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DRUG-METABOLIZING ENZYMES IN RAT, MOUSE, PIG AND HUMAN MACROPHAGES AND THE EFFECT OF PHAGOCYTIC ACTIVATION

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Abstract—Aryl hydrocarbon hydroxylase (AHH) activity mediated by cytochrome P-450 is present in pig hepatic microsomes [10 nmol · 3 mg protein^{-1} · hr^{-1}]. AHH activity was detectable in both hepatocytes and Kupffer cells isolated from pig liver biopsy material. These cells were isolated from needle or wedge biopsy material by collagenase perfusion and incubation with collagenase at 37°. The two cell types were separated from the resulting cell suspension as previously described for whole liver. Kupffer cells were enriched by adherence and were cultured for 24 hr prior to harvesting. Cells were harvested, and cell viability was determined. AHH activity was assayed in Kupffer cell and hepatocyte homogenates. Kupffer cell AHH activity was approximately one-eighth the level detected in hepatocytes. To determine whether this enzyme was present in other macrophages, monocytes were isolated from 10 ml of heparinized peripheral blood using Ficoll–Hypaque and were enriched by adherence. After 24 hr in culture, cell viability was assessed and monocytes were identified by cytochemical staining. AHH activity was detectable in pig monocyte homogenates, and the AHH level was similar to that in pig Kupffer cells. AHH was also easily detectable in human monocytes. This macrophage AHH activity was compared with AHH activity in rat monocytes, mouse Kupffer cells and mouse peritoneal macrophages. Monocyte AHH was relatively stable in cell culture but decreased rapidly upon storage at −70°. Macrophage AHH activity was depressed following phagocytic activation *in vitro* by latex beads with a concomitant increase in heme oxygenase activity.

The macrophages are members of the reticuloendothelial system. In the present study, peritoneal macrophages, peripheral blood monocytes and liver Kupffer cells were used. The major metabolic function of the macrophages is the metabolism of hemoglobin from red blood cells to bilirubin [1]. However, drug-metabolizing enzymes such as aryl hydrocarbon hydroxylase (AHH) have been detected in Kupffer cells [2] and peripheral blood monocytes [3–6].

Though the contribution of macrophage drug-metabolizing enzymes to the total enzyme pool is probably quite small, a measure of their activity may indicate the status of the drug-metabolizing enzymes in the liver. In this study we compared the drug-metabolizing enzymes in peripheral blood monocytes with the enzymes in other macrophages and in whole liver. In addition, we investigated whether the monocyte enzymes behave like the liver macrophage enzymes in response to phagocytic activation.

MATERIALS AND METHODS

Chemicals. Dextran sulfate (500,000 daltons mol. wt) and latex beads (0.45 μm) were obtained from the Sigma Chemical Co., St. Louis, MO.

Animals. Male Swiss mice (25–30 g) were obtained from the Jackson Laboratories, housed on clay chips, and fed Purine mouse chow.

Male Sprague–Dawley rats (200 g) were obtained from Canadian Hybrid Farms, Nova Scotia, and fed Purina rat chow.

Pigs were obtained from Thomas Farms, Nova Scotia, housed in our facility for at least 1 week prior to testing, and fed Purina pig chow.

Human subjects were normal volunteers age 19–67 (eight males and eleven females) having no history of liver disease and normal liver function tests on SMAC (sequential multiple analysis).

Treatments. Human subjects (N = 3) received sodium amytal (200 mg/kg for 10 days). Consent was obtained and protocol was approved by the Dalhousie University Ethics Committee.

Liver biopsy. Needle biopsies were obtained percutaneously from pigs using the Menghini technique. Wedge biopsies were obtained from pigs under general anesthetic.

Blood sampling. Peripheral blood samples (10 ml) were obtained from human subjects by venipuncture and from rats by cardiac puncture with pooling of blood samples from two to three rats. Blood was obtained from pigs via the jugular vein using a portocatheter (Pharmacia) which was pocketed in the dorsal neck region with the catheter tunneled around the neck and cannulated into the jugular vein or alternatively by cranial vena cava venipuncture.

