Biochem. J. (1984) **217**, 85–92
Printed in Great Britain

A comparison of isometallothionein synthesis in rat liver after partial hepatectomy and parenteral zinc injection

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(*Received 18 May 1983/Accepted 31 August 1983*)

The time course of hepatic zinc-isometallothionein synthesis was studied in the regenerating liver and compared with that produced after the parenteral injection of zinc (6mg of Zn^{2+}/kg). In the regenerating liver, zinc levels rose rapidly after partial hepatectomy and reached a maximum at approx. 14h before declining to approximately normal levels at 48h post-operation. During this 48h period most of the zinc was incorporated into metallothionein. Purification of the latter into the charge-separable isometallothioneins (i.e. MT1 and MT2) showed that, in the regenerating liver, there was an unequal distribution of zinc between the two isoproteins. Thus at operation the endogenous thionein had an MT2/MT1 ratio of 1; after regeneration this ratio increased, and all times during the time course there was more MT2 than MT1. In contrast, the intraperitoneal injection of zinc produced a biphasic uptake of zinc into the liver with maxima at 10h and 32h. During the first phase of zinc uptake, metallothionein synthesis increased rapidly and, unlike the regenerating liver, the MT2/MT1 ratio of 1 remained constant. Thereafter, this ratio increased in a manner analogous to that exhibited by the regenerating liver. Half-life determinations for thionein disappearance/degradation shows that MT2 and MT1 were degraded with half-lives ($t_{\frac{1}{2}}$) of 26.18h and 16.44h respectively in the regenerating liver and 14.75h and 9.3h after zinc injection. Thus thionein disappearance/degradation in the regenerating liver was slower than that seen after zinc injection. However, in both situations MT2 was always removed at a slower rate than MT1. Calculation of the rates of thionein synthesis (assuming the above disappearance rates were constant throughout the time course) showed that, in the regenerating liver, the rate of MT2 synthesis was approximately twice that of MT1. This was not the case after zinc injection, where both isometallothioneins were synthesized in equal amounts. These results demonstrate that the rates of synthesis of MT2 and MT1 can be altered according to the metabolic status of the cell and suggest a specific role for MT2 during liver regeneration.

Metallothionein is an inducible low-M_r metallothionein originally isolated as a cadmium- and zinc-containing protein from horse kidney (Margoshes & Vallee, 1957). In the liver and other tissues there are at least two isometallothioneins (MT1 and MT2), which have the same M_r and similar amino acid composition (see Webb, 1979), but differ in overall negative charge [see, e.g., Fig. 4 of Cain & Holt (1979)]. Piscator (1964) originally suggested that thionein was a "protective detoxifying mechanism" for cadmium, but later studies have shown that, in both the young and adult animal, the protein is involved in the homoeostatic control of zinc and copper [see, for a review, Webb & Cain (1982)]. The exact functions of metallothionein in cell metabolism are unknown, although it is apparent, in the liver at least, that there is a close association between cell growth and zinc-thionein synthesis. In the rat, for example, hepatic thionein is undetectable at 16 days gestation, but increases rapidly thereafter to a maximum at 2 days after birth. The total hepatic zinc-thionein content then remains fairly constant until about day 15, after

Abbreviations used: MT1 and MT2, isometallothioneins 1 and 2.

which it declines steadily to the adult level at 26 days of age (Mason *et al.*, 1981). Bell (1980) believes that, in the newborn liver, the thionein is acting as a general storage site for zinc during neonatal development. An analogous situation occurs in the adult regenerating liver, since after partial hepatectomy there is an increase in liver zinc and a concomitant synthesis of thionein (Ohtake *et al.*, 1978). The accumulation of zinc-thionein occurs before the increase in DNA synthesis which is essential to the growth process. The specific functions of zinc-thionein in cell growth are poorly understood, and the simplest suggestion is that the protein acts as a reservoir of essential zinc which is used to supply zinc-dependent processes.

This may occur by direct cation donation as demonstrated *in vitro* by Udom & Brady (1980), or alternatively the cation may be made available by thionein degradation, which in the case of zinc-thionein has a half-life ($t_{\frac{1}{2}}$) of approx. 18h (Feldman & Cousins, 1976). However, these mechanisms do not explain the need for two apparently similar isometallothioneins, and it is interesting that Ohtake & Koga (1979) reported that only one form is synthesized after partial hepatectomy. This could indicate a specific role for this isometallothionein in cell regeneration. In the present study we have examined the time course of isometallothionein synthesis after partial hepatectomy and compared this with that seen after the parenteral injection of zinc. The results show that both MT1 and MT2 are produced in the regenerating liver, albeit in different amounts.

