,736	717	9	8·66
Pellet 30,000 *g*	13	1,449	219	26	8·97	35	1,451	300	25	24·12
Supernatant 105,000 *g*	12	6,370	55	3	1·88	55	7,813	86	2	7·03
Pellet 105,000 *g*	10	858	505	traces	11·65	36	1,152	704	traces	31·25
Ribosomes	4	666	466	traces	6·00	12	1,110	700	traces	10·8

Quantitities are expressed in μg/g of wet weight.

The lentil root ribosomes are particles consisting of approximately 40 per cent RNA and 60 per cent protein, which is in agreement with observations on other plant ribosomes.[28,29] Treatment of the roots with IAA leads to an increase in the ribosome content of the cells (Table 1). In the ribosomal fraction there is high peroxidase activity.[7] Treatment of the roots with IAA causes a considerable increase in peroxidase activity of this fraction.

Since the specific content in peroxidases of all the various fractions (peroxidase/proteins) can be considerably increased in the presence of IAA, it is important to know whether the peroxidase (or the peroxidases) is associated with the ribosomes or with impurities (for

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[27] J. H. CHERRY, *Ann. N.Y. Acad. Sci.* **144**, 154 (1967).
[28] T. C. HSIAO, *Biochim. Biophys. Acta* **91**, 598 (1964).
[29] S. T. BAYLEY, *J. Mol. Biol.* **8**, 231 (1964).

example, membranes) present in the preparations. The results of Table 1 suggest that the former idea is correct. Indeed, the purest ribosome preparations always possess peroxidase activity and are characterized by a protein/RNA ratio which is almost that of pure plant ribosomes. The absence of membranes in these ribosomal preparations can be proved in another way. The purified (Table 1) ribosomes are submitted to an analytical ultra-centrifugation. One can easily discern the presence, on Schlieren diagrams, of two constituents (Fig. 1A) possessing, at infinite dilution, sedimentation constants of 80 S and 125 S (Fig. 1B).

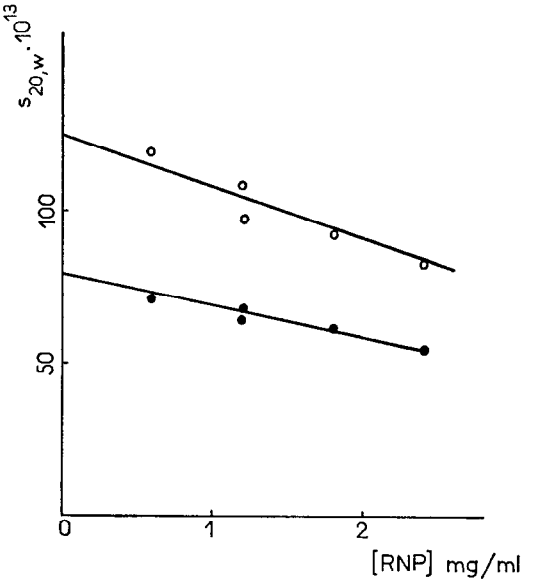

FIG. 1B. DETERMINATION OF THE SEDIMENTATION CONSTANTS OF LENTIL ROOT RIBOSOMES AT INFINITE DILUTION.

The measurements are made under conditions identical to those of Fig. 1A.

These latter particles probably correspond to two ribosomes associated with a messenger RNA fragment.[29] In the preparations studied, no ribosomes are associated with membrane fragments. As these preparations are rich in particulate peroxidases, it is logical to conclude that these haemoproteins are associated with the ribosomes and not with membranes. Moreover, the absence of membranes can be confirmed by examining the preparations under an electron microscope.*

If one supplies the roots (control or IAA-treated) with a labelled amino acid glycine-2-^{14}C (125 μc/l.) or leucine-^{14}C (125 μc/l.) one obtains, in sedimenting the ribosomes on a linear sucrose gradient, the results shown in Fig. 2. As expected, the peroxidase activity is not evenly distributed along the gradient but is localized in the fractions containing ribosomes. Also the distribution of the incorporated radioactivity depends on the nature of the labelled amino acid. In the case of the glycine-^{14}C, the incorporation is very high in the ribosome-containing fractions. Leucine-^{14}C, on the other hand, is incorporated not only into these fractions but also into lighter particles. As we have already stated, IAA activates biosynthesis of ribosomes and of particulate peroxidases, and also the incorporaion of a ^{14}C-amino acid to these same fractions. Under the action of IAA, the incorporation increase at the level of

* We wish to thank F. Marty (Cytology Laboratory, Centre de Marseille-Luminy) who carried out this work for us.

