Biochemical Pharmacology, Vol. 30, pp. 341–347.

0006-2952/81/040341–07 $02.00/0

EFFECTS OF HYPEROXIA ON PULMONARY METABOLISM OF NEWBORN RATS AND MODIFICATION BY CHLORPHENTERMINE

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(*Received* 10 *March* 1980; *accepted* 29 *July* 1980)

Abstract—Continuous exposure of newborn rats to 95% oxygen for 3 days decreased the incorporation of thymidine into lung DNA with a return to control values after 5–7 days of exposure. In general, hyperoxia increased the activities of pulmonary glutathione peroxidase, glutathione reductase, and lactic dehydrogenase without associated significant alterations in glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and pyruvate kinase. Although hyperoxygenation elevated both lung lactate and pyruvate levels, a fall in the ratio of lactate to pyruvate was noted. Daily, oral administration of 20 mg/kg chlorphentermine significantly enhanced the incorporation of thymidine into DNA throughout the course of the experiment. Whereas anorectic drug treatment decreased lactic dehydrogenase activity, increases were seen in the activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione peroxidase, and glutathione reductase. Under our experimental conditions, the chlorphentermine-stimulated rise in newborn lung lactate and pyruvate levels was associated with a lower ratio of lactate to pyruvate. In newborns exposed simultaneously to continuous 95% oxygen and daily chlorphentermine, this drug modified the effects seen with oxidant alone as reflected by significant stimulation in DNA synthesis, elevation in glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase as well as depression in lactic dehydrogenase and pyruvate kinase. While concurrent O_2 and chlorphentermine administration, in general, did not markedly alter glutathione reductase when compared to O_2 alone, combined treatment produced a greater rise in glutathione peroxidase. In comparison to corresponding O_2 controls, simultaneous oxidant and drug exposure increased the ratio of lactate to pyruvate and lactate levels without an associated marked change in tissue pyruvate. Our results indicate that, in simultaneous oxidant- and anorectic drug-treated newborns, the metabolic responses seen in lung, in general, resembled those produced by chlorphentermine alone and were opposite to those induced by 95% oxygen.

The morphologic and metabolic consequences resulting from hyperoxia in newborn lung differ from the responses seen in adults. Yam *et al.* [1] reported that exposure of mature rats to 96–98% oxygen for 3 days produced thickened alveolar septa, edema, alveolar hemorrhage, and accumulation of macrophages in the lung. These histopathological changes were associated with no marked alteration in the activities of pulmonary superoxide dismutase (SOD), glutathione peroxidase (GP), and glutathione reductase (GR). In contrast, Yam *et al.* [1] demonstrated that the minimal alveolar edema seen in neonatal lung obtained from rats exposed to hyperoxia for 5 days was accompanied by elevation of the activities of SOD, GP, and GR. Thus, it was suggested that increases in these enzymes may provide a metabolic basis for the enhanced tolerance seen in newborns to oxygen-induced pulmonary injury. In addition to the glutathione system, other metabolic changes indicative of oxidant-inflicted pulmonary damage in adults include increases in DNA content and in glucose-6-phosphate dehydrogenase (G6PDH), lactic dehydrogenase (LDH), and lactate levels [2, 3]. In the present paper, to compare the metabolic responsiveness of newborns with the known effects of pulmonary oxygen toxicity in adults, the influence of hyperoxia also was examined on neonatal lung lactate and pyruvate levels, on the activities of pyruvate kinase (PK), LDH, G6PDH, GP, and GR, as well as on the incorporation of thymidine into DNA.

Recently, Karabelnik *et al.* [4] reported that daily administration of chlorphentermine (20 mg/kg) for 1 week resulted in accumulation of hypertrophic macrophages in pulmonary alveoli of adult rats. In contrast, Kacew *et al.* [5, 6] found that daily treatment with 20 mg/kg of chlorphentermine for 1 week produced no apparent morphologic alterations in newborn rat lung, suggesting that neonatal pulmonary tissue was less susceptible to the cytologic actions of this agent. Although newborn lung of chlorphentermine-treated rats appeared ultrastructurally to be similar to control tissue, 20 mg/kg of this anorectic drug increased the incorporation of thymidine into DNA. A second aim of this study, thus, was to determine whether chlorphentermine affected other metabolic variables in newborns, such as pentose shunt glycolysis and the glutathione system, which are indices associated with nucleic acid synthesis [7] and with enhanced pulmonary tolerance to chemical-induced injury [1].

