- Polgár, A., van Niel, C. B. & Zechmeister, L. (1944). *Arch. Biochem.* **5**, 243.
- Starr, M. P. & Saperstein, S. (1953). *Arch. Biochem. Biophys.* **43**, 157.
- Strain, H. H. (1954). *Arch. Biochem. Biophys.* **48**, 458.
- van Niel, C. B. (1947). *Leeuwenhoek ned. Tijdschr.* **12**, 156.
- Wallcave, L. & Zechmeister, L. (1953). *J. Amer. chem. Soc.* **75**, 4495.
- Weedon, B. C. L. (1955). *Chem. & Ind.* p. 1219.
- Zechmeister, L. (1944). *Chem. Rev.* **34**, 267.
- Zechmeister, L. & Cholnoky, L. von (1936). *Ber. dtsch. chem. Ges.* **69**, 422.
- Zechmeister, L. & Wallcave, L. (1953*a*). *J. Amer. chem. Soc.* **75**, 4493.
- Zechmeister, L. & Wallcave, L. (1953*b*). *J. Amer. chem. Soc.* **75**, 5341.

Some Studies on Catalase in Mouse-Liver Slices

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Recent work from this laboratory has shown that liver catalase in the mouse is associated with a complex system which influences the level of its activity. So far, hormones, trace metals and riboflavin have been shown to be components of this system, and some indications regarding their possible interrelations have been obtained (Adams, 1952, 1953, 1955). Furthermore, a tumour constituent has been shown to depress catalase activity (Nakahara & Fukuoka, 1949, 1950; Adams, 1950, 1951*a*; Greenfield & Meister, 1951), and it has been suggested that this substance acts by interfering with the stimulating action of hormones on catalase activity (Adams, 1951*b*). In an attempt to open a new approach to the general problem of the nature of the system which controls catalase activity, it was decided to study the behaviour of the enzyme in liver slices. The present paper describes some results obtained by incubating slices in saline media.

EXPERIMENTAL

Animals. The early part of this work was done with the 'Schofield' stock albino strain of mice previously used in catalase studies from this laboratory, but the outbreak of 'Tyzzer's disease' referred to in a previous paper (Adams, 1955) necessitated a change over to other strains. Two such strains have been used, namely, Schneider albino mice (kindly supplied by the National Institute of Medical Research, Mill Hill, London), and a strain of Swiss albinos obtained commercially. Both these strains appear to be free from liver infection. Their catalase activity is similar to that of the albino strain previously used, and they show a marked sex difference in catalase level (greater in males than in females). In the Swiss strain, however, the catalase level in both sexes is rather higher than is usual in the other two strains. The mice were fed *ad lib.* on rat cubes (obtained commercially) and water.

Medium for incubation of slices. A phosphate medium of the following composition was used: NaCl (0.12*M*), KCl (0.006*M*), MgSO₄ (0.0012*M*), Na₂HPO₄ (0.02*M*), glucose

(0.01*M*), and potassium citrate (0.001*M*). The pH was brought to 7.0 by adding HCl.

Estimation of liver-catalase activity. Catalase was estimated as previously described, by titration of the excess hydrogen peroxide remaining after allowing 25 ml. of 0.025*M*-H₂O₂ 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, and catalase results are expressed in arbitrary units/mg. of N (Adams, 1950, 1952).

Preparation and treatment of liver slices. Mice were decapitated, and the livers removed and separated into the individual lobes. One lobe from each liver was then cut with a tissue chopper (McIlwain & Buddle, 1953), giving slices of about 250 μ . in thickness. The slices were separated under ice-cold medium, and approximately 20 mg. (not weighed accurately) was placed in glass tubes which had a flat bottom (diam. 2.3 cm.). Each tube contained 1.5 ml. of medium, and slices for two or three sets of duplicate tubes were prepared from each liver. The tubes were then placed in a bath maintained at 38°, and shaken at 120 oscillations/min. Normally a slow stream of oxygen was passed continuously into the tubes. In experiments where anaerobic conditions were required, the slices were placed in Warburg flasks (gas phase, 100% N₂) and double quantities of slices and medium were used.