Materials and methods

Animals and chemicals

Male Wistar LAC/P rats (180–200g) were used for all experiments and were maintained on a standard laboratory diet (MRC-41B).

All chemical reagents were of AnalaR and Aristar grade and were obtained from the sources detailed previously (Cain & Holt, 1979). Sephadex G-75 and DEAE-cellulose DE52 were obtained from Pharmacia, London W5 5SS, U.K. Benzylpenicillin was purchased from Glaxo, Greenford, Middx., U.K., and was dissolved in iso-osmotic NaCl (saline) (5000units/ml).

Animal treatments

Rats were partially hepatectomized under diethyl ether anaesthesia by the removal of approximately two-thirds of the liver as described by Higgins & Anderson (1931). Each animal received 500 units of benzylpenicillin intraperitoneally, before the abdomen was closed. Sham-operated animals were treated in exactly the same manner, except that the liver, after exteriorization, was relocated without hepatectomy. At appropriate times after the operation the animals were killed by decapitation and the livers removed, samples taken for metal analysis and the remaining tissue frozen in liquid N_2 before storage at $-20°C$.

Parenterally injected rats were given 6mg of Zn^{2+}/kg body weight at $ZnCl_2$ in saline either intraperitoneally or intravenously. The animals were then killed and the dissected livers treated as described for the partially hepatectomized animals.

Liver fractionation

The frozen liver samples from two to three rats were thawed, pooled and homogenized in 2–3vol. of ice-cold 10mM-ammonium formate, pH8.0. The homogenate was then centrifuged as previously described (Cain & Holt, 1979) to yield a 'cytosol' fraction which was sampled for metal analysis. The remaining cytosol was then analysed for its isometallothionein content.

Isometallothionein analysis

Zinc-isometallothioneins were assayed by gel-filtration and anion-exchange chromatography essentially as described previously (Cain & Holt, 1979). Briefly, the cytosol fractions were chromatographed on Sephadex G-75 by using either a 80cm × 2.5cm or 80cm × 5cm column (depending on cytosol volume) equilibrated in 10mM-ammonium formate buffer, pH8.0 (5°C). The crude metallothionein (a mixture of MT1 and MT2) was then eluted at a flow rate of 20ml/h (80cm × 2.5cm) or 60ml/h (80cm × 5cm) and collected in 4ml fractions, which were analysed for Zn^{2+} and Cu^{2+}. Those fractions corresponding to the MT peak (V_e/V_o = 1.8–2.0) were pooled, assayed for metals and then applied without preconcentration to a DEAE-cellulose DE 52 anion-exchange column (30cm × 1.5cm) equilibrated in 10mM-Tris/HCl buffer, pH8.0. MT1 and MT2 were then eluted with 400ml of a 10–200mM-Tris/HCl (pH8.0) gradient at a flow rate of 30ml/h and the eluate collected in 3ml fractions, which were assayed for Zn^{2+} and Cu^{2+}. In some cases (where the amount of thionein was low) a smaller column (20cm × 1.5cm) was used and eluted with a 200ml gradient; 1ml fractions were collected.

Analytical methods

Zn^{2+} and Cu^{2+} were determined by atomic-absorption spectrophotometry with a Perkin–Elmer 460 instrument. Tissue samples or cytosol fractions were wet-ashed with a $HNO_3/HClO_4$ mixture (4:1, v/v), the ash redissolved in 5% HCl and then analysed for metals. Column samples were aspirated directly into the spectrophotometer without pretreatment.

Results

The effect of partial hepatectomy on the zinc concentration of the liver was monitored for 48h after operation. As Fig.1 shows, the zinc level began to rise within 6h after the operation and reached a maximum of approx. 20μg of Zn^{2+}/g at 14h. Thereafter the zinc concentration gradually declined to near normal levels at 48h. The sham-operated animals showed a much smaller increase of 7μg of Zn^{2+}/g, which reached a plateau at about 20h and remained relatively constant up to at least 48h. Fig. 2 shows the increase in cytosolic and metallothionein-bound zinc after partial hepatectomy. Approx. 70% (13–14μg/g) of the increased hepatic zinc was located in the cytosol bound as metallothionein. A similar situation was observed in the sham-operated animals, except that a much smaller percentage of cytosolic zinc was bound to metallothionein. These results are in agreement with the data of Ohtake *et al.* (1978) and clearly show that partial hepatectomy leads to a marked increase in liver zinc and the concomitant synthesis of metallothionein. Copper also binds to zinc-thionein (Bremner & Davies, 1975),

