The Intracellular Redistribution of Catalase During the Incubation of Mouse-Liver Slices

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Adams & Berry (1956) found that when mouse-liver slices were incubated at 38° in a phosphate saline medium the catalase activity of the system approximately doubled after 2 hr., when determined in the way described, and tentatively suggested that the additional catalase might be produced by the breakdown of an inactive complex of the enzyme with an inhibitor. However, subsequent attempts to find such a dissociable complex have been unsuccessful (Adams & Burgess, 1958).

Recently Adams & Burgess (1957) showed that the catalase activity measurable in liver homogenates depended largely on the homogenization technique. The homogenization of liver in an all-glass pestle homogenizer with about 12 pestle strokes gave a suspension virtually free of intact cells, but containing an abundance of large granules. In such a suspension 20–30% of the total catalase activity was found in solution; and this soluble catalase was referred to by these authors as the extra particulate cytoplasmic fraction. The remaining catalase, present in the large granule fraction, was not measurable until the granules were disrupted by prolonged homogenization, or by the addition of a non-ionic detergent (Triton X100). Both nuclei and small granules contain little or no catalase activity (found in rats by Euler & Heller, 1949, and by Ludewig & Chanutin, 1950; and confirmed in mice by Adams & Burgess, 1957). The unbroken large granules had a small but measurable catalase activity (about 5% of the total), attributed by Adams & Burgess (1957) to a slight granule permeability to the hydrogen peroxide substrate.

The homogenization technique used for slices by Adams & Berry (1956) was such that their measurements would have been principally of the extra particulate cytoplasmic catalase fraction. We therefore decided to find out whether the granule fraction was the source of the additional catalase appearing in the extra particulate cytoplasmic fraction during the incubation of slices.

EXPERIMENTAL

Animals. Young adult female Swiss albino mice (obtained commercially) were used throughout. The diet of the animals consisted of commercial rat cubes and water (both *ad libitum*).

*Triton X*100. This non-ionic detergent, kindly given to us by Charles Lennig and Co., was used at a final concentration of 1% to disrupt large granules and liberate their catalase activity.

Homogenization. This was effected by a Ten Broeck grinder of the type described by Adams & Burgess (1957).

Preparation of catalase fractions from liver slices. Liver slices were prepared and incubated at 38° in phosphate saline as described by Adams & Berry (1956), with the following modifications: (1) about 40 mg. of slices were incubated in 2·5 ml. of saline; (2) oxygen was not passed through the tubes: leaving them open to the atmosphere was found to be adequate; (3) 0·25 ml. of 0·1 M-ethanol was added to the homogenates (giving a final concentration of 0·01 M) in order to prevent losses of catalase through the formation of an inactive complex of catalase with hydrogen peroxide ('complex II', Chance 1950; Adams & Burgess, 1958).

Tubes were removed at intervals and the slices homogenized. Twelve pestle strokes were used, and liver treated in this way will be referred to as a '12-stroke homogenate' (Adams & Burgess, 1957). When required, the large granules were separated from the homogenate by centrifuging at 10 000 *g* for 20 min. The supernatant [containing the extra particulate cytoplasm (EPC) catalase fraction] was separated from the pad of granules, which was then resuspended in the same volume of water containing Triton and ethanol. Thus prepared, the large-granule fraction contained the nuclei, and the supernatant contained the small granules.

Catalase measurements were made on the following preparations from slices: (1) 12-stroke homogenate (i.e. EPC fraction and unbroken granules); (2) EPC fraction; (3) granule fraction (after the addition of Triton); (4) 12-stroke homogenate after the addition of Triton (i.e. total catalase). The small catalase activity due to unbroken granules was normally obtained by the difference between (1) and (2).