Although daily chlorphentermine administration at a dose of 20 mg/kg failed to produce any apparent

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ultrastructural changes, simultaneous exposure of newborns to 95% O_2 and this anorectic drug resulted in the presence of hypertrophic histiocytes in lung [8]. At present, the metabolic mechanism(s) underlying this observed interaction on pulmonary morphology of O_2 and chlorphentermine is unknown. The third aim of this study was, therefore, to determine whether concurrent drug and oxidant treatment did indeed affect pulmonary metabolism differently from O_2 or chlorphentermine alone.

MATERIALS AND METHODS

Animals. Female rats of the Sprague–Dawley strain with 1-day-old litters were purchased from Canadian Breeding Farm and Laboratories, St. Constant, Quebec. All animals were maintained on Master Laboratory Chow and had free access to water throughout the course of the experiment.

Experimental procedures. Chlorphentermine hydrochloride was administered daily by gastric intubation at a dose of 20 mg/kg to 1-day-old rats for a period of either 3, 5 or 7 days. Corresponding controls received an equal volume (50 μl) of physiological saline. For the experiments in which neonatal rats were exposed to hyperoxia, animals were maintained for either 3, 5 or 7 days in a spherical, airtight, plexiglass chamber filled with 95% oxygen at a rate of 3 l/min. The oxygen concentration was monitored with a Beckman model OM-11 gas analyzer. The carbon dioxide level was less than 0.5% throughout the exposure period and was monitored continuously with a Beckman model LB-2 gas analyzer. The temperature varied from 22 to 25°, and the relative humidity from 55 to 70%. Corresponding control pups were placed in an identical chamber that was ventilated with compressed air at a flow rate of 3 l/min. To reduce any effects due to differences in nursing ability as well as to avoid oxygen intoxication of the mothers, nursing mothers were exchanged between litters every 24 hr. In addition, groups of newborns were simultaneously exposed to 95% oxygen and administered chlorphentermine for either 3, 5 or 7 days.

Enzyme assays. In preliminary experiments, newborn lungs were perfused with 0.15 M KCl, pH 7.4, to determine whether blood contamination affected enzymic activities. In our experimental conditions, no significant difference was seen in the enzymic activities of perfused control lungs as compared to the values obtained in unperfused pulmonary tissue. Hence, for determination of enzymatic activities, unperfused pulmonary tissue was excised from eight pups from each group and immediately frozen in liquid nitrogen. The lungs were weighed and 5% homogenates were prepared in 0.15 M KCl, pH 7.4. To obtain supernatant fluids the homogenates were centrifuged at 100,000 *g* for 45 min at 0° in a refrigerated Beckman L5-50 ultracentrifuge. Lactate dehydrogenase [9], pyruvate kinase [10], G6PDH [11], 6-phosphogluconic acid dehydrogenase (6PGDH) [11], GR [12], and GP [13] activities were assayed in the supernatant fluids. All enzyme assays were carried out under strictly linear kinetic conditions at 37°. The activities of GR, GP, G6PDH, and 6PGDH were calculated as micromoles of substrate metabolized per hour per gram tissue, whereas LDH and PK were calculated as micromoles of substrate metabolized per minute per gram lung. Enzyme activities were expressed as specific activity per milligram protein. A similar magnitude of response was noted when enzyme activities were calculated as micromoles per minute or per hour per lung.

Glycolytic metabolites. For determination of lactate and pyruvate, lungs were excised and immediately frozen in liquid nitrogen. The lungs were weighed and 15% homogenates were prepared in 5% trichloroacetic acid (TCA). The homogenates were neutralized with 0.2 M K_2CO_3, centrifuged at 1500 *g* for 10 min at 0°, and the supernatant fluids obtained were used for metabolite analysis. Lung lactate was measured according to the method of Hohorst [14] and expressed as micromoles per 100 g tissue. Pyruvate was determined according to the method of Von Korff [15] and expressed as micromoles per 100 g tissue.

