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Atrophy of Leydig cells in the testes of men with longstanding chronic bronchitis and emphysema

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ABSTRACT The total volume of Leydig cells in the testes of men coming to necropsy with a history of chronic bronchitis and emphysema of at least 15 years' duration, and with morphological evidence at necropsy of the cardiopulmonary effects of hypoxia, was significantly less than the volume in matched controls (0·98 (SD 0·39) and 0·93 (0·42) ml *v* 1·66 (0·54) and 1·72 (0·52) ml for left and right testes respectively; $p < 0·005$). This atrophy may be a consequence of hypoxic inhibition of pituitary synthesis or release of luteinising hormone.

Introduction

Hypoxaemia as a consequence of chronic bronchitis and emphysema, idiopathic pulmonary fibrosis, and alveolar hypoventilation causes disturbances in the function of the hypothalamic-pituitary-gonadal axis in men.[1-7] This is reflected in a depression of serum concentrations of testosterone, an effect also seen during exposure of unacclimatised men to the hypobaric hypoxia of natural high altitude.[8] After one month's exposure to prolonged continuous hypobaric hypoxia, the testes of rats contain a significantly smaller volume of Leydig cells than do normoxic controls, a morphological change that is manifest functionally as a depression in concentrations of serum testosterone.[9,10] The aim of this study was to ascertain whether longstanding chronic bronchitis and emphysema with their attendant hypoxaemia induces changes in the population of Leydig cells in man.

Methods

SELECTION OF CASES

Ten male subjects with chronic bronchitis and emphysema were selected from those coming to necropsy at the University Department of Pathology at the Royal Liverpool Hospital by application of the following criteria: (1) a clinical history of chronic bronchitis and emphysema lasting at least 15 years, including documentation of the presence of arterial hypoxaemia on successive occasions during this period; (2) morphological changes of chronic bronchitis and emphysema and their sequelae in the lungs at necropsy and at the subsequent histological examination of bronchopulmonary tissue; (3) a right ventricular fresh weight after trimming of fat at necropsy of over 80 g.

Certain features served to exclude cases that fulfilled the above criteria. These included: (1) atheromatous stenosis of over 50% of the luminal cross sectional area of the lower abdominal aorta or the testicular arteries, changes that might have led to testicular ischaemia; (2) local disease in the area of the testes, including hernia or a history of herniorrhaphy and varicocele; (3) appreciable cardiopulmonary disease other than that due to chronic bronchitis and emphysema, including left ventricular hypertrophy or other morphological evidence of systemic hypertension, or a history or evidence at necropsy of disordered endocrine function; (4) prescription of any drug capable of affecting testicular function, particularly spironolactone, an aldosterone inhibitor with antiandrogenic side effects.

Details of the 10 subjects with chronic bronchitis and emphysema chosen for study are shown in the table. Equal care was taken in the selection of 15 control subjects, and the excluding features described above were strictly applied. No subject with a clinical history or evidence at necropsy of systemic hypertension or appreciable pulmonary disease was included. The mean age of both groups was 69 years. All subjects had at some time smoked from 1 to 40 cigarettes a day for 5–25 years; those with chronic bronchitis and emphysema had not been heavier smokers than had the control subjects, according to

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Details of the subjects with chronic bronchitis and emphysema

Subject No	Age (y)	Duration* of disease (y)	Treatment†	Left ventricular weight (g)	Right ventricular weight (g)	Emphysema and pulmonary vascular changes‡
1	55	20	Frusemide aminophylline salbutamol	136	90	Centrilobular emphysema of both upper and left lower lobes; intimal fibroelastosis and medial muscular hypertrophy of muscular pulmonary arteries
2	62	15	Frusemide aminophylline salbutamol ipratropium bromide	151	81	Gross panacinar emphysema of all lobes; medial muscular hypertrophy of pulmonary arteries
3	81	>20	Frusemide	140	81	Panacinar emphysema of all lobes
4	78	15	None	151	85	Panacinar emphysema of upper lobes with dense apical fibrosis
5	60	16	Frusemide aminophylline salbutamol	162	85	Centrilobular emphysema of upper lobes; intimal fibroelastosis of muscular pulmonary arteries
6	51	>20	Frusemide aminophylline digoxin verapamil	206	154	Panacinar emphysema of all lobes; considerable intimal fibroelastosis and medial muscular hypertrophy of muscular pulmonary arteries
7	78	>20	Frusemide	159	84	"Widespread" centrilobular emphysema
8	86	>20	Aminophylline salbutamol bumetanide temazepam baclofen sodium cromoglycate quinine sulphate	140	80	Centrilobular emphysema of both upper lobes
9	73	>20	Cefuroxime	138	80	Centrilobular emphysema of both upper lobes
10	70	18	Frusemide aminophylline	189	82	Panacinor emphysema of upper lobes; intimal fibroelastosis of muscular pulmonary arteries

