simply determined by the washout time, but depended upon the substance inducing aggregation. The rate of redispersion following DNP-induced aggregation was twice as slow as that for the catecholamine concentration inducing just-maximal aggregation. In addition, the rate of redispersion following DNP-induced aggregation slowed during the course of an experiment. Preparations were studied typically for 3–4 h. Experiments were terminated when the melanophores would not disperse following a 25-min perfusion with normal saline. The cells at that time were not completely unresponsive, because individual melanosomes at the edge of the aggregated melanosome mass displayed a degree of movement, although they never entered a cellular process.

In the presence of tolazoline hydrochloride (TOL), an α-sympathetic receptor blocking agent[9], the catecholamines no longer aggregated the melanophores[10,11]. TOL had no effect upon the aggregating properties of DNP or NaCN (figure, C), suggesting that these latter 2 agents are acting directly at the source of metabolic energy for the intracellular translocation mechanism.

The effects observed in these experiments suggest that a metabolic energy pool of limited size is available for melanosome migration in melanophores, and that it is specifically necessary for centrifugal migration. Once exhausted, centripetal migration necessarily follows. It has been suggested that microtubules are a permanent substrate upon which the melanosomes migrate, because their presence in the cellular processes does not correlate with the position of the melanosomes[12]. The observation of lucent areas surrounding microtubules in dispersed melanophores[13] suggests that a contractile element might be present in association with the microtubules. The motive force for melanosome migration might be provided through the action of that element acting against the fixed microtubular array. It is interesting to note that similar concentrations of DNP stopped protoplasmic streaming in the slime mold *Physarum polycephalum*[14] and particle extrusion by filipodia in 3T3 cells[15]. The intracellular components inactivated in both experiments were thought to be microfilaments. Interference with microfilament integrity in melanophores by cytochalasin B has been shown to inhibit centrifugal melanosome migration in *Rana pipiens*[16]. It is tempting to speculate that microfilaments[17] or filamentous-like materials are the likely candidates as the site of metabolic activity required for centrifugal melanosome migration in teleost melanophores.

9 M. Nickerson and N. K. Hollenberg, in: Physiological Pharmacology, vol. 4, p. 243. Ed. W. S. Root and F. G. Hofmann. Academic Press, New York 1967.
10 B. L. Reed and B. C. Finnin, in: Pigmentation: Its Genesis and Biologic Control, p. 285. Ed. V. Riley. Appleton-Crofts, New York 1972.
11 R. Fujii and Y. Miyashita, Comp. Biochem. Physiol. *51C*, 171 (1975).
12 D. B. Murphy, Ann. N. Y. Acad. Sci. *253*, 692 (1975).
13 M. Schliwa and J. Bereiter-Hahn, Z. Zellforsch. *147*, 107 (1973).
14 H. Komnick, W. Stockem and K. E. Wohlfarth-Bottermann, Z. Zellforsch. *109*, 420 (1970).
15 G. Albrecht-Buehler and R. M. Lancaster, J. Cell Biol. *71*, 370 (1976).
16 J. McGuire and G. Moellmann, Science *175*, 642 (1972).
17 R. Fujii and R. R. Norales, Am. Zool. *9*, 453 (1969).

The effects of chronic peroxide intake on the peroxide metabolism enzyme activities of rat organs[1]

B. Matkovics[2] and R. Novák

Biological Isotope Laboratory, 'A. J.' University of Szeged, H–6701 Szeged (Hungary), 25 April 1977

Summary. In rats receiving a dilute aqueous solution of hydrogen peroxide for a prolonged period, the activity of the peroxide metabolising enzymes, i.e. superoxide dismutase, peroxidase and catalase, is significantly increased in most tissues.

