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Resolution of pulmonary hypertension and other features induced by chronic hypoxia in rats during complete and intermittent normoxia

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Herget, J, Suggett, A J, Leach, E, and Barer, G R (1978). *Thorax*, **33**, 468–473. **Resolution of pulmonary hypertension and other features induced by chronic hypoxia in rats during complete and intermittent normoxia.** Rats subjected to 10% O_2 (hypoxic rats) for various periods and recovery regimens were compared with control animals with respect to pulmonary artery pressure (Ppa), right ventricular hypertrophy (RVH), and muscularisation of small pulmonary vessels. Mean Ppa was measured in anaesthetised animals spontaneously breathing air and rose from 16 mmHg in controls to 36 mmHg in rats exposed to hypoxia for three weeks. Ppa had returned to normal after 20 weeks' recovery in air. RVH regressed a little more quickly, but muscularisation of small pulmonary vessels was still apparent after 20 weeks. Some hypoxic rats were subjected to an intermittent normoxic recovery regimen for either 40 or 80 hours a week in air, the remainder in 10% O_2. Some reduction in RVH probably occurred after six weeks on the 80-hour regimen, but there was no reduction in Ppa or muscularisation of small pulmonary vessels. These results suggest that the pulmonary hypertension of chronic alveolar hypoxia resolves very slowly and is probably related to structural changes in the pulmonary vessels. Their relevance to human cor pulmonale and intermittent long-term oxygen treatment for these patients is discussed.

We have shown that rats kept in a hypobaric or a normobaric hypoxic chamber develop many of the features found in human hypoxic disease and in man at high altitude (Hunter *et al*, 1974; Leach *et al*, 1977a). In an attempt to simulate the situation in patients with hypoxic cor pulmonale on long-term O_2 treatment, we subjected the hypoxic rats to periods in air (intermittent normoxic regimen) and showed that resolution of right ventricular hypertrophy (RVH) and muscularisation of small pulmonary vessels was very slow (Leach *et al*, 1977a). In the present study we have measured pulmonary artery pressure (Ppa) in similar rats and have further examined RVH and muscularisation of small pulmonary vessels. A preliminary account has been published (Leach *et al*, 1977b).

Methods

Litters of male Wistar albino rats (Tucks's) were obtained when 21 days old. They were allowed to adapt to the laboratory for one week and then half of each litter were placed in the hypoxic chamber and half were kept in the same room in air as controls.

The chamber was a modification of that described by Cryer and Bartley (1974). It was normobaric, and its O_2 concentration was kept at 10%. The carbon dioxide concentration (0·2–0·6%), temperature, and humidity were always slightly higher inside the chamber. Details have been described (Leach *et al*, 1977a).

Ppa was measured while the rats breathed air spontaneously under urethane anaesthesia (1·5 g/kg with occasional supplements). The method was described by Herget and Paleček (1972). A specially shaped catheter inside an introducer, attached to an electromanometer (S E

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Laboratories), was inserted into the right ventricle via the external jugular vein. The catheter alone was then manoeuvred into the pulmonary artery while the pressure was watched on an oscilloscope. When Ppa was observed, the introducer was withdrawn. Ppa was recorded on an ultraviolet recorder (S E Laboratories). Herget and Paleček (1972) give the arguments for the validity of measuring Ppa by this method in the rat and show that there is no deterioration in the circulatory condition of the rat over many hours with the catheter in position. In the rat there are large fluctuations in Ppa with respiration; therefore the electrical mean of both cardiac and respiratory variations was recorded.

The rats were killed after Ppa measurements. The heart was removed, and the right ventricle (RV) and left ventricle and septum (LV+S) were separately weighed. Phosphate buffered 10% formol-saline was introduced through a tracheal cannula at 20 cm H_20 pressure for about 5 minutes. The trachea was then tied off, and both lungs were placed in more buffered formol-saline for further fixation. Transverse sections of the lung at the hilar region were stained by Humberstone's modification of the Gomori stain for elastic tissue. Muscularisation of pulmonary arterioles was assessed from these sections by counting the percentage of vessels 50 μ diameter or less with a double elastic lamina round half or more of its circumference situated distal to respiratory bronchioles—that is, next to alveolar ducts and alveoli. We recorded the percentage of thick-walled (with double elastic laminas) peripheral vessels (%TWPV); the word "vessel" is used rather than arteriole because small venules cannot be distinguished from arterioles in this method. Details of these procedures and the validation of the vessel counting method have been described (Leach *et al*, 1977a). The counting method is preferred to measurements of medial thickness because the muscularisation in hypoxia consists of a peripheral extension of the muscular arterial coat to smaller vessels; hence by measuring the medial thickness of muscular vessels one does not compare the same population in control and hypoxic animals.