Monocyte isolation. Monocytes were isolated from blood samples after mixing with RPMI-1640 medium and layering over histopaque (Sigma), according to the method of Boyum [7]. After washing isolated monocytes with RPMI-1640 medium, the pellet was resuspended in Leibovitz (L-15) medium, supplemented with 10% fetal calf serum and 10% antibiotic antimycotic solution, and plated on tissue culture plates. Monocytes were purified by adher-

ence according to the method of Pennline [8]. The medium was then removed and replaced with fresh supplemented L-15 medium. The cells were then incubated at 37° for 24 hr, after which the cells were more macrophage-like in appearance. Monocytes were identified by staining for non-specific esterase [9]. Cell monolayers were washed with 1.15% KCl and harvested by scraping. The cell viability was determined by trypan blue exclusion. Due to the high stringency of the washes, the resulting cell population was >95% viable and contained <1% contamination with other cell types. A typical monocyte preparation yielded 4×10^6 monocytes/10 ml blood, and reaction mixtures contained approximately 2×10^6 cells. The cells were homogenized prior to protein assay and enzyme assay.

Kupffer cell and hepatocyte isolation. Kupffer cells and hepatocytes were isolated from wedge or needle biopsy specimens taken from pigs using a modification of the method of Peterson and Renton [10] by gravity settling and differential centrifugation as diagrammed in Fig. 1. Kupffer cells were then plated

