THE X-IRRADIATION OF HAPLOID AND DIPLOID STRAINS OF YEAST AND ITS ACTION ON CELL DIVISION AND METABOLISM*

by

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LATARJET AND EPHRUSSI[1] and ZIRKLE AND TOBIAS[2] found that X-irradiation affected differently the multiplication of haploid and diploid yeast, the haploid yeast being more sensitive. While 5,000 r inhibited cell division by 50 per cent in haploid yeast, 54,000 r were required to produce the same inhibition in diploid yeast. LUCKE AND SARACHEK[3] also found poliploid strains of yeast more resistant to X-irradiation, as well as to ultraviolet irradiation[4]. The mechanism of this very interesting relation between ploidy and resistance of cell multiplication to X-ray inhibition is not known. The present experiments were performed in an attempt to elucidate the mechanism of the inhibition.

EXPERIMENTAL

The initial experiments were carried on with the two samples of yeast (*Saccharomyces cerevisiae*) kindly provided by Dr. ZIRKLE: the haploid yeast, SC-7, and the diploid, SC-6. Master stocks of yeast were prepared on Difco potato-dextrose-agar slants and were kept at —30°C. Cultures were transferred every two months. One loopful from the slant was introduced into a 125 ml Erlenmeyer flask containing 50 ml of liquid medium (1% dextrose, 0.5% Difco yeast extract, and 0.3% Difco peptone) and was incubated for 8 hours at room temperature. At the end of this time, the yeast culture was transferred to a large flat-bottomed flask of 2,500 ml capacity, containing 500 ml of the same medium. The flasks were incubated at room temperature (25–26°C), while continuously shaken, for 16 hours. The haploid yeast was always checked by X-irradiation before use. The experiments concerning the effect of X-irradiation upon cell multiplication were performed according to the technique of WOOD[5] in Dr. ZIRKLE's laboratory. For the experiment on metabolism, the cells were washed four times with metal-free, glass-distilled water, and were suspended in water for transfer into the Warburg vessels. The buffer was the universal buffer of THEORELL AND STENHAGEN[6] at a concentration of 0.01 *M*. The final concentration of the substrates was 0.01 *M*. The cell-free extracts of yeast were prepared by crushing the frozen cells in the bacterial press of HUGHES[7] and then centrifuging the suspension of crushed cells in the Spinco preparative ultracentrifuge at 40,000 rpm for 30 minutes. The clear supernatant fluid was used to test enzyme activity, which was measured according to the methods previously reported[8,9]. Carboxylase activity in the cell-free extracts was measured manometrically in the presence of 0.05 *M* phosphate buffer pH 6.0, and 30 micromoles of K pyruvate at 25°. Carboxylase was also measured in the dried yeast, the Q_{CO_2} values being referred to mg dry weight per hour. Cytochrome oxidase in the crushed cell suspensions was determined either manometrically, according to KEILIN AND HARTREE[10], or spectrophotometrically, by measuring the reduction of cytochrome *c* in the presence of HCN

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(0.003 *M*). Catalase activity was measured at 0° by titration of H_2O_2 with thiosulfate. The protein content of cell-free extracts was determined with the micro Kjeldahl apparatus[11].

The other yeast samples used were the haploid strains YO-2022 and YO-2521 kindly sent by Dr. R. K. MORTIMER from Dr. C. A. TOBIAS' laboratory at the University of California, and H 13778 from Mrs. CARL C. LINDEGREN of Southern Illinois University. The diploid yeast YO 22 × 21 was prepared at Dr. LINDEGREN's laboratory by mating YO-2022 and YO-2521. Single cell isolations of the diploid were kindly performed by Mrs. LINDEGREN. X-irradiation of yeast for the experiments on fermentation activity were performed in flat lucite vessels at 0°C. The X-ray machine was a G.E. MAXIMAR of 100 kv, 5 ma. with a 5 mm Al filter at a dose rate of 950r/min.

Glucose oxidation and fermentation by haploid (SC-7) and diploid (SC-6) yeast

The haploid and diploid yeast used by ZIRKLE AND TOBIAS were quite different in their metabolic behavior. The haploid yeast was found to belong to the group of fermenting yeast (yeast cells which do not take up O_2 in the presence of glucose) whereas the diploid yeast belonged to the group of respiring yeast (cells which take up O_2 in the presence of glucose). Fermentation (CO_2 formation) was more active in the haploid yeast (Table I).