PROTOCOL OF EXPERIMENTS

Four separate experiments were performed; the groups treated in the same way are pooled and the numbers are given in table 1. At the start of experiments all animals were 28 days old. There were four groups of rats: (*a*) controls, (*b*) rats kept in 10% O_2 for varying periods (hypoxic rats), (*c*) rats kept in 10% O_2 for three weeks and then allowed to recover in air for varying periods, and (*d*) rats kept in 10% O_2 for three weeks and then subjected to an intermittent-normoxic regimen for six or 12 weeks; they were taken out of the chamber for eight or 16 hours a day, five days a week (total 40 or 80 hours a week) but spent the remainder of the time in 10% O_2.

Table 1 *Numbers of rats subjected to various treatments and measurements*

Treatment	*Duration (weeks)*	*Measurement—number of rats*				
		Ppa	*RV*	*V*	*RV*†	*V*†
Controls	3	12	27	23	14	14
Controls	9		8	8	8	8
Controls	15				6	6
Controls	23	5	8	8		
10% O_2	1		10	6		
10% O_2	1·4		8			
10% O_2	3	15	20	20	20	19
10% O_2	5	4	7	7		
10% O_2	9	2	4	4	5	5
10% O_2	11	4	7	7		
Air recovery*	6	7	7	7	21	20
Air recovery*	12				11	11
Air recovery*	20	6	8	8		
40-hour normoxia*	6	4	9	9	5	5
80-hour normoxia*	6	5	5	5	11	11
80-hour normoxia*	12				10	10

*All after an initial period of three weeks in 10% O_2; all tests began when rats were 28 days old.
†From Leach *et al* (1977a).
Ppa=Pulmonary artery pressure.
RV=Right ventricle weight.
V=Count of thick-walled lung vessels.

STATISTICS

Means and SEM are given. Means are compared by Student's *t* test. Where groups are pooled, means are compared between all groups treated similarly. When only one experiment was performed, treated animals are compared only with their own controls to allow for differences between litters. The groups compared are shown in tables 2 and 3.

Results

PULMONARY ARTERY PRESSURE

Figure 1 shows the Ppa in all groups of rats. Mean Ppa of the control group (of 2 different ages because results were similar) was 15·8±0·6 mmHg, while the mean Ppa in all hypoxic rats was 35·8± 2·0 mmHg ($P<0.001$); all hypoxic rats are pooled because there was no difference in Ppa between those exposed for three, five, nine, or 11 weeks. Ppa in the group that had recovered for six weeks in air after three week's hypoxia was 26·9± 1·6 mmHg, which is still significantly higher than the control group ($P<0.001$) but significantly lower than that of rats just removed from the chamber

Table 2 *Recovery from right ventricular hypertrophy. Statistical comparison of RV/LV+S between different groups*

Groups compared				
Group 1	*n*	*Group 2*	*n*	P
6 weeks' air recovery	28	3 weeks' hypoxia	40	<0·001
6 weeks' air recovery	28	9 weeks' controls	16	<0·001
*12 weeks' air recovery	11	3 weeks' hypoxia	12	<0·001
*12 weeks' air recovery	11	15 weeks' controls	6	NS
*20 weeks' air recovery	8	3 weeks' hypoxia	8	<0·001
*20 weeks' air recovery	8	23 weeks' controls	8	<0·05
40-hour normoxia, 6 weeks	14	3 weeks' hypoxia	40	NS
40-hour normoxia, 6 weeks	14	9 weeks' controls	16	<0·001
80-hour air normoxia, 6 weeks	16	3 weeks' hypoxia	40	<0·01
80-hour air normoxia, 6 weeks	16	9 weeks' controls	16	<0·001
*80-hour air normoxia, 12 weeks	10	3 weeks' hypoxia	40	<0·001
*80-hour air normoxia, 12 weeks	10	15 weeks' controls	6	<0·001

All recovery groups exposed first to three weeks in 10% O_2.
All recovery groups were compared with pooled three weeks' hypoxic group.
Recovery groups were compared with pooled control group of comparable age (eg 20 weeks' air recovery group were same age as 23 weeks' controls) if they were a pooled group. If they were from a single experiment (asterisk) they were compared with their litter-mate controls only. This procedure avoided bias due to differences between litters.

