Br. J. exp. Path. (1973) **54**, 388

THE EFFECT OF ISOPRENALINE AND PILOCARPINE ON (a) BRONCHIAL MUCUS-SECRETING TISSUE AND (b) PANCREAS, SALIVARY GLANDS, HEART, THYMUS, LIVER AND SPLEEN

J. STURGESS* AND L. REID

From the Department of Experimental Pathology, Cardiothoracic Institute, Brompton Hospital, London S.W.2

Received for publication March 7, 1973

Summary.—The effect was followed in the rat of 6 or 12 injections of isoprenaline (IPN), at a dose of 10 or 25 mg, and pilocarpine (PCP) at a dose of 10 mg.

In some respects the effects are similar, in others strikingly dissimilar. IPN and PCP each increase bronchial submucosal gland size and the number of goblet cells previously thought not to be under nervous control. Isoprenaline increases goblet cells containing acid glycoprotein, the PCP all types: IPN increases small acini in the gland, PCP large ones. The IPN effect was apparent even under germ-free conditions. After 12 injections of PCP the secretory cells appeared " exhausted " and relatively empty of secretion.

A similar picture was seen in the pancreas and the salivary glands—hypertrophy after IPN or 6 injections of PCP, exhaustion after 12 of PCP. In the heart, IPN caused an increase in ventricular weight (the right more affected than the left), increase in fibre size and a minor degree of myocardial damage; PCP caused only dilatation. After 6 injections, both IPN and PCP reduced thymic weight; this had recovered after 12 injections. The effect of PCP seems to be at least in part directly on discharge; IPN seems to affect synthesis.

This is the first demonstration of goblet cell increase by drug effect. These changes are considered in relation to control of mucus secretion and to their relevance to cystic fibrosis.

INCREASE in the number of goblet cells in the airway epithelium and in the size of the submucosal glands are the basis for mucus hypersecretion, leading to sputum production in chronic bronchitis and cystic fibrosis (MRC Definition, 1965; Reid, 1954, 1960; Andersen, 1938). Similar changes have been produced in experimental animals. In the rat goblet cell number has been increased by exposure to sulphur dioxide (Reid, 1963; Lamb and Reid, 1968, 1969) and by tobacco smoke (Lamb and Reid, 1969) and gland size by exposure to tobacco smoke (Jones, Bolduc and Reid, 1973). These irritants have produced a similar effect in other species (Mawdesley-Thomas, Healey and Barry, 1971) and other irritants such as nitrous oxide have a similar effect in the rat (Freeman and Haydon, 1964).

The present experiments were undertaken to trace the effect of isoprenaline on the mucus-secreting structures in the bronchial tree, an aspect not previously investigated. Pilocarpine was chosen for comparison since organ culture studies had demonstrated that this drug increased the rate of mucus secretion by human

* Present address: Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto 2, Canada.

submucosal glands (Sturgess, 1970; Sturgess and Reid, 1972*a*, *b*). Both drugs have been found to affect the glands and goblet cells of the tracheobronchial tree, findings not previously reported. Changes in other organs, including the secretory cells from the salivary glands, pancreas and heart were also investigated.

MATERIALS AND METHODS

Experimental animals.—Specific pathogen-free albino rats, 220–240 g in weight, were obtained from 2 sources—Anticimex (Sweden) and Carworth Europe (England). Rats were given food and water *ad libitum*. One experiment was carried out on germ-free rats under aseptic conditions at Allington Farm, Porton, Wilts, with the series of injections given by Dr J. S. Patterson and Mr R. Cook.

Administration of drugs.—Isoprenaline (IPN) was given to 32 rats, pilocarpine (PCP) to 24, and normal saline as control to 27. Injections of 0·5 ml in volume were given daily to each rat of either isoprenaline sulphate (10 or 25 mg) or of pilocarpine nitrate (10 mg). A series of experiments consisted of either 6 or 12 injections.

Each of 4 germ-free animals received 6 injections of 10 mg of IPN—those given higher doses died: 4 rats were used as controls.