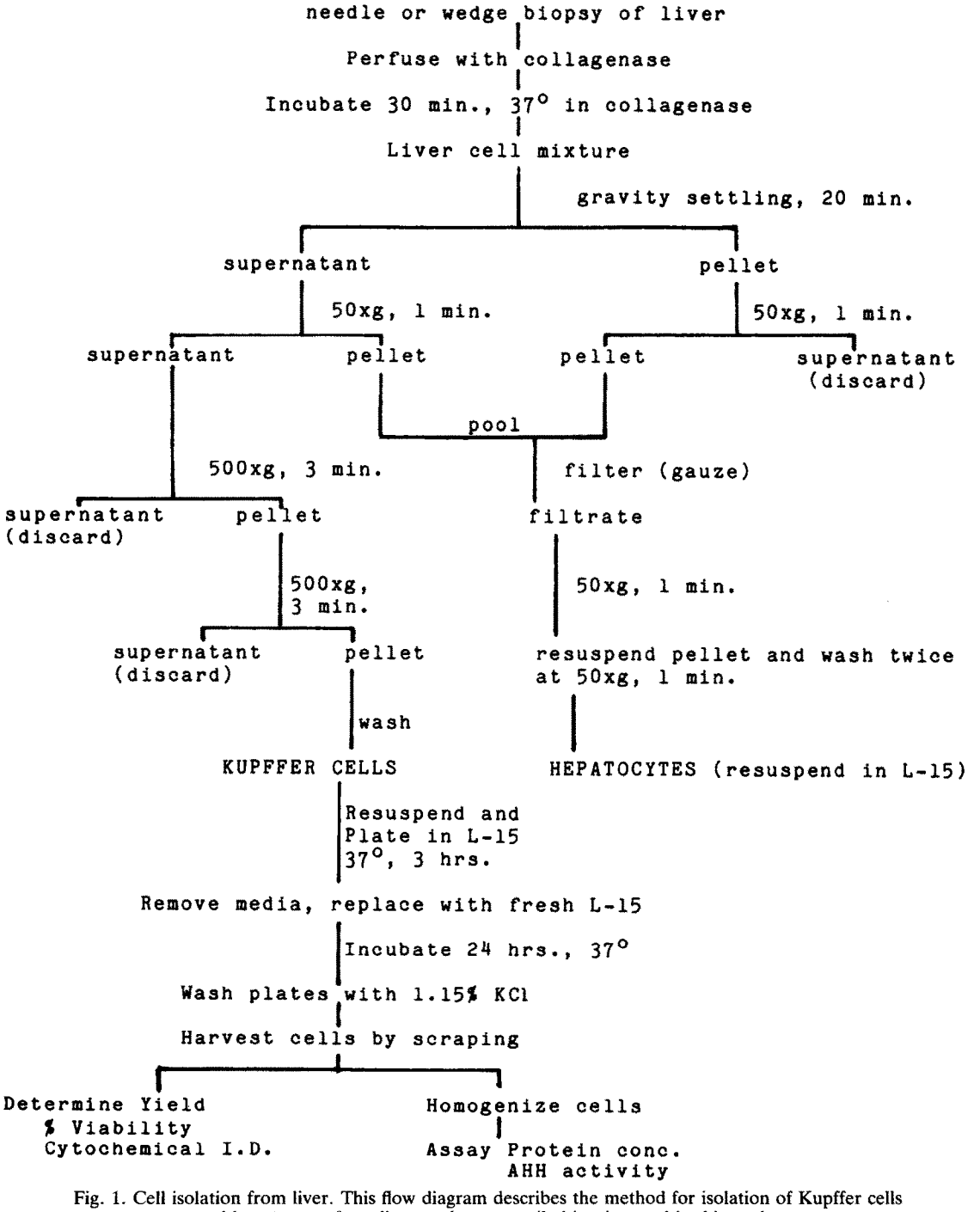

Fig. 1. Cell isolation from liver. This flow diagram describes the method for isolation of Kupffer cells and hepatocytes from liver wedge or needle biopsies used in this study.

onto tissue culture plates in supplemented L-15 medium at 37° for 3 hr. The L-15 medium was then replaced, and cell monolayers were incubated at 37° for 24 hr. Kupffer cell monolayers were washed with 1.15% KCl and then harvested by scraping. The cells were assessed for viability by trypan blue exclusion and identified by phagocytic activity [11]. A typical preparation of Kupffer cells yielded 3×10^7 cells/g liver. Cells were >95% viable and not contaminated with other cell types (<1%). The cells were homogenized and assayed for protein and enzyme activity. The adherence step improved the selection for Kupffer cells from other non-adherent cells, thus increasing the relative AHH activity per mg cellular protein compared to earlier studies [10].

Peritoneal macrophage isolation. Peritoneal macrophages were obtained from mice as described previously [12] using a modification of the method of Stuart [13] to obtain resident peritoneal macrophages. The peritoneal macrophage exudate was layed over histopaque, and the resulting monocyte preparation was washed and then enriched by adherence to petri dishes. The monolayers obtained were 99% pure.

Macrophage in vitro *experiments.* Macrophages (monocytes and peritoneal macrophages) were activated by incubation with 0.1% latex beads (0.46 μm diameter, Sigma) for 2 hr at 37°, while Kupffer cells and peritoneal macrophages were activated by incubation with dextran sulfate (50 μg/ml) for 2 hr at 37°, as previously described by Peterson and Renton [12, 14]. Monolayers were then rinsed to remove latex and washed with 1.15% KCl prior to harvesting.

Freezing experiments. Monocytes were isolated from peripheral blood as described above. Pellets obtained after the 10 min spin at 400 *g* were then resuspended in 3 ml of freezing medium. The freezing medium consisted of 70% RPMI-1640, 20% fetal calf serum (FCS) and 10% dimethyl sulfoxide (DMSO). Aliquots (1 ml) were frozen gradually to −70° by placing samples first in an insulated container. After 1, 9 and 42 days, samples were then centrifuged, and pellets were washed with fresh RPMI-1640 medium and finally suspended in supplemented L-15 medium and plated. After 4 hr, the medium was replaced with fresh L-15 medium and plates were incubated at 37° for 24 hr prior to harvest.

