Effect of Cobalt on the Synthesis and Degradation of Hepatic Catalase *in vivo*

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(Received 20 May 1974)

1. The administration of CoCl_2 to rats caused a decrease in hepatic catalase activity as well as a decrease in the amount of catalase protein as measured by immunological assay. The mitochondrial enzyme decreased progressively over 2 days, whereas the cytosol enzyme decreased over 12 h and then remained essentially unchanged for 2 days after a single injection of cobalt. 2. Incorporation of [^{14}C]glycine into catalase haem was dramatically decreased by a single injection of cobalt, but that into catalase protein remained essentially unaltered. 3. Incorporation of [^3H]leucine into liver protein increased in rats in a steady state receiving a daily injection of cobalt, which was in contrast with a marked inhibition observed in 5-amino[^3H]laevulinate incorporation. 4. The initial rate of [^3H]leucine incorporation into mitochondrial and cytosol catalase did not alter or was slightly depressed in the cobalt-treated animals, whereas the incorporation of 5-amino[^3H]laevulinate into mitochondrial and cytosol catalase was conspicuously decreased, indicating that haem synthesis was limiting catalase formation. 5. The degradation rate of catalase protein, as measured by a double-labelling method, was not changed by the cobalt treatment.

In rabbit reticulocytes, the syntheses of haem and globin are co-ordinately regulated: globin synthesis is markedly stimulated by haem and Fe^{2+} ions (Bruns & London, 1965; Rabinovitz & Waxman, 1965). There have been contradictory reports about the effect of cobalt on the synthesis of globin by reticulocytes; Schulman & Jobe (1968) and Schulman (1970) observed that globin synthesis was inhibited, whereas Morell *et al.* (1959) and Waxman (1970) showed stimulation at cobalt concentrations at which haem synthesis is inhibited. On the other hand, Tephly and co-workers have shown that cobalt inhibits the syntheses of hepatic microsomal cytochromes *P*-450 and *b*₅ (Tephly & Hibbeln, 1971) and that the incorporation of labelled 5-aminolaevulinate into microsomal haem was impaired by CoCl_2 treatment, whereas that of labelled leucine into microsomal protein was stimulated (Tephly *et al.*, 1973). We observed that cobalt administration to rats *in vivo* depressed haem synthesis in liver by decreasing the activities of the haem-synthesizing enzymes (M. Nakamura, unpublished work). These observations led us to investigate whether there is co-ordinate regulation of haem and apoprotein syntheses in liver. We have studied catalase, a haemoprotein, the biosynthesis of which has been extensively investigated (Higashi & Peters, 1963; Lazarow & de Duve, 1973*a,b*).

In the present paper, the effect of cobalt administration to rats on the biosynthesis and degradation of hepatic catalase was investigated by the use of

immunochemical and labelling techniques. We found that the decrease in the rate of haem synthesis in the cobalt-treated animals was limiting catalase synthesis and that rate of protein degradation was not very important.

Experimental

Animals

Male Wistar rats (230–250 g), obtained from the Institute of Experimental Animals, Kyushu University, were used.

Subcellular fractionation

Rats were killed by decapitation at time-intervals specified in the Figures and Tables, and the livers were rapidly removed and perfused with ice-cold saline (0.9% NaCl). Tissue homogenates (16%, w/v) were prepared in 0.25 M-sucrose containing 5 mM-Tris-HCl buffer and 0.5 mM-EDTA, pH 7.5, in a Potter-Elvehjem-type homogenizer with a Teflon pestle. Subcellular fractions were obtained as described by Nakamura *et al.* (1973).

Protein concentration was determined by the method of Lowry *et al.* (1951), with bovine serum albumin (type V, Armour Pharmaceutical Co., Kankakee, Ill., U.S.A.) as standard. Catalase activity and radioactivity are expressed per mg of protein of

homogenate or subcellular fraction unless otherwise stated.