Assessment of drug treatment

Histological examination.—Twenty-four hours after the last injection, each rat was anaesthetized with sodium pentobarbitone (0·5 mg/g body weight). The trachea was exposed and ligated so that the lungs could be inflated with neutral buffered formol saline. Both lungs and trachea were removed and fixed as described by Lamb and Reid (1968) so that the the trachea was straight and did not retract. Other organs—the heart, liver, pancreas, thymus, spleen and parotids, sublingual and submaxillary salivary glands—were removed and fixed in neutral buffered formol saline. After 24 hours' fixation, each organ was weighed and pieces taken for microscopical examination. The left ventricle and septum of the heart were weighed together but separately from the right ventricle, as described by Fulton, Hutchinson and Jones (1952). Tissues were dehydrated in graded alcohols, cleared in chloroform and embedded in paraffin wax. Sections, 4 μm thick, were cut and stained either with Erlich's haematoxylin eosin (H & E), with periodic acid Schiff (PAS) or with both alcian blue (AB) and PAS (AB/PAS), since in this combination the first identified acid glycoprotein as blue, the latter neutral glycoprotein as red (Jones and Reid, 1973*a*, *b*).

Organ culture.—The effect on normal rat trachea of IPN and PCP, each at a concentration of 10 μg/ml, was tested in organ culture. Glands from control, IPN and PCP treated animals were also compared in organ culture. Before formalin injection the upper 5 mm of trachea was cut transversely into 1·2 mm rings that were incubated for 4 hours using the techniques already described (Sturgess and Reid, 1972*a*) with tritium-labelled glucose (TRA-85, Radiochemical Centre, England) added to the culture fluid to give a concentration of 10 μCi/ml. After incubation tissues were fixed in neutral buffered formol saline and autoradiographs of 4 μm tissue sections, stained with PAS, were prepared (Sturgess and Reid, 1972*a*). For each treatment, material from the trachea of 2 rats was studied.

The secretory activity of the rat tracheobronchial gland was assessed by tracing the progress of radioactive tracers through the mucous cells as has been described for human bronchial gland (Sturgess and Reid, 1972*a*). The percentage of mucous cells discharging labelled mucus into the gland lumen at 4 hours was taken to be the Secretory Index (SI) of the gland.

Measurement of gland size.—The rest of the trachea was divided into 3 rings, each about 10 mm long. Serial transverse sections, 5 μm thick, were cut from each ring and at 40 μm intervals 2 consecutive sections were mounted, one being stained with H & E and one with AB/PAS. Twenty such pairs were prepared from each ring—these always included at least one whole gland; 120 sections were studied from each trachea.

In each cross section of trachea gland size was measured using the convention described by Lamb and Reid (1968); the length of the gland parallel to the circumference of the trachea and the maximum width of the gland was measured perpendicular to the surface epithelium. The depth of the gland was estimated by assessing the distance through the series of sections that the same gland could be traced.

Acinar diameter.—The diameter of each secretory acinus, whether lined with mucous or serous cells, in the tracheobronchial gland was measured as described by de Haller and Reid (1965). The mean diameter and the frequency distribution of different size tubules in each gland were calculated.

Cell size.—In the tracheal submucosal glands the height of the mucous and serous cells was estimated as the perpendicular distance between basement membrane and cell apex; the diameter of the nucleus was measured along the same axis. The size of the mucous and serous cells and their nuclei were calculated from 10 adjacent microscope fields in each of a series of 20 sections.

Goblet cell quantitation.—The number of goblet cells—in this context this includes all secretory cells, recognizable as such, by light microscope—was estimated in 3 regions of the tracheobronchial tree—trachea, proximal, and peripheral airways. Four types of goblet cell were characterized in the epithelium according to their size and staining affinity with AB/PAS. Some cells had only an apical zone of secretory granules, that stained either magenta with PAS having no reaction with AB (small PAS +ve), or both magenta with PAS and blue with AB (small AB +ve). Cells with the classic goblet shape and secretory granules extending to the cell base either stained with PAS only (full PAS +ve) or with both PAS and with AB (full AB +ve). The number of goblet cells of each type was recorded for a 6 mm length of airway epithelium cut in longitudinal section (Lamb and Reid, 1968).

RESULTS

A. *Tracheobronchial Tree*

Isoprenaline

Goblet cells.—In the trachea (Fig. 1) and at all levels in the bronchial tree the goblet cell number was increased by IPN. The increase correlated with the dose and number of injections. Only AB +ve goblet cells increased in number. After the 10 mg dose the main increase was in small AB +ve cells whereas after the 25 mg dose large AB +ve increased also. Under germ-free conditions the increase in goblet cell number and in gland size reported below was confirmed, establishing that these changes were produced by the drug and not by increasing susceptibility to infection.