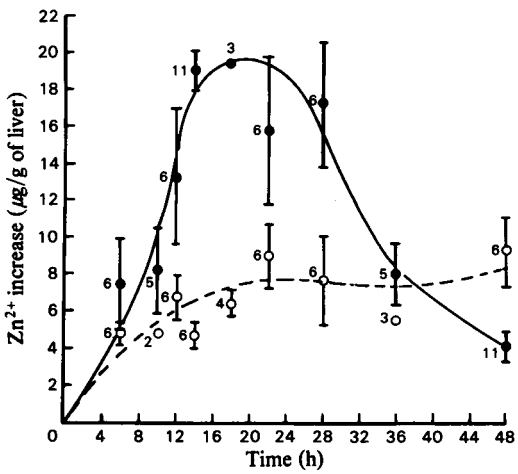

Fig. 1. *Increase in liver zinc concentration after partial hepatectomy or sham operation*

Rats were operated on as described in the Materials and methods section. At various time intervals the animals were killed and the livers removed and sampled for zinc and copper. The zinc increase was calculated with respect to the normal metal concentration, which was determined from the tissue removed at the time of hepatectomy and was 28.26 ± 0.34 (S.E.M.)μg/g (n = 68). The results are then given as the means ± S.E.M. with the numbers referring to the number of animals. ●, Partially hepatectomized animals; ○, sham-operated animals.

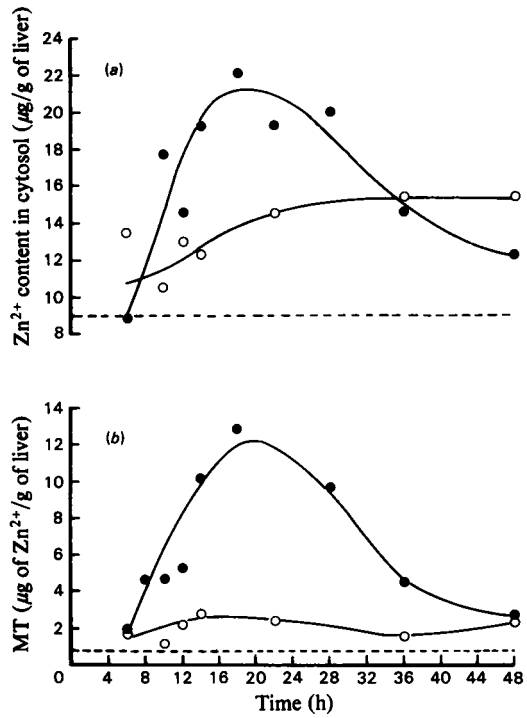

Fig. 2. *Increase in zinc concentration in cytosol and total metallothionein concentration after partial hepatectomy and sham operation*

Frozen tissues from two to three rats were pooled and the cytosol and crude metallothionein fraction (i.e. Sephadex G-75-purified fraction) prepared as described in the Materials and methods section. In the case of the 14 and 48h time points the symbols represent the mean results for two separate pooled preparations. Closed and open symbols refer to partially hepatectomized and sham-operated animals respectively. The cytosol results are shown in (*a*) and the metallothionein data in (*b*); in each case the broken horizontal line refers to the normal level of cytosolic and metallothionein (MT) zinc as determined on two separate preparations derived from pooled liver samples removed at the time of operation.

and Fig. 3 shows that there was a marked increase in hepatic copper during the regenerative process. Unlike the zinc, however, the copper concentration rose rapidly after the operation and reached a peak at 14h, after which it declined until 20h, before it increased again; at 48h it was still rising. The initial increase in hepatic copper coincided with the elevated levels of zinc and thionein synthesis. Fig. 3(*b*) shows that most of the copper during this period was located in the cytosol bound as metallothionein (i.e. a zinc/copper-thionein). After 20h, however, the increase in total copper