Measurement of catalase activity

Rats were injected subcutaneously once with either 3% (w/v) cobaltous chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) solution at a dose of 60 mg/kg body wt. or 0.5 ml of iso-osmotic saline (controls). Hepatic subcellular fractions were extracted with sodium deoxycholate (final concentration, 0.5%). Catalase activity was assayed spectrophotometrically by the method of Beers & Sizer (1952) and is expressed as a first-order rate constant (k ; min^{-1}) in a final volume of 2 ml (Nakamura *et al.*, 1973). The same extract was also used for immunological determination of catalase.

Preparation of anti-catalase antibody

Anti-catalase antibody was prepared as described previously (Nakamura & Minakami, 1973). In the Ouchterlony double-diffusion test, the antibody gave a single precipitation line with purified catalase and sodium deoxycholate extract of liver but no reaction with rat serum. A single batch of antibody was used throughout the experiments.

Determination of catalase content by immunotitration

The diluted samples (0.2 ml, 10–20 k/0.2 ml) were added to series of diluted anti-catalase antibody (0.2 ml). The mixtures were incubated at room temperature for 60 min and at 4°C overnight. The immunoprecipitate was sedimented and the catalase activity remaining in the supernatant (20 μ l) was assayed by the spectrophotometric method. Catalase content was determined by extrapolation of the linear portion of the titration curve to zero catalase activity and it was expressed as ml of undiluted anti-catalase antibody required to completely sediment catalase activity.

Isotope experiments

(a) *Incorporation of [2- ^{14}C]glycine into haem and protein moieties of catalase.* Rats were treated with cobalt or iso-osmotic saline as described above; 2 h after the injection, 80 μCi of [2- ^{14}C]glycine per rat was administered intraperitoneally in a volume of 0.5 ml. The animals were killed 4 h later. Immunoprecipitate of catalase was prepared from deoxycholate extract of liver homogenate as described above and the supernatant was assayed for catalase activity to ensure that the supernatant was found to be devoid of catalase activity. The precipitates were then washed three times with ice-cold 0.25 M-sucrose containing 0.05% sodium deoxycholate. When the

extracts were tested with control serum, precipitates were below 1% of those recovered with anti-catalase antibody. The washed precipitates were dissolved in 5 ml of 1 M-acetic acid (immunoprecipitate fraction). A measure of the radioactivity incorporated into the protein of catalase was obtained by collecting the precipitate formed after the addition of 7 ml of cold 1% (v/v) HCl-acetone solution to 0.2 ml of dissolved precipitate. The precipitates were washed twice with the same solution and twice with 6% (w/v) trichloroacetic acid; they were then dissolved in 0.25 ml of 1 M-NaOH and 1.0 ml of 0.01 M-NaOH was added (apocatalase fraction). Scintillation fluid [4 g of 2,5-diphenyloxazole/litre of 33% (v/v) Triton X-100-toluene solution] was added to the dissolved precipitate and the radioactivity in the resulting clear mixture was determined in a Horiba liquid-scintillation spectrometer LS-500. The efficiency of counting was 87%. Carrier haemoglobin was added to the immunoprecipitate dissolved in 1 M-acetic acid. The haem was purified by the method of Schiefer (1969) (haem fraction), and counted for radioactivity at infinite thinness in a windowless gas-flow Geiger counter (Aloka SC-5) with a background of 12–13 c.p.m. The radioactivity of the haem was calculated from the recovery of the carrier haem.

(b) *Incorporation of L-[4,5- ^3H]leucine and 5-amino-[2,3- ^3H]laevulinate into catalase.* CoCl_2 (3%) was injected subcutaneously at a dose of 30 mg/kg body wt. every day for 4 days. The control animals were similarly injected with 0.5 ml of iso-osmotic saline. The rats were starved for 16 h after the last injection and then each was given an intraperitoneal injection of either 145 μCi of L-[4,5- ^3H]leucine or 68 μCi of 5-amino[2,3- ^3H]laevulinate and was killed at time-intervals specified in the Figures. The washed immunoprecipitate was dissolved in 0.05 M-NaOH and a 2 ml sample was counted for radioactivity in 15 ml of the scintillation fluid containing Triton X-100. The efficiency of counting was 24%. The protein in the crude fraction was precipitated with 6% (w/v) trichloroacetic acid to measure the radioactivity incorporated into liver protein; it was washed three times with the same solution. The radioactivity was measured as described above for the immunoprecipitate.