Table 3 *% TWPV and statistical comparison between groups*

Groups compared		*% TWPV*	*No of rats*	P
3 weeks' controls	⎫	11·5±0·5	37	<0·001
3 weeks' hypoxia	⎭	22·5±0·9	39	
6 weeks' air recovery	⎫	18·0±0·6	27	<0·001
3 weeks' hypoxia	⎭	22·5±0·9	39	
6 weeks' air recovery	⎫	18·0±0·6	27	<0·001
9 weeks' controls	⎭	11·1±0·8	16	
*12 weeks' air recovery	⎫	14·7±0·8	11	<0·001
15 weeks' controls	⎭	8·4±0·7	6	
*20 weeks' air recovery	⎫	16·4±1·1	8	<0·001
23 weeks' controls	⎭	9·7±0·9	8	
40 hours' normoxia, 6 weeks	⎫	24·8±1·3	14	NS
3 weeks' hypoxia	⎭	22·5±0·9	39	
80 hours' normoxia, 6 weeks	⎫	20·0±0·7	16	NS
3 weeks' hypoxia	⎭	22·5±0·9	39	
*80 hours' normoxia, 12 weeks	⎫	18·7±1·2	10	NS
3 weeks' hypoxia	⎭	22·2±1·3	11	

Pooled recovery groups are compared with pooled control or hypoxic groups.
Groups from a single experiment (asterisk) are compared only with their litter-mate controls or hypoxic rats.
All recovery groups exposed first to three weeks in 10% O_2.

(P<0·02). In the group that had recovered for 20 weeks in air after three weeks' hypoxia Ppa was 15·0±1·1 mmHg, which was not significantly different from the three or 23-week controls (which were not significantly different from each other). The groups subjected to the intermittent normoxic regimens after three weeks' hypoxia showed a

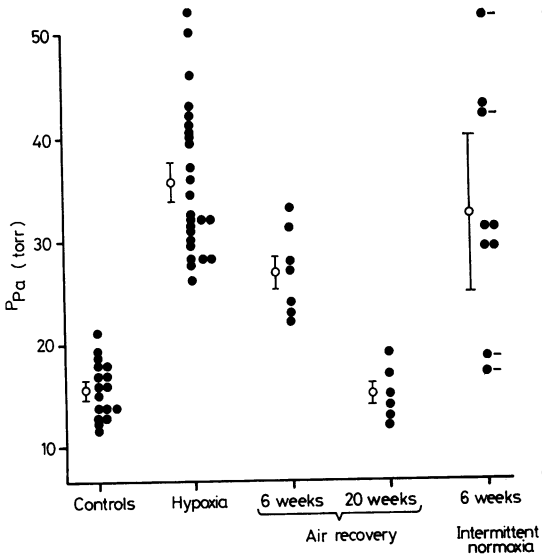

Fig 1 *Pulmonary artery pressure measurements for control rats (three and 23 weeks), hypoxic rats (three, five, nine, and 11 weeks in 10% O_2), rats alllowed to recover in air for six and 20 weeks after three weeks' hypoxia and rats submitted to intermittent normoxic regimens. Control and hypoxic groups of different durations are combined because values were similar. Forty- and 80-hour normoxic recovery groups are combined, but values from 40-hour group are marked ●—. Individual values and means ±SEM are shown.*

wide scatter, but the mean was 32·4±3·8 mmHg, not significantly different from the hypoxic group. Rats subjected to the 40 and 80 hours/week normoxic regimen are pooled. There were only four rats from the 40-hour regimen, two with high and two with low Ppa (as indicated in fig 1); the low values suggest some circulatory deterioration.

RIGHT VENTRICULAR HYPERTROPHY

Figure 2 shows the changes in the right ventricle caused by hypoxia in all groups including those from the earlier study (Leach *et al*, 1977a). We have recorded the ratio RV/LV+septum (RV/LV+S) as this is a commonly used measurement, and we showed in the earlier study that though not constant in control rats, it declines slowly as body weight increases. Figure 2 shows the regression line and 95% confidence limits for the large group of control animals examined earlier (Leach *et al*, 1977a). Against this background are plotted the mean RV/LV+S ratios for the groups exposed to 10% O_2 for 1, 1·4, 2, 3, 5, 9, and 11 weeks, together with the regression line for these values. All groups showed gross RVH relative to body weight