TABLE I

RESPIRATION (O_2 UPTAKE) AND FERMENTATION (CO_2 PRODUCTION) OF THE HAPLOID AND THE DIPLOID YEAST FROM ZIRKLE AND TOBIAS

Figures give μl of O_2 uptake or CO_2 production per mg dry weight per hour (blank subtracted). Substrate, glucose, 30 μM. Temp. 25°C.

Measurement	*pH*	*Yeast*	
		Haploid SC-7	*Diploid SC-6*
O_2 uptake	2.8	3.1	51.3
CO_2 production in air	2.8	81.0	38.6
CO_2 production in N_2	2.8	47.0	84.6
O_2 uptake	6.0	5.2	77.6
CO_2 production in air	6.0	74.6	63.5
CO_2 production in N_2	6.0	63.3	124.5
CO_2 production in air	5.3	234.0	100.0
CO_2 production in N_2	5.3	203.0	130.0

The haploid yeast was extremely unstable. When freshly cultured in liquid medium it had the typical characteristics of fermenting yeast, and cell multiplication was very sensitive to X-irradiation—to the same degree as reported by ZIRKLE AND TOBIAS. On repeated growth under aerobic conditions it changed into a respiring yeast and became more resistant to X-irradiation. Glucose oxidation and resistance to X-irradiation seemed to go together. In this respect this strain of yeast resembled the "petites colonies" of yeast studied by EPHRUSSI[12] and SLONIMSKI[13], which changed when grown under aerobic conditions from fermenting into respiring yeast.

Effect of X-irradiation on glucose fermentation

BARRON AND GASVODA[14] found that, whereas bacterial multiplication (*Cornynebacterium creatinovorans*) was half-inhibited with 7,000 r, half-inhibition of glucose oxidation required 15,000 r. The same behavior was found with the haploid and diploid yeasts. X-ray doses sufficient to produce half-inhibition of cell multiplication in haploid yeast had no effect at all on the CO_2 production in the presence of glucose. Half-

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inhibition of fermentation was produced on irradiation with 100,000 r, *i.e.*, 30 times the dose necessary to inhibit cell multiplication. The same relationship was found in the diploid cells; both cell multiplication and fermentation were more resistant to X-irradiation. Irradiation with 100,000 r produced only 10% inhibition of fermentation (Table II).

TABLE II

EFFECT OF X-IRRADIATION ON THE FERMENTATION OF GLUCOSE BY HAPLOID AND DIPLOIS YEAST (*Saccharomyces cereviscae*)

Yeast irradiated in water suspension. KH_2PO_4 added afterwards to make 0.01 *M*. Glucose, 0.01 *M*. Gas phase, N_2. Tempt. 28°C. Measurements soon after irradiation.

X-Ray dose	*CO_2 production*			
	Haploid (SC-7)		*Diploid (SC-6)*	
	Control cmm	*X-Ray cmm*	*Control cmm*	*X-Ray cmm*
10,000	210	180	—	—
25,000	313	246	317	320
50,000	220	160	300	325
100,000	240	122	420	380

Ploidy and respiration in yeast cells

In order to see whether the differences in metabolic behavior of the strains of yeast obtained from Dr. ZIRKLE were due to their chromosome number, other hapliod and diploid yeasts were studied. In the meantime, OGUR[15] reported that in respiring yeast the O_2 uptake per cell in the presence of glucose increased with ploidy, whereas it was essentially ploidy independent when calculated to dry weight or total nitrogen. Of the four haploid strains of yeast studied, one, H–13778, was a respiring cell, as shown by the large O_2 uptake on addition of glucose, pyruvate, and acetate. The other three (SC–7, H–YO–2022) were fermenting cells. The diploid yeast SC–6 was a respiring cell, whereas the other 20x22, obtained by mating two fermenting haploids, was a fermenting cell. These differences were not due to differences in cell permeability, for the same results were obtained at pH 2.8, where both pyruvate and acetate are largely present as the undissociated acid which is readily permeable (Table III). The complete inhibition of respiration of fermenting yeast on addition of acetate remains to be explained.

TABLE III

OXIDATION OF GLUCOSE, PYRUVATE, AND ACETATE BY HAPLOID AND DIPLOID YEAST

Figures give μl O_2 uptake per mg dry weight per hour. Tempt. 25°C.