(c) *Degradation of catalase protein.* Rate constants of degradation were measured by the double-labelling method of Glass & Doyle (1972). Rats were injected daily for 4 days with 3% (w/v) cobalt chloride solution at a dose of 30 mg/kg body wt., the control rats being injected with 0.5 ml of iso-osmotic saline. Each rat was then starved for 16 h, and then given an intraperitoneal injection of 30 μCi of L-[1- ^{14}C]leucine containing 0.48 μmol of L-leucine. Food was restored 4 h later. At 3 days after the initial isotope injection, the starvation regimen was repeated, and the next day the animals were injected with 225 μCi of L-

[4,5- ^3H]leucine containing $0.48\mu\text{mol}$ of L-leucine, the same amount of leucine as in the initial isotope injection so as not to change the free leucine pool in the liver. The animals were killed 4h after the final injection of isotope. Preparation of material for radioactive counting was carried out as described above. Counting of the double isotope was done in a Beckman liquid-scintillation system LS 250 equipped with automatic quenching compensation. Channels were chosen such that there were no ^3H counts in the ^{14}C channel, but 11 % of the ^{14}C counts were in the ^3H channel with 'narrow ^3H window'. The efficiency of counting was 23 % for ^3H and 58 % for ^{14}C . Sufficient counting was done to ensure a counting error of 5 % or less. Efficiencies were determined with an external standard ratio.

Determination of free leucine pool

Rats were killed 30min after [^3H]leucine injection ($145\mu\text{Ci}/\text{rat}$) and the liver was homogenized as described above. Tyrosine ($3\mu\text{mol}$) was added to 8ml of liver homogenate to calculate the recovery of leucine. Free amino acids were extracted from the homogenate and concentrated by the method of Loftfield & Harris (1956). Amino acids were separated on a Hitachi 034 amino acid analyser and the split stream was collected in fractions. The ninhydrin-reactive material and ^3H were measured. The free leucine concentration in liver was calculated from the recovery of tyrosine.

Reagents

All chemicals used were of analytical grade. The radiochemicals, [2- ^{14}C]glycine (sp. radioactivity, $47.7\text{mCi}/\text{mmol}$) and L-[4,5- ^3H]leucine (sp. radioactivity, $32.0\text{Ci}/\text{mmol}$) were obtained from Daiichi Pure Chemicals, Tokyo, Japan. 5-Amino[2,3- ^3H]laevulinate hydrochloride (sp. radioactivity, $22\text{Ci}/\text{mmol}$) and L-[1- ^{14}C]leucine (sp. radioactivity, $62\text{mCi}/\text{mmol}$) were purchased from CEA, Gif-sur-Yvette, France and The Radiochemical Centre, Amersham, Bucks., U.K., respectively.

Results

Effect of cobalt on hepatic catalase activity

To study the effect of CoCl_2 on hepatic catalase activity of rat, the activity of this enzyme in both the mitochondrial and cytosol fractions was measured from 12h to 3 days after a single cobalt injection (Fig. 1). Catalase activities in both fractions were decreased by cobalt administration; the mitochondrial activity decreased progressively over 2 days, reaching a minimum of 52% of the control, but the cytosol activity decreased to 33 % of the control within 12h and then remained unchanged until 2 days later. After 3 days the catalase activities of both fractions tended to recover.

