The Mechanism of the Action of Androgenic Hormones on Mouse-Liver Catalase

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Adams & Burgess (1957) showed that 70–80% of the catalase activity in mouse liver was present in the large granules, the remainder being in the extraparticulate cytoplasm. The homogenization technique used before 1957 was such that the catalase measured in the suspensions was principally extraparticulate cytoplasm fraction (Adams & Burgess, 1957). Adams (1952) found that the catalase activity in such suspensions was higher when they were prepared from male mice than from female mice, and that castration of males reduced their liver catalase activity to the female level. Injection of testosterone into normal females or castrated males elevated their liver-catalase activity to about the normal male level. Adams & Burgess (1957) confirmed that the sex difference in activity was associated with the extraparticulate cytoplasm catalase fraction, and showed that the level of catalase activity in the granules was the same in both sexes. Their data suggested that different catalases were present at the two liver sites, one reacting to hormonal stimulation, the other not. Catalase activity in the erythrocytes was found to be the same in both sexes (Adams, 1956), resembling the liver-granule enzyme in this respect.

However, although many fractionation studies have been made [e.g. in rats by Greenfield & Price (1956)] no definite evidence has been found that more than one catalase is present in liver. Spectroscopic and immunological evidence obtained in a variety of species (Bonnichsen, 1947; Chance, 1950, Deutsch & Seabra, 1955) has also suggested the presence of only a single catalase in liver, which resembles closely the erythrocyte enzyme. It appears therefore that the catalase present in the granules and extraparticulate cytoplasm of liver and in erythrocytes are closely similar or identical enzymes; this conclusion provides no indication why only the extraparticulate cytoplasm fraction of liver should have a sex difference in activity.

Recently Adams & Burgess (1959*b*) showed that during the incubation of liver slices catalase migrated from the large granules into the extraparticulate cytoplasm. Catalase also migrated from the large granules into the surrounding medium when they were incubated in 0.25-M

sucrose solution. No evidence could be found that this catalase migration was the result of rupture of, or damage to, the granule membranes, and it was concluded that the membranes of the large granules are permeable to catalase.

It seemed possible therefore that liver contains a single catalase, which is produced in the large granules and is then distributed between the large granules and the extraparticulate cytoplasm by migration through the granule membranes. If this hypothesis is correct, the catalase level in the extraparticulate cytoplasm will depend broadly on the granule catalase level, the permeability of the granule membranes and on the rate of catalase destruction in or loss from the extraparticulate cytoplasm. The term 'permeability' may be defined for the purpose of this paper as being that property of a membrane which governs the rate of transfer of catalase from one side to the other. At present it is not known whether the catalase transfer can occur in both directions or whether factors other than simple diffusion are involved in the process.

Such a catalase system would probably be very complex kinetically, and at present no attempt at any strict analysis can be contemplated. However, taking the simplest possible view, it may be considered that the relative levels of catalase in granules and extraparticulate cytoplasm will provide an approximate index of granule permeability.

The purpose of this paper has been to test this hypothesis by making comparative measurements of the extraparticulate cytoplasm and granule-catalase levels after the injection of various substances, and to discover whether the action of androgenic hormones is possibly to increase the permeability of the granule membranes to the enzyme.

EXPERIMENTAL

Animals. Young adult mice of the CBA strain and of the AKR Laboratory Animals Bureau substrain have been used. These two strains have similar liver-catalase activities, and the availability of sufficiently large groups has been the main factor deciding which strain has been used for which experiment. CBA mice have been bred in this Laboratory by brother-sister mating. AKR substrain mice have been

similarly bred here, or obtained from the Laboratory Animals Bureau. The diet of the animals consisted of commercial rat cubes and water (both without restriction).

Triton X 100. This non-ionic detergent was used at a final concentration of 0.25% to disrupt large granules and liberate their catalase activity into solution.