The slices were prepared for catalase estimation by tipping the contents of each vessel (i.e. slices and medium) into a Ten Broeck grinder (a glass-pestle homogenizer) and homogenizing. Part of the suspension (2 $\times$ 0.1 ml.) was then taken for duplicate determinations of enzyme activity and part (1.0 ml.) for nitrogen estimation.

After setting up the vessels and adding the liver slices and medium, a catalase estimation was done on the contents of one vessel from each mouse. The catalase level obtained at this point (i.e. after slicing but before incubation) will be referred to subsequently as the initial level. Tests made on slices and on uncut liver showed that substantially the same enzyme levels were obtained in each case. The initial level therefore corresponds to the catalase activity of the liver as normally measured. The remainder of the vessels were incubated as described above, and removed (in duplicate) at intervals (usually 2 and 4 hr.) for further catalase estimations.

Hormone. Testosterone (crystalline B.P.) was kindly given to us by Organon Laboratories Ltd. Quantities of 3 mg. were weighed out, moistened with glycerol, and injected subcutaneously.

Operative procedure. Adrenalectomy was carried out as previously described (Adams, 1952).

RESULTS

Mouse-liver slices were incubated as already described, and their catalase activity was determined at intervals. Since the slices were homogenized in the medium in which they were incubated, the measurements were of the total catalase in the system. After incubation for 1 hr. the total catalase activity had risen appreciably above the initial level, and this rise continued for a further hour. Little or no further change occurred, except that the level often began to fall again in experiments lasting 4 hr. or more. This might have been due to bacterial contamination of the system, since no

precautions were taken to ensure sterility. Generally, therefore, experiments were terminated after 4 hr., and in any event they did not extend beyond 6 hr. The results of catalase estimations obtained with a large number of mice of all three strains are summarized in Fig. 1. Fig. 1 shows a sex difference in the initial catalase level in all three strains (greater in males than in females, see Adams, 1952) and indicates that this difference is maintained during incubation, the rise in activity being the same for males and females.

A number of experiments were then performed to determine some of the conditions necessary for the development of this additional catalase activity.

Composition of the medium

The results given in Fig. 1 were obtained with the saline-glucose-citrate medium described in the Experimental section. However, in the early stages of the investigation a few experiments were done to

Fig. 1. Change in catalase activity of mouse-liver slices during incubation (○, males, ●, females). Catalase activities are given as arithmetic means±s.e.m. The number of animals in each group is given by the small figures above the standard error limits. (a) Schofield albinos, (b) Swiss albinos, (c) Schneider albinos.

Table 1. Effect of the addition of glucose (0.01 M), and glucose (0.01 M) with citrate (0.001 M), to the phosphate saline, on the catalase level obtained after incubation of mouse-liver slices

Schofield mice were used. Results are given in arbitrary units/mg. of N.

		Catalase levels after incubation for time shown					
Sex	Initial catalase level	Saline only		Saline + glucose		Saline + glucose + citrate	
		2 hr.	4 hr.	2 hr.	4 hr.	2 hr.	4 hr.
Female	73	170	100	160	129	220	205
Female	79	146	100	214	136	205	200
Female	120	248	125	263	238	241	234
Male	147	257	140	276	211	324	314
Male	158	262	125	292	180	294	226

determine whether the addition of substrates was necessary. Slices were incubated in saline medium only, in saline medium with glucose, and in saline medium with glucose and citrate. The results in Table 1 show that rises in catalase level were observed after incubation for 2 hr. with glucose and citrate absent, but the level fell again after incubation for a further 2 hr. With glucose present the 2 hr. readings were, in general, higher than when it was absent, and the subsequent fall was reduced. The further addition of citrate resulted in an even smaller fall after 2 hr.

Effect of temperature

Liver slices from individual mice were divided into two groups, which were incubated at 38° and at 20° respectively. The usual rise in catalase activity was observed in the slices incubated at 38°, but there was little change in the slices incubated at the lower temperature (Table 2). In this latter group, however, there seemed to be a tendency for a small rise in catalase level after incubation for 4 hr.

