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MIXED-FUNCTION OXIDASES AND THE ALVEOLAR MACROPHAGE

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Abstract—Biphenyl 4-hydroxylase, benzpyrene hydroxylase and D-(+)-benzphetamine *N*-demethylase activities in subcellular fractions of the alveolar macrophage were investigated and found to be extremely low. Cytochrome P-450 was absent from the microsomal fraction but both NADPH-cytochrome *c* reductase and NADH-cytochrome *c* reductase activities were present. A b-type cytochrome, which was probably cytochrome b_5, was detected in the microsomal fraction. Glutathione *S*-aryltransferase and UDP-glucuronyl transferase activities were absent from the soluble and microsomal fractions respectively. These results indicate that alveolar macrophages may not play a significant role in the detoxication of foreign compounds by the lung.

THE LUNG contains mixed-function oxidases (MFOs) capable of oxidizing numerous foreign compounds.[1,2] As with the liver, the MFOs of the lung appear to be concentrated in the microsomal fraction prepared from the homogenized organ.[3] The lung is a structurally complex organ consisting of many different cell types,[4] and it is important to know which of these are associated with the MFO activities since such information might lead to a greater understanding of the response of the lung to inhaled foreign compounds. Amongst the diverse cell types is the alveolar macrophage whose primary role appears to be one of defense, especially since its phagocytic ability enables the macrophage to clear inhaled particulate matter from the alveoli. The defensive role of the alveolar macrophage, as far as particulate matter is concerned, is well documented (Refs. 5 and 6), but as yet little is known concerning its ability to detoxify inhaled toxic chemicals. This communication reports our investigation into the MFOs of the alveolar macrophage.

MATERIALS AND METHODS

Adult, male, New Zealand white rabbits weighing between 2 and 3 kg were used throughout the study (supplied by Arrows Rabbit Farm, Inc., Statesville, N.C.). The rabbits were killed by injection of air into the marginal ear vein. The upper part of the trachea was dissected free and clamped shut with a hemostat. The lungs and trachea were dissected out intact and immediately placed on ice. Alveolar cells were washed from the lungs using ice-cold Hanks' balanced salt solution (BSS) (about 40 ml) according to the method of Myrvik *et al.*[7] The lung washes were pooled (about 240 ml/pair of lungs) and then centrifuged at 1000 *g* for 10 min. The sediment was washed three times by resuspension in 30-ml vol. of BSS and resedimentation at 1000 *g* for

10 min. The cells were finally suspended in BSS and counted, using a hemocytometer (yield of mononuclear cells = $41 \times 10^6 \pm 8 \times 10^6$ (3) (S.E.M.)/pair of lungs).

The cells were examined by both light and electron microscopy. When stained with hematoxylin and eosin the cell preparations appeared to consist of primarily alveolar macrophages (90%) and a smaller, granulated, mononuclear cell type (9%) which stained more intensely than the alveolar macrophage. These differences were not so apparent when the preparations were examined using the Wrights stain; differences were noted in size but the cytoplasm of most cells had a basophilic appearance containing numerous, small, acidophilic granules. Very few polymorphonuclear leukocytes were found (0·4%). Examination of the cell preparations by electron microscopy showed that most cells were morphologically well preserved.

The cells were disrupted by sonication at 80 W (Sonifier Cell Disruptor model W185, Heat Systems–Ultrasonics, Inc.) for three 10-sec intervals with ice-cooling. The suspension was cooled for 2 min between sonications. Cell disruption was complete as shown by microscopic examination.

Preparation of microsomes. The macrophage sonicate was centrifuged at 10,000 *g* for 20 min. After removing the supernatant by aspiration, the 10,000 *g* pellet was resuspended in 1·15% KCl buffered with 0·02 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7·5, at 5°, to give a final protein concentration (determined by the method of Lowry *et al.*[8]) of 4 mg/ml. The 10,000 *g* supernatant was centrifuged at 138,000 *g* for 60 min. The 138,000 *g* supernatant (referred to in the text as the macrophage soluble fraction) was carefully removed by aspiration and the pellet (macrophage microsomes) was resuspended in 1·15% KCl–0·02 M HEPES buffer, pH 7·5, to a final concentration of 4 mg protein/ml.