Preparation of large-granule samples from whole liver. These were prepared by homogenizing unsliced liver (12 pestle strokes) in phosphate saline, or in 0·25 M-sucrose, both containing ethanol (1 g. of liver in 10 ml. of medium). The sucrose homogenates were brought to pH 7·0 by the addition of sodium bicarbonate. The pH was measured with a Pye pH meter and glass electrode, standardized according to the maker's instructions. In some experiments the homogenates were centrifuged at 800 *g* for 10 min. to remove nuclei and debris. The granules were then left in contact with the soluble components of the liver during the incubation. In other experiments the granules were separated from the nuclei-free suspension by centrifuging at 10 000 *g* for 20 min. and resuspending in sufficient fresh medium to restore the original fluid volume.

Estimation of liver-catalase activity. Catalase was estimated, as previously described, by titration of the excess of hydrogen peroxide remaining after allowing 25 ml. of 0·025M-hydrogen peroxide to stand in contact with a measured sample of liver homogenate for 4 min. at 0°. Corresponding determinations of total nitrogen were made by the Kjeldahl method, catalase results being expressed in arbitrary units/mg. of N. The method, the arbitrary units used and the reasons for referring catalase activity to nitrogen are described by Adams (1950, 1952). The catalase activities in the various fractions were referred to the nitrogen content of the whole homogenate from which they were prepared.

Method of counting large granules. (*a*) Liver slices. Samples of 12-stroke homogenates of slices were diluted in phosphate saline to give a liver concentration of about 0·1% (w/v). (*b*) Isolated granules. These were prepared in 0·25M-sucrose and diluted in the same medium until the suspension contained the granules from about 1 mg. of liver in 1 ml. In both cases three separate counts, each of about 120 granules, were made in a Neubauer haemocytometer, and the mean was taken.

RESULTS

Liver slices were incubated at 38° and catalase was estimated in the various fractions after 1, 2 and 4 hr. The results, in Fig. 1, show that whereas the total catalase activity remained constant over the incubation period, the activity in the EPC fraction rose and that in the granule fraction fell by the same amount. The catalase activity in the 12-stroke homogenate rose in parallel with that of the EPC fraction, indicating that the contribution due to the unbroken granules remained constant during the incubation.

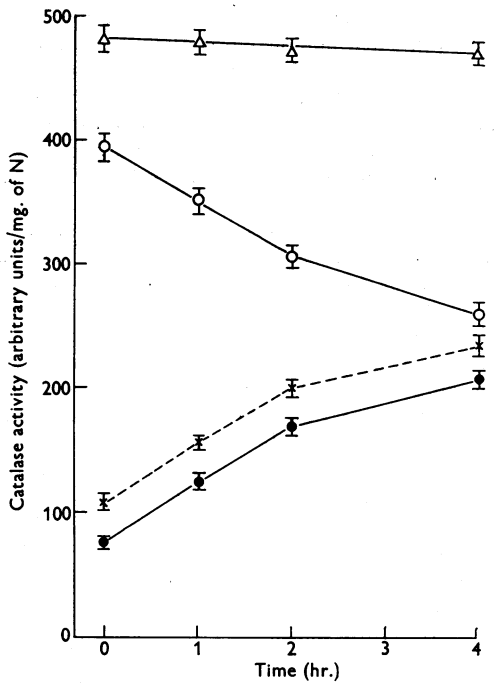

Fig. 1. Catalase activities in mouse-liver slices during incubation for 4 hr. at 38° in phosphate saline. △, Total catalase; ○, granule fraction; ●, EPC fraction; ×- - -×, 12-stroke homogenate. Results are given as arithmetic means (six experiments) ±S.E.M.

These results suggested very strongly that the catalase appearing in the EPC fraction was derived from the granule fraction. This might have occurred in three ways. First, the granules may be permeable to catalase, with the result that the enzyme migrates through the intact granule wall. Secondly, the granules may become more fragile during incubation, with the result that they are broken up to a much greater extent than before by the 12 strokes of homogenization. Thirdly, the granules may lyse during the incubation, liberating their enzyme content into the EPC.

Experiments were performed in order to distinguish between these possibilities. Counts were made on 12-stroke homogenates prepared from liver slices before and after incubation: the results are given in Table 1. After incubation for 2 hr., when most of the catalase release occurred, no change was seen either in number or appearance. After 4 hr., however, the granules were less refractile and difficult to count, and the number visible had decreased.