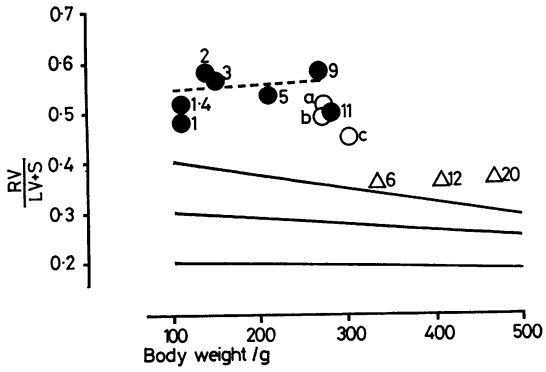

Fig 2 *Right ventricle/left ventricle +septum weight ratios related to body weight. Regression line and 95% confidence limits are shown for a large series of control rats from a previous study (Leach* et al, *1977a).* ●*=Mean values of this ratio for groups of rats kept in 10% O_2 for the number of weeks in the adjacent figure; dashed line is the regression line for these groups.* △*=Mean ratios for groups of rats allowed to recover in air for the number of weeks shown in adjacent figure.* a, b *and* c*=Mean ratios for rats subjected to intermittent normoxic regimens after three weeks in 10% O_2.* a*=six weeks of 40 hours' normoxia/week,* b*=six weeks of 80 hours' normoxia/week, and c=12 weeks of 80 hours' normoxia/week).*

(fig 2) and relative to controls, although they were always lighter than controls due to retardation of body growth. Control ratios are not plotted on fig 2 for simplicity, as they all fell within the confidence limits of the regression line for controls. Figure 2 also shows RV/LV+S for the air recovery and intermittent normoxic recovery groups, and table 2 shows the significance of the difference of these values from the appropriate control and hypoxic groups. The ratios of the 6-, 12-, and 20-week air recovery groups were still slightly outside the confidence limits for controls, although they were all significantly less than in hypoxic rats; only one group, the 12-week recovery group, had values not significantly different from its controls. The ratios of the groups exposed to intermittent normoxia were all significantly greater than controls. The "40-hour" intermittent normoxic group had a ratio not significantly different from the hypoxic group, but the groups subjected to six and 12 weeks of the 80-hour normoxic regimen had ratios significantly less than the hypoxic group. They were compared with the large group removed from the chamber after three weeks' hypoxia. It can be seen from fig 2, however, that the reduction in RV/LV+S is small and that the value for the group exposed to hypoxia for 11 weeks is close.

Figure 3 relates the Ppa in all groups to RV/LV +S. There is a significant correlation ($r=0{\cdot}73$, $P<0{\cdot}001$).

PERCENTAGE OF THICKED-WALLED PERIPHERAL VESSELS

We have pooled together the %TWPV measured in this with the previous study (Leach *et al*, 1977a). Table 3 shows the significance of the difference between values for the various groups. The mean % TWPV was 11·5±0·5 for all three-week control rats and 22·5±0·9 in rats exposed to 10% O_2 for three weeks. Older controls were similar to three-week controls. Rats exposed to hypoxia for 1·4, 2, 3, 5, 9, and 11 weeks all gave similar values, but those exposed for only one week had a value not different from controls. After three weeks' hypoxia and six weeks' recovery in air % TWPV was 18± 0·6; after three weeks' hypoxia and 12 and 20 weeks' recovery in air the % TWPV were 14·7± 0·8 and 16·4±1·1 respectively, both of which values were still significantly higher than those of corresponding control groups. The % TWPV for groups exposed to 10% O_2 for three weeks and then allowed six weeks of the 40-hour intermittent normoxic regimen, six weeks of the 80-hour regimen, and 12 weeks of the 80-hour regimen were, respectively, 24·8±1·3, 20±0·7, and 18·7±1·2. None of these values were significantly lower than that of the rats tested directly after three weeks in 10% O_2.

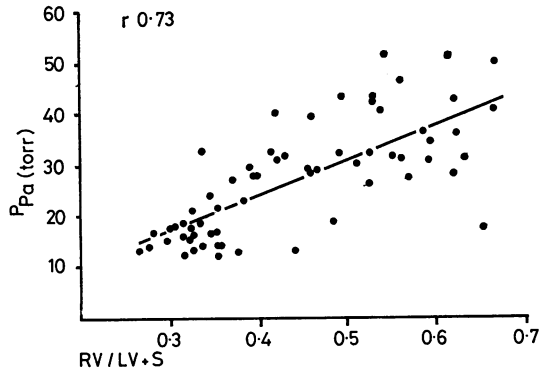

Fig 3 *Relation between pulmonary artery pressure and RV/LV+S for all groups and regression line.*

Figure 4 relates Ppa in all groups to the % TWPV ($r=0{\cdot}63$, $P<0{\cdot}001$).