Microsomes were also prepared from homogenates of unlavaged lungs (from a minimum of three rabbits), lavaged lungs, as well as liver, for comparative purposes. The lungs were minced using a domestic meat grinder and then homogenized (1 g of lung plus 2 ml of 0·25 M sucrose buffered with 0·05 M HEPES, pH 7·5) in a motor-driven Potter homogenizer using twelve passes of the loosely fitting plastic pestle. Liver was homogenized in the same manner except that only four passes of the pestle were used. Microsomes were prepared in the same manner as described for the macrophages except that after resuspension in KCl–HEPES they were sedimented once more. These washed microsomes were resuspended in KCl–HEPES to a final protein concentration of 4 mg/ml.

Since the effect of sonication on the MFOs of lung microsomes was not known and the absence of MFO activity in the alveolar macrophage (see Results) might be attributed to sonication effects, it was necessary to investigate such treatment. Consequently, washed microsomes from homogenates of lavaged lung were subjected to preparation and sonication conditions identical to those to which the macrophages had been exposed. The lung microsomes were suspended in Hanks' solution (1·2 mg microsomal protein/ml) to the same volume, in an identical vessel as occupied by the macrophage suspension, and then sonicated as described for the macrophage cell suspension. These microsomes were then sedimented as described for the macrophage microsomes and like the macrophage microsomes were not rewashed. Microsomes were also prepared from lavaged-lung mince which had been homogenized only by sonication. Sonication times as used for disrupting macrophages gave only poor breakdown of the minced-lung tissue and so were extended (4 × 15 sec interval, 80 W,

with ice-cooling). Sonication was carried out in 0·25 M sucrose buffered with 0·05 M HEPES, pH 7·5.

Determination of MFO activities. The incubation mixtures consisted of 0·17 M HEPES buffer (pH 7·55 at 37°), 3·3 mM NADPH, 4·2 mg/ml carboxymethyl cellulose (used as a carrier for substrate in the study of biphenyl and benzpyrene only) and various substrates in a volume of 1·5 ml. Biphenyl and benzpyrene (added as suspensions in 2·5% carboxymethyl cellulose) concentrations in the incubation mixtures were 16 and 10 mM respectively. D-(+)-benzphetamine hydrochloride was added in aqueous solution (concentration in the incubation mixture was 5 mM) and formaldehyde formed was measured according to the method of Nash.[9] In all formaldehyde determinations, correction was made for incubated blank values, i.e. apparent formaldehyde formed in the absence of substrate.[10] 4-Hydroxybiphenyl was measured according to the fluorescence assay of Creaven *et al.*,[11] and the hydroxylation of benzpyrene was measured using the fluorescence method of Wattenberg *et al.*[12]

NADPH-, NADH- and succinate-cytochrome c *reductases.* NADPH-cytochrome *c* reductase was assayed according to the method of Williams and Kamin[13] but with the following modifications: the reaction was carried out in a total volume of 2·4 ml of 0·31 M HEPES buffer, pH 7·6, containing 2·5 mg cytochrome *c*, 240 μmoles KCl, 5 μmoles KCN and 0·4 mg protein. The reaction was started by the addition of 2 μmoles NADPH and was carried out at 37° in 1-cm cuvettes and monitored at 550 nm using a Gilford 2400 recording spectrophotometer. The assay of NADH-cytochrome *c* reductase was carried out under the same conditions as NADPH-cytochrome *c* reductase but was started by the addition of 2 μmoles NADH instead of NADPH. Both reactions were maximally stimulated.

Succinate-cytochrome *c* reductase was assayed in 1-cm cuvettes at 37° using the Gilford 2400 recording spectrophotometer according to the method of Singer and Kearney.[14] The reaction was carried out in a total volume of 2·7 ml 0·028 M sodium phosphate buffer, pH 7·6, containing 2·5 mg cytochrome *c*, 10 mg bovine serum albumin, 5 μmoles KCN and 0·4 mg protein. The reaction was started by the addition of 9 μmoles sodium succinate.