Fig. 1. Effect of cobalt on catalase activity

Rats were injected with CoCl_2 ($60\text{mg}/\text{kg}$ body wt.) and were killed at time-intervals as specified in the Figure. Catalase was assayed spectrophotometrically. Points and bars are the mean values $\pm$ S.E.M. of three animals. At points without bars the S.E.M. are not shown because of low values, except after 12h (the control) and 3 days (the cobalt-treated and the controls) where the points are mean values of two animals. Control: ○, mitochondrial; △, cytosol. Cobalt-treated: ●, mitochondrial; ▲, cytosol.

Effect of cobalt on catalase protein

To determine whether the decrease in catalase activity in cobalt-treated animals is related to the decrease in the content of the enzyme, immunological analysis was carried out. In Fig. 2, minimum amount of anti-catalase antibody required to completely precipitate the catalase activity is plotted against the catalase activity. It demonstrates that the catalase activity was proportional to the amount of the enzyme in the control animals and the same amount of antibody was required to completely sediment the catalase activity in mitochondrial extract as was required for the cytosol fraction.

The amount of catalase protein was determined immunologically with the samples used in Fig. 1. The changes in catalase protein content shown in Fig. 3 were closely similar to those of the catalase activities in Fig. 1.

Fig. 2. Amount of anti-catalase antibody to precipitate catalase activity in cytosol and mitochondrial extract

Mitochondrial extract or cytosol were diluted to obtain catalase activity (k in 0.2ml) specified in the Figure. The samples (0.2ml) were added to series of diluted anti-catalase antibody. Catalase content was determined as described in the Experimental section and it was expressed as ml of undiluted anti-catalase antibody required to completely sediment catalase activity. $\circ$, Mitochondrial; Δ , cytosol.

Incorporation of [^{14}C]glycine into haem and protein moieties of catalase

We next considered the question of whether the haem and protein moieties of catalase were co-ordinately synthesized or not. As shown in Table 1, the incorporation of [^{14}C]glycine into catalase apoprotein fraction in the cobalt-treated rats did not differ from that of the control, whereas that into catalase haem in the cobalt-treated animals was markedly decreased by 92%. This observation may indicate that cobalt inhibited the haem synthesis and this would have led to the decrease in the content of catalase by depressing catalase apoprotein synthesis or stimulating catalase degradation or both.

Incorporation of [^3H]leucine into total proteins

The animals treated with cobalt (30mg/kg body wt.) daily for 4 days were found to be essentially in a steady state (see under 'Control experiments for half-life determination') and this condition was used in the following experiments. The cobalt treatment did not alter the time-course of [$4,5\text{-}^3\text{H}$]leucine incorporation into the trichloroacetic acid-soluble fraction, although the incorporation into total liver proteins in the cobalt-treated animals increased as

Fig. 3. Effect of cobalt on catalase content

The amount of catalase protein was determined immunologically with the samples used in Fig. 1. It was expressed as ml of undiluted antibody required to completely precipitate catalase activity per mg of protein of the samples. Points and bars are the mean values $\pm$ S.E.M. as indicated in Fig. 1. Control: $\circ$, mitochondrial; Δ , cytosol. Cobalt-treated: $\bullet$, mitochondrial; $\blacktriangle$, cytosol.

shown in Fig. 4. This increase may be explained by the specific radioactivity of the hepatic free leucine pool which is shown in Table 2. A similar acceleration of the incorporation was observed when mitochondrial and cytosol proteins were measured separately (not shown in the Figure).

Incorporation of [^3H]leucine into catalase apoprotein

Fig. 5 shows the time-course of [$4,5\text{-}^3\text{H}$]leucine incorporation into mitochondrial and cytosol catalase. The radioactivity of cytosol catalase in cobalt-treated animals was the same as that in the controls 30min after the injection of radioactive leucine, but thereafter declined more markedly than that of the controls; 5h later the radioactivity in the cytosol catalase in the cobalt-treated animals was 64% of that in the controls. On the other hand, the incorporation into mitochondrial catalase of the cobalt-treated animals was 67% of that in the controls even 30min

Table 1. Incorporation of [2-¹⁴C]glycine into the haem and protein moieties of catalase in homogenate from rats treated with cobalt

Rats were treated as described in Fig. 1; 2h after the cobalt injection 80 μ Ci of [2-¹⁴C]glycine were intraperitoneally administered to the rats. The animals were killed 4 h later and the radioactivities of the haem and protein moieties of catalase were determined as described in the Experimental section. The values are means $\pm$ s.e.m. of four animals.