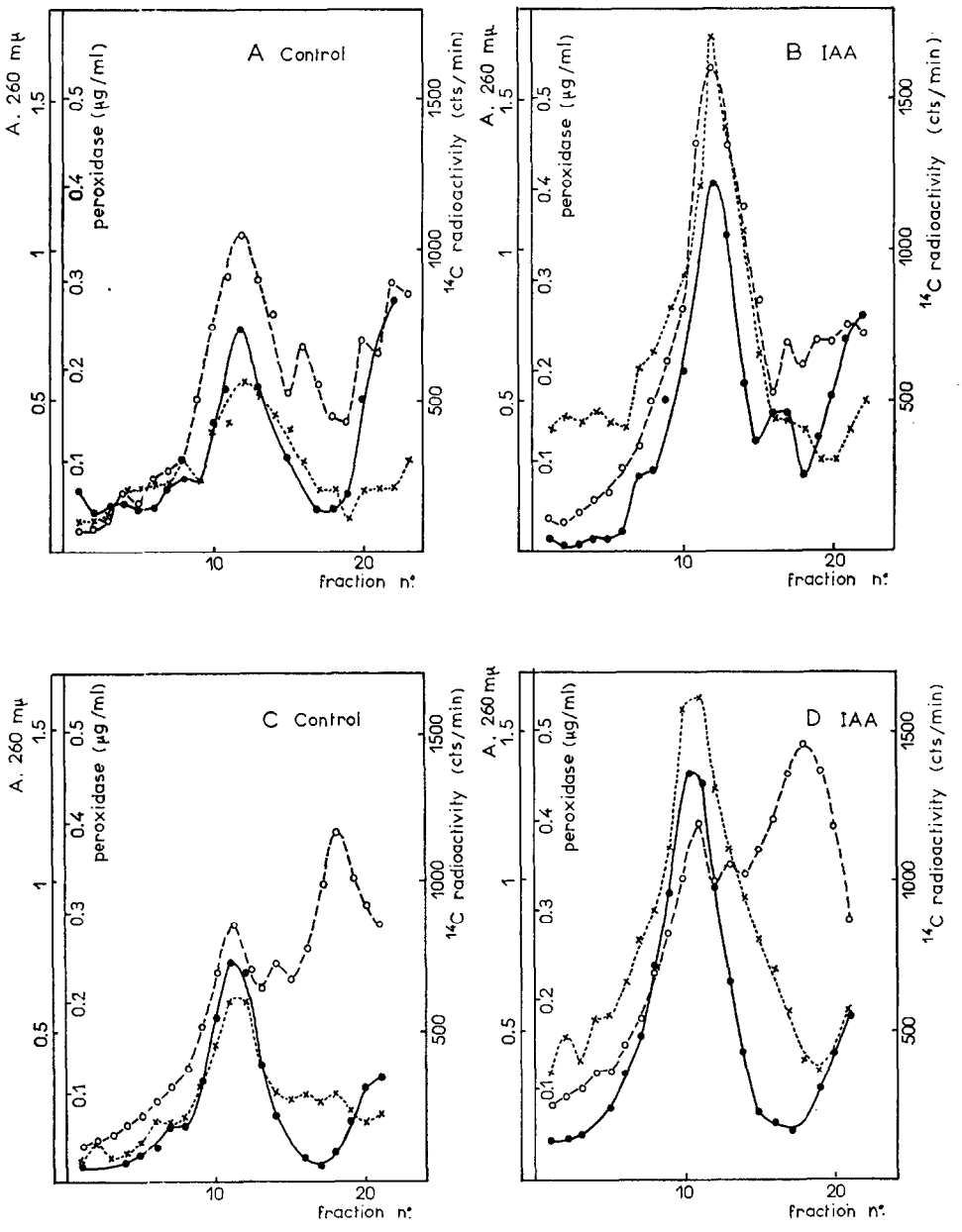

FIG. 2. SEDIMENTATION OF LENTIL ROOT RIBOSOMES ON A LINEAR SUCROSE GRADIENT.

The "control" (A) and "treated" (B) with IAA $2{\cdot}5 \times 10^{-4}$ M, for 16 hr, are incubated for 2·5 hr with 125 μc/l. of ^{14}C-2-glycine. The "control" (C) and "treated" (D) with IAA, $2{\cdot}5 \times 10^{-4}$ M, for 16 hr, are incubated for 2·5 hr with 125 μc/l. of leucine-^{14}C. Centrifugation is carried out at 40,000 rev/min for 60 min using a MSE 3×5 ml rotor in a linear sucrose gradient (5–27 per cent). One applies 0·2 ml of a ribosome suspension corresponding to 1 g of fresh tissue to each gradient. The absorption of the collected fractions (10 drops each) is determined using a Jean and Constant spectrophotometer after suitable dilution. Aliquots are taken to measure the peroxidase activity and to estimate the radioactivity. (●——●: Absorbance at 260 nm; o——o: radioactivity; ×------×: peroxidase.)

the ribosomes is greater in the case of glycine than in that of leucine. This is perhaps connected with the fact that glycine participates in haem biosynthesis.[30]

In order to study these ribosome-associated peroxidases one treats these particles with EDTA, 0·05 M, pH 6·8. The prepration is centrifuged 2 hr at 105,000 × *g*. The supernatant is applied to carboxymethyl–Sephadex (CM–Sephadex C-50) column equilibrated in an ammonium phosphate buffer, 0·03 M, pH 6·8.[5] Upon elution, one obtains an acid peroxidase. Two basic peroxidases are retained on the column and can be eluted with NaCl (Fig. 3).

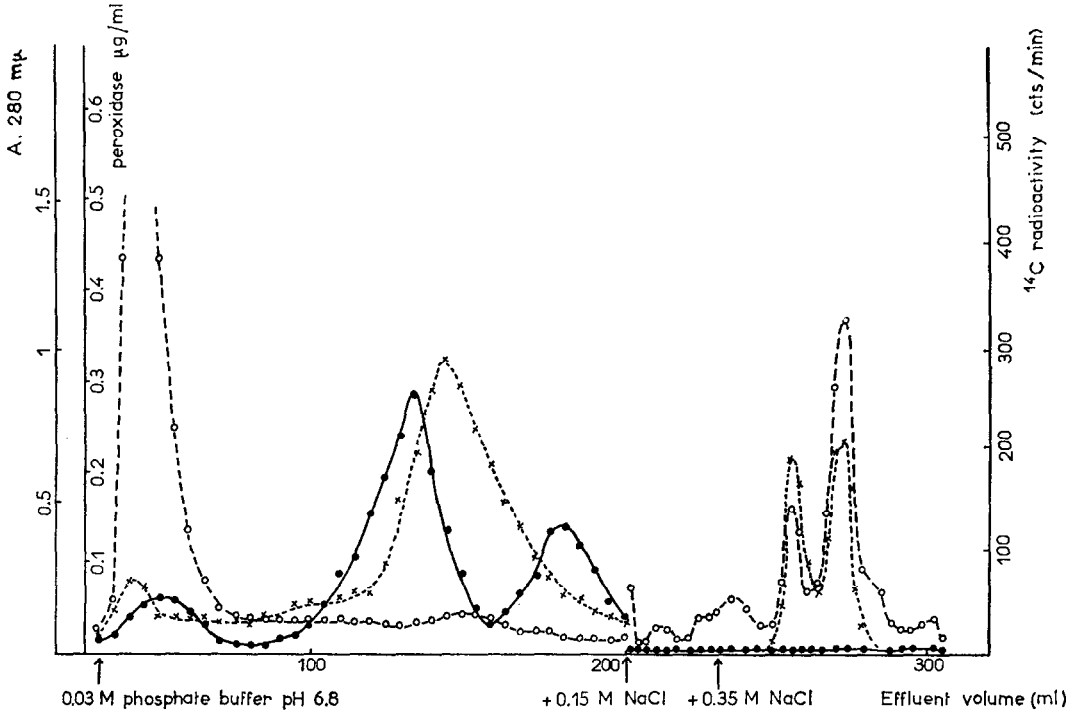

FIG. 3A

FIG. 3. CHROMATOGRAPHY ON CM–SEPHADEX C-50 OF THE RIBOSOME-ASSOCIATED PEROXIDASES OF LENTIL ROOTS (FIG. 3A AND FIG. 3B).