Homogenization

Slices from individual mice were divided into two groups. One group was incubated at 38° in the normal way. The other was homogenized in a Ten Broeck grinder, and the resulting suspension incubated at 38°. The ratio of liver weight to medium volume was the same in the suspension as in the vessels containing slices. Little or no rise in catalase level was seen in the suspensions, as shown in Fig. 2.

Table 2. *Catalase levels in mouse-liver slices after incubation at 38° and at 20°*

Swiss female mice were used. Results are given in arbitrary units/mg. of N.

Initial catalase level	Catalase levels after incubation for time shown			
	At 20°		At 38°	
	2 hr.	4 hr.	2 hr.	4 hr.
129	138	154	250	297
131	121	177	208	215
140	130	115	248	260
126	139	155	271	244
120	135	128	235	190

Table 3. *A comparison of the catalase rises obtained by incubating mouse-liver slices under aerobic and under anaerobic conditions*

Results are given in arbitrary units as arithmetic means $\pm$ s.e.m. Citrate was omitted from the standard medium.

Mice	Initial catalase level	Catalase levels after incubation for time shown			
		Gas phase 100% O ₂		Gas phase 100% N ₂	
		2 hr.	4 hr.	2 hr.	4 hr.
Swiss males (9)	161 $\pm$ 9.5	278 $\pm$ 11	259 $\pm$ 10	183 $\pm$ 14	216 $\pm$ 13
Swiss females (7)	125 $\pm$ 7.4	246 $\pm$ 13	226 $\pm$ 11	197 $\pm$ 10	204 $\pm$ 10

Anaerobic conditions

Slices were incubated anaerobically at 38° and catalase was estimated at intervals and compared with the catalase of slices incubated in the normal way. In this experiment citrate was omitted from the medium. The results are given in Table 3 and

Fig. 2. Catalase levels in mouse-liver slices during incubation, compared with those of corresponding homogenates. The points represent individual livers and the crosses the arithmetic mean values of the groups. $\circ$, Male liver slices; $\square$, male liver homogenates; $\bullet$, female liver slices; $\blacksquare$, female liver homogenates. The circles at 0 hr. represent the initial catalase levels. Continuous lines refer to slices, dotted lines to homogenates.

show that a rise in catalase was still obtained under anaerobic conditions, although it was smaller than that obtained aerobically.

Sodium/potassium ratio in the medium

It has been shown that a large potassium leakage from liver slices occurs when they are placed in media with a high $[Na^+]/[K^+]$ ratio (Buchanan, Hastings & Nesbitt, 1949). Slices were therefore incubated in a medium in which this ratio was substantially lowered. These conditions were attained by substituting KCl for NaCl in the medium

Table 4. *Effect of a high potassium medium on the rise in mouse-liver catalase activity obtained on incubation*

The medium was prepared by substituting KCl for NaCl in the standard formula. Six Swiss female mice per group were used. Results are given in arbitrary units as arithmetic means $\pm$ S.E.M.

Fig. 3. Change in catalase activity during incubation of liver slices from normal female mice (●), and female mice pretreated with testosterone implants (○). The points represent individual livers and crosses the arithmetic mean values of the groups.

hitherto used. However, a rise in catalase level to the same extent was obtained in both media (Table 4).

The results have already indicated that the sex difference in the liver catalase level of normal mice is maintained on incubation *in vitro*, approximately the same catalase rise being obtained in both males and females. Injection of testosterone into females elevates their catalase level (Adams, 1952), and liver slices from mice so treated were incubated, and the catalase levels obtained were compared with those of untreated controls. In this experiment, the mice received single subcutaneous implants of 3 mg. of testosterone moistened with glycerol, and 6 days were allowed to elapse before the catalase estimations were made. The testosterone implant raised the initial catalase activity from an average of 84 units to an average of 134 units, and this difference was approximately maintained during the rise in activity following incubation of the liver slices (Fig. 3).

Fig. 4. Change in catalase activity during incubation of liver slices from normal female mice (●) and adrenalectomized females (Δ). The points represent individual livers and crosses the arithmetic mean values of the groups.