DNA synthesis. To measure the incorporation of thymidine into DNA, portions of lung were removed from eight pups from each group 90 min after an intraperitoneal injection, in a final volume of 0.1 ml per rat of 0.5 μCi [2-^{14}C]thymidine (sp. act. 54.7 mCi/mmole). The method described by Witschi and Saheb [16] was then used to determine the incorporation of thymidine into DNA, and the data are expressed as disintegrations per minute per milligram DNA. The diphenylamine method of Burton [17] was employed to determine tissue DNA content, and the concentration of RNA was measured by the orcinol procedure of Volkin and Cohn [18]. Protein was determined according to the Folin phenol method of Lowry *et al.* [19]. The pulmonary levels of protein, RNA, and DNA are expressed as milligrams per gram tissue.

Drugs and chemicals. All reagents were of the purest grade available and were dissolved in double glass-distilled water. Chlorphentermine (H. Lundbeck & Co., Copenhagen, Denmark) was dissolved in physiological saline; [^{14}C]thymidine was purchased from New England Nuclear (Montreal, Quebec, Canada); and 100% oxygen was purchased from the Central Oxygen Co. (Ottawa, Ontario, Canada). All other biochemicals used for various assays were obtained commercially from the Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Statistical analysis. All data were analyzed statistically using Student's *t*-test; significant differences between the mean values are indicated when the P value was < 0.05.

RESULTS

Nucleic acids and protein. Data in Table 1 show the effects of chlorphentermine and/or oxygen on newborn lung nucleic acids and protein. Although daily chlorphentermine administration for 3 days did not produce a marked change in pulmonary RNA, a significant fall was noted after 5 days, followed by a return to control levels after 1 week. Irrespective of the duration of exposure, hyperoxia decreased RNA levels significantly. Simultaneous treatment with 95% oxygen and chlorphentermine, however, resulted in a significant rise in RNA levels when

Table 1. Effects of chlorphentermine and/or oxygen on newborn lung nucleic acids and protein*

Treatment group	Exposure period (days)	Metabolic variable RNA (mg/g)	DNA (mg/g)	Protein (mg/g)
Saline and air	3	2.68 ± 0.25	5.72 ± 0.66	72 ± 5
	5	3.13 ± 0.32	5.08 ± 0.40	75 ± 4
	7	2.75 ± 0.18	5.88 ± 0.50	71 ± 2
Chlorphentermine and air	3	2.30 ± 0.40	6.78 ± 0.66	75 ± 2
	5	2.21 ± 0.17†	10.20 ± 0.70†	60 ± 4†
	7	2.69 ± 0.20	9.45 ± 1.21†	77 ± 7
Saline and oxygen	3	1.55 ± 0.14†	3.84 ± 0.23†	52 ± 2†
	5	2.00 ± 0.24†	3.92 ± 0.44†	63 ± 3†
	7	2.08 ± 0.22†	4.74 ± 0.40	79 ± 3
Chlorphentermine and oxygen	3	3.03 ± 0.28‡	8.56 ± 1.36‡	69 ± 5‡
	5	4.23 ± 0.40‡	9.92 ± 0.78‡	52 ± 3‡
	7	3.64 ± 0.31‡	11.96 ± 0.78‡	62 ± 3‡

* Each value is the mean ± S.E.M. of eight animals in each group. Newborn pups were given daily 20 mg/kg chlorphentermine for either 3, 5 or 7 days by the oral route while corresponding controls received physiological saline. Groups of pups were also exposed to 95% oxygen and given saline daily for either 3, 5 or 7 days. In the simultaneous experiment, pups were exposed to 95% oxygen and given daily 20 mg/kg chlorphentermine for either 3, 5 or 7 days.

† Statistically significant difference ($P < 0.05$) when compared with the corresponding value for saline and air.

‡ Statistically significant difference ($P < 0.05$) when compared with the value for corresponding control saline and oxygen.

compared to the corresponding control, oxygen and saline. Results in Table 1 also show that, whereas chlorphentermine treatment for 5 and 7 days increased DNA levels, a decrease was seen in lungs obtained from 3- and 5-day hyperoxic newborns. As in the case of RNA, concurrent exposure to 95% oxygen and chlorphentermine elevated levels of DNA significantly in comparison to corresponding controls. Daily treatment with chlorphentermine or 95% oxygen significantly decreased or produced no change in newborn lung protein (Table 1). Although an elevation of pulmonary protein was noted 3 days after drug and oxidant exposure, a significant fall occurred after 5 and 7 days when compared to corresponding controls.