Slices were incubated for 2 hr., and the rate of catalase liberation on progressive homogenization was compared with that from unincubated slices. The results, in Table 2, show that although after 12 strokes of homogenization more catalase was present in the homogenate from incubated than from unincubated slices, on further homogenization there was no evidence of increased fragility of the granules from the incubated slices. Over the successive intervals of homogenization given at

Table 1. *Granule counts and corresponding catalase activities after incubation of liver slices at* 38°

Slices were homogenized with 12 strokes of a Ten Broeck grinder. Both catalase activities and granule numbers are expressed in arbitrary units/mg. of N.

Time of incubation (hr.) ...	0	2	4
Catalase	105	201	216
	136	205	261
	130	246	273
	142	236	302
	124	229	262
Average	126	224	263
Number of large granules ($\times 10^{-7}$)	226	267	200
	241	256	172
	241	255	114
	313	285	280
	269	266	219
Average	258	266	211

Table 2. *Rate of catalase liberation during progressive homogenization of 12-stroke homogenates of liver slices before incubation and after incubation for 2 hr.*

Catalase activities are given as arbitrary units/mg. of N. Homogenization is expressed as pestle strokes of a Ten Broeck grinder.

Pestle strokes of grinder ...	12	50	100	200	300
Before incubation	91	163	247	327	394
	109	176	262	333	384
	89	146	208	279	354
	91	136	182	278	320
	82	145	175	250	340
Average	92	151	215	293	359
After incubation	259	266	292	332	372
	252	279	292	313	364
	136	177	217	289	330
	157	181	234	279	321
	190	246	280	310	335
Average	199	230	263	305	345

12–300 strokes gave 267 units from unincubated slices, 146 units from incubated slices, a ratio of 1·83:1. This ratio was essentially constant over the homogenization range.

Strokes	12–50	50–100	100–200	200–300
Unincubated / Incubated	1·90	1·94	1·85	1·65

Table 3. *Catalase activities in liver large granules and the surrounding medium, when incubated in phosphate saline at* 38°

Catalase activities are given in arbitrary units/mg. of N.

Time of incubation (hr.)	0	1	2	4
1. (*a*) In liver supernatant phosphate saline (after removing nuclei by centrifuging)				
Granule catalase	170	170	150	155
	130	120	115	115
	190	190	175	175
Supernatant catalase	72	59	52	66
	60	59	49	50
	67	62	56	56
(*b*) Without the preliminary removal of nuclei				
Granule catalase	400	390	385	385
Supernatant catalase	72	70	70	68
2. (*a*) After resuspending nuclei-free granules in fresh phosphate saline				
Granule catalase	190	190	185	185
	175	160	160	160
Supernatant catalase	10	10	10	12
	10	9	11	11
(*b*) After centrifuging granules + nuclei and resuspending in fresh phosphate saline				
Granule catalase	480	470	470	475
Supernatant catalase	10	15	15	15

the foot of Table 2, the ratio (catalase produced from unincubated slices)/(catalase produced from incubated slices) remained constant.

Experiments with isolated granules

Granule samples prepared from 12-stroke homogenates in phosphate saline were incubated at 38°, and their catalase content, and that of the surrounding medium, were measured at intervals. As shown in Table 3, no additional catalase appeared in the medium, and the catalase content of the granules was unchanged. This was found whether the granules were incubated in 12-stroke homogenates, in such homogenates after the removal of nuclei or after resuspension in fresh phosphate saline. The lower activities of the granule fractions after removal of nuclei (Table 3) was due to some loss of granules through their clumping in the relatively concentrated saline suspensions and consequent sedimentation with the nuclei.

In further experiments granules were prepared and incubated at 38° in 0·25 M-sucrose. A rapid liberation of catalase into this medium was observed. The rate was greater when the granules were incubated in contact with the soluble components of the liver than when they were first resuspended in fresh sucrose (Fig. 2). Incubation at 25° or at 0° (granules not resuspended) greatly reduced the catalase liberation (Fig. 3).