Cellular and microsomal drug metabolism. Macrophages were harvested by scraping. Macrophages and hepatocytes were homogenized and assayed for AHH activity using the method of Cantrell and Bresnick [2]. AHH activity is expressed as nmol 3-hydroxybenzo[α]pyrene formed per 3 mg cellular protein per hr. Cellular protein concentration was determined by the Biorad method of Bradford [15] using bovine serum albumin as a protein standard. Heme oxygenase activity was assayed on the supernatant fractions from macrophage homogenates, obtained after centrifugation at 15,000 *g* for 10 min, using the method of Tenhunen *et al.* [16] and was expressed as nmol per mg cellular protein per hr. Aminopyrine *N*-demethylase activity was determined in macrophage homogenates using the radiometric method of Poland and Nebert [17] and expressed as pmol HCHO per mg protein per hr. Cytochrome P-450 was measured in macrophage homogenates solubilized in 4% Lubrol in 0.1 M (pH 7.4) phosphate buffer using the method of Omura and Sato [18], and expressed as pmol per mg protein. Microsomes were prepared from liver biopsy material, and AHH activity was measured using established methods as previously described [10].

Statistics. An unpaired Student's *t*-test was utilized to compare two variables, and a Student–Newman–Keul's test was used when more than two variables were compared [19].

RESULTS AND DISCUSSION

AHH activity was assayed in macrophage homogenates from human monocytes, pig monocytes, rat monocytes, pig Kupffer cells, mouse Kupffer cells and mouse peritoneal macrophages. The results showed that AHH activity was of a similar magnitude from all macrophage sources and in all species studied (Fig. 2). For the first time our results compare the macrophage AHH activity in two different macrophages of the same animal. Pig monocyte AHH activity was 0.97 ± 0.4 nmol 3-OHBP · (3 mg protein)$^{-1}$ · hr^{-1} (N = 11) and was not significantly different from pig Kupffer cell AHH activity (1.15 ± 0.63, N = 7) Pig liver microsomal AHH activity was 10 ± 4 nmol 3-OHBP · (3 mg protein)$^{-1}$ · hr^{-1} (N = 4), and pig hepatocyte AHH activity was 8.2 ± 2.2 (N = 6). The level of pig hepatocyte AHH activity was approximately 8-fold higher than the level of pig Kupffer cell AHH activity. This is consistent with other reports relating the level of mouse liver hepatocyte AHH activity to the level of liver macrophage AHH activity [2, 10]. In this study the murine Kupffer cell AHH activity was 1.98 ± 0.59 nmol 3-OHBP · (3 mg protein)$^{-1}$ · hr^{-1} (N = 4) and was not significantly different from the murine peritoneal macrophage AHH activity (0.98 ± 0.3, N = 4). In addition, these results indicate that AHH activities in peripheral blood monocytes of humans (1.23 ± 0.2, N = 19), pigs (0.97 ± 0.40, N = 11) and rats (1.6 ± 0.6, N = 5) were not significantly different. Variability between individual animals was evident, but the standard errors decreased with increasing sample size. The level of pig liver microsomal AHH activity was very similar to the level of AHH activity reported in microsomes prepared from human liver biopsy material [20]. The specific activity of AHH in monocytes reported here [0.41 nmol · (mg protein)$^{-1}$ · hr^{-1}] represents approximately one-fifth of the specific activity of AHH reported in liver microsomes [2.05 nmol · (mg protein)$^{-1}$ · hr^{-1}] [20]. If one considers the contribution of monocytes to the total AHH drug-metabolizing capacity using the results reported here and the microsomal AHH activity reported by Brodie *et al.* [20], one finds that the total blood monocyte AHH activity would be 0.123 μmol (i.e. 0.246 nmol/10 ml blood × 5 litre blood volume) while total liver AHH activity would be 61.5 μmol (2.05 nmol/mg protein × 20 mg/g liver × 1500 g liver). Thus, the monocyte AHH activity may represent 0.2% of the liver AHH activity.