Adrenalectomy reduces catalase activity (Adams, 1952). Liver slices from a group of adrenalectomized females were incubated, 3 days being allowed to elapse between the operations and the catalase estimations. The initial catalase level in the adrenalectomized group was considerably lower than in the controls. On incubation of liver slices from these animals the rise in catalase level in the adrenalectomized group was, if anything, slightly lower than in the normals. Substantial rises were, however, seen in both groups (Fig. 4).

Tumour-bearing animals

It then became of interest to determine whether tumour growth (which depresses liver-catalase activity) had any effect on the *in vitro* rise. Sarcoma 37 tissue was implanted subcutaneously into a large group of Schneider male mice, and allowed to grow. The tumour-bearing animals were then killed, and liver slices from them incubated in the usual way. Catalase activities were determined after incubation for 2 and 4 hr., and the results were averaged. The initial level was then subtracted, to give the rise in level during incubation. The tumours were dissected out and weighed approximately. In Table 5, the mice are arranged in five groups: controls, and mice bearing tumours weighing 0.9–1.9, 2.0–2.9, 3.0–3.9 and 4.0–4.9 g. The results show that the initial liver catalase activity was reduced very sharply in the first tumour-bearing group, but the catalase rise during liver-slice incubation was substantially unchanged. With tumours weighing 2.0–4.0 g. a slower but progressive fall in initial catalase level was observed, and this trend was paralleled in the rise obtained during incubation. Growth of the tumours to above 4 g. in weight resulted in sharp reductions in both the initial catalase level and the rise during incubation.

Since injections of tumour homogenate also depress catalase activity (Adams, 1950) a group of Swiss males was injected subcutaneously on each of 3 consecutive days with 50 mg. of homogenized Sarcoma 37, and catalase levels were determined after incubation of liver slices, on the fourth day. The results (Table 6) agree with those obtained from tumour-bearing animals in suggesting that the initial level is more tumour-sensitive than the rise during incubation. Liver slices were then incubated in the presence of Sarcoma 37 homogenate. The tumour was grown subcutaneously and a quantity of fresh tissue was homogenized in the saline medium with a Ten Broeck grinder. The resulting suspension was made to a concentration of 100 mg. of tissue/ml. This was added in 0.1 ml. quantities to a group of vessels, together with liver slices and additional saline medium to bring the fluid volume to the usual 1.5 ml. The slices were then incubated in the normal way, together with a corresponding group in

Table 5. *Catalase levels before and after incubation of liver slices from mice bearing transplanted Sarcoma 37 tumours, compared with those of normal controls*

Schneider male mice were used. Results are given in arbitrary units/mg. of N.

Tumour wt. (g.)	(a) Initial liver catalase level	(b)	
		Level after incubation of slices (average of readings at 2 and 4 hr.)	Rise in catalase during incubation (b) – (a)
0	142	260	118
(Controls)	138	217	89
	149	231	82
	125	208	83
	133	198	65
	172	254	82
	151	235	84
	138	218	80
	Mean 144	229	85
0.9–1.9	67	151	84
	95	176	81
	87	221	134
	94	178	84
	82	147	65
	98	187	89
	Mean 87	177	90
2.0–2.9	81	142	61
	52	130	78
	103	169	66
	Mean 79	147	68
3.0–3.9	63	128	65
	54	147	91
	61	184	123
	82	130	48
	76	123	46
	60	85	25
	Mean 66	132	66
4.0–4.9	33	60	27
	22	41	19
	50	89	39
	64	110	46
	50	81	31
	Mean 44	76	32

Table 6. *Effect of daily injection for three days of Sarcoma 37 (50 mg./day) on the catalase activity obtained by incubation of liver slices*

Swiss male mice were used. Catalase levels were measured on the fourth day, and are expressed in arbitrary units/mg. of N as arithmetic means $\pm$ S.E.M.