UDP-glucuronyl transferase. UDP-glucuronyl transferase was measured at 37° using *p*-nitrophenol as substrate.[15] The reaction was carried out in 1·5 ml 0·08 M Tris–HCl buffer, pH 7·4, containing 1·0 mg microsomal protein/ml, 1·2 mM UDPGA and 0·2 mM *p*-nitrophenol. After 15 min the reaction was terminated by the addition of 5·0 ml 95% ethanol and then centrifuged. Aliquots (2·0 ml) of the supernatant were added to 2·0 -ml vol. of 0·1 N NaOH and the absorbance was measured at 400 nm.

Glutathione S-*aryltransferase.* Glutathione *S*-aryltransferase was measured according to the method of Booth *et al.*[16] using 1,2-dichloro-4-nitrobenzene as substrate.

Spectral studies. All microsomal difference spectra were measured at room temperature using a Shimadzu model MPS-50L spectrophotometer. Cytochrome P-450 was measured using the dithionite-difference method of Omura and Sato[17] and an extinction coefficient of 100 mM^{-1} cm^{-1} was employed to calculate the specific content in terms of nanomoles of cytochrome P-450.[18]

RESULTS

Before discussing results it seems relevant to remind the reader that the term "microsomes" as defined by Siekevitz[19] "describes the high-speed pellet (from 100,000

TABLE 1. MIXED-FUNCTION OXIDASE ACTIVITIES OF LUNG MICROSOMES AND SUBCELLULAR FRACTIONS OF THE ALVEOLAR MACROPHAGE FROM MALE RABBITS*

	Biphenyl 4-hydroxylase (nmoles 4-hydroxybiphenyl formed/min/mg protein)	Benzpyrene hydroxylase (fluorescence units† min/mg protein)	D-(+)-benzphetamine *N*-demethylase (nmoles formaldehyde produced/ min/mg protein)
Macrophage microsomes	0·14 ± 0·06 (5)‡	0·02 ± 0·01 (3)	0·29 ± 0·09 (5)
Macrophage, 10,000 *g* pellet	0·05 ± 0·02 (3)	0·003 ± 0·003 (3)	0·16 ± 0·08 (3)
Macrophage soluble fraction	0·01 ± 0·004 (3)	0 (2)	0·15 ± 0·09 (3)
Lung microsomes	4·54 ± 0·48 (5)	2·44 ± 0·28 (3)	7·66 ± 0·89 (5)
Lavaged-lung microsomes	5·08 ± 0·79 (4)	1·58 ± 0·48 (3)	9·38 ± 1·24 (4)
Lavaged-lung microsomes (sonicated)	5·86 ± 1·27 (3)	2·22 (2)	10·83 ± 1·28 (3)
Microsomes from sonicated lavaged-lung	3·29 (2)	1·30 (2)	4·70 (1)
Liver microsomes	3·24 ± 0·40 (4)	5·06 ± 1·79 (3)	6·50 ± 1·21 (4)
Lung microsomes + macrophage microsomes	5·13 ± 0·84 (3)	2·25 ± 0·54 (3)	10·30 (2)
Lung microsomes + macrophage 10,000 *g* pellet	5·21 ± 0·86 (3)	2·23 ± 0·38 (3)	9·99 (2)
Lung microsomes + macrophage soluble fraction	4·68 ± 0·76 (3)	1·97 ± 0·40 (3)	10·47 (2)

* Macrophage subcellular fractions and microsomes from pooled lungs and livers were prepared as described in Materials and Methods. "Lavaged-lung microsomes" were prepared from lungs, from which macrophages had been washed. "Lavaged-lung microsomes (sonicated)" were microsomes which had been prepared from lavaged-lungs and then sonicated in BSS. "Microsomes from sonicated lavaged-lung" were prepared from lavaged-lungs which had been disrupted by sonication. Incubations were carried out with protein concentrations of 1 mg/ml of incubate except in those cases where the activity of lung microsomes was determined in the presence of macrophage subcellular fractions. In these cases the concentration of lung microsomes in the incubates was 1·0 mg protein/ml and the concentration of the macrophage protein was also 1·0 mg/ml giving a total protein concentration of 2·0 mg/ml. However, MFO activities are expressed, not in terms of total protein, but as product formed per min per milligram of lung microsomal protein.