The H_2O_2-producing superoxide dismutase (SOD; EC 1. 15.1.1), and the 2 most active H_2O_2-decomposing enzymes, peroxidase (P; EC 1.11.7) and catalase (C; EC 1.11.1.6) form the group of peroxide metabolizing enzymes (PME)[3,4]. Use of this collective term is justified, among others, by the fact that it is widely assumed that these 3 enzymes are the first appearance of forms of the protein-bound Cu and Fe in the course of evolution. All 3 enzymes are of great importance in aerobic life; they take part in the dismutation of the toxic radicale formed from molecular oxygen, the superoxide anion (O_2^-) formed by addition of 1 electron, and the peroxy radical (O_2^{2-}) formed by uptake of 2 electrons[5]. The aim of our experiments was to establish whether the levels of the PME in the various tissues are affected by chronic oral intake of reagent H_2O_2.

Materials and methods. Examinations were carried out on the CFY inbred rat strain, animals of the same age and sex being compared. The experimental animals were reared under identical conditions, with the exception that 1 group received 0.5% H_2O_2 added to the drinking water from the age of 1 month. Water-drinking rats were taken as controls. At the age of 3 months, rats from both groups were killed, and the activities of the PME in tissue homogenizates were determined[3,4]. The protein contents of the homogenizates were measured with the Folin phenol reagent by the method of Lowry et al.[6].

Results and discussion. The results are listed in the table. In the table, one column gives the results of the control measurements, with the $X \pm S$ values. The enzymatic activites have in all cases been calculated for 1 g wet tissue weight ($n = 10$).

1 Properties of enzymes. Serial publication, Part XV.
2 Mailing address: P.O. Box 539, H–6701 Szeged, Hungary.
3 B. Matkovics, R. Novák, Hoang Duc Hanh, L. Szabó, Sz. I. Varga and G. Zalesna, Comp. Biochem. Physiol. *56B*, 31 (1977).
4 B. Matkovics, R. Novák, Hoang Duc Hanh, L. Szabó and Sz. I. Varga, Comp. Biochem. Physiol. *56B*, 397 (1977).
5 I. Fridovich, in: Horizons in Biochemistry and Biophysics, p. 1. Ed. E. Quagliariello. Addison-Wesley Publ. Co., London 1974.
6 O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).

SOD, P and C values in normal rats; effect of chronic H_2O_2 drinking

Organs	SOD Units/g w.t.wt Controls	SOD H_2O_2-treated	P Units/g w.t.wt Controls	P H_2O_2-treated	C Units/g w.t.wt Controls	C H_2O_2-treated
Liver	4000 ± 600	10,408 ± 750	0	0	4.80 ± 0.43	25.250 ± 2.455
Kidney	1120 ± 151	1692 ± 240	361 ± 33	1200 ± 105	0.36 ± 0.03	3.300 ± 0.295
Spleen	560 ± 50	470 ± 240	963 ± 91	4250 ± 418	2.40 ± 0.21	0.730 ± 0.062
Testes	960 ± 63	1100 ± 53	407 ± 41	–	0.41 ± 0.03	0.150 ± 0.011
Whole brain	240 ± 24	397 ± 38	120 ± 10	140 ± 13	0.04 ± 0.004	0.012 ± 0.001
Lung	210 ± 20	895 ± 85	872 ± 86	2020 ± 201	0.26 ± 0.02	0.520 ± 0.050
Pancreas	310 ± 31	352 ± 33	136 ± 13	480 ± 46	0.195 ± 0.010	0.255 ± 0.023
Muscles						
Heart	480 ± 47	950 ± 93	2690 ± 300	1615 ± 155	0.245 ± 0.023	0.725 ± 0.065
Skeletal	300 ± 27	530 ± 50	105 ± 10	750 ± 69	0.11 ± 0.009	0.092 ± 0.009
Haemolysate*	696 ± 68	787 ± 80	11,666 ± 1000	17,900 ± 1340	4.12 ± 0.04	6.98 ± 0.07

* Units/ml. W.t.wt, wet tissue weight; n = 10; C-activity-values are given in B.units/g w.t.wt. All values are X ± SD.

