polymerising tendency, the reactivity of the substance is shown by the behaviour on the anion exchanger. Using a fresh column the monomer is eluated in a quite different spectral form than that shown in Fig. 1. This form is denoted as D in Fig. 2. Also the supposed polymeric forms give the new spectrum after elution. It has been shown that some of these reactions depend on a combination of the molecule with metal ions. Thus it seems to be a parallelism between these reactions and the polymerisation of actin, which is dependent upon the presence of bivalent cations.

There are many interesting features about this substance, but it is necessary to know much more about it in order to give a full explanation of all these reactions and to make a study of its role in muscle contraction. This investigation is going on, and a more detailed description together with more results will be published later.

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INTRACELLULAR DISTRIBUTION OF FUMARASE AND ACONITASE IN YEAST

by

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HIRSCH[1] has reported recently that fumarase and aconitase are mainly associated with the non-sedimentable fraction of cell-free yeast extracts. These extracts were prepared from a pure culture of *S. cerevisiae* by mechanical shaking with glass beads at 560 cycles/min. The period of shaking was unspecified, but about 1 hour seems to be the practice in that laboratory[2].

From our studies (to be published shortly) on dehydrogenases in cell-free yeast extracts (prepared by mechanical shaking at 5,600 cycles/min for 10–90 secs), we found that the distribution of certain dehydrogenases varied with the disintegration period: for very short periods, even the "soluble" dehydrogenases (ethanol, malic and lactic) had high activities in the granular portion of extracts. With increasing disintegration periods, these enzymes tended to migrate to the non-sedimentable supernatant.

Similar experiments were done with fumarase and aconitase, because it seemed possible that HIRSCH's failure to observe high activities for these enzymes on the granules was due to excessive disintegration periods. Cell-free extracts prepared by 10, 30 and 90-sec shaking[3] of commercial baker's yeast were fractionated as follows: 30 min centrifugation at 3500 *g* gave "heavy" granules. Recentrifugation of the supernatant for 30 min at 10,000 *g* gave "light" granules. Both sediments were washed once with 25 to 50 vol. 0.9% KCl. Fumarase and aconitase activities were measured spectrophotometrically[4]. Specific activities (increase in optical density at 240 mμ/min/mg dry wt. $\times$ 1000) were measured at room temperature (about 20°) and pH 7.0, with 10^{-2} *M* substrate. Our values multiplied by 10 can be compared approximately with those of HIRSCH.

Both types of granules have high fumarase and aconitase activities when prepared from 10-sec extracts (Table I). 90-sec granules possess almost no measurable aconitase or fumarase activity. Since the acceleration of a mechanical shaker is proportional to the square of the number of cycles/min, our machine is about 100 times more efficient than that used by HIRSCH. Our 90-sec granules are therefore roughly equivalent to 150-min granules prepared on the slower shaker.

Table I also shows that, even when washed with 0.9% KCl, considerable amounts of both enzymes are detached from the granules in the short time required for washing. Therefore, in extracts prepared by long-term shaking, the activities of the resulting granules would be further lowered.

The very high activities of granules obtained after 10-sec disintegrations make it probable that in the intact yeast cell much of the fumarase and aconitase activity is associated with intracellular

TABLE I

THE EFFECT OF DISINTEGRATION PERIOD ON THE DISTRIBUTION OF FUMARASE AND ACONITASE IN CELL-FREE YEAST EXTRACTS

Activities measured at 240 mμ in a Unicam SP. 500 spectrophotometer: Enzyme, phosphate buffer pH 7.0 (10^{-2} *M* final) and L-malate or citrate (10^{-2} *M* final) in quartz cells, final vol. 3.0 ml. Measurements at about 20°. Blanks do not contain substrate. Specific activity = change of optical density/mg dry wt./min × 1000.

Disintegration period (sec)	*Enzyme*	*Specific activities of fractionated extracts*				
		Whole extract	*Heavy granules*	*Light granules*	*Washings of heavy granules*	*Washings of light granules*
10	Fumarase	110	130	160	70	85
30	Fumarase	120	36	54	88	121
90	Fumarase	129	5	4	86	120
10	Aconitase	40	17	25	16	15
30	Aconitase	42	9	14	34	40
90	Aconitase	54	2	1	22	21

granules, which some authors have called mitochondria[5,6,7,8]. This would be in accordance with the well-known distribution in animal cells of enzymes concerned with respiration.

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MECHANISM OF HYDROGEN TRANSFER IN MODEL DEHYDROGENASE SYSTEMS

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It is well known that in spite of the low redox potential of coenzyme I[1], the reduced coenzyme shows very little tendency to autoxidise in the absence of intermediaries. Although the ΔF of the reaction is very favourable there appears to be an equally important factor preventing the interaction

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