*All except subject 2 had documentation of episodes of arterial hypoxaemia on several occasions during their illness.
†Drugs prescribed for at least one year before death.
‡All subjects had muscularised pulmonary arterioles and intimal longitudinal muscle in muscular pulmonary arteries, and all had some degree of hypertrophy and hyperplasia of bronchial mucous glands with squamous metaplasia of bronchial epithelium.

the clinical histories. In no case was the interval between death and necropsy greater than 18 hours.

FIXATION AND EXAMINATION OF PULMONARY TISSUE

Pairs of lungs were fixed in 10% formol-saline, all those from the bronchitic subjects and 10 of the 15 pairs from the control subjects by intratracheal perfusion. Sections from representative blocks of tissue were stained with haematoxylin and eosin and by an elastic and Van Gieson method. The degree of emphysema was not accurately assessed, since this was unnecessary for the purposes of this study.

MEASUREMENT OF TOTAL LEYDIG CELL VOLUME

Testes were removed at necropsy and weighed, and their dimensions were measured in three diameters with a pair of callipers. This was repeatable, with practice, with an error of only ± 1 mm. The formula for the calculation of the volume of an ellipsoid, to which the geometry of the testis closely approximates, was then applied. In this formula $V = 4/3\ \pi r_1 r_2 r_3$, where V is the volume of the ellipsoid and r_1, r_2, and r_3 are its three radii. The results of this method correlate very closely with those obtained by water displacement.[11] The testes were sliced into blocks of tissue 3–4 mm thick, fixed for 24 hours in Bouin's solution, and embedded in paraffin wax. Sections of 4 μm thickness were cut and stained with haematoxylin and eosin, and the proportion of each testis comprising Leydig cells was assessed by point counting,[12 13] an eyepiece graticule being used on which

were etched 25 randomly positioned points. At a magnification of ×100 5000 points were counted. Three components were recognised: Leydig cells, seminiferous tubules, and interstitial tissues with blood vessels and non-Leydig interstitial cells. From the value for the percentage volume of Leydig cells in each testis and by reference to the overall testicular volume, the total volume of Leydig cells in millilitres was calculated for each organ.

Results

All the bronchitic subjects in this study had right ventricular hypertrophy, and all had the changes of hypoxic pulmonary vascular disease. In three cases there was also hypertrophy of the medial muscle of muscular pulmonary arteries. The mean right ventricular weight for the 15 control subjects was 51 (SD 7·4) g.

Testicular weights and volumes in the two groups did not differ significantly. Mean (SD) weights and volumes for the testes of the controls were: right—22·4 (3·9) g, 24·0 (3·7) ml; left—21·5 (4·2) g, 22·9 (4·2) ml. The corresponding figures for the bronchitic subjects were: right—19·5 (4·9) g, 21·2 (4·8) ml; left—19·3 (4·9) g, 20·6 (4·6) ml. The mean total volumes of Leydig cells in the right and left testes of the bronchitic subjects, however, were 0·93 (0·42) and 0·98 (0·39), whilst the figures for the controls were 1·72 (0·52) and 1·66 (0·54) ml (fig 1), the difference being significant ($p < 0.005$ by Student's t test). In some of the bronchitic subjects the loss of Leydig cells was obvious even on cursory examination (fig 2). An additional relevant finding was the absence of aggregates of apparently hyperplastic Leydig cells from the testes of subjects with chronic bronchitis. These were common in most of the control cases, and are considered to be a feature of the aging testis.[14] There was no difference in the cytological appearance of the Leydig cells of the