Animals	Haem (c.p.m./mg of immunoprecipitate)	Radioactivity (d.p.m./mg of immunoprecipitate)	
		Immunoprecipitate	Apocatalase
Control	47.1 $\pm$ 5.4	2783 $\pm$ 180	2480 $\pm$ 138
Cobalt-treated	3.8 $\pm$ 0.4	2591 $\pm$ 175	2368 $\pm$ 169

Fig. 4. Incorporation of [³H]leucine into total protein and into the trichloroacetic acid-soluble fraction

Rats were injected daily for 4 days with 30 mg of CoCl₂/kg body wt. and then with 145 μ Ci of [³H]leucine 16 h after the last cobalt injection. The animals were killed at time-intervals specified in the Figure. Points and bars are the mean values $\pm$ s.e.m. of four animals. Total protein: $\square$ — $\square$, control; $\blacksquare$ — $\blacksquare$, cobalt-treated. Trichloroacetic acid-soluble fraction: $\square$ --- $\square$, control; $\blacksquare$ --- $\blacksquare$, cobalt-treated.

Table 2. *Effect of cobalt on specific radioactivity of hepatic free leucine*

Rats were treated as described in Fig. 4. The animals were killed 30 min after the [^3H]leucine injection ($145\mu\text{Ci}/\text{rat}$). The radioactivity and the concentration of free leucine were determined as described in the Experimental section. Free leucine concentration is calculated from the recovery of tyrosine added. The recovery of leucine was more than 94%. The values are means $\pm$ S.E.M. of four animals.

	Free leucine		
	(nmol/mg of protein)	(c.p.m./mg of protein)	(c.p.m./nmol)
Control	3.83 ± 0.19	196 ± 5	51.2
Cobalt-treated	3.24 ± 0.32	193 ± 13	59.6

Fig. 5. *Incorporation of [^3H]leucine into mitochondrial and cytosol catalase*

Rats were treated as described in Fig. 4. Catalase in each fraction was isolated immunochemically and counted for radioactivity as described in the Experimental section. Points and bars are the mean values $\pm$ S.E.M. of four animals. Control: $\circ$, mitochondrial; $\triangle$, cytosol. Cobalt-treated: $\bullet$, mitochondrial; $\blacktriangle$, cytosol.

after the injection and the ratio remained essentially unaltered.

In connexion with the possible transfer of a haemless intermediate of catalase from the cytosol to the

peroxisomes (Lazarow & de Duve, 1973b), the radioactivity in a sample of liver homogenate was calculated from the protein content of cytosol and mitochondrial fractions (Nakamura *et al.*, 1973). The decrease in the cytosol radioactivity in control rats between 30 min and 3 h after injection is less than the increase in the mitochondrial radioactivity, whereas in the cobalt-treated animals the former is about two times that of the latter.

Incorporation of 5-amino[2,3- ^3H]laevulinate

The time-courses of 5-amino[2,3- ^3H]laevulinate incorporation into trichloroacetic acid-soluble and -insoluble fractions of homogenate are shown in Fig. 6. No difference in the incorporation into the trichloroacetic acid-soluble fraction was observed between the cobalt-treated animals and the controls. The incorporation into the trichloroacetic acid-insoluble fraction in the cobalt-treated animals was lower than that in the controls, indicating an inhibition of haem synthesis. A similar pattern was obtained both in mitochondrial and cytosol fractions (not shown in the Figure).