In all the results described above, the macrophage

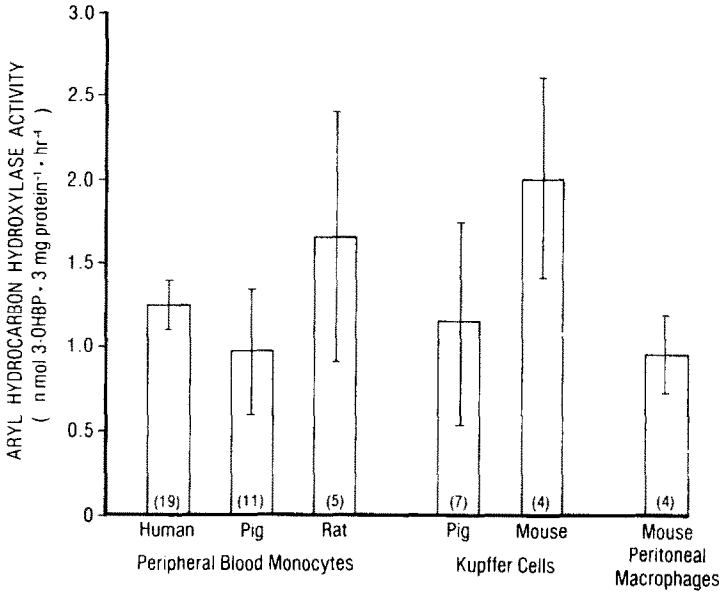

Fig. 2. Aryl hydrocarbon hydroxylase (AHH) activity in macrophages from different sources. Macrophages were obtained, as described in Materials and Methods, from pigs, mice, rats and humans. The macrophages included resident peritoneal macrophages, Kupffer cells, and peripheral blood monocytes. AHH was assayed on homogenates from cultured cells and expressed in nmol 3-hydroxybenzo[α]pyrene formed per 3 mg cellular protein per hr, as mean ± SE for the number of animals indicated in parentheses.

AHH activity was assayed on freshly harvested cells. Samples of freshly isolated macrophages were then frozen to determine if the macrophage enzyme was stable and could be stored at −70° in the freezer using a standard freezing medium consisting of 70% RPMI, 20% FCS, 10% DMSO. Monocyte AHH activity rapidly decreased under these conditions such that only 65% of the AHH activity was detectable after only 1 day of storage at −70° and less than 5% of the control monocyte AHH activity remained after 9 days at −70° using this freezing medium (Fig. 3). Trypan blue exclusion tests revealed that the decreased monocyte. AHH activity was not due to loss of cell viability. Rapid loss of monocyte AHH activity from storage at −70° suggests that freshly harvested monocytes should be used in the assay. Blood samples, after mixing with L-15 supplemented with FCS (10%) antibiotic antimycotic (10%), did retain total activity after storage at 4° for 4 hr. This now allows us to transport blood from surrounding areas (i.e. those within 4 hr of the University). Measurement of human monocyte AHH activity was found to be highly reproducible when assessed on samples obtained on two occasions over a 1-week, 2-week and 4-week interval [5].

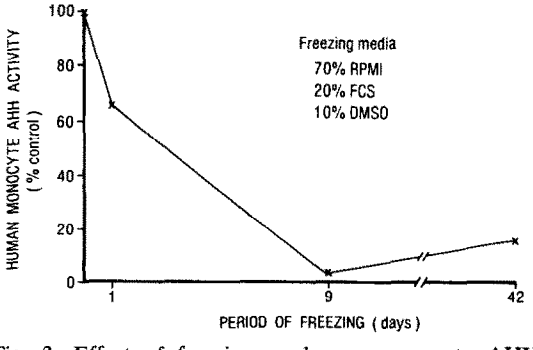

Fig. 3. Effect of freezing on human monocyte AHH activity. Monocytes were isolated from peripheral blood and frozen and thawed as described in Materials and methods. Cells were frozen to −70° in 70% RPMI-1640 medium with 20% FCS and 10% DMSO. Results are expressed as percent control human monocyte AHH activity. Typical control human monocyte AHH activity was 1.23 ± 0.2 (mean ± SE) nmol 3-hydroxybenzo[α]-pyrene formed · (3 mg cellular protein)$^{-1}$ · hr^{-1}.