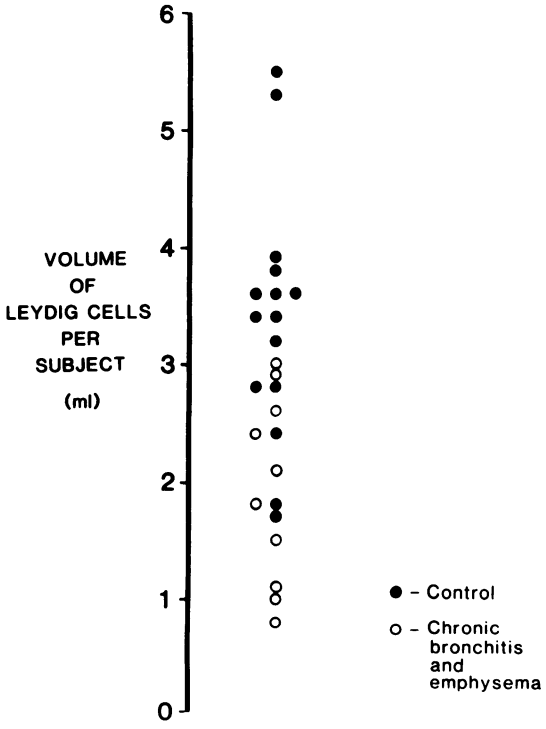

Fig 1 *Total volumes of Leydig cells in the testes of 10 men with longstanding chronic bronchitis and emphysema and in those of 15 matched controls. The difference between the two groups is significant ($p < 0.005$, Student's t test).*

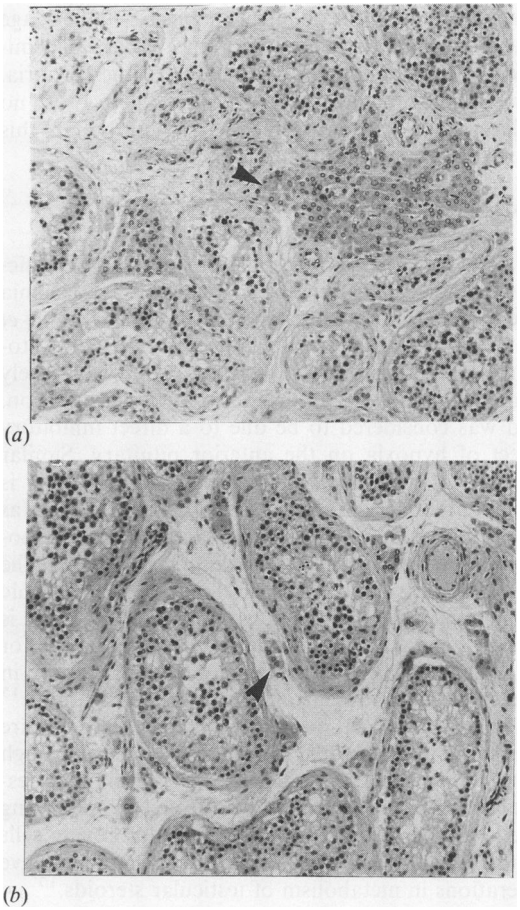

Fig 2 *Testicular tissue from (a) a control subject and (b) a subject with a 15 year history of chronic bronchitis and emphysema. In (a) clusters of Leydig cells are readily seen between the tubules, whereas in (b) they are infrequent (arrowheads). In both there is some age related thickening of the tunica propria of the seminiferous tubules. (Haematoxylin and eosin.)*

bronchitic and the control subjects. Other age changes, such as focal obliteration of groups of seminiferous tubules and thickening of the tunica propria, were found in both groups, and there was no difference in spermatogenic activity, although this showed wide variation from case to case.