Homogenization of liver. The preparation of a 12-stroke homogenate has been previously described (Adams & Burgess, 1957). In the present work ethanol (0.01M) has been present in all catalase-containing solutions and suspensions to prevent losses in activity through the formation of the catalase-hydrogen peroxide complex II (Chance, 1950; Adams & Burgess, 1959a). Catalase measurements were made of the following preparations: (1) 12-stroke homogenate + Triton (giving total catalase); (2) extraparticulate cytoplasm (EPC) fraction (supernatant after centrifuging a 12-stroke homogenate at 10 000 g for 20 min.); (3) granule fraction [sediment from (2) after resuspending it in Triton solution to the original volume].

Estimation of liver-catalase activity. Catalase was estimated as previously described (Adams, 1950, 1952). The catalase activities of the various fractions have been referred to the nitrogen content of the whole homogenate from which they were prepared. One advantage of this is that the ratio of the catalase activities in granules and EPC is independent of the concentration of any other tissue component.

Chemical substances. (Cysteine hydrochloride, thiourea and methylbis-(β -chloroethyl)amine (NM) were separately dissolved in sodium chloride solution (0.9%) and brought to pH 7.0 by the addition of sodium bicarbonate solution, the pH being measured with a Pye pH meter and glass electrode standardized according to the maker's instructions. They were then injected intraperitoneally.

Individual doses of testosterone, 17-methyltestosterone and 3'-methyl-4-dimethylaminoazobenzene (MDAB) were weighed on a torsion balance, each dose then being placed separately on a siliconized white tile. Each dose was moistened with 0.9% sodium chloride and then implanted intraperitoneally with a trocar and cannula.

RESULTS

Normal animal. Table 1 gives the total, granule and EPC liver-catalase activities found in male and female AKR and CBA mice. The results show that the granule-catalase activity was very similar in males and females in each strain, whereas the EPC catalase level was much higher in the males than in

the females. The sex difference in EPC catalase activity was much higher in untreated AKR and CBA mice than in the stock albino strain used for the previous hormone experiments (Adams, 1952, 1956). Thus the granule/EPC catalase distribution ratio was about 4.9 in the females and 3.4 in the males.

Effect of the injection of non-androgenic substances. Fig. 1 shows the effect of the injection of single doses of cysteine on the total, granule and EPC catalase levels, and on the granule/EPC catalase distribution ratio, of CBA female mice. The results show that depressions of total catalase activity occurred within 24 hr., followed by a return to normal. The extent of the depression and the time needed for recovery increased with increasing dose of cysteine. The shaded areas on the graphs are in fact approximately proportional to dose. Little or no change was seen in the granule/EPC distribution ratio.

Cysteine is not a direct inhibitor of catalase: however, when solutions of catalase and cysteine were mixed, losses in activity occurred due to the combination of the enzyme with small quantities of hydrogen peroxide produced by auto-oxidation (complex II, Chance, 1950). To examine the possibility that the catalase loss in the livers of cysteine-treated animals was due to complex II formation *in vivo*, samples of the homogenates prepared from the 48 hr. group given the largest dose of cysteine were incubated at 38° for 2 hr. Adams & Burgess (1959b) found that complete regeneration of catalase activity from solutions containing complex II was achieved under these conditions in the presence of ethanol. However, no rise in activity was observed in the liver homogenates made from the cysteine-treated animals (Table 2).

The effect of the implantation of two doses of MDAB into AKR females, and the larger dose into AKR males, is shown in Fig. 2. Once again catalase activity was rapidly depressed, the amount of the depression (extent $\times$ duration) being approximately proportional to dose in the two cases. The granule/EPC distribution ratio was only slightly affected in either sex except on the third day after the injection of the smaller dose into females, when the

Table 1. *Liver-catalase activities and intracellular distribution ratio (granule/extra particulate cytoplasm) in normal male and female CBA and AKR mice*

Catalase activities are expressed in arbitrary units/mg. of N as arithmetic means $\pm$ s.e.m. Eight animals were used in each group.