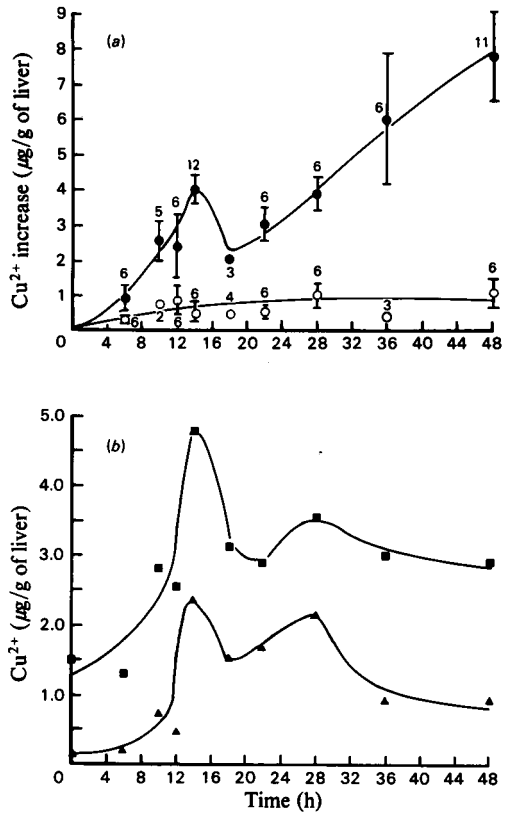

Fig. 3. *Increase in copper concentration in the liver after partial hepatectomy*
The experimental details are described in Figs. 1 and 2. In (*a*) the total copper concentration is shown after partial hepatectomy (●) and sham-operation (○). The cytosolic (■) and metallothionein (▲) copper after partial hepatectomy is shown in (*b*).

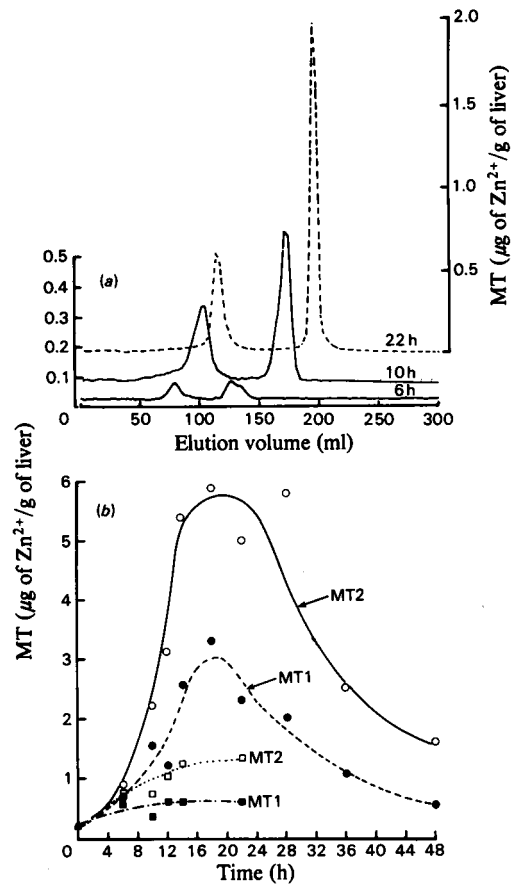

Fig. 4. *Isometallothionein synthesis after partial hepatectomy and sham-operation*
The crude metallothionein fractions isolated by Sephadex G-75 filtration (see Fig. 2) were separated into the purified isometallothioneins MT1 and MT2 by anion-exchange chromatography as described in the Materials and methods section. In (*a*), representative DEAE-cellulose DE 52 elution profiles obtained from partially hepatectomized animals are shown (n.b.: right-hand scale refers to 22h time point). The full time course of synthesis and degradation of MT1 (closed symbols) and MT2 (open symbols) in both sham-operated and hepatectomized animals is presented in (*b*). The latter are shown as circles and the former are depicted as squares. The endogenous levels are shown as the zero time point.

was not equalled by the thionein-bound metal, which returned to more normal levels.

Anion-exchange chromatography of the crude metallothionein showed that both isometallothioneins were synthesized (see Fig. 4*a* for representative elution profiles) after partial hepatectomy. The time course of isometallothionein synthesis and disappearance is shown in Fig. 4(*b*), from which it is apparent that the levels of MT2 were greater than those of MT1 at all times. Thus at 0h the endogenous MT2/MT1 ratio was 1.0; after hepatectomy there was a preferential accumulation of MT2, and at 18h the ratio had increased to 1.8. Thereafter the isometallothioneins gradually disappeared (presumably due to protein degradation), but not at the same rate, and as a result the MT2/MT1 ratio continued to rise; by 48h it was 2.8. A similar pattern was also observed with the sham-operated animals. In contrast the ratio of the copper content of the two forms was approximately unity at operation and did not vary appreciably throughout the regenerative process.