Granules were then prepared and incubated in 0·25 M-sucrose, and counts were made in parallel with catalase estimations during incubation for 4 hr. at 38°. No change was seen in either the number or microscopical appearance of the granules (Table 4).

Evidence that the decomposition of hydrogen peroxide is due to catalase

Since the breakdown of hydrogen peroxide may be caused by substances other than catalase we felt that evidence on this point should be included.

Samples of granules were prepared in phosphate

saline, and homogenized with 300 strokes. As Table 5 shows, almost complete inhibitions of catalase were obtained by heating, exposure to 1% sulphuric acid, and by the action of 0·1 mM-sodium azide or hydroxylamine.

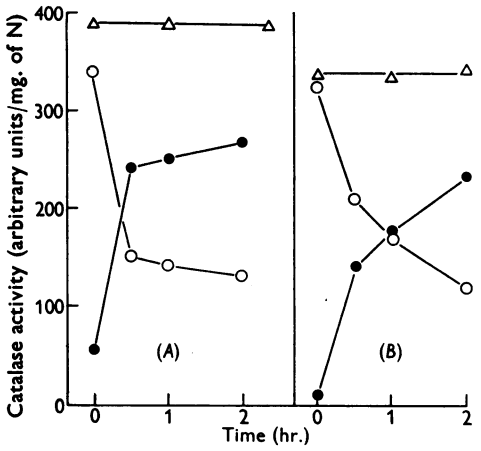

Fig. 2. Catalase liberation from liver large-granule suspensions incubated at 38° in 0·25 M-sucrose. △, Total catalase; ○, granule catalase; ●, supernatant catalase. (*A*) In the presence of the soluble components of the liver. (*B*) After centrifuging and resuspending in fresh sucrose.

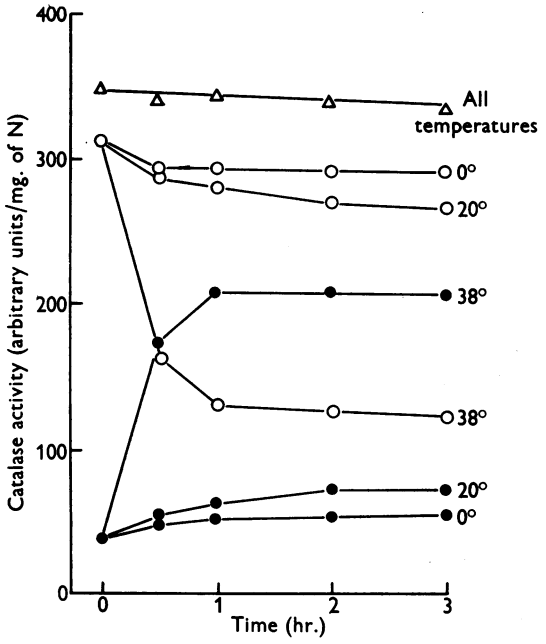

Fig. 3. Effect of temperature of incubation on catalase liberation from liver large granules in 0·25 M-sucrose. Nuclei were separated by a preliminary centrifuging, but the granules were not resuspended. △, Total catalase; ○, granule catalase; ●, supernatant catalase.

DISCUSSION

During incubation, the total catalase activity of liver slices remained constant, but catalase was transferred from the granules to the EPC. Isolated granules also liberated catalase into the surrounding medium when incubated at 38° in 0·25 M-sucrose, the results closely resembling those obtained during incubation of slices. When the granules were incubated in sucrose at 20° the catalase liberation was greatly reduced. Adams & Berry (1956) also observed that only small amounts of catalase appeared in the medium when slices were incubated at 20°. The liberation of catalase in

Table 4. *Granule counts after incubation at* 38° *in* 0·25 M-*sucrose, together with corresponding catalase activities (averaged)*

Both granule numbers and catalase activities are given/mg. of N. Nuclei were separated by a preliminary spin.