† One fluorescence unit is the fluorescence of a quinine-sulphate solution (0·3 μg/ml) in 0·1 N sulphuric acid (Ex. 400 nm; Em. 525 nm).

‡ Mean ±S.E.M. (N) where N is the number of separate tissue pools measured.

to 250,000 *g* for 60–120 min) resulting when the supernatant fluid from the mitochondrial fraction is sedimented". Due to a preoccupation with liver microsomes and the localization of MFOs in the endoplasmic reticulum of the liver cell, the terms microsomes and endoplasmic reticulum have become, erroneously, almost synonymous.

The MFOs biphenyl 4-hydroxylase, D-(+)-benzphetamine *N*-demethylase and benzpyrene hydroxylase (Table 1) are present in microsomes from liver and lung homogenate but present to only a minor degree (if at all) in the microsomal fraction of the alveolar macrophage. This may be merely an apparent result arising because of some peculiarity of the alveolar macrophage or because of the manner of preparation of the microsomes. We have investigated a number of possibilities that might account for low or absent activities.

Since the MFO activities are absent from the alveolar macrophage 10,000 *g* pellet and the soluble fraction, their absence from the microsomal fraction is not simply due to a subcellular distribution different from that of liver. The method of preparation of the microsomes should also be considered, as it is possible that sonication of the macrophage may inactivate enzymes. However, sonication of washed microsomes prepared from lavaged-lung did not reduce the specific activities of the hydroxylases investigated. MFO specific activities (per milligram of protein) in microsomes prepared from sonicated lung mince were lower than in microsomes from lung mince homogenized using a Potter–Elvehjem homogenizer. However, when the activities of biphenyl 4-hydroxylase and benzpyrene hydroxylase are expressed per nanomole of cytochrome P-450 (Table 2), the difference between the two methods of homogenization is not significant. Also, the specific activity (per milligram of protein) of the mitochondrial inner membrane marker succinate-cytochrome *c* reductase[20] is higher in microsomes from sonicated lung mince (Table 3), and consequently the lower MFO

TABLE 2. CYTOCHROME P-450 CONTENT OF LUNG MICROSOMES AND SUBCELLULAR FRACTIONS OF ALVEOLAR MACROPHAGES FROM MALE RABBITS*

	Experiment (nmoles cytochrome P-450/mg protein)		
	1	2	3
Macrophage microsomes	0·02	0	0
Macrophage 10,000 *g* pellet		0·01	
Macrophage soluble fraction		0·09	
Lung microsomes		0·30	0·23
Lavaged-lung microsomes†	0·63	0·35	0·27
Lavaged-lung microsomes (sonicated)†	0·54	0·32	0·31
Microsomes from sonicated lung mince†		0·19	0·18
Liver microsomes		1·02	1·05

* Cytochrome P-450 content was determined using the dithionite difference method. Experiments 1, 2 and 3 were carried out on macrophages, lavaged-lungs and livers pooled from fifteen, seventeen and fifteen rabbits respectively. Lungs were in each case pooled from three rabbits.

† See footnote to Table 1.

activities of these microsomes is probably due to protein-containing fragments of other subcellular organelles (in particular mitochondria). From such considerations it seems unlikely that disruption of the macrophages by sonication has inactivated the MFO activities.

TABLE 3. SUCCINATE-CYTOCHROME *c* REDUCTASE ACTIVITIES OF LUNG MICROSOMES AND SUBCELLULAR FRACTIONS OF THE ALVEOLAR MACROPHAGE*

	Experiment (nmoles cytochrome *c* reduced/min/mg protein)	
	1	2
Macrophage microsomes	12·4	9·1
Macrophage 10,000 *g* pellet	26·9	26·9
Macrophage soluble fraction	0	0
Lung microsomes	6·8	4·2
Lavaged-lung microsomes	5·7	3·7
Lavaged-lung microsomes (sonicated)	4·1	2·9
Microsomes from sonicated lavaged-lung	14·2	8·0
Liver microsomes	5·2	1·9

* Experiments 1 and 2 were carried out on tissue pools prepared from seventeen and fifteen rabbits, respectively. Lung and liver microsomes were from pools of three rabbits. Succinate-cytochrome *c* reductase was measured as described under Materials and Methods. The reaction was carried out at 37° in 1-cm cuvettes. The reaction mixture had a total volume of 2·7 ml and consisted of 0·028 M sodium phosphate buffer, pH 7·6, 0·093 mg cytochrome *c*/ml, 1·85 mM KCN, 3·3 mM sodium succinate and 0·15 mg protein/ml.