The metabolic state of the regenerating liver is quite different from that of the normal non-growing adult liver and it was essential to compare the time course of isometallothionein synthesis in the

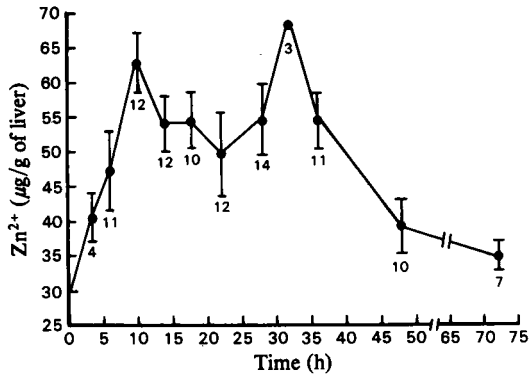

Fig. 5. *Hepatic zinc uptake after an intraperitoneal injection of zinc*

Rats were injected with 6mg of Zn^{2+}/kg body wt. intraperitoneally. At the indicated times the animals were killed and the livers removed and sampled for zinc. The results are expressed as the mean ± S.E.M.; the normal level is given as the zero time point.

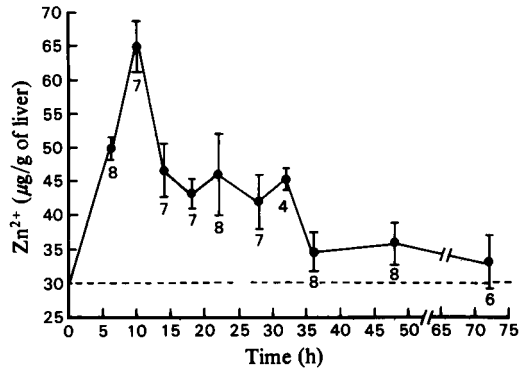

Fig. 7. *Hepatic zinc uptake after an intravenous injection of zinc*

Rats were injected with 6mg of Zn^{2+}/kg body wt. intravenously. At the indicated times the animals were killed and the livers removed and sampled for zinc. The results are expressed as the mean ± S.E.M.; the normal zinc level is given as a broken line.

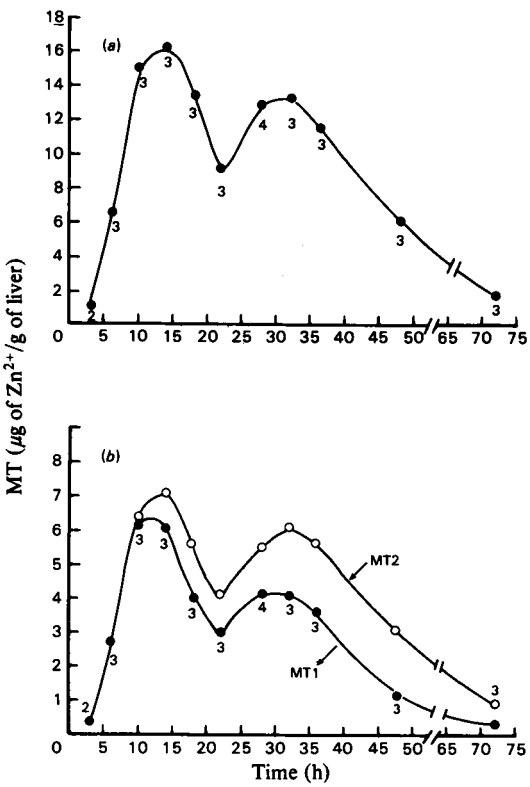

Fig. 6. *Metallothionein synthesis after zinc injection*

Crude metallothionein (*a*) and the purified isometallothioneins (*b*) were prepared as described in Fig. 3 from pooled livers (two to three livers per preparation) of rats injected with zinc (see Fig. 4). The results are presented as the means of (usually) three separate preparations.

former with the latter. Consequently, thionein synthesis was studied in rats injected intraperitoneally with 6mg of Zn^{2+}/kg body weight. Preliminary experiments showed that this dose resulted in hepatic zinc concentrations comparable with those achieved after partial hepatectomy. Fig. 5 shows the time course of zinc uptake in the liver after the injection of the cation, and it can be seen that the increase in hepatic zinc followed a biphasic curve with peaks at 10h and 32h respectively. A similar profile was observed in the cytosol (results not shown) and metallothionein fractions (Fig. 6*a*). The latter was further purified by anion-exchange chromatography and the isoproteins separated. Fig. 6(*b*) shows that, up to 10h, equivalent amounts of MT1 and MT2 were synthesized. Thereafter, however, the ratio of MT2/MT1 gradually increased (compare with partial hepatectomy, Fig. 4) up to a value of 2.5 at 72h. This biphasic response was only seen after the intraperitoneal injection of zinc. Thus the same dose given intravenously resulted in a single component uptake curve with a maximum at 10h after injection (Fig. 7).