Incorporation of 5-amino[^3H]laevulinate into catalase haem

In contrast with leucine incorporation into cytosol catalase, the radioactivity of cytosol catalase labelled with 5-amino[^3H]laevulinate did not decline in both the cobalt-treated animals and the controls as shown in Fig. 7. The radioactivity in the cytosol catalase of the cobalt-treated rats was lower than that of the control 30 min after the injection and was 20% of the control after 5 h. The radioactivity in the mitochondrial catalase in the cobalt-treated rats was 45% of that in the controls 30 min after injection and the ratio remained almost unchanged.

Control experiments for half-life determination

We carried out two control experiments to show that the assumptions inherent in the use of the

Fig. 6. Incorporation of 5-amino[³H]laevulinate into the trichloroacetic acid-insoluble and -soluble fractions of homogenate

Rats were treated with cobalt as described in Fig. 4 and received an injection of 68 μ Ci of 5-amino[³H]laevulinate 16 h after the last cobalt injection. The animals were killed at time-intervals specified in the Figure. Points and bars are the mean values $\pm$ S.E.M. of four animals. Trichloroacetic acid-insoluble fraction: $\square$ — $\square$, control; $\blacksquare$ — $\blacksquare$, cobalt-treated. Trichloroacetic acid-soluble fraction: $\square$ --- $\square$, control; $\blacksquare$ --- $\blacksquare$, cobalt-treated.

double-isotope technique for the half-life determination are valid. First, we examined whether the animals were in a steady state during the double-labelling experiments. Table 3 shows the hepatic catalase activities and rates of [4,5-³H]leucine incorporation into liver proteins when rats were injected daily for either 4 days or 8 days with either cobalt or isotonic saline. The results indicate that the animals were essentially in a steady state during the double-labelling experiments in both the cobalt-treated animals and the controls. Next we examined the ratio of

³H to ¹⁴C when rats were injected with [4,5-³H]leucine and [1-¹⁴C]leucine simultaneously. The ratios varied from 6.7 to 6.9, which was not identical with the initial ratio of 7.5 (Table 4). Accordingly these ratios were used for the calculation of the degradation rate.

Half-lives of hepatic proteins and catalase

Tables 5 and 6 show the degradation rates and half-lives of liver proteins and catalase in various sub-cellular fractions. The mean half-lives of proteins in

Table 3. Comparison of catalase activity and [^3H]leucine incorporation between rats injected with cobalt for 4 days and those injected for 8 days

Rats were injected daily for either 4 days or 8 days with 30 mg of CoCl_2/kg body wt.; they were then injected with $38\mu\text{Ci}$ of [^3H]leucine 16 h after the last cobalt injection and killed 4 h later. The values are means $\pm$ S.E.M. obtained from four animals. n.d., not determined.

Fractions	Catalase activity after: (k/mg of protein)		[^3H]Leucine incorporation after: (c.p.m./mg of protein)	
	4 days of injection	8 days of injection	4 days of injection	8 days of injection
Homogenate				
Control	27.7 ± 1.5	26.4 ± 0.8	628 ± 6.5	538 ± 22.0
Cobalt-treated	11.2 ± 0.4	8.0 ± 0.3	718 ± 55.5	617 ± 60.5
Mitochondrial				
Control	61.3 ± 4.4	67.8 ± 5.9	719 ± 32.5	635 ± 11.0
Cobalt-treated	25.9 ± 2.0	21.5 ± 1.9	749 ± 43.0	666 ± 42.2
Microsomal				
Control	2.70 ± 0.06	n.d.	1213 ± 19.5	983 ± 43.3
Cobalt-treated	1.14 ± 0.10	n.d.	1130 ± 16.5	880 ± 97.5
Cytosol				
Control	14.6 ± 0.5	16.0 ± 0.1	644 ± 38.5	579 ± 11.3
Cobalt-treated	4.5 ± 0.5	3.3 ± 0.2	770 ± 54.0	694 ± 52.4

Fig. 7. Incorporation of 5-amino[^3H]laevulinate into mitochondrial and cytosol catalase

Rats were treated as described in Fig. 6. Catalase in each fraction was isolated immunochemically. Points and bars are the mean values $\pm$ S.E.M. of four animals. At points without bars, the S.E.M. are not shown because of low values. Control: $\circ$, mitochondrial; Δ , cytosol. Cobalt-treated: $\bullet$, mitochondrial; $\blacktriangle$, cytosol.