1·5 ml of a mixture of ribosomal peroxidases (1·25 mg of proteins/ml) are placed on the CM–Sephadex C-50 column. Columns (2·4 × 50 cm) are equilibrated with 0·03 M, pH 6·8, ammonium phosphate buffer. The same buffer (200 ml) is used for elution. The retained proteins are eluted by increasing concentrations of NaCl (0·15 M and 0·35 M). The arrows show where changes in elution conditions are made. The fraction volume is 5 ml. The elution rate is 12 ml/hr. The peroxidases are solubilized from ribosomes of the control (A) and treated (B) (with IAA $2{\cdot}5 \times 10^{-4}$ M for 7·5 hr) preparations. 2·5 hr before the end of the experiment one adds ^{14}C-leucine (0·125 μc/ml final concentration) to each preparation. (●——●: Absorbance at 280 nm; o——o: radioactivity; × ------ × : peroxidase.)

If the ribosomes have been extracted from roots previously treated with a ^{14}C-amino acid, one will not see any notable incorporation of the label in the proteins of the fractions containing the acid peroxidase. Incorporation to the fractions containing the basic peroxidases, on the other hand, is considerable. IAA treatment of the roots increases both their content

[30] J. C. WRISTON, L. LACK and D. SHEMIN, *J. Biol. Chem.* **215**, 603 (1955).

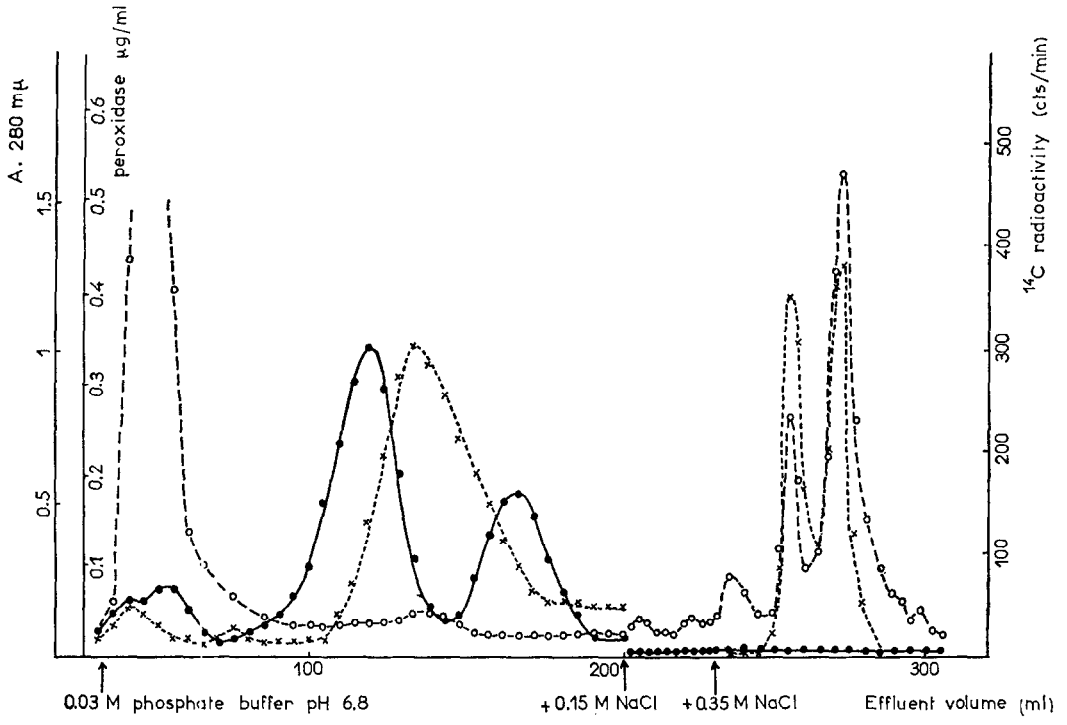

FIG. 3B

in basic peroxidases and the incorporation of label into the fractions containing these haemoproteins. This result is not observed in the case of the acid peroxidase. It is important to determine whether, in the experimental conditions used, these three peroxidases are specifically associated with the ribosomes or whether they are also present in the "soluble fraction". A lentil root homogenate, previously treated with EDTA, is brought to 80 per cent saturation with ammonium sulphate. The residue is collected, dialysed against 0·03 M pH 6·8 ammonium phosphate buffer, and then applied to a carboxymethyl-Sephadex column. The peroxidases eluted with 0·03 M pH 6·8 ammonium phosphate buffer are collected. Those still on the column (Fig. 4) are then eluted with 0·5 M NaCl. The combined peroxidase fractions, on electrophoresis, were found to have seven isozymes (Fig. 5).

The results of Fig. 5 enable us to identify peroxidases P_4, P_5 and P_6 as the "ribosomal" peroxidases R_2, R'_1 and R_1, respectively. Electrophoresis of the 105,000 × *g* supernatant (Table 1) shows appreciable quantities of only four peroxidases (Fig. 5B). Peroxidases P_4, P_5 and P_6 are present, but in small amounts. These results therefore prove that peroxidases R_1, R'_1 and R_2 associated with the ribosomes are specifically fixed on these particles and are not present in the "cytoplasmic" fraction.