Time of incubation (hr.)	0	1	2	4
Granules after centrifuging and resuspending in fresh sucrose soln.				
Granule catalase (averaged)	320	215	170	105
Supernatant catalase (averaged)	62	164	212	256
Granule numbers ($\times 10^{-7}$)	346	300	346	305
	234	211	235	226
	274	258	269	281
Granules in liver supernatant containing 0·25 M-sucrose				
Granule catalase	350	160	150	145
Supernatant catalase	64	250	255	255
Granule numbers ($\times 10^{-7}$)	351	351	384	375

Table 5. *Inhibition of granule catalase by heat and by various agents*

Except where otherwise stated the experiments were done at room temperature (20°) and the inhibitors were allowed to act for 2–5 min. Catalase activities are expressed in arbitary units/mg. of N.

(*a*) Heating to 100° for 2 min.

Control	Treated	Inhibition (%)
200	1	99
185	1	99

(*b*) Inhibitor

	Concentration			Inhibition
	0	1%	0·1 M	(%)
Sulphuric acid	198	1	—	99
	215	2	—	99
Sodium azide	220	—	4	98
	203	—	3	98
Hydroxylamine	220	—	4	98
	203	—	15	95

sucrose solutions seems to agree with some results of Greenfield & Price (1956), who stated that during the preparations of samples of mitochondrial catalase some enzyme was 'eluted' from the particles by the sucrose solutions used.

During the incubation of granule suspensions in sucrose no diminution in the number of granules present could be seen, and their microscopical appearance was unchanged, even after 4 hr. This was also true for granule suspensions made from slices incubated for 2 hr., although after 4 hr. the granules were difficult to count and the number present was apparently reduced. Since, however, the catalase transfer occurred mainly in the first 2 hr. there was no correlation with granule disappearance. The results given in Table 2 also suggest that after incubation for 2 hr. the same number of granules was present, but with less catalase in each.

It is also quite clear that no catalase was liberated from granules prepared and incubated in the phosphate saline medium. This suggests that the ionic balance of the surrounding medium is of considerable importance in regulating catalase transfer. Further, there seems no obvious reason why granules should lyse or suffer membrane damage within incubated slices, or during incubation in sucrose solution, but not when incubated in phosphate saline. The constant low catalase activity shown by unbroken granules from incubated slices (Fig. 1) also suggested that incubation did not cause a general damage to the membrane.

It seems reasonable to conclude that under certain conditions the membranes of liver large granules are permeable to catalase.

SUMMARY

1. During incubation of liver slices at 38° in phosphate saline, catalase disappears from the large granules and an equal amount appears in the extra particulate cytoplasm.

2. When granule preparations are incubated in phosphate saline no catalase is liberated. Incubation in 0·25 M-sucrose results in a transfer of catalase to the medium.

3. No evidence has been found that the granules become lysed or more fragile during incubation.

4. It is concluded that during incubation of liver slices in phosphate saline, catalase migrates from the large granules into the extra particulate cytoplasm, probably through the intact granule membrane, and that a similar process occurs when isolated granules are incubated in 0·25 M-sucrose.

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The Uptake of Vitamin B_{12} by Rat-Liver Slices

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This work has been carried out as an extension of our observations with isolated rat intestine in which we have shown there are two systems for the uptake of vitamin B_{12}. One of these is highly specific, and the other shows competition with pseudovitamin B_{12} (Latner & Raine, 1958). In the hope that studies of other tissues might shed light on the mechanism of uptake of vitamin B_{12}, we have extended our observations to liver slices. Liver seemed appropriate in view of the facts that it is the main store of body vitamin B_{12} and embryologically it is developed from the foregut and might therefore retain a mechanism of uptake which is similar to that in the intestine. Such slices could be studied without intrinsic factor being present, a state of affairs impossible with isolated small intestine from normal animals.

While this work was in progress it was reported that a pig intrinsic-factor preparation had increased the uptake of vitamin B_{12} by rat-liver slices