Table 4. Ratios of $^3\text{H}/^{14}\text{C}$ of liver cell fractions from rats receiving simultaneous injections of [^3H]leucine and [^{14}C]leucine

Two rats were treated for 4 days as described in Fig. 4 and then received simultaneous injections of $10\mu\text{Ci}$ of [^{14}C]leucine and $75\mu\text{Ci}$ of [^3H]leucine. The animals were killed 4 h later.

Fractions	Specific radioactivity (d.p.m./mg of protein)		Ratio of $^3\text{H}/^{14}\text{C}$
	^{14}C	^3H	
Homogenate			
Control	1065	7127	6.69
Cobalt-treated	1204	8201	6.81
Mitochondrial			
Control	1212	8269	6.82
Cobalt-treated	1330	8908	6.70
Microsomal			
Control	1822	12610	6.92
Cobalt-treated	1816	12491	6.88
Cytosol			
Control	1027	7034	6.85
Cobalt-treated	1237	8497	6.87

each subcellular fraction of the control animals were in close accord with the observations of previous authors who used [$\text{guanidino-}^{14}\text{C}$]arginine (Glass & Doyle, 1972). Appreciable increases in the half-lives of the proteins were observed when the animals were treated with cobalt.

Table 5. Mean half-lives of liver cell fractions from rats treated with cobalt

Rats were treated daily with cobalt as described in Table 3 and then they were given an injection of 30 μ Ci of [1- 14 C]leucine; 4 days later each rat received 225 μ Ci of [4,5- 3 H]leucine. The animals were killed 4 h after the second isotope injection. The 3 H incorporations are corrected by the ratios of 3 H/ 14 C given in Table 4. The values are means $\pm$ S.E.M. of three animals. a is the experimental 3 H/ 14 C ratios given in Table 4.

Fractions	10 ⁻³ $\times$ Radioactivity (d.p.m./mg of protein)		$\frac{^3\text{H}}{^{14}\text{C}} \times \frac{1}{a}$	k_d (day ⁻¹)	$t_{\frac{1}{2}}$ (days)
	^{14}C	^3H			
Homogenate					
Control	1.43 $\pm$ 0.06	21.9 $\pm$ 0.5	2.29	0.207	3.3 (3.2)*
Cobalt-treated	2.13 $\pm$ 0.14	24.0 $\pm$ 1.3	1.66	0.127	5.5
Mitochondrial					
Control	1.38 $\pm$ 0.07	24.3 $\pm$ 0.6	2.58	0.237	2.9
Cobalt-treated	1.81 $\pm$ 0.07	20.9 $\pm$ 1.2	1.72	0.136	5.1
Microsomal					
Control	1.40 $\pm$ 0.02	32.4 $\pm$ 0.2	3.34	0.320	2.3 (2.5)*
Cobalt-treated	1.99 $\pm$ 0.11	30.0 $\pm$ 0.2	2.19	0.196	3.5
Cytosol					
Control	1.52 $\pm$ 0.10	21.3 $\pm$ 0.8	2.04	0.178	3.9 (3.7)*
Cobalt-treated	2.19 $\pm$ 0.15	22.2 $\pm$ 0.9	1.47	0.096	7.2

* Values from Glass & Doyle (1972).

Table 6. Half-lives of catalases from rats treated with cobalt

Rats were treated as described in Table 5. Catalase was isolated immunochemically from each cell fraction and counted for radioactivity as described in the Experimental section. The 3 H incorporations are corrected by the ratios of 3 H/ 14 C given in Table 4. The values are means $\pm$ S.E.M. of three animals. a is the experimental 3 H/ 14 C ratios given in Table 4.