	Initial catalase level	Catalase levels after incubation	
		(Average of 2 and 4 hr. readings)	Rise on incubation
Controls	171 $\pm$ 8.6	259 $\pm$ 3.9	88 $\pm$ 7.6
Tumour-injected	102 $\pm$ 3.8	177 $\pm$ 6.4	75 $\pm$ 5.4

Table 7. *Catalase in liver slices after incubation in the presence of 10 mg. of Sarcoma 37 (as homogenate) per vessel, compared with catalase in slices incubated in the absence of tumour*

Swiss female mice were used. Results are given in arbitrary units/mg. of N, the nitrogen readings being corrected for the tumour contribution.

Initial catalase level	Catalase levels after incubation for time shown			
	Tumour homogenate present		No tumour homogenate	
	2 hr.	4 hr.	2 hr.	4 hr.
93	207	201	222	231
87	183	200	215	188
84	211	178	213	170
110	227	201	200	196

which the tumour homogenate was omitted. The nitrogen estimations were corrected for the presence of tumour tissue by determining its nitrogen content separately and subtracting it from the total. No catalase activity could be detected in the tumour homogenate. Table 7 shows that the presence of tumour homogenate had no effect either on the initial catalase level or on the levels found during incubation.

DISCUSSION

The results have shown that there is a potential catalase activity in mouse liver which is not detected by estimating the level of enzyme in a freshly prepared homogenate. This additional activity is present in homogenates prepared from liver slices which have been incubated for approximately 2 hr. in a suitable medium at 38°, and is of the order of 70–120% of the initial activity. The two most likely explanations of the appearance of this additional catalase are the presence in liver tissue either of a store of precursors from which fresh enzyme is rapidly synthesized, or of a 'reserve' of catalase in the form of an inactive complex capable of dissociation to give free enzyme. The present preliminary results do not enable a decision to be made between these two alternatives, although *prima facie* a dissociable complex might be considered to be the more simple explanation. However, the dissociation of such a complex might be expected to occur readily in a dilute homogenate, but, as has been shown, no alteration in catalase activity was observed when such homogenates were incubated. This suggests that the breakdown of such a complex can take place only under the influence of some accessory system, for example, enzymically, or alternatively that a supply of energy is necessary. Many of the other experiments have also indicated that some metabolic activity is

involved in the production of the additional enzyme. It appears that, under aerobic conditions, oxidizable substrates are necessary for full development, that a much smaller rise is obtained under anaerobic conditions, and that the rise is virtually abolished by incubating the slices at 20°.

The sex difference in initial catalase level found in the three strains of mice seems to be maintained during the development of the additional activity. This result has been consistently obtained, although the same difference at a generally higher level of activity is less significant statistically. The same result was also obtained with liver slices from females treated with testosterone, the elevation in initial level being maintained on incubation. It seems therefore that although males and females have different initial catalase levels, their livers can liberate the same amount of additional enzyme.

Adrenalectomy, which reduced the initial enzyme level substantially, did not greatly affect the rise of activity on incubation.

The experiments with tumour-bearing animals suggest that the initial catalase level is much more sensitive to early tumour growth than the activity released by incubation. However, the interpretation of these results must await further study. It is nevertheless of interest that the addition of tumour homogenate to incubation vessels had no detectable effect on the catalase activity of the liver slices.

SUMMARY

1. Incubation of mouse-liver slices at 38° in a suitable medium results in the development of additional catalase activity.
2. This effect is not observed in homogenates under the same conditions or in slices incubated at 20°. It is smaller under anaerobic conditions.
3. Although males have a higher initial liver-catalase activity than females, the rise in enzyme activity during incubation of slices occurs to the same extent in both sexes.
4. Tumour growth reduces the initial liver catalase activity much more than it reduces the increase of activity obtained on incubation.
5. Incubation of liver slices in the presence of tumour homogenate had no effect either on the initial catalase activity or on the development of the additional activity.