We have reported previously that phagocytosis of latex by murine Kupffer cells or peritoneal macrophages depresses drug-metabolizing enzymes in hepatocytes, in coculture [12, 14]. The AHH enzyme in the peritoneal macrophages themselves was also depressed following phagocytosis of latex (unpublished observation).

To determine if the AHH enzyme detectable in human monocytes reacted in a similar fashion, isolated monocytes were incubated with 0.1% latex for 2 hr at 37°. After 2 hr at 37°, 90–95% of the monocytes have engulfed latex beads [21, 22]. The monocytes were then harvested and AHH activity was assayed. The monocyte AHH activity was depressed significantly to 30% of control levels following phagocytosis of latex beads (Fig. 4). Similar depression was observed in AHH activity of Kupffer cells and peritoneal macrophages following phagocytic activation by latex beads or dextran sulfate (Fig. 5). These results indicate that murine Kupffer cells and murine peritoneal macrophage AHH activities were depressed to a similar extent following incubation with dextran sulfate and that murine peritoneal

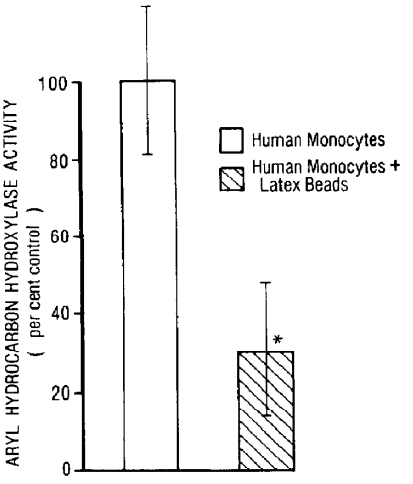

Fig. 4. Effect of phagocytic activation by latex beads on monocyte aryl hydrocarbon hydroxylase (AHH) activity. Monocytes were isolated from human peripheral blood and incubated with latex beads (0.1%) for 2 hr, as described in Materials and Methods. Results are expressed as percent of control AHH activity (see legend of Fig. 3 for control values). The mean and SE and statistical analysis were done using the raw data. Key: (*) statistically significant difference ($P < 0.05$) compared to control.

macrophage AHH activity was also depressed after phagocytic activation by latex beads.

Depression of AHH activity is often followed by increased (induced) heme oxygenase activity [23]. Other investigators have measured heme oxygenase activity in macrophages, e.g. Kupffer cells, and reported its induction following treatment with zymosan [24]. In our study heme oxygenase was detectable in pig and human monocytes and was about the same in these two species (Table 1). Also,

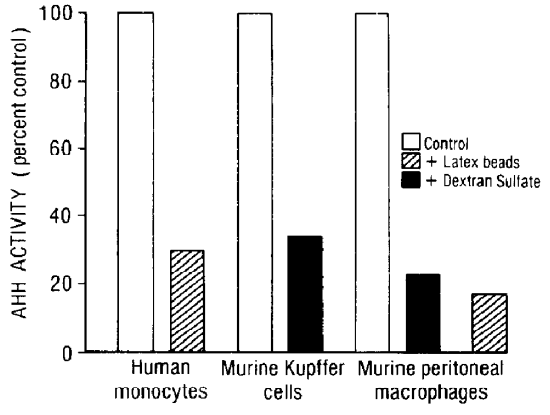

Fig. 5. Effect of phagocytic activation by latex beads on AHH activity in different macrophages. Monocytes, Kupffer cells and resident peritoneal macrophages were isolated, as described in Materials and Methods. Results are expressed as percent of control macrophage AHH activity. Control values were 1.23 ± 0.2 for human monocytes, 1.98 ± 0.59 for murine Kupffer cells and 0.98 ± 0.3 (nmol 3-hydroxybenzo[α]pyrene formed per 3 mg cellular protein per hr) for murine peritoneal macrophages.