Submucosal gland size.—IPN caused an increase in the mean gland width, length and depth of the submucosal glands, the effect being significantly greater with increase in dose and number of injections (Fig. 2). Similar increases were found when the largest glands from each rat were compared.

Acinar diameter.—IPN caused an increase in the mean acinar diameter of both mucous and serous acini (Fig. 3) because of increased cell size (see below) as well as widening of the lumen. The effect on the maximum acinar diameter was greater than on the mean, suggesting that new small acini were formed. This was confirmed by analysis of the distribution of acinar diameter in a control animal and a treated animal after 12 injections of 25 mg of IPN (Fig. 4).

Cell size.—IPN produced hypertrophy of both the mucous and serous cells. The mean height of the former increased from 10 to 14 μm after 12 injections of 25 mg, the mean height of the serous cell from 9 to 11 μm. The mean size of the mucous cell nucleus increased by a maximum of 25% as dose and number of injections increased. Only slight nuclear hypertrophy was seen in the serous cell and was similar, regardless of dose or number of injections.

Staining characteristics.—IPN altered the staining characteristics of both mucous and serous cells. After H & E, basophilia increased throughout the mucous cell and in the basal and lateral regions of the serous cell. With AB/PAS a larger part of the mucous cell stained blue and this more intensely while, in the serous

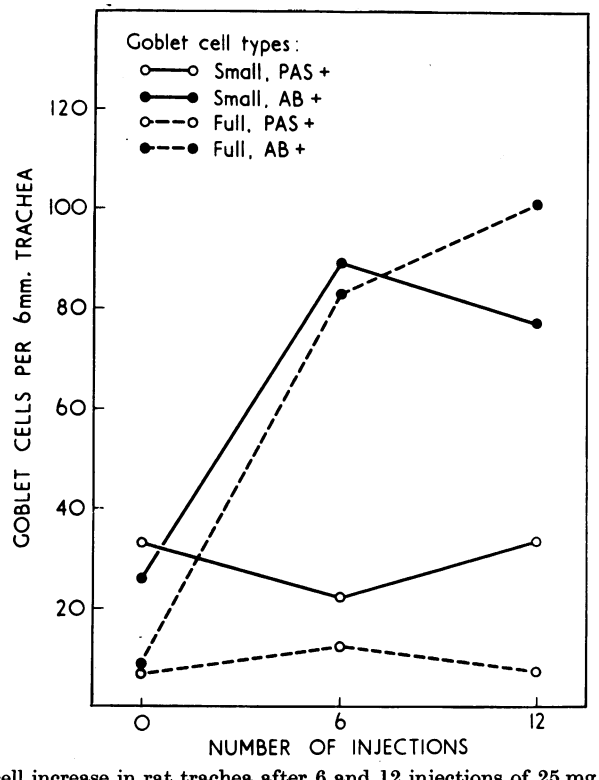

FIG. 1.—Goblet cell increase in rat trachea after 6 and 12 injections of 25 mg of isoprenaline. Small and full AB +ve goblet cells (contain acid glycoprotein) increase, with little change in PAS +ve cells (contain neutral glycoprotein). Each point, mean value of 6 rats.

cell, secretory granules stained more intensely with PAS and some stained blue with AB (in the control animals no blue granules were seen in this cell). After IPN the goblet cells were not only more numerous but more conspicuous since they contained a larger volume of intracellular mucus secretion whose granules stained more intensely with AB/PAS.

Pilocarpine

Goblet cells.—PCP caused an increase in the total number of goblet cells in the trachea (Fig. 5) and the bronchial tree. After 6 injections both the small PAS and AB +ve cells showed a slight increase; after 12 injections a larger increase was seen, all cell types participating but particularly the small PAS +ve cells. In all goblet cell types the volume of intracellular secretion increased, the secretory granules appeared larger, more conspicuous and with increased affinity for PAS stain, even in those cells that also stained with AB. The increase induced by PCP in goblet cell number and in gland size occurred also when the experiment was carried out under germ-free conditions.