Thionein levels are regulated by the rates of synthesis and disappearance (degradation). In the case of cadmium-thionein, the rate of synthesis is equalled by the rate of protein degradation, and thus although there is a continual turnover of the protein (t_i = 3–6 days), the level of thionein-bound cadmium remains constant (Cain & Holt, 1979). In contrast, after zinc administration, thionein synthesis (as measured by [^{35}S]cysteine incorporation and metallothionein mRNA production) reaches a maximum at 5–7h and then returns to normal (endogenous) levels by 20h (Squibb *et al.*, 1977;

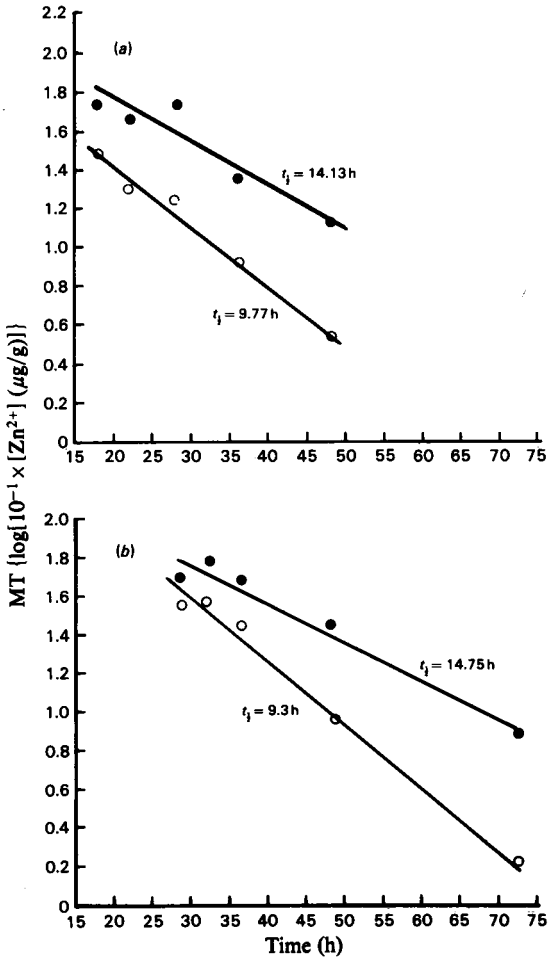

Fig. 8. *Determination of degradation rates ($t_{\frac{1}{2}}$) for zinc-isometallothioneins after partial hepatectomy (a) and zinc injection (b)*

The degradation rates were determined from semi-logarithmic plots of thionein-bound zinc versus time. Lines of best fit and half-times ($t_{\frac{1}{2}}$) were calculated by a least-squares computer program and are indicated in the Figures. The data for partial hepatectomy and zinc-injection experiments are taken from Fig. 4 and Fig. 6 respectively, and all values are corrected for the endogenous MT1 and MT2 levels. ○, MT1; ●, MT2.

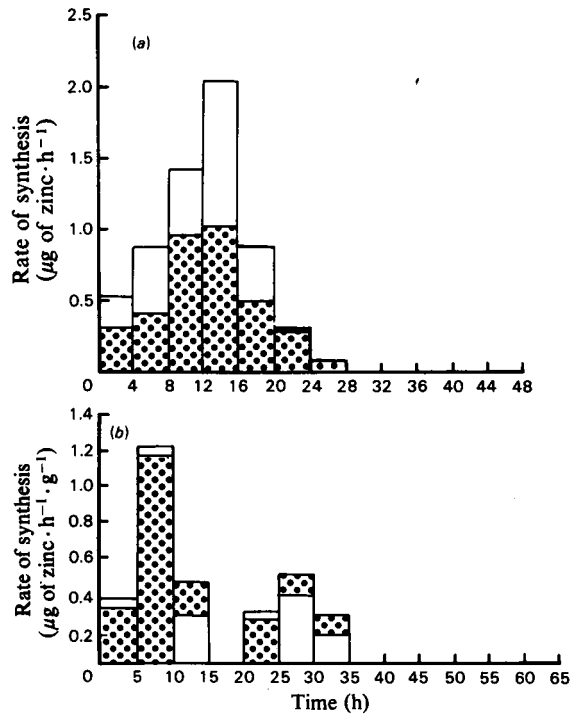

Fig. 9. *Rates of isometallothionein synthesis after partial hepatectomy (a) and zinc injection (b)*

Rates of synthesis were calculated on the assumption that the degradation rates were constant throughout the time course of isometallothionein accumulation. In the case of the partial hepatectomy the rates of synthesis were calculated from the total-metallothionein-content-versus-time curves and using the $t_{\frac{1}{2}}$ values of 16.44h and 26.18h for MT1 and MT2 respectively. For the zinc-injected animals the data were calculated from Fig. 6(*b*) by using the $t_{\frac{1}{2}}$ determined in Fig. 8(*b*). The shaded and open histograms refer to MT1 and MT2 respectively. In both cases the quantification of MT1 and MT2 is based on the zinc content of the isometallothioneins.