Discussion

In a series of clinical investigations of hypothalamic-pituitary-testicular function in men with hypoxaemia due to chronic bronchitis and emphysema, Semple *et al*[1-5] showed that serum concentrations of testosterone were profoundly depressed. This was closely related to the degree of arterial oxygen desaturation, and was considered to be due to a direct inhibitory effect of hypoxia on the anterior pituitary. Similar depression of serum testosterone concentrations is seen in other conditions causing hypoxaemia, such as idiopathic pulmonary fibrosis[6] and alveolar hypoventilation due to the "Pickwickian syndrome."[7] The present study shows that in longstanding chronic bronchitis and emphysema with hypoxaemia there is loss of Leydig cells from the testis. Mean values for total Leydig cell volume from control subjects are in agreement with those from previous investigations,[15] although the normal range appears to be wide.[11] Care was taken to match the groups for age since, although testicular size, spermatogenesis, and synthesis of testosterone do not appear to be affected by increasing age,[16] an increase in the total volume of Leydig cells in the testes of old men has been reported,[14] as have alterations in metabolism of testicular steroids.[17]

A right ventricular weight of 80 g is usually taken as indicative of unequivocal right ventricular hypertrophy, although the normal range depends on body weight and to a lesser extent on height.[18] Most of the bronchitic subjects studied died with an artificially increased "lean" body mass as a result of systemic oedema, and therefore 80 g was taken as the upper limit of normal provided that this was still above the upper limit of normal calculated from body height. Although this is a less accurate means of assessing ventricular hypertrophy than calculation from body weight,[18] it served to prevent inclusion of any patient with a right ventricular weight greater than 80 g by virtue of body size alone. In the absence of any other cause, such as cardiac valvular disease or cardiomyopathy, it was considered to reflect the presence of pulmonary arterial hypertension as a consequence of chronic hypoxia, although other factors may have contributed.[19]

All of the 10 bronchitic subjects had muscularised pulmonary arterioles, and three showed arterial muscular hypertrophy. The latter change is uncommon in hypoxic pulmonary vascular disease, in contrast to other types of pulmonary hypertension, probably because the increase in pulmonary arterial pressure is not so great.[19] All patients except subject 2, who had had only one documented episode of hypoxia, had suffered many episodes of arterial hypoxaemia as judged by measurement of arterial blood gases on several occasions.

The method of measuring total Leydig cell volume has been used widely in the study of both human[11 14 15] and non-human[9 20] testes and provides accurate results, even when overall testicular volume is measured in the living subject and the proportional Leydig cell volume is calculated from a single biopsy specimen.

The smaller population of Leydig cells in the testes of the subjects with chronic bronchitis and emphysema is typical of the atrophy of endocrine tissues that occurs when there is a decrease in their stimulation, and would be expected as a consequence of diminished levels of gonadotrophins, particularly luteinising hormone. The latter has as its primary effect in the male the stimulation of steroidogenesis by testicular Leydig cells,[16] and levels of serum luteinising hormone have been shown to be decreased in men with depressed levels of testosterone due to hypoxaemia during exacerbations of chronic bronchitis and emphysema[5] and in subjects with idiopathic pulmonary fibrosis[6] and alveolar hypoventilation.[7] An analogous picture is seen in unacclimatised subjects exposed acutely to the hypobaric hypoxia of natural high altitude, where there is a decrease in serum levels of luteinising hormone[21] and testosterone.[8] In both circumstances the effects are transient, and in subjects with chronic bronchitis and emphysema, hormone levels return to normal when the exacerbation remits and hypoxia abates. Morphological effects in the relevant tissues might not therefore be expected except after either repeated episodes of short term hypoxia or in circumstances of chronic unremitting hypoxia. The subjects investigated in the present study were selected only if they had had clearly documented disease for at least 15 years, but at what point during the natural history of chronic bronchitis and emphysema with intermittent or chronic hypoxia does atrophy of Leydig cells begin? This is of some importance since, although concentrations of testosterone return rapidly to normal after an acute episode of hypoxaemia,[4] this would be unlikely to occur so readily in cases in which considerable numbers of Leydig cells had actually been lost from the testes. This has obvious implications, particularly in the younger patient with severe disease.

This investigation provides morphological evidence of one aspect of the changes in endocrine function that are increasingly recognised in patients with

chronic bronchitis and emphysema and supports the findings of previous functional studies.

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