CBA mice	Total activity	Granule activity	EPC activity	Granule/EPC ratio
♂	625 $\pm$ 10.5	480 $\pm$ 7.9	140 $\pm$ 4.1	3.4
♀	575 $\pm$ 10.2	475 $\pm$ 8.4	98 $\pm$ 2.8	4.8
AKR mice				
♂	640 $\pm$ 12.0	490 $\pm$ 11.2	140 $\pm$ 3.9	3.5
♀	600 $\pm$ 10.2	500 $\pm$ 9.0	100 $\pm$ 3.1	5.0

Fig. 1. Action of single doses of cysteine on the liver-catalase activity and intracellular-catalase distribution (granule/extraparticulate cytoplasm; G/EPC) of CBA female mice. Δ , Total catalase; $\circ$, granule catalase (G); $\bullet$, extraparticulate-cytoplasmic catalase (EPC). (a) 20 μ moles. (b) 50 μ moles. (c) 125 μ moles. Controls at 0 days. The same control group was used for all three graphs. Twelve control and eight treated mice were used in each group. Results in this and subsequent figures are given as arithmetic means $\pm$ s.e.m.

Table 2. Total catalase activity in the livers of mice before and after incubation of homogenates with 0.01M-ethanol for 2 hr. at 38°

(a) CBA female mice 48 hr. after the injection of 125 μ M-cysteine; (b) AKR female mice 48 hr. after the injection of 1 μ mole of NM. Results are given in arbitrary units/mg. of N as arithmetic means $\pm$ s.e.m. Eight animals were used in each group, with twelve controls.

	Control catalase activity	Catalase activity of liver of treated animals	
		Before incubation with ethanol	After incubation with ethanol
(a)	540 $\pm$ 11.2	425 $\pm$ 12.5	430 $\pm$ 11.0
(b)	590 $\pm$ 10.5	210 $\pm$ 11.5	220 $\pm$ 12.1

ratio rose to 5.7. However, this result may be slightly aberrant since the larger dose gave a distribution ratio of only 5.4 after 3 days. Thiourea (10 μ moles) injected into male and female CBA mice affected their catalase activity in

a similar way to MDAB. The results were almost identical with those obtained with the higher dose of MDAB.

Fig. 3 shows the results of the injection of various single doses of NM into female AKR mice and of one single dose into males. This substance was extremely toxic; the mice receiving the largest dose (1 μ mole) did not survive beyond 48 hr., and at this time had lost about 70 % of their liver-catalase activity. The intermediate dose (0.2 μ mole) resulted in the death of some of the treated animals. As with the substances already described the depressions in catalase activity were related to dose. However, the granule/EPC catalase distribution ratio was altered from 5.0 in the females and 3.3 in the males to a new value of 4.2-4.4 in both sexes.

Erythrocyte-catalase measurements were made on the group of female mice 48 hr. after the injection of the largest dose of NM, when their liver-catalase activity was about 30 % of normal (Fig. 3), and their erythrocyte-catalase activity was

found to be unchanged. Samples of liver homogenate from these mice were also incubated with ethanol (cf. experiments with cysteine, described above). As shown in Table 2 no regeneration of catalase activity was observed.

Incubation of homogenates with methylbis-(β -chloroethyl)amine in vitro. Liver homogenates were prepared with 300 strokes of a Ten Broeck grinder, which liberates most of their catalase activity (Adams & Burgess, 1957). They were centrifuged at 10 000 *g* for 20 min. and the supernatants were then incubated with various concentrations (up to 0.01 M) of NM at 0° for 2 hr. The NM was dissolved in water and brought to pH 7.0 with sodium bicarbonate before use. As shown in Table 3, little inhibition was seen.

Effect of the injection of androgenic hormones. In preliminary experiments it was found that testosterone administered to female mice of the two strains used caused a rise in EPC-catalase level, but to a point below the normal male level. Further experiments showed that 17-methyltestosterone would elevate the EPC catalase level in females to, or to a little above, the male level.