The first 2 columns compare the SOD activities in control rats and rats which have been drinking H_2O_2 solution for 2 months.

The data reveal that, with the exception of the spleen, drinking H_2O_2 solution increases the SOD activity to a considerable extent. For example, this increase is more than 250% of the control value in the liver.

The second 2 series of columns compare the tissue peroxidase activity changes. The P activities are also increased, with the one exception of heart muscle.

However, the largest activity changes are observed in catalase as shown in the table. For instance, after prolonged H_2O_2 intake, the C-activity increase is about 6 times the control value in liver, and more than 9 times in kidney. In contrast, the C-activity decreases in spleen (to about $^1/_3$), testis, brain and skeletal muscle.
In general, therefore, it can be said that prolonged intake H_2O_2 solution brings about an induction of the PME in most organs. However, no explanation can be given of why catalase in spleen, testis, brain tissue and skeletal muscle do not fit this general picture.

Variation of glutamate decarboxylase activity and γ-amino butyric acid content of wheat embryos during ripening of seeds[1]

L. Galleschi, C. Floris and I. Cozzani[2]

Istituto di Botanica, Università di Pisa, via Luca Ghini 5, I–56100 Pisa (Italy), 8 March 1977

Summary. GAD activity and γ-ABA content of wheat embryos at 7 ripening stages were verified with the aim of studying the metabolic activity of embryo during dehydration and quiescence of caryopsis. Data showed that in the early stage of ripening GAD activity is very low, increases rapidly at dough-stage, remaining constant up to waxy-stage, and decreases in the last fully-ripe embryos. γ-ABA content appears to be roughly parallel to the variations of GAD activity.

Studies on the metabolism of γ-aminobutyric acid (γ-ABA) in plants demonstrated that this amino acid is formed from L-glutamic acid by way of L-glutamate-1-carboxylyase, EC 4.1.1.15 (GAD). γ-ABA, in turn, enters the dicarboxylic acid cycle as succinic acid, after the action of the 2 succeeding enzymes γ-aminobutyrate-α-ketoglutarate aminotransferase (EC 2.6.1.19) and succinic semialdehyde dehydrogenase (EC 1.2.1.16)[3,4]. Some data are also available on the variations of GAD activity and γ-ABA content in plants[5–7]. A parallel increase of both were reported to occur in leaves during senescence[8–10]. In the present paper we report the variations of GAD activity and of γ-ABA content occurring in wheat embryos during the development of the seed. The aim was to study some of the metabolic processes which occur during the ripening of wheat caryopsis and to obtain preliminary information correlating these processes with the onset of seed quiescence that is associated with the cessation of mitotic activity and with the increasing dehydration during ripening.

Materials and methods. In the period from May to June 1976, seeds of durum wheat c.v. Cappelli at different stages of ripening were collected and their relative humidity was measured with a hygrometric balance (Ultrax-Simplex). Embryos (embryo axis plus scutellum) were isolated from ice-cold seeds, sterilized with 1% NaClO,

1 This work was supported by a grant from the Consiglio Nazionale delle Ricerche.
2 Laboratory of Biochemistry, Faculty of Science, University of Pisa, Italy.
3 R. O. D. Dixon and L. Fowden, Ann. Bot. *25*, 513 (1961).
4 J. G. Streeter and J. F. Thompson, Pl. Physiol. *49*, 579 (1972).
5 P. Weinberger and C. Godin, Can. J. Bot. *42*, 329 (1964).
6 J. M. Lawrence and D. R. Grant, Pl. Physiol. *38*, 561 (1963).
7 J. F. Thompson, C. R. Stewart and C. J. Morris, Pl. Physiol. *41*, 1578 (1966).
8 P. Weinberger and K. A. Clendenning, Can. J. Bot. *30*, 755 (1952).
9 P. Lahdesmaki, Physiologia pl. *21*, 1097 (1968).
10 J. M. Lawrence and D. R. Grant, Pl. Physiol. *38*, 561 (1963).