Incorporation of thymidine into DNA. The influ-

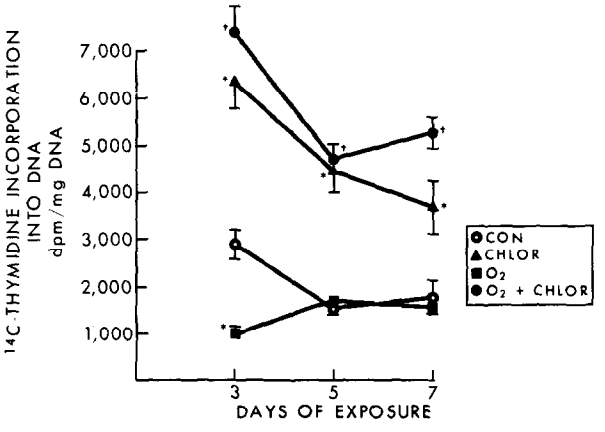

Fig. 1. Time-course of oxygen and/or chlorphentermine-induced changes in the incorporation of thymidine into newborn lung DNA. Each point is the mean ± S.E.M. of eight pups in each group. One set of animals was given chlorphentermine (20 mg·kg^{-1}·day^{-1}, p.o.) for either 3, 5 or 7 days; these are designated CHLOR. Corresponding controls received physiological saline and are shown as CON. A third set of pups, exposed continuously to 95% O_2 for 3, 5 or 7 days, was administered physiological saline and is designated as O_2. The last group of animals, which received chlorphentermine (20 mg·kg^{-1}·day^{-1}, p.o.) and was maintained in a 95% O_2 environment for 3, 5 or 7 days, is shown as O_2 + CHLOR. An asterisk (*) indicates a statistically significant difference ($P < 0.05$) when compared with the respective control (saline + air). A dagger (†) indicates a statistically significant difference ($P < 0.05$) when compared with the corresponding control (O_2 + saline).

ence of chlorphentermine and/or oxygen on the incorporation of thymidine into pulmonary DNA of newborns is illustrated in Fig. 1. Daily chlorphentermine administration produced a 2-fold significant increase in the incorporation of thymidine into newborn lung DNA throughout the treatment period. In contrast, exposure to 95% oxygen for 3 days resulted in a significant decrease in the incorporation of thymidine into DNA and this was followed by a return to approximately control values after 5 and 7 days. When compared to corresponding control values, data in Fig. 1 demonstrate that chlorphentermine markedly modified the effects of hyperoxia. Indeed, simultaneous drug and oxygen treatment produced a 2.5- to 7-fold increase in the incorporation of thymidine into DNA compared to oxygen and saline.

Metabolites and enzymes involved in glycolysis. Data in Fig. 2 illustrate that chlorphentermine decreased significantly the activity of pulmonary LDH 3, 5 and 7 days after treatment. In contrast, hyperoxia for 3 and 5 days produced a significant rise in LDH activity. Simultaneous exposure to 95% oxygen and anorectic, however, resulted in a marked depression of LDH activity compared to the corresponding control values. In contrast to changes in LDH, the drug-induced decrease in pulmonary PK activity after 3 days was followed by a significant rise after 1 week. Exposure to 95% oxygen failed to markedly alter PK activity at all times studied. As in the case of LDH, concurrent chlorphentermine and oxygen treatment depressed the activity of lung PK when compared to the saline and oxygen control. Results in Fig. 2 also show that daily chlorphentermine administration significantly increased the activities of the pentose shunt glycolytic enzymes G6PDH and 6PGDH throughout the treatment period. Exposure to 95% oxygen did not alter markedly the activities of G6PDH and 6PGDH except in the case of G6PDH where a significant increase was seen after 3 days. In comparison to oxygen and saline, simultaneous chlorphentermine and oxygen treatment significantly elevated G6PDH and 6PGDH activities, except after 3 days in the case of G6PDH.

Results in Table 2 demonstrate that daily chlorphentermine treatment increased significantly the concentrations of pulmonary lactate and pyruvate at all times examined. Similarly, hyperoxia for 3, 5 or 7 days produced a significant rise in the levels of lung lactate and pyruvate. The observed increase in lactate and pyruvate induced by oxygen or chlorphentermine was associated with a lower lactate/pyruvate ratio, indicating that, proportionately, the rise in pyruvate was higher than in lactate. Data in Table 2 also show that simultaneous exposure to 95% oxygen and chlorphentermine significantly elevated lactate levels without a marked alteration in pyruvate, at all times studied. Thus, concurrent drug and oxidant treatment increased the lactate/pyruvate ratios compared to corresponding control values.