In order to discount the possibility that upon disruption the macrophages released an inhibitor of the MFOs or an enzyme capable of degrading the MFO system, washed microsomes from lung homogenate were incubated with each of the three subcellular fractions of the macrophages. Little effect of macrophage fractions was seen on the lung MFOs studied (Table 1).

As would be expected from the low MFO activities, the cytochrome P-450 content of alveolar macrophage microsomes was also very low (Table 2). With liver microsomes, NADPH-cytochrome *c* reductase appears to participate in the transfer of reducing equivalents from NADPH to the terminal oxidase cytochrome P-450.[21–24] The specific activity of NADPH-cytochrome *c* reductase in lung and liver microsomes is similar (Table 4) and its presence explicable in terms of its accepted association with cytochrome P-450. NADPH-cytochrome *c* reductase activity is present in alveolar macrophage microsomes although relative to the protein content its activity is lower than in microsomes from lung and liver. The reductase may not be associated with cytochrome P-450 since the levels of that cytochrome in alveolar macrophage microsomes are extremely low or absent.

The specific activity of NADH-cytochrome *c* reductase in microsomes from the alveolar macrophage was lower than in lung or liver microsomes. The microsomal fraction of many tissues from many species is rich in NADH-cytochrome *c* reductase.[25]

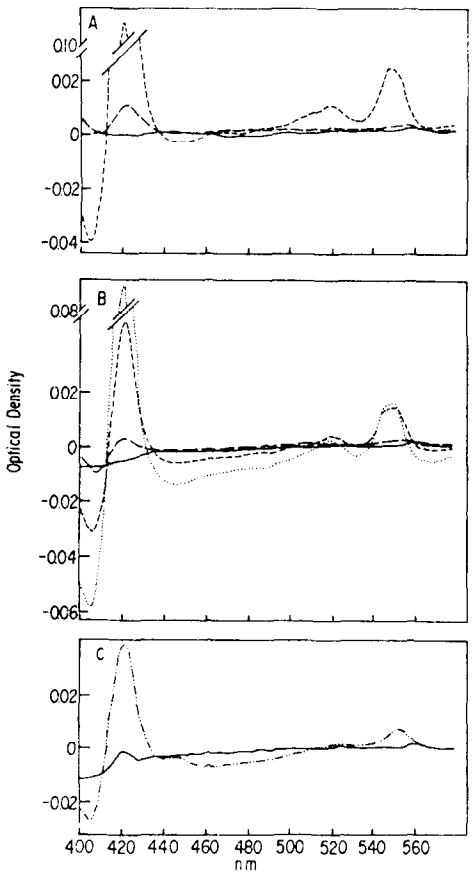

FIG. 1. Reduction of cytochrome b_5 in alveolar macrophage microsomes by NADH and NADPH. The sample and reference cuvettes contained 2·0 mg of microsomal protein/ml of 0·1 M HEPES buffer (pH 7·4). Difference spectra were recorded with a Shimadzu MPS-50L spectrophotometer. The sample and reference were balanced to points of equal light absorption (baseline ———). (A) NADH was added to the sample cuvette (— — —). Equal amounts of air-oxidized cytochrome b_5 were then added to both the sample and reference cuvettes (– – – –). (B) NADPH was added to the sample cuvette (— — —). Equal amounts of air-oxidized cytochrome b_5 were then added to both the sample and reference cuvettes (– – – –). NADH was then added to the sample cuvette (· · · · · ·). (C) Dithionite-scrubbed CO was bubbled through the microsomal suspension for 2 min. Equal amounts of the microsomes were then added to the sample and reference cuvettes. After establishing the baseline, a small amount of solid dithionite was added to the sample cuvette (– · · – · · –).