Discussion

Both Abraham *et al* (1971) and Ressl *et al* (1974) showed that chronic hypoxia in rats was associated

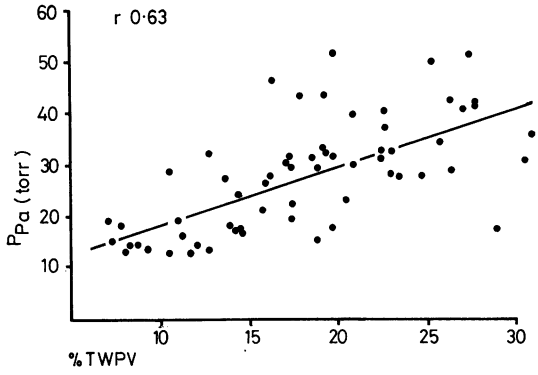

Fig 4 *Relation between pulmonary artery pressure and % thick-walled peripheral vessels for all groups and regression line.*

with increased right ventricular pressure and that this was reversible when they were returned to air.

We have now measured Ppa in chronically hypoxic rats. When exposed to 10% O_2, which is equivalent to an altitude of 5850 m, the Ppa rose to 36 mmHg. This more than twice normal value may be attributed to the structural changes in the pulmonary vessels because there was no stimulus to hypoxic vasoconstriction at the time of measurement. It is in accord with the observation that pulmonary hypertension at high altitude is reduced but not to normal when O_2 is breathed. Ressl *et al* (1974) also observed only a partial reduction of the raised right ventricular pressure of chronically hypoxic rats when they were given O_2 to breathe.

We previously showed that pulmonary vascular resistance, measured in isolated perfused lungs ventilated with air, is significantly increased in chronically hypoxic rats (Hunter *et al*, 1974). The increased Ppa and resistance is probably attributable to the thickening of small peripheral vessels on the arterial side of the vascular bed. Two alternative explanations cannot be excluded; that high Ppa is due, even during air breathing, to high vascular tone or that it is due to high cardiac output. The latter seems improbable because in man cardiac output is not increased at high altitude but often reduced, and even were it increased in rats during hypoxia there seems no reason why it should persist on removal from the chamber. Recently McMurtry *et al* (1977) have shown that normoxia partially reduces pulmonary hypertension in chronically hypoxic calves but that a β-adrenoreceptor agonist reduces the pulmonary hypertension nearly to normal. Thus the residual hypertension during air or oxygen breathing could be due to high vascular tone from a non-hypoxic cause.

When the rats returned to air pulmonary hypertension resolved very slowly compared with its development in hypoxic environment. In man Peñaloza *et al* (1962) showed that near-normal Ppas were recorded in high-altitude natives after two years at sea level. We have added to our previous measurements of RVH and % TWPV. The larger numbers show that the % TWPV as well as RV/LV+S are significantly reduced after six weeks' recovery in air. This emphasises the very slow resolution of vascular changes. Others using different methods of assessment have recorded faster resolution (Abraham *et al*, 1971; Ressl *et al*, 1974; Heath *et al*, 1973). We think our method of categorising small vessels detects changes in very small vessels whose medial thickness could not be measured (Leach, 1978). It has proved applicable to man in hypoxic cor pulmonale (Scott, 1976) and given results similar to those in the rat model of this disease.

RV/LV+S was slightly and significantly reduced on the 80-hour intermittent normoxic regimen compared with that of rat hearts weighed after three weeks' hypoxia, but the reduction was small and probably within the range of variability of values for rats kept continuously in the chamber for longer periods (fig 2).

Ppa and % TWPV were not reduced by the intermittent normoxic regimens. These were designed to simulate the treatment of patients with hypoxic cor pulmonale on the MRC trial of long-term oxygen treatment. The value and limitation of the rat model for comparison with human hypoxic disease was previously evaluated (Leach *et al*, 1977a). It is likely that certain principles may be applied to the human condition. One of these is that even with perfect normoxia, rarely produced in the human disease, resolution of hypoxic changes in pulmonary vessels is likely to be very protracted.

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