Submucosal gland size.—Pilocarpine produced an increase in gland size, including length (Fig. 6), width and depth. Little change was detected in animals after

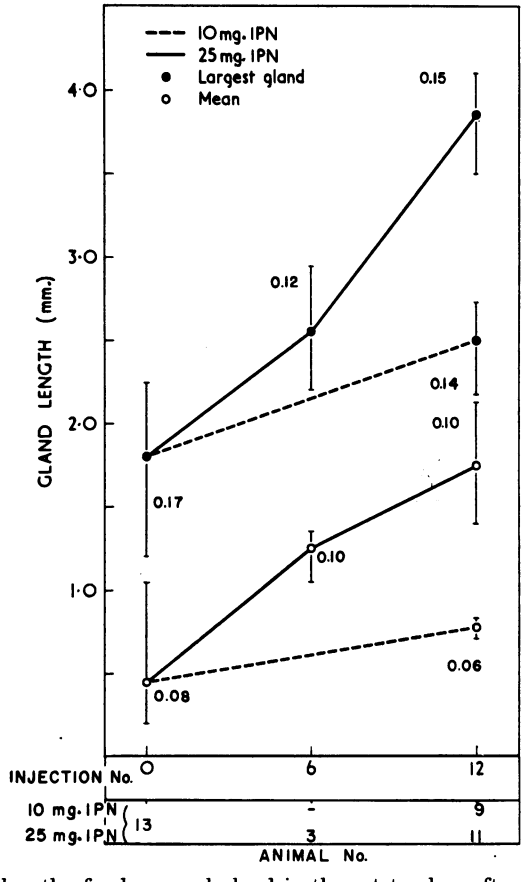

FIG. 2.—Increase in length of submucosal gland in the rat trachea after injections of 10 mg and 25 mg of isoprenaline. The increase in the largest gland is greater than the mean from each rat. Each point, mean gland length; vertical line, the range; the figure, the s.e. mean.

6 injections of pilocarpine, whereas after 12 injections the mean length of gland in the trachea increased as well as the size of the largest glands.

Acinar diameter.—Acinar diameter was also increased (Fig. 7), the largest acini of the glands were significantly greater than in the control animals ($P = < 0{\cdot}001$) but the difference in the mean acinar diameter did not achieve significance. Comparison of the frequency distribution of acini in the glands showed that the main effect of PCP was to increase the proportion of larger tubules (Fig. 8).

Cell size.—PCP produced hypertrophy of both the mucous and serous cells; for mucous cells the increase in mean height was from 9 μm in the control to 13 μm after 6 injections, and to 15 μm after 12 injections: for the serous cell the increase was from 9 to 12·5 μm after 6 injections, decreasing to 11 μm after 12 injections.

After 6 injections of PCP, the nuclear size of both mucous and serous cells was increased in height, the mucous cell showing a more striking change (60%)

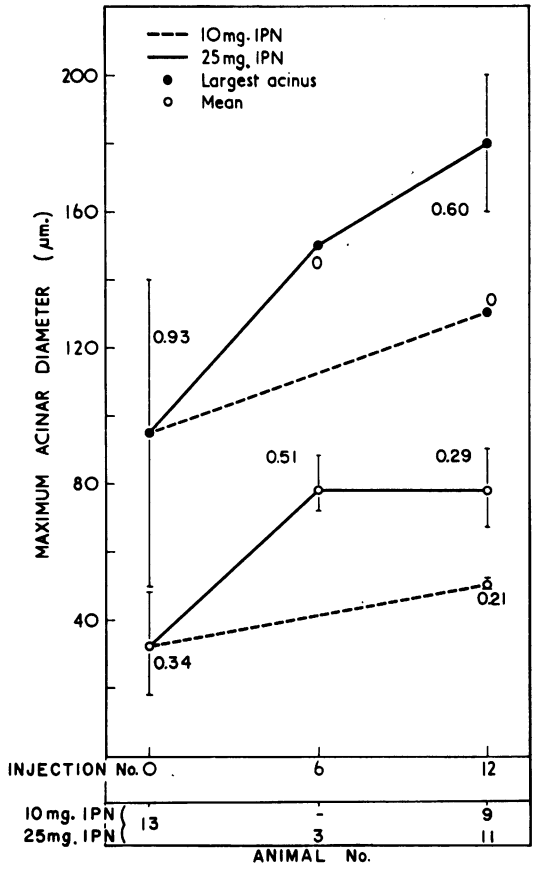

FIG. 3.—Increase in maximum acinar diameter after injections of 10 mg or 25 mg of isoprenaline. The mean diameter of the largest acinus on each animal increases with increasing number of isoprenaline injections whereas the mean for each section increases after 6 injections, with no further change after 12 injections. Each point, mean acinar diameter; vertical line, the range; the figure, the s.e. for each group.