Glutathione system enzymes. The influence of chlorphentermine and/or oxygen on the activities of pulmonary GR and GP is illustrated in Fig. 3. Daily administration of chlorphentermine for 3, 5 or 7 days elevated significantly the activities of newborn lung GP and GR. A similar increase in GR activity occurred at all times studied after oxygen exposure. In the case of GP, only exposure to 95% oxygen for 5 days resulted in a significant rise in enzymic activity. When compared to corresponding control values, concurrent drug and oxygen treatment produced a significant decrease in GR after 3 days without marked alterations after 5 or 7 days. In contrast, simultaneous oxygen and chlorphentermine exposure significantly elevated GP activity throughout the course of this study.

DISCUSSION

In a recent study of lung ultrastructure, Kacew and Narbaitz [8] found evidence of interstitial edema in the alveolar walls of newborn rat lung exposed to

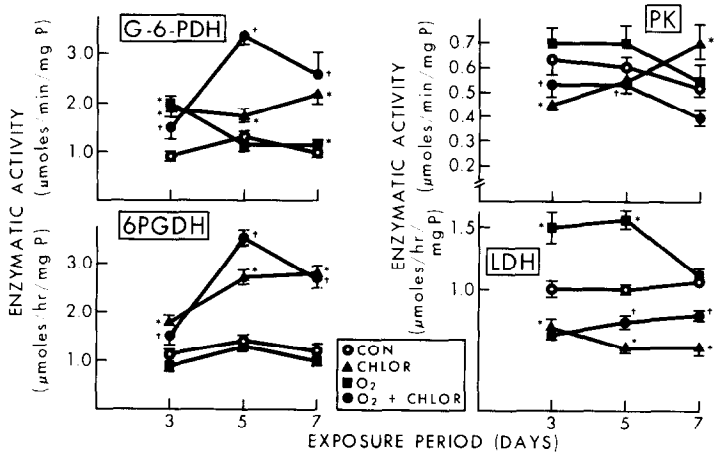

Fig. 2. Time-course of oxygen and/or chlorphentermine-induced alterations in pulmonary glycolytic enzyme activities of newborn rats. Each point is the mean ± S.E.M. of eight pups in each group. For details concerning treatment, see legend to Fig. 1. An asterisk (*) indicates a statistically significant difference ($P < 0.05$) when compared with the respective control (saline + air). A dagger (†) indicates a statistically significant difference ($P < 0.05$) when compared with the corresponding control (O_2 + saline).

Table 2. Effects of chlorphentermine and/or oxygen on lactate and pyruvate levels and on the ratio of L/P in newborn lung*

Treatment group	Exposure period (days)	Metabolic variable		
		Lactate (μmoles/100 g)	Pyruvate (μmoles/100 g)	Lactate/Pyruvate ratio
Saline and air	3	101.2 ± 5.9	31.2 ± 5.4	3.3
	5	136.9 ± 4.9	29.2 ± 2.5	4.7
	7	113.0 ± 5.3	30.6 ± 1.7	3.7
Chlorphentermine and air	3	158.7 ± 17.6†	68.7 ± 6.3†	2.3
	5	175.5 ± 13.9†	65.7 ± 4.1†	2.7
	7	146.8 ± 8.8†	44.4 ± 6.7†	3.3
Saline and oxygen	3	162.4 ± 8.5†	68.7 ± 6.3†	2.6
	5	171.7 ± 10.1†	58.9 ± 3.5†	2.9
	7	168.3 ± 18.1†	51.2 ± 5.2†	3.3
Chlorphentermine and oxygen	3	317.8 ± 21.1‡	54.2 ± 5.7	5.9
	5	355.7 ± 10.3‡	51.3 ± 8.2	6.9
	7	334.6 ± 24.5‡	46.0 ± 5.1	7.3

* Each value is the mean ± S.E.M. of eight animals in each group. Newborn pups were given daily 20 mg/kg chlorphentermine for either 3, 5 or 7 days by the oral route while corresponding controls received physiological saline. Groups of pups were also exposed to 95% oxygen and given saline daily for either 3, 5 or 7 days. In the simultaneous experiment, pups were exposed to 95% oxygen and given daily 20 mg/kg chlorphentermine for either 3, 5 or 7 days.