Fig. 2. Action of single doses of 3'-methyl-4-dimethyl-aminoazobenzene on the liver-catalase activity and intracellular-catalase distribution of AKR mice. Δ , Total catalase; $\circ$, granule catalase; $\bullet$, EPC catalase. (a) Females; (b) males. (1) 10 μ moles. (2) 20 μ moles. Numbers of animals in the groups are given by the figures at the head of the s.e. limits.

The action of single injections of 17-methyltestosterone on the liver-catalase activity of female CBA mice is shown in Fig. 4. So far as total catalase activity is concerned, the results closely resembled those obtained with cysteine (Fig. 1), i.e. transient catalase depressions proportional to dose. However, unlike cysteine, striking effects were seen on the granule/EPC-catalase distribution ratio. This changed from the female value to the male value 48 hr. after the injection, and independently of dose. With the largest two doses the new ratio was maintained for several days, during which time the granule catalase returned to normal and the EPC activity rose to the male level.

Testosterone produced results on injection (Fig. 5) similar to those obtained with 17-methyltestosterone. As previously mentioned, however, the EPC-catalase activity, although elevated, did not reach the male level, the granule/EPC-catalase distribution ratio falling to a value intermediate between that of males and that of females.

Fig. 6 shows the effect of the injection of 17-methyltestosterone into males. Over the first few days the results closely resembled those obtained after the injection of cysteine, thiourea and MDAB, i.e. a transient fall in catalase activity in both fractions, with little or no alteration in the granule/EPC distribution ratio. From the sixth to the eighth days the EPC-catalase level fell, but not the granule level, the distribution ratio approaching the female value. By the eleventh day the EPC level returned to normal.

DISCUSSION

All the substances tested caused losses in total catalase activity after injection, the activity usually returning to normal after 4-7 days. The losses were approximately proportional to the dose of injected material. The results suggested that the erythrocyte catalase remained unchanged under the applied conditions and that the losses in liver catalase were not due either to direct inhibition or to the formation of complex II (Chance, 1950).

It seems reasonable to suppose that the losses in total catalase activity are due to hydrogen peroxide produced during the metabolism of the injected material, and are consequently of a non-specific nature.

The catalase activity after the injection of the highest doses of the steroids has shown a tendency to rebound to a higher than normal level. This rebound, coupled with the other results presented, suggests that the turnover rate of catalase is high, and that the synthetic mechanisms are capable of responding rapidly to a sudden demand. Also, Adams & Burgess (1959b) found that 70 % of their catalase could migrate from isolated granules to the

Fig. 3. Action of single doses of methylbis-(β -chloroethyl)amine on the liver-catalase activities and intracellular-catalase distribution of AKR mice. Δ , Total catalase; $\circ$, granule catalase; $\bullet$, EPC catalase. (a), (b) and (c), female mice receiving 0.05, 0.2 and 1.0 μ mole respectively; (d) male mice receiving 0.2 μ mole. Twelve control and eight treated animals were used in each group. The same control group was used for graphs (a), (b) and (c).

Table 3. Action of methylbis-(β -chloroethyl)amine on liver catalase *in vitro* during incubation for up to 1 hr. at 0°

A 300-stroke homogenate of liver in water (Adams & Burgess, 1957) was centrifuged at 10 000 *g* for 20 min., and the supernatant diluted tenfold with water. Samples were mixed with aqueous solutions of NM, brought to pH 7.0 with bicarbonate, to give the final concentrations stated.

Time of incubation (min.)	Concn. of NM (mm)			
	0 (Control)	1	5	10
	Catalase activity (arbitrary units/mg. of N)			
10	519	525	570	548
	546	558	540	540
60	540	575	545	520
	556	560	550	510

surrounding medium within 30 min. The system would therefore seem well fitted to deal with the sudden intracellular production of hydrogen peroxide.