The results of Fig. 3 imply that the two basic peroxidases R_1 and R'_1 are much more rapidly synthetized than the acid R_2 peroxidase. However, an incorporation of labelled amino acid into the fractions containing the basic peroxidases (Fig. 3) does not prove that it is these haemoproteins rather than contaminating proteins of similar charge that have incorporated the label. These two isoperoxidases have therefore been purified further by molecular sieving on Sephadex G-100 (Fig. 6). The RZ (Reinheitszahl) (ratio of absorbancies

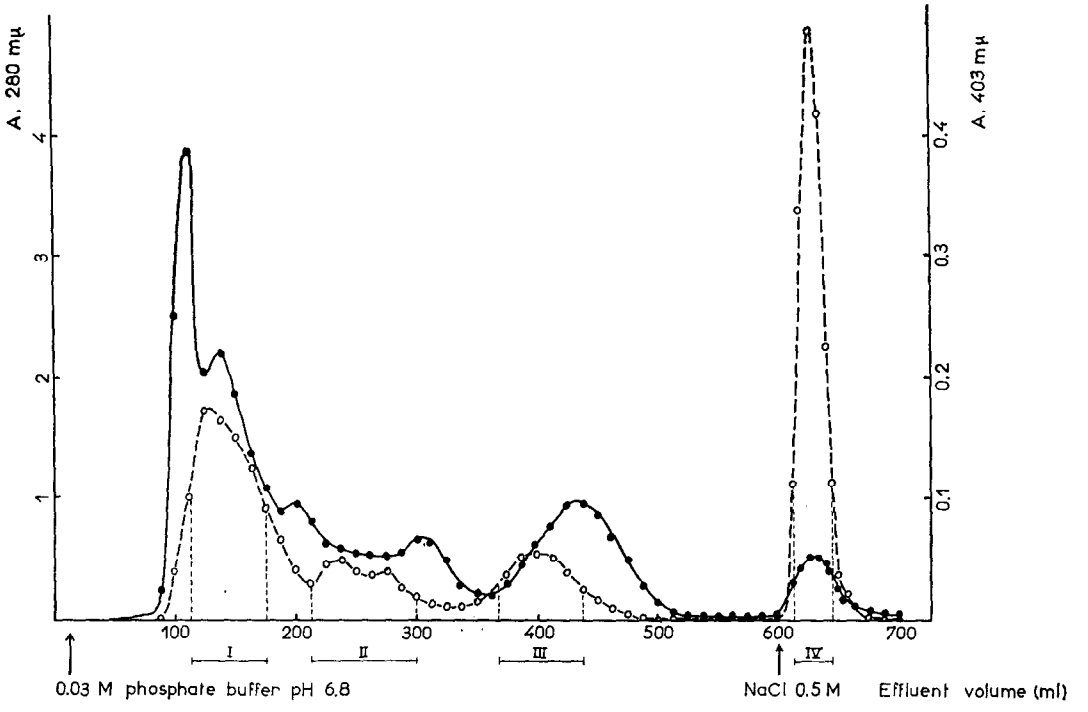

FIG. 4. CHROMATOGRAPHY ON CM–SEPHADEX C-50 (3·8 cm × 40 cm) OF AN HOMOGENATE OF LENTIL ROOTS TREATED WITH EDTA, 0·05 M, pH 6·8.

The acidic peroxidases come off under circumstances similar to those described in Fig. 3. The basic peroxidases are eluted by NaCl, 0·5 M. The arrow indicates the change in elution conditions. 25 ml of the extract (12 mg of proteins/ml) were applied. The fraction volume is 5 ml. The elution rate is 20 ml/hr. The fractions indicated by I, II, III, and IV on the figure were combined. (●——●: Absorbance at 280 nm; o——o: absorbance at 403 nm.)

TABLE 2. SOME PHYSICOCHEMICAL CHARACTERISTICS OF RIBOSOME-ASSOCIATED PEROXIDASES R_1, R_1' AND R_2 OF LENTIL ROOTS.

Peroxidase	Migration speed (mm/hr)	RZ (ratio)	Molecular weight
R_1	+4·4	3·1	33,400
R_1'	+1·2	2·7	45,000
R_2	−2·7	3·2	57,000

Migration speeds: determined in 0·03 M, pH 6·8, ammonium phosphate buffer. Molecular weights: determined by molecular sieving. RZ = Reinheitszahl in the 0·03 M pH 6·8, ammonium phosphate buffer.

at the Soret peak and at 280 nm) are 3·1 and 2·7 respectively. These preparations are homogeneous by the criterion of electrophoresis on polyacrylamide gel. They can therefore be considered as being highly purified and are always labelled by ^{14}C-amino acid (Fig. 6).

These results prove therefore that while the roots were in contact with the labelled amino acid, the acid peroxidase R_2 was not synthetized whereas the basic peroxidases were. The regulation mechanisms of these two groups of haemoproteins are therefore different and IAA specifically activates the synthesis of the two basic peroxidases.