Substrate	*pH*	*Haploid Yeast*				*Diploid Yeast*	
		SC-7	*H-13778*	*H-YO-2521*	*H-YO-2022*	*SC-6*	*D-20 × 21*
None	2.8	4.8	9.9	0.5	0.4	10.1	0.7
Glucose	2.8	7.9	41.5	0	0	51.3	1.7
Pyruvate	2.8	4.8	39.3	0	0.7	19.3	0.4
Acetate	2.8	0	26.1	3.1	0.6	26.1	0.2
None	6.0	7.7	10.5	1.0	2.8	10.5	0.4
Glucose	6.0	12.2	62.9	0.7	6.8	77.6	2.8
Pyruvare	6.0	5.3	33.0	0.5	1.9	24.5	0
Acetate	6.0	4.7	28.0	0.5	1.7	27.5	0

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Aerobic glucose fermentation in fermenting haploid yeast varied widely at both pH values, 2.8 and 5.1–6.0. Anaerobic fermentation was either lower or slightly higher than aerobic fermentation. In respiring yeast anaerobic fermentation was twice as much as earobic fermentation (Table IV).

TABLE IV

GLUCOSE FERMENTATION BY HAPLOID AND DIPLOID YEAST

(aerobic cultures, 16–18 hours, washed five times with water or with 0.1 *M* NaCl). Figures give μl CO_2 produced.

Yeast	*pH 2.8*		*pH 5.1–6.0*	
Haploid				
SC-7 (F)	81	47	154.3	133.1
H-YO-2022 (F)	66.9	—	41.3	60.5
H-YO-2521 (F)	101.3	—	77.4	113.6
Diploid				
SC-6 (R)	38.6	84.6	81.5	172.7
D-21X22 (F)	100	—	86.9	160.0

Enzymes in cell-free extracts

In order to see whether the difference between the respiring and fermenting yeast was due to the distribution of the dehydrogenases which take part in these reactions, or to the electroactive catalysts which transfer electrons to molecular oxygen, it was decided to measure the activity of some enzymes concerned with fermentation and oxidation of glucose. In the cell-free extracts of respiring yeast, hexokinase, carboxylase, glucose phosphate dehydrogenase, isocitric dehydrogenase, condensing enzyme (synthesis of citric acid from acetate, coenzyme A, and oxalacetate) had greater activity, whereas alcohol dehydrogenase was more active in the fermenting yeast. The enzymes for ribose-5-phosphate metabolism were more active in respiring yeast. The large increase in carboxylase activity in cell-free extracts of respiring yeast was not found when the enzyme was measured in dried yeast powder. There was no difference in the catalase activity (Table V).

TABLE V

ACTIVITY OF SOME ENZYMES IN CELL-FREE EXTRACTS OF HAPLOID AND DIPLOID YEAST

Enzyme activity expressed in units, where one unit = 0.01 micromole of reactant used or produced per mg protein per min. *F* = fermenting yeast; *R* = respiring yeast.

Enzyme	*Haploid, F.*	*Diploid, F.*	*Diploid, R.*
1. Hexokinase	71.9	97	162.3
2–3. Aldolase-triosephosphate dehydrogenase	0.564	1.64	0.844
4. Carboxylase	16.6	20.8	41
5. Enolase	1.25	3.1	—
6. Glucose phosphate dehydrogenase	2.86	3.36	6.01
7. Ribose-5-phosphate utilization	4.6	7.1	18
8. Alcohol dehydrogenase	12.42	19.9	3.1
9. Isocitric dehydrogenase	1.09	1.35	3.156
10. Condensing enzyme	1.08	—	5.50

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Lack of oxidation of acetic acid is not due therefore to lack of enzymes required to initiate this process. The difference between the fermenting and respiring yeast must lie in the activity of the enzyme which transfers electrons to atmospheric oxygen, mainly cytochrome oxidase. (This was shown beautyfully by EPHRUSSI and his co-workers in their studies on fermenting mutant yeasts[12–13]). All the cytochromes of baker's yeast, including cytochrome oxidase, were observed spectroscopically on thick suspensions of respiring yeast (Diploid SC–6). Cytochrome oxidase was lacking in the fermenting haploid in manometric and spectrophotometric measurements.