The hypothesis given in the introduction to this paper was, briefly, that catalase is produced within the large granules and then migrates into the EPC. The results obtained have shown clearly that the

granule catalase may be destroyed and regenerated very rapidly, and since most of the enzyme is found in the granules they must be at least the major site of catalase production. Catalase migration cannot easily be demonstrated *in vivo*, although its occurrence *in vitro* has been shown (Adams & Burgess, 1959*b*). However, the effects of the non-androgenic substances suggest very strongly that the catalase levels in granules and EPC are interdependent. Thus changes in granule-catalase level have always been associated with similar changes in EPC-catalase level, over the whole range of doses and time. After the injection of cysteine, thiourea and MDAB, the granule/EPC-catalase distribution ratio remained at the control value in both males and females. There seem only two possible explanations of this result. Either there is a single catalase which migrates in the way already proposed, or there are two separate catalase systems, one in the granules and one in the EPC, which are affected to the same proportional extent, independently of substance, dose and time. The pattern obtained after the injection of NM provided evidence in favour of the first alternative. In the females, the granule/EPC distribution ratio was lowered, from the control value of 5.0 to a

new value of about 4.2 which was maintained independently of dose and time, and, in the males, the distribution ratio was raised from the control value of 3.3 to about 4.4, i.e. to about the same value as in the females. These results are not easily explained in terms of separate granule and EPC-catalase systems. They may, however, be readily explained by supposing that part of the action of NM is to alter the rate of catalase migration from granules to EPC, whereas cysteine, MDAB and thiourea do not alter the rate of migration.

An explanation of the effects of androgens on catalase may be given in similar terms. The granule/EPC distribution given in Table 1 would be expected if (1) the EPC-catalase level depended on the granule-catalase level and (2) the rate of catalase migration was higher in the males. The action of testosterone and 17-methyltestosterone on the total catalase activity of females closely resembled that of cysteine, for example. However, within 48 hr. after the injection of 17-methyltestosterone in any of the doses used, the granule/EPC-catalase distribution ratio changed to the male value, and

remained there during the subsequent rise in EPC level.

Testosterone gave similar results. However, the granule/EPC distribution ratio changed to a value intermediate between the normal male and female values, reflecting the fact that the EPC-catalase activity, although elevated, did not reach the male level.

The injection of 17-methyltestosterone into males resulted, over the first 4 days, in a fall followed by a rise in catalase activity in both fractions, without change in the distribution ratio. This result closely resembles those obtained with cysteine, thiourea and MDAB in both males and females. From the sixth to the eighth day the granule/EPC distribution ratio approached the female value. It seems probable that this was due to a temporary suppression of the endogenous androgen secretion by the large administered dose.

The data presented, when taken in conjunction with the evidence that the large-granule membranes are permeable to catalase (Adams & Burgess, 1959*b*), are consistent with the following: (1) Catalase, largely produced in the granules, is

Fig. 4. Action of single doses of 17-methyltestosterone on the liver-catalase activity and intracellular-catalase distribution of CBA female mice. Δ , Total catalase; $\circ$, granule catalase; $\bullet$, EPC catalase. (a) A comparable group of normal male mice; (b), (c) and (d), female mice receiving 8.0, 3.0 and 0.8 μ moles respectively. The same control group was used for (b), (c) and (d). Numbers of animals in the groups are given by the figures at the head of the s.e. limits.

Fig. 5. Action of a single dose of 8 μ moles of testosterone on the liver-catalase activity and intracellular-catalase distribution of CBA female mice. Δ , Total catalase; O, granule catalase; $\bullet$, EPC catalase. Twelve control and eight treated animals were used in each group.

distributed between the granules and EPC by migration through the granule membranes. (2) Androgenic hormones influence the intracellular distribution of catalase by increasing the rate of migration. (3) It is reasonable to suppose that the increased rate of migration is the result of an increased permeability of the granule membranes, brought about by the hormones. (4) Methyltestosterone increases the granule permeability to a greater extent than testosterone.