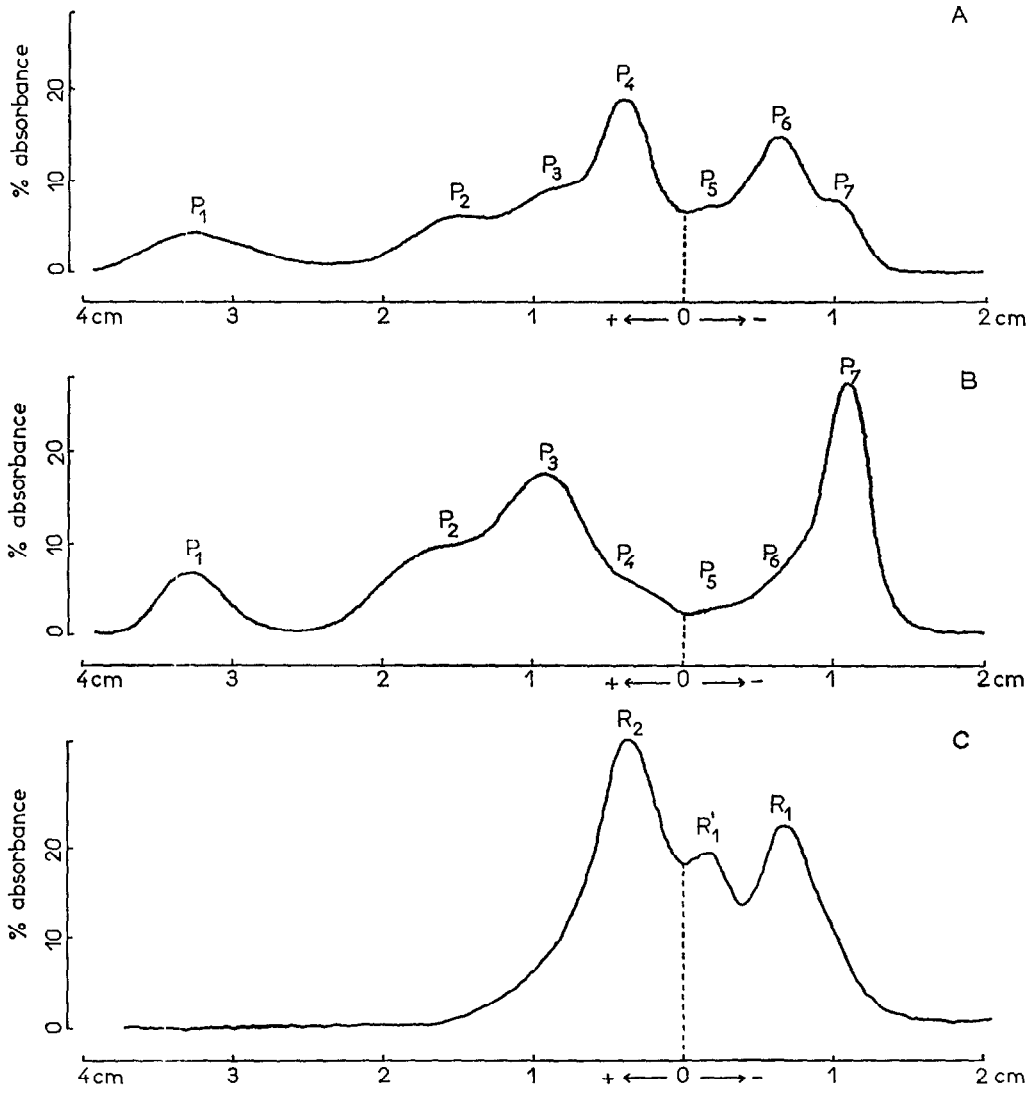

FIG. 5. ELECTROPHORESIS PROFILES OF A LENTIL ROOT EXTRACT.

A. Electrophoresis profile obtained from an homogenate of tissues treated with EDTA, pH 6·8, 0·05 M. B. Electrophoresis profile obtained from the ultracentrifugation supernatant (105, 000 *g* for 3hr) of an homogenate of tissues not treated with EDTA. C. Electrophoresis profile of the peroxidases solubilized from the lentil root ribosomes using EDTA, 0·05 M, pH 6·8. Time of electrophoresis 90 min, 0·03 M, pH 6·8, ammonium phosphate buffer.

Pyridine haemochromogen spectra of these peroxidases are identical to that of the protohaematin IX. Protohaematin IX is therefore the prosthetic group of these peroxidases. Their molecular extinction coefficients in the Soret and visible bands were determined by the Keilin and Hartree method.[8] The spectral characteristics of these three haemoproteins are shown in Fig. 7. Their peroxidase activities, determined using guaicol as electron donor, are very different (Fig. 8).