† Statistically significant difference ($P < 0.05$) when compared with the corresponding value for saline and air.

‡ Statistically significant difference ($P < 0.05$) when compared with the value for corresponding control saline and oxygen.

95% oxygen for 3 days. Continuation of hyperoxygenation for 5 or 7 days did not increase the intensity of this edema in newborns. Our present data show that under the same conditions, with the same tissue, there was a significant reduction in the incorporation of thymidine into newborn lung DNA. This decrease in DNA synthesis subsequently returned to control values after 5 and 7 days of oxygen exposure. Northway *et al.* [20] also found that exposure of newborn mice to 96–100% oxygen for 72 hr resulted in a fall in incorporation of thymidine into DNA. When the oxygen exposure was continued from 96 to 144 hr, however, there was a significant increase in mouse pulmonary DNA synthesis [21]. Since the decrease in incorporation of thymidine into DNA seen in this study was followed by a return to control values at 1 week, it is conceivable that, unlike in the mouse, a longer duration of hyperoxia may be necessary to elevate pulmonary DNA synthesis in newborn rats. In contrast to neonates, hyperoxia for 96 hr significantly elevated the RNA and protein content of adult rat lung [22, 23]. The observation that newborn lung RNA and protein levels were decreased by oxygen, while the reverse occurred in adults, is in

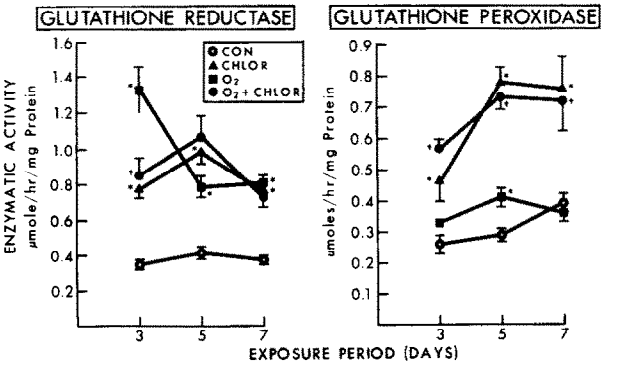

Fig. 3. Influence of oxygen and/or chlorphentermine on the activities of glutathione reductase and glutathione peroxidase in newborn rat lung. Each point is the mean ± S.E.M. of eight pups in each group. For details concerning treatment, see legend to Fig. 1. An asterisk (*) indicates a statistically significant difference ($P < 0.05$) when compared with the respective control (saline + air). A dagger (†) indicates a statistically significant difference ($P < 0.05$) when compared with the corresponding control (O_2 + saline).

accord with the findings that neonatal responsiveness to oxidant exposure differs from that seen in mature animals [1].

Our data also show that daily, oral administration of 20 mg/kg chlorphentermine elevated significantly the incorporation of thymidine into DNA of newborn lung as early as 3 days after initiation of treatment. Both an increase in pulmonary DNA levels and incorporation of thymidine into DNA was noted after 5 and 7 days of anorectic administration. Previously, Kacew *et al.* [5, 6] found that the 20 mg/kg chlorphentermine-induced rise in newborn lung DNA levels and synthesis seen after 1 week was associated without any apparent change in tissue morphology. As the dose of anorectic was increased to 40 and 60 mg/kg, there was a dose-related elevation in thymidine incorporation into DNA accompanied by an accumulation of pulmonary hypertrophic macrophages. Kacew *et al.* [6] suggested that estimation of DNA synthesis was a more sensitive indicator than morphologic examination in the case of chlorphentermine-induced effects on newborn lung. Results in the present study also demonstrate that, in addition to direct biochemical actions on lung, chlorphentermine modifies the oxygen-inflicted effects on pulmonary metabolism. Anorectic drug, administered concurrently with hyperoxia, increased DNA levels and incorporation of thymidine into DNA, effects opposite to those observed with O_2 alone. Although the significance of these biochemical findings with respect to organ functions such as gas exchange or regulation of endogenous substrate levels remains to be elucidated, the possibility exists that the observed effects of chlorphentermine and/or 95% oxygen on DNA synthesis may be due to differences in the uptake of thymidine into lung.