This theory also provides a plausible explanation of phenomena described in previous publications. Firstly, the absence of a sex difference in erythrocyte-catalase level (Adams, 1956). Hormones could not influence catalase distribution in the erythrocyte in the way suggested for liver. Secondly, copper and manganese deficiency in females resulted in an elevation of EPC-catalase activity resembling that observed after the injection of testosterone (Adams, 1953). This may be explained by supposing that trace metals are also

Fig. 6. Action of a single dose of 8 μ moles of 17-methyltestosterone on the liver-catalase activity and intracellular-catalase distribution of male CBA mice. Δ , Total catalase; O, granule catalase; $\bullet$, EPC catalase. The numbers of animals in the groups are given by the figures at the head of the s.e. limits.

implicated in the control of the permeability of granule membranes.

SUMMARY

1. A study has been made of the effect of the injection of a number of substances, including androgenic hormones, on the total catalase activity and the intracellular-catalase distribution between large granules and extraparticulate cytoplasm in the livers of mice.

2. All the substances tested produced depressions of catalase activity, approximately proportional to dosage, usually followed by a return to normal after a few days.

3. These catalase losses are probably of a non-specific nature, and it is suggested that they may be due to hydrogen peroxide produced during the metabolism of the injected material.

4. Cysteine, thiourea and 3-methyldimethylaminoazobenzene caused losses in catalase activity without markedly affecting the intracellular-catalase distribution.

5. Males and females have a different intracellular-catalase distribution. In males the granule/extraparticulate-cytoplasmic distribution ratio is about 3.3, in females it is about 4.9.

6. A nitrogen mustard altered the catalase distribution ratio in males and females to the same value, intermediate between their normal values.

7. 17-Methyltestosterone injected into females caused a change in the catalase distribution ratio to the male value. Testosterone behaved similarly, but the distribution ratio was altered to a point intermediate between the male and female values.

8. 17-Methyltestosterone injected into males did not affect the distribution ratio.

9. It is concluded that the catalase levels in the granules and extraparticulate cytoplasm are interdependent; that catalase probably migrates from the granules into the extraparticulate cytoplasm; and that androgenic hormones influence the rate of migration in a way which suggests that they increase the permeability of the granule membranes to catalase.

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The Behaviour of Liver Mitochondria Isolated from Rats with Different Body Temperatures after Limb Ischaemia or after Injection of 3:5-Dinitro-*o*-Cresol

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The liver is one of the main sites of heat production in the body (Federov & Shur, 1942; Birnie & Grayson, 1952) and the heat formed there mainly arises during the oxidations carried out by the mitochondria. It is therefore possible that changes in hepatic heat production might reflect alterations within the mitochondria or be due to variations in the amount of substrate being fed to mitochondria whose potentialities had remained unaltered.

The changes in liver temperature on simple exposure of the rat to different environmental temperatures are quite small (Stoner, 1958*a*), although the changes in overall metabolic rate may be large and rapid (Depocas, Hart & Héroux, 1957). Exposure to cold increases heat production but, according to Panagos, Beyer & Masoro (1958), mitochondrial changes occur only as the rat becomes acclimatized.

Much greater changes in liver temperature occur in rats which have been injured by limb ischaemia, particularly if the environmental temperature is

also altered (Stoner, 1958*a*). The possibility of mitochondrial changes in these rats is of special interest as several workers (Strawitz & Hift, 1956*a, b*; Packer, Michaelis & Martin, 1958; Hift & Strawitz, 1958) have sought to relate the metabolic decline and death of the injured rat to alterations in the mitochondria.

Fatal doses of 3:5-dinitro-*o*-cresol, which has the same biochemical action as 2:4-dinitrophenol, produce very high liver temperatures in the rat (Stoner, 1956) and the acute effects of this compound have also been investigated.

Experience in the production of a highly reproducible preparation of liver mitochondria (Aldridge, 1957) has allowed us to seek a possible association between acute changes in temperature (heat production) and the biochemical behaviour of the isolated mitochondria. It was found that except in rats treated with 3:5-dinitro-*o*-cresol large changes in liver temperature were unaccompanied by similar changes in the behaviour of the isolated mitochondria.