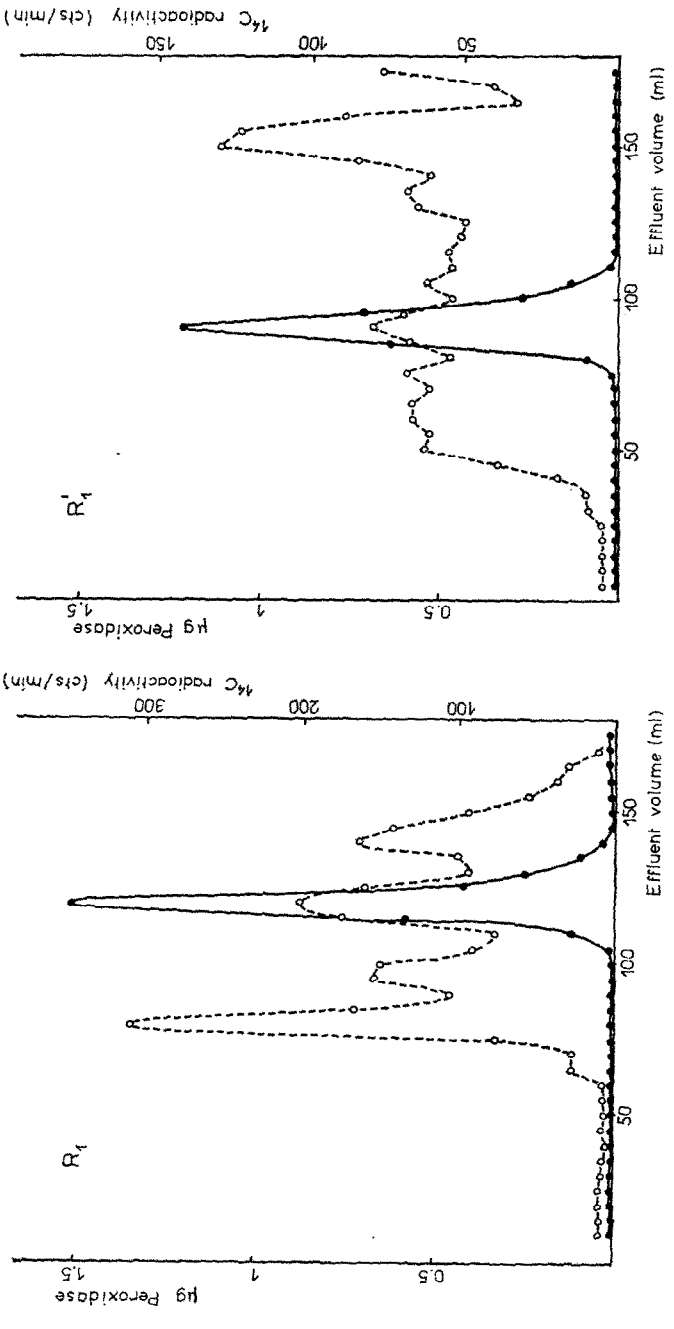

FIG. 6. PURIFICATION OF PEROXIDASES R_1 AND R'_1 BY MOLECULAR SIEVING ON SEPHADEX G-100.
One places on the columns 1 ml of a protein solution containing 6 μg of peroxidase R_1 or 4 μg of peroxidase R'_1 (labelled with leucine-^{14}C) in a 0·1 M, pH 6, sodium acetate. The columns (1·1 × 170 cm) are equilibrated with a sodium acetate buffer, 0·1, M pH 6, containing NaCl, 0·4 M. The volume of each fraction is 2·5 ml. The elution rate is 5 ml/hr. (●——●: Peroxidase; o-----o: radioactivity.)

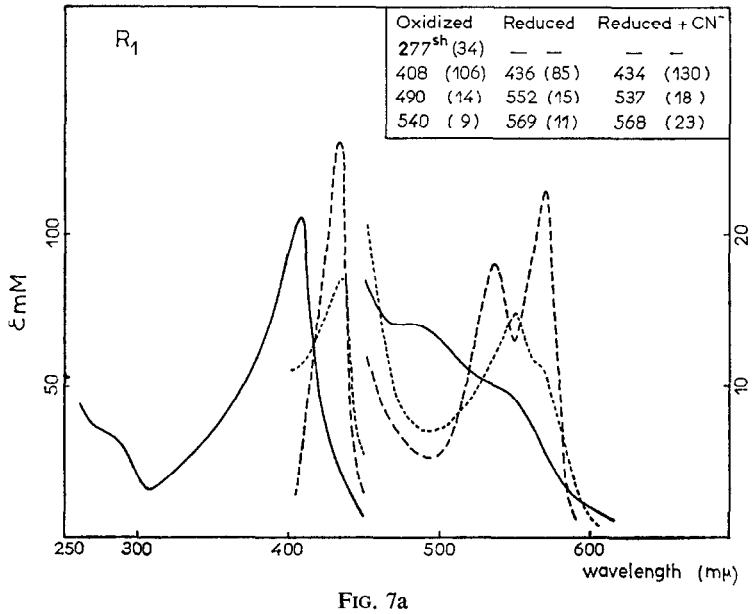

FIG. 7a

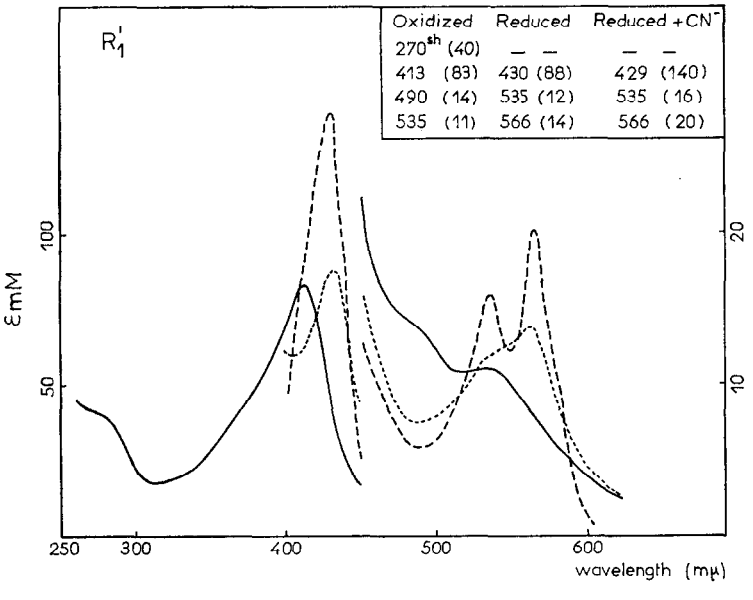

FIG. 7b

FIG. 7. ABSORPTION SPECTRA OF THE VARIOUS RIBOSOME-ASSOCIATED PEROXIDASES OF LENTIL ROOTS. In each table the values in brackets correspond to the millimolar extinction coefficients at an absorption peak or at a shoulder (sh.). All the spectra are recorded in 0·03 M, pH 6·8, ammonium phosphate buffer. (——: oxidized; ------: reduced; ————: reduced + CN^-.)