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REFERENCES

- Adams, D. H. (1950). *Brit. J. Cancer*, **4**, 183.
 Adams, D. H. (1951a). *Brit. J. Cancer*, **5**, 115.
 Adams, D. H. (1951b). *Brit. J. Cancer*, **5**, 409.
 Adams, D. H. (1952). *Biochem. J.* **50**, 486.
 Adams, D. H. (1953). *Biochem. J.* **54**, 328.
 Adams, D. H. (1955). *Biochem. J.* **60**, 568.
 Buchanan, J. M., Hastings, A. B. & Nesbitt, F. N. (1949). *J. biol. Chem.* **180**, 435.
 Greenfield, R. E. & Meister, A. (1951). *J. nat. Cancer Inst.* **11**, 997.
 McIlwain, H. & Buddle, H. L. (1953). *Biochem. J.* **53**, 412.
 Nakahara, W. & Fukuoka, F. (1949). *Gann*, **40**, 45.
 Nakahara, W. & Fukuoka, F. (1950). *Gann*, **41**, 47.

Endogenous Respiration and Polysaccharide Reserves in Baker's Yeast

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In the two following papers an attempt is made to assess quantitatively the Pasteur effect in baker's yeast, to study the effect of a wide range of inhibitors on it, and to determine whether it is related to phosphorylation rates. The present preliminary paper deals with the endogenous metabolism of the same yeast, so that it may be determined to what extent the endogenous metabolism must be taken into account in assessing the results of the experiments on glucose breakdown.

Meyerhof (1925) first observed that the respiratory quotient (R.Q.) of yeast respiring in the absence of added substrate is far below unity (about 0.85), and gave reasons for believing that the R.Q. in the presence of glucose is 1.00. Lundsgaard (1930) demonstrated that in the presence of glucose the apparent R.Q. is 1.00, and remains at that value even in circumstances where there is no possibility of any contribution being made to the carbon dioxide production by a simultaneous fermentation, namely in the presence of 10^{-3} M sodium iodoacetate.

More recently Stier & Stannard (1936) and Spiegelman & Nozawa (1945) have reported that yeast of many varieties, including pure strains of *Saccharomyces cerevisiae*, show an endogenous respiration with an R.Q. of 1.00. This is, of course, taken to imply that the oxidation is one of carbohydrate, i.e. that the yeast is oxidizing its stored polysaccharide; but the same authors show beyond doubt that all the cultures they used are unable to ferment their stored polysaccharide. As yeast contains all the enzymes necessary for both processes, it is difficult to see why the one takes place and the other does not.

MATERIALS AND METHODS

Yeast. A single brand of commercial pressed baker's yeast was used (City Brand Pure Yeast, J. Mann and Co., Leeds). This was made into a suspension in water containing

15 g. fresh wt./100 ml.; for manometric measurements 0.5 or 1.0 ml. was used, according to the metabolic activity of the yeast, in a total volume of 3.0 ml., and for phosphorylation measurements 2.0 ml. in a total of 3.0 ml. (1.0 ml. contained roughly 40 mg. dry wt.)

Measurement of respiration and fermentation. Standard methods, with Warburg manometers, were employed. All manometric measurements were made at 25°, and lasted 1 hr. unless otherwise stated. Whenever a comparison was made between polysaccharide determinations and manometric results, the latter were corrected for processes occurring during the equilibration of the manometers.

All experiments were carried out in 0.016M aminotris-hydroxymethylmethane (tris) buffer at pH 6.5; it was confirmed that there was no change in the CO₂ content of the buffer during fermentation experiments. Aerobic measurements were made in air; the N₂ for the anaerobic experiments was purified by passage over heated copper.

Measurement of polysaccharide content of yeast. The contents of the manometer vessels (3.0 ml.) were treated with 2.0 ml. of 10% trichloroacetic acid and centrifuged. The deposit of yeast cells was hydrolysed in 2.0 ml. of 2N-HCl at 100° for 90–120 min., neutralized and made up to 15 ml., and the glucose content of a measured sample determined by the method of Hanes (1929). The initial polysaccharide content of the yeast was also determined in the same way. The values obtained (total material capable of yielding reducing sugars on acid hydrolysis) will be referred to as 'polysaccharide' and will be expressed as the equivalent amount of glucose.

Table 1. *Respiratory quotient of the endogenous respiration of yeast in different nutritional states*

Figures in parentheses indicate the range of individual values.

Treatment of yeast	No. of samples	R.Q.
Impoverished	6	0.86 (0.82–0.90)
Normal	14	0.86 (0.83–0.91)
Enriched	8	0.83 (0.76–0.90)