It has been shown that cytochrome b_5 is involved in this activity[26] although a microsomal NADH-cytochrome *c* reductase not involving cytochrome b_5 is also present in the liver system.[27] The NADH-cytochrome *c* reductase activity measured was non-specific, but from the difference spectra of the reduced versus oxidized macrophage microsomes (Fig. 1), cytochrome b_5 is probably involved. Reduction with either NADPH or NADH gave rise to identical spectra. The Soret band at 422 nm is not due to mitochondrial contamination since reduction with succinate did not give rise to a difference spectrum. Treatment of the microsomes with CO and reduction of the sample cuvette contents with dithionite considerably enhanced the Soret band. There

TABLE 4. NADPH- AND NADH-CYTOCHROME *c* REDUCTASE ACTIVITIES OF LUNG MICROSOMES AND SUBCELLULAR FRACTIONS OF ALVEOLAR MACROPHAGES*

	NADPH-cytochrome *c* reductase (nmoles cytochrome *c* reduced/min/mg protein)	NADH-cytochrome *c* reductase (nmoles cytochrome *c* reduced/min/mg protein)
Macrophage microsomes	43 ± 3 (3)†	223 ± 64 (3)
Macrophage 10,000 *g* pellet	13 ± 2 (3)	125 ± 17 (3)
Macrophage soluble fraction	2·6 ± 1·6 (3)	19 ± 15 (3)
Lung microsomes	127 ± 19 (3)	586 ± 74 (3)
Lavaged-lung microsomes	117 ± 37 (3)	525 ± 35 (3)
Lavaged-lung microsomes (sonicated)	105 ± 30 (3)	583 ± 116 (3)
Microsomes from sonicated lavaged-lung mince	58 (2)	335 (2)
Liver microsomes	143 ± 27 (3)	805 ± 58 (3)

* Microsomes and other subcellular fractions were prepared as described under Materials and Methods. The reactions were carried out at 37° in 1-cm cuvettes containing 2·4-ml vol. of incubation mixture consisting of 0·31 M HEPES buffer, pH 7·6, 1·04 mg cytochrome *c*/ml, 2·1 mM KCN, 100 mM KCl, 0·17 mg protein/ml, and 0·83 mM NADPH or NADH. The reaction was monitored at 550 nm.

† Mean ±S.E.M. (N) where N is the number of separate experiments performed.

TABLE 5. GLUTATHIONE *S*-ARYLTRANSFERASE AND UDP-GLUCURONYL TRANSFERASE ACTIVITIES OF RABBIT LUNG, LIVER AND ALVEOLAR MACROPHAGE SUBCELLULAR FRACTIONS*

	Glutathione *S*-aryltransferase† (nmoles GSH conjugated/min/mg protein)	UDP-glucuronyl transferase‡ (nmoles *p*-nitrophenyl-glucuronide formed/min/mg protein)
Macrophage soluble fraction	0	0
Macrophage microsomes		0
Macrophage 10,000 *g* pellet		0
Lung soluble fraction	1·58	
Lung microsomes		0·08
Lavaged-lung soluble fraction	1·75	
Lavaged-lung microsomes		
Lavaged-lung microsomes (sonicated)		
Soluble fraction from sonicated lavaged-lung	1·31	
Microsomes from sonicated lavaged-lung		0·64
Liver soluble fraction	4·89	
Liver microsomes		5·99

* Macrophages were washed from the lungs of seventeen rabbits. Lavaged-lung, sonicated lavaged-lung and liver subcellular fractions were obtained from the same pool of rabbits. Lung subcellular fractions were obtained from a separate pool of three rabbits.

† Glutathione *S*-aryltransferase activity was measured using the method of Booth *et al.*[16] with 1,2-dichloro-4-nitrobenzene as substrate.

‡ UDP-glucuronyl transferase activity was measured as described under Materials and Methods using *p*-nitrophenol as substrate.

is little doubt that a cytochrome b_5 reductase is present in the macrophage microsomes since added cytochrome b_5 was reduced by either NADH or NADPH (Fig. 1).