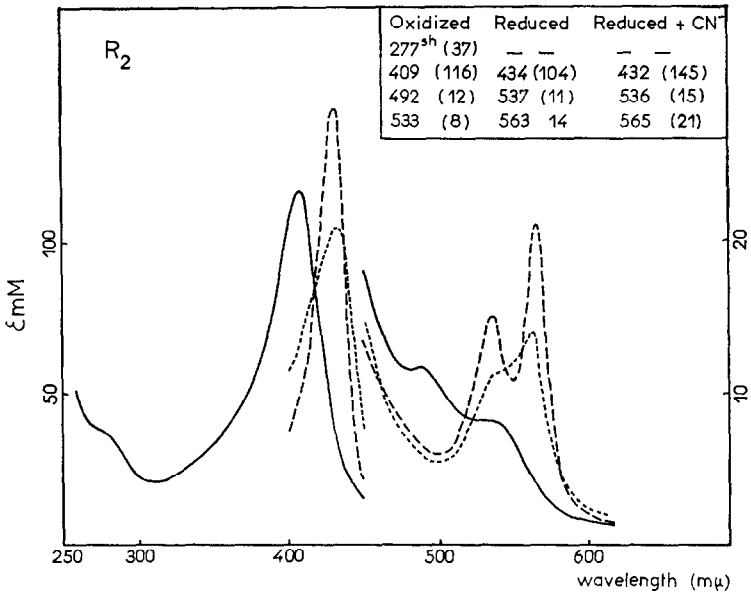

FIG. 7c

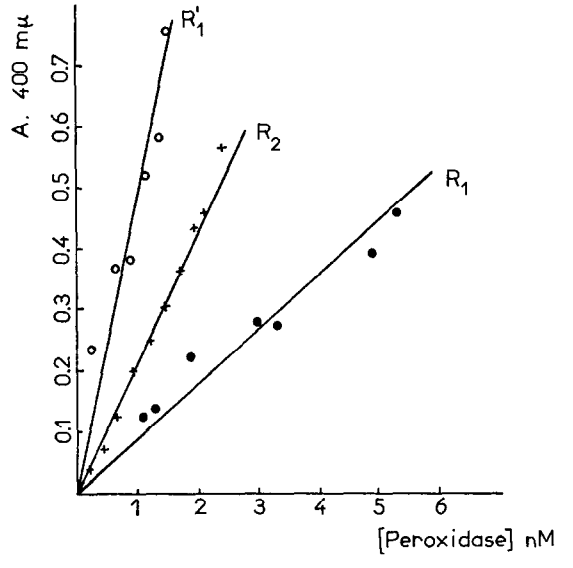

FIG. 8. COMPARISON OF THE PEROXIDASE ACTIVITIES OF THE HAEMOPROTEINS R_1, R'_1 R_2.

The activity is estimated by the Jermyn and Thomas method.[42] The quantities of reagents used are 10 μmoles of H_2O_2, 1·4 μmoles of guaicol, and 0–5 $\mu\mu$moles of peroxidase R_1 or 0–1·5 $\mu\mu$moles of peroxidase R'_1, or 0–2·5 $\mu\mu$moles of peroxidase R_2. The final volume of the reaction mixture (pH 6·8, 0·06 M, ammonium phosphate buffer) is 2 ml. The reaction is stopped after 10 min with 1 ml of NaOH N.

The molecular weights of the three peroxidases have been estimated by molecular sieving through a Sephadex G-100 column previously calibrated with proteins of known molecular weight. One thus obtains the values of $33{,}400 \pm 2500$ (peroxidase R_1), $45{,}000 \pm 2500$ (peroxidase R_1'), and $57{,}000 \pm 2500$ (peroxidase R_2). Table 2 contains some of the physicochemical properties of these three haemoproteins.

DISCUSSION

Our demonstration of ribosome-bound peroxidases can be related to analogous observations on ribosomes and microsomes of animal and plant origin.[7, 31–33] It is probable that these haemoproteins are not ribosomal constituents. They might represent only a contaminant of these particles. But this contamination is highly specific. Of the seven peroxidases that can be demonstrated in the root extracts by electrophoresis, only R_1, R_1' and R_2 can be shown in the ribosomal fractions. The haemoproteins R_1, R_1' and R_2, on the other hand, are present only in very small quantities in the "cytoplasmic" fractions. It is therefore clear that peroxidases R_1, R_1' and R_2 are specifically associated with the ribonucleoprotein particles. One of these three peroxidases R_1' presents analogies with cytochromes of the b type (reduced β band at 566 nm). It can be considered to be a "peroxidase 566".[34, 35, 5] It has, in particular, two of the characteristics of haemoproteins of this type: (i) the molecular extinction coefficient of the Soret band is higher in the case of ferroperoxidase than in that of ferriperoxidase; (ii) the addition of CN^- to ferroperoxidase does not produce a shift of the bands but a heightening of them.[34] All the "566" type peroxidases studied until now are of a highly basic type. From this point of view, haemoprotein R_1' is therefore a remarkable exception.

Numerous authors have isolated peroxidases in homogenates of various plant tissues,[1–6] some in a highly purified form.[2, 5] However, Keilin and Hartree[8] demonstrated, a long time ago, the transformation of one form of horseradish peroxidase into another having a different electrophoretic mobility. From this observation they concluded that only one peroxidase exists in horseradish roots and that the various components discovered in homogenates correspond to transformations of a single haemoprotein. This opinion is not shared by the majority of the authors who assume, implicitly or explicitly, that the isoenzymes present in plant homogenates do in fact exist in the intact cells.[1, 2, 4] Until now there existed no experimental argument enabling a choice between these two hypotheses. The fact that peroxidase R_1 and R_1' have very different turnovers from that of peroxidase R_2, and that the biosyntheses of these haemoproteins are under different control mechanisms, proves that at least two isoperoxidases really exist in the cells of lentil roots.

The increase, under the action of auxins, of various enzymatic activities, or of the amount of tissue proteins, has often been described.[16–21] However, these results provide simply an indication and not a proof of *de novo* biosynthesis of an enzyme under the action of a hormone. This can be proved by showing an increase in the enzymatic activity under the hormone action and, an incorporation of a labelled amino acid into the enzyme, and the increase, in absolute value, of this incorporation under hormonal action.

Using these criteria, Varner and his group[16, 25–27] have shown that gibberellic acid controls the *de novo* biosynthesis of α-amylase in the endosperm of barley grain. The results of Figs.

[31] F. IBUKI and S. MATSUSHITA, *Biochim. Biophys. Acta* **40**, 540 (1960).
[32] T. HOSOYA, *J. Biochem.* **53**, 381 (1963).
[33] T. HOSOYA and M. MORRISON, *J. Biol. Chem.* **242**, 2828 (1967).
[34] M. SHIN and W. NAKAMURA, *J. Biochem.* **50**, 500 (1961).
[35] B. HAGIHARA, K. TAGAWA, I. MORIKAWA, M. SHIN and K. OKUNUKI, *Nature* **181**, 1656 (1958).

2, 3 and 6 allows us to conclude, in the same way, that indoleacetic acid controls the *de novo* biosynthesis of the two basic peroxidases associated with the ribosomes.* It should also be stressed that the hormonal control of the biosynthesis of haemoproteins R_1 and R_1' is not necessarily related to the growth of the root. This control appears at IAA concentrations that block cell division and cell elongation. This fact is to be compared with Halevy's observations.[36]

The induction by IAA of a peroxidase biosynthesis shows analogies with the control, by tryptophan, of the tryptophan–pyrrolase content in mammalian hepatic cells.[37] As we have already shown,[38, 39] this parallelism is also found at the level of the mechanism of action of these two haemoproteins. The results obtained agree well, on the whole, with the hypothesis that a phytohormone derepresses genes previously repressed,[40] thus implying the *de novo* biosynthesis of a series of proteins.