Effect of X-irradiation on the multiplication of yeast cells

It has been shown that the haploid and diploid yeast cells of ZIRKLE AND TOBIAS on X-irradiation lost the ability to multiply much more rapidly than the ability to perform catabolic reactions, although the X-ray-sensitive haploids were also more sensitive to X-irradiation when the rate of fermentation was measured. However, ploidy and metabolic pathways were shown to be independent processes. The relation between ploidy and cellular multiplication in respect to resistance to X-ray inhibition was tested on the different strains of yeast collected from different laboratories. On irradiation of the different haploid and diploid yeasts, the great sensitivity of haploid yeast was confirmed (Fig. 1).

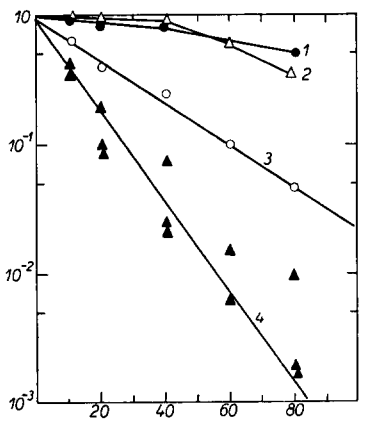

Fig. 1. Effect of X-irradiation on the multiplication of haploid and diploid strains of yeast. X-ray dose, 300 r per min. Abscissa, Time in minutes. Ordinate, log of ratio of number of cells/ml multiplying after irradiation to the number of cells/ml in controls. This is the socalled "surviving fraction". 1. Respiring diploid, DSC-6. 2. Fermenting diploid 21x22. 3. Respiring haploid 13778. 4. Fermenting haploid H-812.

Effect of –SH reagents on yeast fermentation and on cell multiplication

A number of enzymes which take part in yeast fermentation are –SH enzymes[16]. Moreover, fermentation can be completely inhibited by amounts of iodoacetate which do not affect respiration[17]. In intact cells this inhibition depends also on the cell membrane. Glucose fermentation by diploid yeast cells was largely inhibited (92 %) by 0.001 *M* chlorovinyl dichloroarsine, whereas the same concentration of *p*-chloromercuribenzoate (a very efficient inhibitor of –SH enzymes) inhibited only 52 % (Fig. 2). The rapid rate of inhibition with the arsenical, and the slow rate with the mercurial are indication of the rate of penetration through the cell membrane.

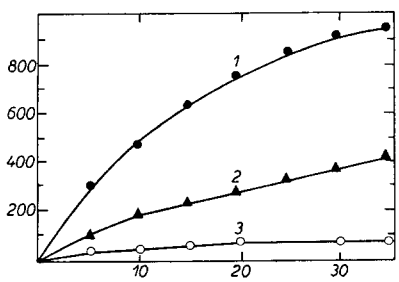

Fig. 2. Effect of –SH inhibitors on the fermentation of glucose (CO_2 production) by diploid yeast, SC-7. Buffer, 0.02 *M* $NaHCO_3$:CO_2. Gas phase: N_2:CO_2; pH 7.4. Glucose concentration, 0.01 *M*. Temp. 25°C. Abscissa, time in minutes. Ordinate, CO_2 production in μl. 1. Control; 2. 0.001 *M* *p*-chloromercuribenzoate; 3. 0.001 *M* chlorovinyl dichloroarsine.

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In striking contrast with the slow rate of inhibition of fermentation was the powerful inhibition of cell multiplication produced by *p*-chloromercuribenzoate. A concentration of 10^{-4} *M* inhibited completely cell division of diploid yeast cells; 10^{-5} *M* produced 72%, and $5 \cdot 10^{-6}$ *M*, 43%. To produce half-inhibition of cell division, a concentration of *p*-chlorormecuribenzoate 500 times less than that necessary to produce half-inhibition of fermentation was enough. Haploid yeast was found to be more sensitive. A concentration of $5 \cdot 10^{-6}$ *M* of the organic mercurial inhibited cell division by 90% (Fig. 3).