The macrophage preparations were contaminated with erythrocytes. However, this contamination did not account, apparently, for the reductase activities observed since "microsomes" (138,000 *g* pellet) prepared from washed erythrocytes were quite inactive as far as the enzymic activities studied were concerned.

The ability of the alveolar macrophage to conjugate foreign materials with glutathione was apparently absent although the lung itself possessed considerable *S*-aryltransferase (Table 5). Glucuronyl transferase was apparently absent from both the macrophage and the lung (Table 5).

DISCUSSION

Alveolar macrophages are phagocytic cells[28] whose primary function is concerned with defense of the lung. The phagocytic properties of these cells are important in their anti-microbial function and ability to remove inhaled particulates from the lung. Many so-called environmental agents gain access to the body via the lungs and often cause damage to the lungs themselves. Some of these compounds, such as benzpyrene, are water insoluble and are inhaled as particulates or adsorbed to particulates. If such particulates are phagocytized by the alveolar macrophage, it would be advantageous for such cells to possess enzyme systems capable of detoxifying foreign compounds. Our studies indicated that the hydroxylating and demethylating ability of the alveolar macrophage was very small, that is, as far as NADPH-dependent mixed-function oxidases similar to those found in the liver are concerned. The lung possesses mixed-function oxidases capable of hydroxylating many foreign compounds,[29] but these do not apparently reside in the alveolar macrophage. Correlating with the lack of measurable MFO activities in macrophage microsomes was the absence of the terminal oxidase cytochrome P-450. Even the conjugating enzymes glutathione *S*-aryltransferase and glucuronyl transferase were apparently absent. Without hydroxylation providing a necessary handle for the introduction of highly polar groups, other conjugating enzyme systems, such as the sulphotransferases, may also be absent.

Alveolar macrophage microsomes possessed a very active NADPH-cytochrome *c* reductase. This reductase in liver microsomes at least, seems to be associated with cytochrome P-450.[21–24] NADPH-cytochrome *c* reductase of macrophage microsomes may be quite different from that present in liver since cytochrome P-450 levels were extremely low or absent in macrophage.

NADH-cytochrome *c* reductase was also present in alveolar macrophage microsomes. Liver and lung microsomes are known to be rich in this activity.[25] NADH-cytochrome b_5 reductase and cytochrome b_5 can reduce cytochrome *c* and thus summate as the experimentally measured NADH-cytochrome *c* reductase,[26,30,31] although a liver microsomal NADH-cytochrome *c* reductase not involving cytochrome b_5 also exists.[27] The observed NADH-cytochrome *c* reductase of alveolar macrophage microsomes probably involves cytochrome b_5 since a b-type cytochrome Soret band was observed in the difference spectra (Fig. 1). As with the liver[32] and lung[33] microsomal systems, the b-cytochrome was reduced by either NADH or NADPH. In view of these results, the observed NADPH-cytochrome *c* reductase activity may be due to the same enzyme(s) giving rise to the NADH-cytochrome *c* reductase activity.

The alveolar macrophages of the lung and the Kupfer cells of the liver, as well as numerous other phagocytic cells of the body, are members of what is generally described as the reticuloendothelial system (RES). Benzpyrene hydroxylase activities of a population of non-parenchymal cells from liver have been investigated by Cantrell and Bresnick[34] and were found to be very low. A relationship between the RES and drug metabolism seems to exist[35,36] but probably does not involve RES MFOs. Although we have found low MFO activity in alveolar macrophages and non-parenchymal cells of liver are also low in benzpyrene hydroxylase activity[34] and the cells studied are part of the RES, extrapolation to macrophages from other tissue sources must be done with caution since considerable biochemical differences can exist.[37]

Lung microsomes are rich in MFOs. These activities must be contained in cells other than the alveolar macrophage. So that a deeper understanding of the effects of environmental agents on the lung may be gained it is important that the nature of these MFO-containing cells be determined. The histochemical aniline hydroxylase staining method of Gangolli and Wright[38] suggests that the cells of the bronchial epithelium[39] may bear at least some of the MFO-system of the lung. If this is the case, then a question arises concerning the fate of foreign compounds phagocytized by the alveolar macrophage.

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