EXPERIMENTAL

The lentils (*Lens culinaris* Med. var. Ronde Blonde Vilmorin) were germinated, after sterilization with 1% calcium hypochlorite. Root growth was effected in aerated distilled water. In 3 days, the roots reached a length of a 4–5 cm. Some seedlings were introduced into an aerated solution of IAA ($2{\cdot}5 \times 10^{-4}$ M) ("treated" samples). Other seedlings remained in aerated distilled water ("control" samples).

Incorporation of Radioactive Precursors and Determination of Radioactivity

The roots (10 g wet wt.) from "control" or "treated" seedlings were removed and incubated for 2·5 hr in aerated solutions (volume 50 ml) of tris-HCl, 0·02 M, pH 7·2, penicillin G, 100 mg/l., glycine-2-^{14}C, 125 μc/l., or leucine-^{14}C, 125 μc/l. The incorporated radioactivity was determined after precipitation of the fractions at 0° by 5% trichloracetic acid in the presence of albumin (0·5 mg), and filtering onto 0·45 μmillipore membranes. The radioactivity was determined with the aid of a Tracerlab gas flow counter.

Cell Fractionation and Ribosome Isolation

The lentil roots (10 g wet wt.) were picked, washed in a 0·2% solution of cemulsol NPT 6 (a non-ionic bactericide), thoroughly rinsed, frozen in liquid N_2, ground in a mortar and homogenized three times for 15 sec in a 10 ml solution of 0·25 M sucrose tris-HCl, 5×10^{-3} M, pH 7, Mg acetate, 10^{-3} M, macaloid,† 2 mg/ml, by means of an Ultra-Turrax apparatus (Janke and Kumkel KG STAUFEN, Germany). The homogenate obtained was filtered through nylon mesh and centrifuged for 10 min at 10,000 *g*. The supernatant (S_0) was then centrifuged for 30 min at 30,000 *g*. The pellets (C_0 and C_1) were resuspended in the grinding medium. The supernatant (S_1) thus obtained was centrifuged once again for 2 hr at 105,000 *g*. The pellet (C_2) corresponds to the ribosomes. This preparation was suspended in 5×10^{-3} M tris-HCl and 10^{-4} M Mg acetate buffer. The ribonucleoprotein particles can be appreciably purified by new centrifugations at 30,000 *g* and 105,000 *g*.[28] This purification, however, causes considerable loss of material (50 per cent). All these operations were carried out at temperatures not exceeding 5°. The ribosomes were analysed on a sucrose gradient by the method of Gros *et al.*[41] The linear concentration gradients of sucrose (5–27 per cent) were made using the Buchler apparatus. The ribosomes were centrifuged at 5° over the 5–27 per cent gradient containing tris-HCl, 5×10^{-3} M, pH 7·2 and magnesium acetate 5×10^{-4} M at 40,000 *t*/min for 1 hr using a MSE No. 2414 rotor. The analytical ultracentrifugation of the ribosomes was performed in a Spinco model E analytical centrifuge. The sedimentation constants were determined by extrapolation at infinite dilution.

Chromatography of the Peroxidases Associated with Ribosomes

The chromatographic isolation of the peroxidases was carried out on carboxymethyl-Sephadex (CM Sephadex) C 50 according to the technique of Mazza *et al.*[5] The columns were equilibrated with an ammonium

* It is probable that IAA also induces the biosynthesis of other proteins in the roots.

† Purified lithofluorosilicate of Na and magnesium.

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phosphate buffer, 0·03 M, pH 6·8. The basic peroxidases which remained fixed on the column were eluted by increasing concentrations (0·15 M and 0·35 M) of NaCl.

Electrophoresis of the Peroxidases

Zone electrophoreses were carried out in a Kohn (Shandon) tank on cellulose acetate film (2·5 × 12 cm) "cellogel" (Chemetron) in ammonium phosphate buffer, 0·03 M, pH 6·8. The electrophoreses took 90 min and were performed at 4° under a regulated current of 2 mA per band. The peroxidases were detected according to the method of Mazza *et al.*[5] The electrophoresis profiles were determined using a Vernon recording densitometer.

Determination of the Peroxidases, Nucleic Acids and Proteins

The peroxidases were determined by the Jermyn and Thomas method.[42] The nucleic acids were extracted by the Ogur and Rosen method.[43] RNA was determined either by measuring the absorbance at 260 nm, or by the Militzer method.[44] DNA determination was effected with the Burton technique,[45] that of the proteins with the Lowry method.[46]

Other Methods

Molecular weights of the peroxidases were evaluated by sieving through a Sephadex G 100 column[47] equilibrated with a sodium acetate buffer, 0·1 M, pH 6, NaCl, 0·4 M. The column (1·1 cm × 170 cm) was calibrated with proteins of known molecular weights. The absorption spectra of the peroxidases were obtained with the aid of a Bausch and Lomb recording spectrophotometer, Spectronic 505. Molecular extinction coefficients were determined with the Keilin and Hartree method.[10]

Acknowledgements—The cemulsol NPT 6, penicillin G, macaloid were the gifts of Melle Bezons (Bezons, 95 France), Specia (Paris VIII°, France), National Lead Company, Baroïd Division (Houston, Texas, U.S.A.), respectively. The technical assistance of Miss Anne-Marie Moustacas was particularly appreciated. This work was carried out with the financial aid provided by the Atomic Energy Commission (Contract No. 9.687/r) to the senior author (J. R.). Thanks are due to Mrs. Grossman for carefully reading the manuscript. We are indebted to Dr. Jacques Reynaud and Miss Jacqueline Savary (Faculté de Médecine et de Pharmacie de Marseille) who kindly carried out ultracentrifugal analyses.

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