Fractions	Radioactivity (d.p.m./mg of immunoprecipitate)		$\frac{^3\text{H}}{^{14}\text{C}} \times \frac{1}{a}$	k_d (day ⁻¹)	$t_{\frac{1}{2}}$ (days)
	^{14}C	^3H			
Homogenate					
Control	270 $\pm$ 4.1	5779 $\pm$ 237	3.20	0.291	2.4 (2.5)*
Cobalt-treated	331 $\pm$ 13.2	7084 $\pm$ 434	3.14	0.286	2.4
Mitochondrial					
Control	436 $\pm$ 25.2	9008 $\pm$ 333	3.04	0.278	2.7
Cobalt-treated	473 $\pm$ 55.0	8516 $\pm$ 307	2.69	0.247	2.8
Cytosol					
Control	355 $\pm$ 4.7	6955 $\pm$ 207	2.86	0.263	2.6
Cobalt-treated	844 $\pm$ 79.1	12660 $\pm$ 924	2.18	0.195	3.6

* Value from Poole *et al.* (1969).

The half-lives of catalase in both the cytosol and mitochondrial fractions were the same (2.5 days), which agreed with that of total catalase protein reported by Poole *et al.* (1969) using [guanidino- 3 H] arginine. The half-life of catalase in the homogenate and in the mitochondrial fraction was not changed by the cobalt treatment. The half-life of cytosol catalase in the cobalt-treated rats was appreciably prolonged, which is hard to explain.

Discussion

Hepatic catalase activity was decreased by the treatment of rats with cobalt. The decrease observed in catalase protein, as determined by immunotitration, was in accord with the decrease in catalytic activity. This result is supported by the observation that the catalase activity did not alter by incubation of liver homogenate with 1 mM-CoCl₂ at 37°C for 1h.

The decrease in catalase protein could not be explained by the effect of cobalt on protein synthesis, since incorporation of radioactive glycine into catalase protein was unaltered by cobalt administration, but its incorporation into catalase haem was dramatically decreased. This observation, together with those in which radioactive leucine and 5-aminolaevulinate were used, imply that the administration of cobalt inhibited haem synthesis and thereby decreased the amount of catalase. This is analogous to the observation by Marty *et al.* (1970) that in vitamin E-deficient rats that partially fail to synthesize haem, haematin enzyme activities including catalase decrease and non-haematin enzyme activities remain unaltered.

Enzyme concentrations in liver are maintained by the balance of synthesis and degradation. When rats were in a steady state with the daily administration of cobalt, the incorporation of radioactive leucine into total liver proteins was the same as that in the control. On the other hand, the incorporation rates of the radioactive leucine over the initial 30 min into catalase in mitochondria and in cytosol were 67% and 100% of the control respectively, which are not sufficiently low to explain the depression in the catalase activity. The half-life of catalase protein as measured by the double-labelling method showed no stimulation of the decay by the cobalt treatment, indicating that the degradation may not be related to the catalase concentration in this case.

Lazarow & de Duve (1973a,b) carried out a thorough investigation on the incorporation of radioactive leucine and 5-aminolaevulinate into rat hepatic catalase. They have shown the appearance of a haemless intermediate of catalase in the cytosol. The rapid increase and the subsequent decrease in the leucine radioactivity in the cytosol first observed by Higashi & Peters (1963) and later by Redman *et al.* (1972) was explained by the transfer of this haemless intermediate to peroxisomes to be completed to the holoenzyme there. We observed that the initial rate of leucine incorporation into the immunoprecipitate in the cytosol was essentially unaltered by cobalt treatment and that the decrease in the cytosol radioactivity between 30 min and 3 h after injection was about twice the increase in the mitochondrial radio-

activity in cobalt treated rats (see Fig. 5). These results urge us to postulate the degradation of the haemless intermediate in conditions when the supply of haem was limiting the formation of holoenzyme.

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