Shapiro *et al.*, 1978). At the latter time, zinc incorporation into thionein is maximal; thereafter the protein (as measured by [^{35}S]cysteine incorporation) and its zinc disappear from the liver at the same rate ($t_{\frac{1}{2}}$ approx. 19h; Feldman & Cousins, 1976). The simultaneous loss of metal and protein has been interpreted by the latter authors to show that zinc-thionein is degraded with the concomitant release of zinc that is not reincorporated into new thionein. A similar correlation was obtained for total metal (copper plus zinc) and ^{35}S disappearance in studies on hepatic zinc/copper thioneins (Bremner *et al.*, 1978). The rate of disappearance of the thionein-bound zinc can therefore be used as a reasonable index of zinc-thionein degradation [see also the Discussion in Feldman & Cousins (1976)]. In Fig. 8, semilogarithmic plots of thionein-bound zinc versus time were used to determine the rates of degradation of the isometallothioneins. The time points used for the semilogarithmic plots were obtained from Fig. 4 and Fig. 6. In the regenerating liver (Fig. 8*a*), MT1 had a shorter half-life [$t_{\frac{1}{2}} = 9.77 \pm 0.7$ (S.E.M.)h] than MT2 ($t_{\frac{1}{2}} = 14.13 \pm 2.85$h). In the case of the zinc-injection experiments the half-lives were determined from the second phase of the uptake

curve and showed that the degradation of MT1 ($t_{\frac{1}{2}} = 9.3 \pm 0.6$h) was significantly shorter than MT2 ($t_{\frac{1}{2}} = 14.75 \pm 1.74$h). Similar values were obtained when the total metal (i.e. zinc plus copper) disappearance was used to determine the rate of degradation. The half-times for zinc-thionein degradation were somewhat lower than previously reported values (Feldman & Cousins, 1976); however, the latter workers determined the degradation time of the crude metallothionein (i.e. Sephadex G-75 preparation) without correcting for contaminating proteins. As discussed by Bremner *et al.* (1978) and Cain & Holt (1979), this can lead to an overestimate of the half-life. This objection does not apply to the anion-exchange-purified proteins.

Discussion

The results presented in the current study confirm the findings of Ohtake *et al.* (1978) that partial hepatectomy leads to an increase in liver zinc and thionein synthesis. In subsequent studies Ohtake & Koga (1979) purified the thionein by gel filtration and anion-exchange chromatography 14h after the partial removal of the liver. They reported that only one form of metallothionein was synthesized and speculated that this isoprotein may have a specific role in liver regeneration. The results described in the present paper do not fully support these findings, since both MT1 and MT2 were isolated at all times throughout the time course of regeneration (Fig. 5). However, it was clear that the rate of accumulation of MT2 was greater than that of MT1. A similar result was observed for the sham-operated animals. Although it is difficult to understand why Ohtake & Koga (1979) failed to identify both isometallothioneins in the regenerating liver, it should be pointed out that the separation of MT1 and MT2 is based on protein charge differences. Consequently any changes in the overall charge of the isoprotein which could occur during the preparative procedures may result in MT1 and MT2 being eluted as a single peak. In this context it is of interest to note that the elution positions of MT1 and MT2 on the anion-exchange column changed during the time course (Fig. 3*a*), indicating that the newly synthesized thioneins were more negatively charged than the endogenous proteins.

The comparison of isometallothionein synthesis in the regenerating liver with that of the normal liver after parenteral zinc injection produced some surprising results. Firstly, the time course of hepatic zinc uptake after intraperitoneal zinc injection followed a biphasic curve, with an initial zinc uptake between 0 and 12h and a further uptake at 22–36h after injection. Other workers studying hepatic zinc uptake after an intraperitoneal injection of zinc have not reported this phenomenon (Davies *et al.*, 1973; Richards & Cousins, 1975; Suzuki & Yamamura, 1980). However, these earlier studies used time courses which concentrated on the initial 24h after injection. Thereafter, the time points were usually placed at 24h intervals (see, e.g., Suzuki & Yamamura, 1980). If similar time points were used in the present study (Fig. 5), the pattern of zinc uptake would be a simple one-component curve reaching a maximum at around 12h and then gradually declining with time [i.e. similar to that previously described by Richards & Cousins (1975) and Suzuki & Yamamura (1980)]. The second phase of hepatic zinc uptake suggests that a proportion of the injected zinc is initially stored in some other tissue and then released into the blood at a later time. In support of this suggestion, Davies & Bremner (1974) reported that the pancreas was initially involved in the hepatic uptake of zinc after an intraperitoneal injection of $ZnSO_4$. It is therefore possible that the rate of uptake and release of zinc into this tissue is dependent on the route of administration, and this may contribute to the second phase of zinc uptake seen in the present study.