Table 1. Heme oxygenase activity in monocytes from human and pig blood

Monocyte source	Heme oxygenase activity [nmol · (mg protein)$^{-1}$ · hr^{-1}]
Human	0.4 ± 0.17
Pig	0.41 ± 0.11

The results are expressed as mean ± SE for the number of individual subjects or animals indicated in parentheses.

incubation of human monocytes with latex beads (0.1%) for 2 hr at 37° resulted in a significant increase in monocyte heme oxygenase activity. Heme oxygenase activity increased 2.2-fold in human monocytes following phagocytic activation with latex beads (Fig. 6).

Phagocytic activation is a primary defense response to infection. Since phagocytic activation depressed monocyte AHH activity, one might hypothesize that monocyte AHH activity is depressed during infection. Preliminary results suggest that this may occur. The monocyte AHH activity was determined in a human subject before, during, and after infection. The pre-infection level of monocyte AHH activity was 0.77 nmol · (3 mg protein)$^{-1}$ · hr^{-1}. The monocyte AHH activity dropped to 0.055 nmol · (3 mg protein)$^{-1}$ · hr^{-1} during the infection and returned to 0.77 nmol · (3 mg protein)$^{-1}$ · hr^{-1} several weeks after the episode of infection. Phagocytic activation also occurs in liver

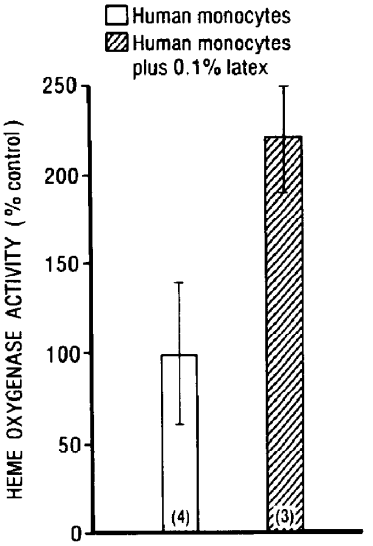

Fig. 6. Effect of phagocytic activation of human monocytes by latex beads (0.1%) on heme oxygenase activity. Monocytes were isolated from peripheral blood, as described in Materials and Methods. Results are expressed as percent of control monocyte heme oxygenase activity. The means and SE and statistical analysis were done using the raw data. Control values were 0.4 ± 0.17 nmol · (mg cellular protein)$^{-1}$ · hr^{-1} (mean ± SE) Human monocyte heme oxygenase activity was statistically significantly different following incubation with latex compared to control ($P < 0.05$).

disease as a result of engulfment of necrotic hepatocytes by Kupffer cells [25]. Recent results suggest that macrophage AHH activity is depressed in patients with liver disease compared to normal volunteers [5].

All of the AHH activities reported in this study are constitutive and are not the result of treating donors or cells with any conventional AHH inducers of the "3-methylcholanthrene" type. As such, the levels of constitutive AHH activity are consistent with those reported by others [2] and are lower than the levels of AHH activity reported in freshly isolated Kupffer cells after induction *in vivo* with 3-methylcholanthrene [2], or in monocytes in culture after induction with benzanthracene [6].

Both aminopyrine *N*-demethylase and cytochrome P-450 were non-detectable in monocytes isolated from either pig or human blood. After 10 days of treatment with sodium amytal, a recognized inducer of "phenobarbital-type" P-450, the level of cytochrome P-450 in human subjects was within the detectable range at 96.3 ± 10.4 pmol/mg protein ($N = 3$). No change was observed in monocyte AHH activity following treatment with sodium amytal.