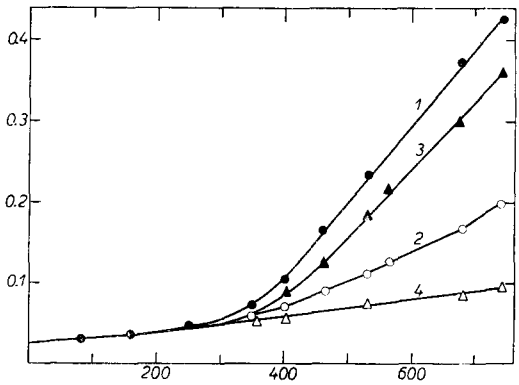

Fig. 3. Effect of *p*-chloromercuribenzoate on cell division of haploid and diploid yeast. *p*-ClHg benzoage concentration, $5 \cdot 10^{-6}$ *M*. Abscissa, time in minutes. Ordinate, cell multiplication as expressed by turbidity measurement. 1. Diploid, control (SC-6). 2. Diploid with *p*-Cl-Hg benzoate. 3. Haploid control (SC-7). 4. Haploid with *p*-Cl-Hg benzoate.

DISCUSSION

A great deal of confusion has been introduced by measuring effects of ionizing radiation on cell division and presenting them as "cell death". The experiments presented here have demonstrated that X-ray doses sufficient to inhibit completely cell division had no effect at all on cell metabolism as measured by glucose oxidation and fermentation. The irradiated cells were not dead. They had lost only the power of cell division. Of all processes of cellular activity, cell division seems to be the most sensitive to inhibition by ionizing radiations. The mechanism of this inhibition is independent of any direct action or "hit" between the ionizing track and a "special site" in the cell because it is affected by oxygen[18]. The mechanism of action must be sought, therefore, on the chemical action of the free-radicals OH and O_2H produced by irradiation of oxygen-saturated water. The great efficiency of ionizing radiations for the oxidation of –SH groups[19], and the important role of –SH groups in cell division[20] explain in our opinion the mechanism of the inhibition of cell division. RAPKINE discovered the importance of –SH groups in 1931[21], and the important new finding of MAZIA[22] have put RAPKINE'S hypothesis on more firm ground. According to them, the soluble non-protein –SH groups in the fertilized sea urchin eggs decrease during the first 30 minutes after fertilization, while the protein –SH groups increase. In the second phase up to cell division, the soluble –SH groups increase while the protein –SH groups decrease. The orderly process of chromosome splitting and division, as well as the various phases of cell division, seems to be processes of oxidation-reduction between the soluble –SH groups and the protein –S–S-bonds, with either reduction of the protein –S–S-bonds and with subsequent increase in length, or intermolecular formation of protein –S–S-protein complexes through transfer of their hydrogen to the soluble –S–S-groups.

The difference between the haploid and diploid yeast cells would be due to the higher chromosome content of the diploid cell. Their orderly structural changes must

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also be due to aggregation by –S–S-bond formation or lengthening by reduction of –S–S-bonds. The small content of –SH groups in chromosomes[23] makes them highly vulnerable to oxidation by ionizing radiations.

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SUMMARY

The diploid and haploid yeast cells used by ZIRKLE AND TOBIAS in their study of inhibition of cell multiplication by ionizing radiations were quite different in their metabolism: The first were respiring cells, and the second, fermenting cells. This difference was not due to lack of dehydrogenases but to lack of cytochrome oxidase in the fermenting cells. No relation was found between ploidy and cell metabolism. The great sensitivity of cell division to X-irradiation was also found towards –SH reagents for these reagents inhibited cell division at concentrations which had no effect on fermentation. The mechanism of this inhibition is postulated to be due to oxidation of –SH groups (protein and non-protein) which are essential for the division process. The greater sensitivity of the haploid yeast seems due to a lesser content of –SH groups in their chromosomes.

RÉSUMÉ

Les cellules de levures diploïdes et haploïdes utilisées par ZIRKLE ET TOBIAS au cours de leur étude de l'inhibition de la multiplication cellulaire par les radiations ionisantes diffèrent profondément par leurs métabolismes: les premières sont des cellules qui respirent, les secondes des cellules qui fermentent. Cette différence n'est pas due à un manque de déshydrogénase mais à un manque de cytochrome oxydase des cellules qui fermentent. Aucune relation n'a été observée entre la ploïdie et le métabolisme cellulaire. La grande sensibilité de la division cellulaire à l'irradiation aux rayons X s'observe également vis à vis des réactifs –SH, car ces réactifs inhibent les divisions cellulaires à des concentrations qui sont sans effet sur la fermentation. Les auteurs supposent que le mécanisme de cette inhibition repose sur l'oxydation des groupes –SH (protéiques et non-protéiques) qui sont essentiels pour le processus de division. La plus grande sensibilité des levures haploïdes semble due à une teneur plus faible en groupes –SH de leurs chromosomes.