The differences in the degradation times for MT1 and MT2 provide an explanation for the changing MT2/MT1 ratios observed in the present study and also by Suzuki & Yamamura (1980). Thus an increase in liver zinc (by either parenteral injection or partial hepatectomy) leads to a marked stimulation of MT1 and MT2 synthesis. The stimulation of synthesis is not maintained and returns to normal levels; thereafter the concentration of both isoforms would be determined by their respective degradation rates and thus the MT2/MT1 ratio increases with time. In the liver of the zinc-injected animals the MT2/MT1 ratio is approximately unity during the synthesis stage (i.e. 0–10h, Fig. 4). This would imply that the rate of synthesis of MT1 is greater than, or at least very similar to, that of MT2. This is obviously not the case in the regenerating liver or sham-operated animals (Fig. 6), where it appears that MT2 has a much greater rate of synthesis. The protein content of the regenerating liver is known to increase dramatically, and Scornik and co-workers (Scornik, 1974; Scornik & Botbol, 1976) believe that the most important factor in this process is a decrease in protein degradation. This does not seem to be the case with the isometallothioneins, since the half-times for degradation were very similar to that observed in the zinc-injected animals (Fig. 8). However, these degradation times are based on the concentration of zinc-thionein in the liver (i.e. μg/g). In the case of the zinc-injected animals there is no change in liver weight and thus the half-times determined in Fig. 8 are a true representation of the degradation rate. In contrast, the regenerating

liver is increasing in weight (approx. 60% from 18 to 48 h) and the half-times determined on a concentration basis (Fig. 8) do not take this into account. Recalculating, the degradation rates, using the value for total isometallothionein content, showed that in the regenerating liver, MT1 and MT2 had half-times of 16.44 ± 0.63 (S.E.M.)h and 26.18 ± 3.8 h respectively. Thus the rate of degradation in the regenerating liver of both isoforms is approx. 60% slower than that observed in the normal liver after zinc injection. This reduction in the degradation rate is similar to that observed for other proteins in the regenerating liver (Scornik & Botbol, 1976). Nevertheless, the relative differences in the degradation rates of MT1 and MT2 are maintained after partial hepatectomy, and it is unlikely that the changes in degradation can explain the differences in the amounts of MT1 and MT2 accumulated during liver regeneration. The simplest explanation is that the rate of synthesis of MT1 is less than that of MT2 in the regenerating liver and obviously further experiments are required to test this. On the other hand, the rates of MT1 and MT2 synthesis can be estimated by assuming a constant degradation rate. The calculated values are shown in Fig. 9 and demonstrate in the regenerating liver that the rate of MT2 synthesis is 2-fold greater than that of MT1 (Fig. 9*a*). By contrast the rates of MT1 and MT2 synthesis after zinc injection are very similar (Fig. 9*b*).

The significance and the mechanisms determining the different rates of synthesis for MT1 and MT2 in the regenerating liver are unknown. However, it is apparent from other studies that Cd-MT1 (Cain & Holt, 1979) and Zn-MT1 (Andersen *et al.*, 1978) are more readily degraded than Cd-MT2 and Zn-MT2 respectively. Furthermore, recent studies (Bremner & Mehra, 1983) have shown that Zn-MT1 is also more readily degraded *in vitro* by lysosomal proteinases. A possible explanation for these findings is that the tertiary structure of Cd or Zn-MT2 is such that the protein is relatively resistant to proteinases. In support of this, ^{113}Cd n.m.r. studies have shown that the cadmium-isoproteins have different conformations (Nicholson *et al.*, 1983). Furthermore, Winge & Miklossy (1982) have shown that the isometallothioneins have different binding affinities for zinc and differ in their ability to reconstitute the apoenzyme of carbonic anhydrase. This is remarkable in view of the marked similarities in the amino acid composition of the two isoproteins (see Webb, 1979). It is possible, therefore, that isometallothioneins have specific functions in the cell which are related to their conformations and that the regenerating liver has a specific requirement for MT2.

We thank Dr. V. J. Cunningham for helpful advice on the calculation of synthesis rates.

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