Glycolysis via the hexose monophosphate shunt is an important indicator of chemical-inflicted injury to lung, in that the pentoses that are generated are used for nucleic acid synthesis and repair. The chlorphentermine-induced increase in the incorporation of thymidine into DNA was accompanied by an elevation in the activities of G6PDH and 6PGDH. A similar stimulation in DNA synthesis associated with a rise in G6PDH was found in adult rats exposed to cadmium aerosol [24, 25]. In a recent study, Bassett and Fisher [26] demonstrated that hyperbaric oxygenation of perfused, adult rat lung at 5 atmospheres of O_2 caused increased lactate and pyruvate production associated with a fall in the L/P ratio, suggesting a shift toward oxidation of cytoplasmic pyridine nucleotides. In the present study, anorectic drug treatment increased lactate and pyruvate levels, but lowered the L/P ratio. In agreement with the suggestion of Bassett and Fisher [26], our data show that chlorphentermine stimulates pentose shunt glycolysis in newborns, possibly through a shift toward oxidation of cytoplasmic pyridine nucleotides; the pentoses thus formed may serve as substrates in the observed increase in DNA synthesis. In contrast to chlorphentermine, the hyperoxic-induced decrease or lack of change in DNA synthesis in newborns was not associated with a marked alteration in pentose shunt glycolysis. The findings that, in newborns concurrently treated with chlorphentermine and hyperoxia, the observed changes in DNA synthesis and in the activities of G6PDH, 6PGDH, and LDH were similar to those seen with the anorectic alone and in a direction opposite to that induced by 95% oxygen suggests that neonatal lung may be more susceptible to the metabolic actions of this drug. With respect to the glycolytic end-products, chlorphentermine given concurrently with O_2 modified the oxygen-induced effect, as evidenced by an increase in the L/P ratio and an accumulation of lactate.

It has been established that one of the functions of the glutathione system, which consists of GP and GR, is to protect the lung from injury [7]. Yam *et al.* [1] reported that exposure of neonatal rats to 96–98% O_2 increased the activity of pulmonary GP and GR and also produced minimal alveolar edema. In contrast, severe morphologic changes inflicted by oxygen in adult lung were not associated with marked alterations in GP and GR. Additional evidence that a rise in pulmonary GP is associated with protection against O_2-induced injury, as evidenced by minimal histologic changes, was provided by Frank *et al.* [27] in oxygen-exposed neonatal rats, mice, and rabbits. They found that exposure to 95% oxygen of neonatal guinea pig and hamster, as well as adult rat, mouse and rabbit, resulted in marked morphologic changes accompanied by insignificant alteration in GP, but that the activities of GP and GR in newborn rat lung were elevated indicating that antioxidant defense mechanisms are dependent on age and species [27]. In contrast to the findings of Yam *et al.* [1], Kimball *et al.* [28] reported that oxygen-induced interstitial edema in adult rats was associated with stimulation in the activities of GP, GR, and G6PDH. Although a discrepancy may exist between these studies of adult lung responsiveness, there is agreement that hyperoxia increased the glutathione antioxidant system in newborns.

In addition to the reduction of oxidants, glutathione is essential for normal cellular function; in particular, it may be essential for cell proliferation. The possibility exists that the chlorphentermine-induced increase in incorporation of thymidine into DNA is related to enhanced glutathione formation and utilization, as reflected by a rise in GP and GR. Although simultaneous drug and oxygen treatment, in general, did not markedly alter GR, when compared with the effect of O_2 alone, the activity of GP was increased in concurrently exposed newborns, indicating a greater utilization of glutathione. It is possible that this enhancement in glutathione utilization was required by the lung for the increase in cell proliferation observed normally in newborns. At present, few studies are available on the interaction between O_2 and drugs in newborns. Our data, however, show that in newborns simultaneously exposed to hyperoxia and chlorphentermine the pulmonary metabolic responses seen resembled, in general, those produced by the anorectic alone and were different from those induced by oxygen. In conditions of concurrent hyperoxia and chlorphentermine exposure, the evidence indicates that pulmonary tissue is more susceptible to the effects of the anorectic.

Acknowledgements—This work was supported by grants from the Medical Research Council of Canada. We wish

to thank Dr. O. Svendsen, H. Lundbeck & Company, Copenhagen, Denmark, for the generous supply of chlorphentermine.

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