In summary, our results show that Kupffer cells with detectable AHH activity can be isolated from liver needle biopsy specimens. For the first time we have assessed both peripheral blood monocyte AHH activity and Kupffer cell AHH activity in the same individual animals (i.e. pigs). The AHH activities in both pig monocytes and Kupffer cells were similar and were also similar to the AHH levels of human and rat monocytes and mouse Kupffer cells and peritoneal macrophages. This macrophage enzyme activity was reproducible but not stable when cells were frozen. Our results also show that the human monocyte AHH enzyme behaves like the liver Kupffer cell enzyme and peritoneal macrophage enzyme in response to phagocytic activation, i.e. the AHH enzyme activity was depressed, and there was a concomitant increase in heme oxygenase activity. Preliminary results suggest that monocyte AHH activity may be depressed due to infection. In conclusion, these results indicate that the AHH activities in macrophages of different sources and from different species are of similar magnitude and are similarly depressed following phagocytic activation.

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REFERENCES

1. D. M. Bisseli, L. Hammaker and R. Schmid, *J. Cell Biol.* **54**, 107 (1972).
2. E. Cantrell and E. Bresnick, *J. Cell Biol.* **52**, 316 (1972).
3. T. C. Peterson and C. N. Williams, *Clin. invest. Med.* **8**, A99 (1985).
4. T. C. Peterson and C. N. Williams, *Clin. invest. Med.* **9**, A51 (1986).
5. T. C. Peterson and C. N. Williams, *Hepatology* **7**, 333 (1987).
6. R. C. Bast, T. Okuda, E. Plotkin, R. Tarone, H. J. Rapp and H. V. Gelboin, *Cancer Res.* **36**, 1967 (1976).
7. A. Boyum, *Scand. J. clin. Lab. Invest.* **97**, 77 (1968).
8. K. J. Pennline, in. *Manual of Macrophage Methodology* (Eds. H. B. Herscowitz, H. J. Holden, J. A. Bellant and A. Ghaffar), Immunology Series Vol. 13, pp. 161–9. Marcel Dekker, New York (1981).
9. L. S. Kaplow, in. *Manual of Macrophage Methology* (Eds. H. B. Herscowitz, H. J. Holden, J. A. Bellant and A. Ghaffar), Immunology Series Vol. 13, pp. 199–207. Marcel Dekker, New York (1981).
10. T. C. Peterson and K. W. Renton, *J. Pharmac. exp. Ther.* **229**, 299 (1984).
11. A. C. Munthe-Kaas, T. Berg, P. O. Seglen and R. Seljelid, *J. exp. Med.* **141**, 1 (1975).
12. T. C. Peterson and K. W. Renton, *Immunopharmacology* **11**, 21 (1986).
13. A. E. Stuart, in *Handbook of Immunology* (Eds. J. A Habeshaw and A. Davidson), Chap. 31. Blackwell Publishers, Edinburgh (1970).
14. T. C. Petersn and K. W. Renton, *Biochem. Pharmac.* **35**, 1491 (1986).
15. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
16. R. Tenhunen, H. S. Marver and R. Schmid, *J. biol. Chem.* **24**, 6388 (1969).
17. A. P. Poland and D. W. Nebert, *J. Pharmac. exp. Ther.* **184**, 269 (1973).
18. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
19. J. H. Zar, *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, NJ (1984).
20. M. J. Brodie, A. R. Boobis, C. J. Bulpitt and D. S. Davies, *Eur. J. clin. Pharmac.* **20**, 39 (1981).
21. R. Matthay, P. Balzer, C. Putman, J. Gee, G. Beck and R. Greenspan, *Invest. Radiol.* **13**, 514 (1978).
22. T. Tanaka, K. Takoya, T. Kunimoto and H. Baba, *Int. J. Immunopharmac.* **1**, 197 (1979).
23. A. Kappas and G. Drummond, *Envĭron. Hlth Perspect.* **57**, 301 (1984).
24. A. Hupka and R. Karler, *J. reticuloendothel. Soc.* **14**, 225 (1973).
25. P. J. Scheuer, *Liver Biopsy Interpretation*, 3rd Ed, pp. 36–59. Williams & Wilkins, Baltimore (1980).