ZUSAMMENFASSUNG

Die von ZIRKLE UND TOBIAS, bei ihren Studien der Hemmung der Zellenfortpflanzung durch ionisierende Strahlungen, benützten diploiden und haploiden Hefezellen waren metabolisch sehr verschieden: erstere waren atmende, letztere jedoch gärende Zellen. Dieser Unterschied war nicht der Abwesenheit von Dehydrogenasen, sondern dem Fehlen von Cytochromoxydase in den gärenden Zellen zuzuschreiben. Es wurde kein Zusammenhang zwischen der einfachen oder doppelten Chromosomgarnitur und dem Zellmetabolismus gefunden. Die Zelldivision reagierte nicht nur auf Röntgenbestrahlung, sondern auch auf –SH-Reagenzien mit grosser Empfindlichkeit, da diese Substanzen die Zellteilung in Konzentrationen hemmten, welche auf die Gärung keinen Einfluss ausübten. Es wird angenommen, dass der Mechanismus dieser Hemmung auf der Oxydation von Protein- und nicht-Protein-SH-Gruppen beruht, welche für die Zelldivision unerlässlich sind. Die grössere Empfindlichkeit der haploiden Hefen scheint auf dem geringeren Gehalt an SH-Gruppen ihrer Chromosomen zu beruhen.

References p. 432.

REFERENCES

1 R. LATARJET AND B. EPHRUSSI, *Compt. rend.*, 229 (1949) 306.
2 R. E. ZIRKLE AND C. A. TOBIAS, *Arch. Biochem. Biophys.*, 47 (1953) 285.
3 W. H. LUCKE AND A. SARACHEK, *Nature*, 171 (1953) 1014.
4 A. SARACHEK AND W. H. LUCKE, *Arch. Biochem. Biophys.*, 44 (1953) 271.
5 T. H. WOOD, *Influence of temperature on X-ray sensitivity of haploid yeast cells*, Univ. of Chicago Thesis, 1953.
6 T. TEORELL AND E. STENHAGEN, *Biochem. Z.*, 299 (1938) 416.
7 D. E. HUGHES, *J. Exp. Pathol.*, 32 (1951) 197.
8 E. S. G. BARRON AND F. GHIRETTI, *Biochim. Biophys. Acta*, 12 (1953) 239.
9 F. GHIRETTI AND E. S. G. BARRON, *Biochim. Biophys. Acta*, 15 (1954) 445.
10 D. KEILIN AND E. F. HARTREE, *Biochem. J.*, 41 (1947) 500.
11 T. S. MA AND G. ZUAZAGA, *Ind. Eng. Chem. Anal. Ed.*, 14 (1942) 280.
12 B. EPHRUSSI, *Harvey Lecturers*, 46 (1950–51) 45.
13 P. SLONIMSKI, *La formation des enzymes respiratoires chez la levure*, Paris, 1953.
14 E. S. G. BARRON AND J. GASVODA, *Argonne Natl. Lab. Quart. Rep.* ANL-4291, p. 86 (1949).
15 M. OGUR, *Arch. Biochem. Biophys.*, 53 (1954) 484.
16 E. S. G. BARRON, *Adv. in Enzymology*, 11 (1951) 201.
17 E. LUNDSGAARD, *Biochem. Z.*, 217 (1930) 162; 220 (1932) 8.
18 A. C. BIRGE AND C. A. TOBIAS, *Arch. Biochem. Biophys.*, 52 (1954) 388.
19 E. S. G. BARRON AND V. FLOOD, *J. Gen. Physiol.*, 33 (1950) 229.
20 J. BRACHET, *Embryologie chimique*, Paris, p. 175 (1944).
21 L. RAPKINE, *Ann. Physiol. physicochim. biol.*, 9 (1931) 383.
22 D. MAZIA, In *Glutathione*, ed. by S. COLOWICK *et al.*, Acad. Press, New York, 1954, p. 209.
23 J. N. DAVIDSON AND R. A. LAWRIE, *Biochem. J.*, 43 (1948) xxix.

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