than the serous (15%). After 12 injections, reduction in nuclear size occurred in both cell types: in the mucous cell the nuclei remained 30% larger than the controls whereas the serous cell nuclei resembled those of control cells.

Staining characteristics.—Six injections of PCP produced an increase in the amount of intracellular secretion in both the mucous and serous cells. With H & E the mucous cells were more basophilic and the cytoplasm vacuolated; in the serous cell an increase in basophilia was seen, particularly in the basal and lateral cell areas. With AB/PAS, increased PAS staining intensity in mucous cells caused a shift from the normal blue to blue–red colours. In the serous cell the PAS +ve granules were slightly bigger and more conspicuous.

After 12 injections, striking cytoplasmic changes were seen in both mucous and serous cells which suggested " exhaustion " of the cells. The nuclei of both cell types were densely granular and with irregular outlines. With H & E the cytoplasm of the mucous cell was still basophilic but showed extensive vacuolization

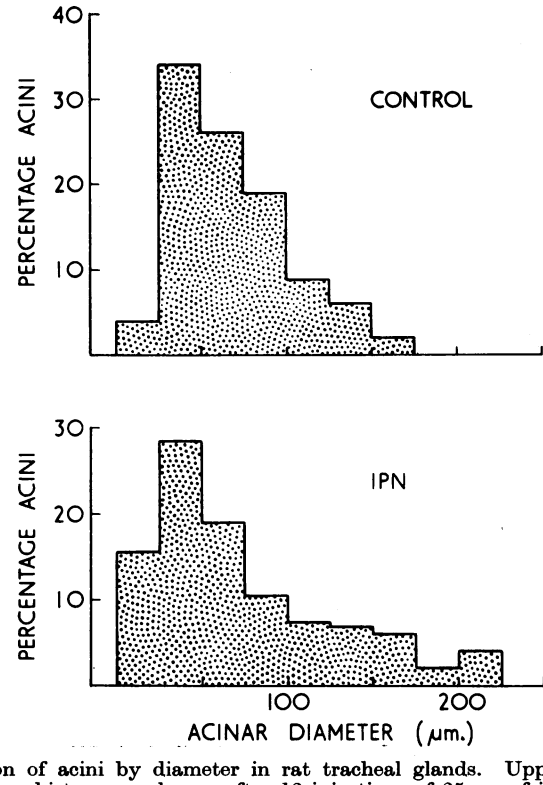

FIG. 4.—Distribution of acini by diameter in rat tracheal glands. Upper histogram, the control gland; lower histogram, change after 12 injections of 25 mg of isoprenaline. This causes an increase in the proportion of both large and small acini.

throughout the cell, particularly in the apical half where secretory granules are found. The serous cell had lost its secretory granules and its cytoplasm was evenly eosinophilic. With AB/PAS the mucous cell stained either blue or blue–red, but the intensity of staining was greatly reduced; in the serous cell granules were seen only occasionally.

Secretory activity of mucous cells in organ culture (after IPN and PCP)

In organ culture of normal rat trachea, the Secretory Index or SI (*i.e.*, percentage of mucous cells discharging labelled mucus into the gland lumen after 4 hours' incubation with radioactive tracer) was 30% in the control preparation. Addition of IPN to the incubation medium raised the SI to 35%, an increase not statistically significant (Sturgess and Reid, 1972*b*). The SI of mucous cells for explants treated with PCP rose to 60%. The radioactive label was moving significantly more rapidly through the cell population in the presence of PCP.

The trachea of rats that had received 12 injections of 25 mg IPN gave, in organ culture, a SI on average 15% higher than that of controls—a difference not statistically significant (no further IPN was added to the culture medium). The trachea from rats which had received 12 injections of PCP were unsatisfactory

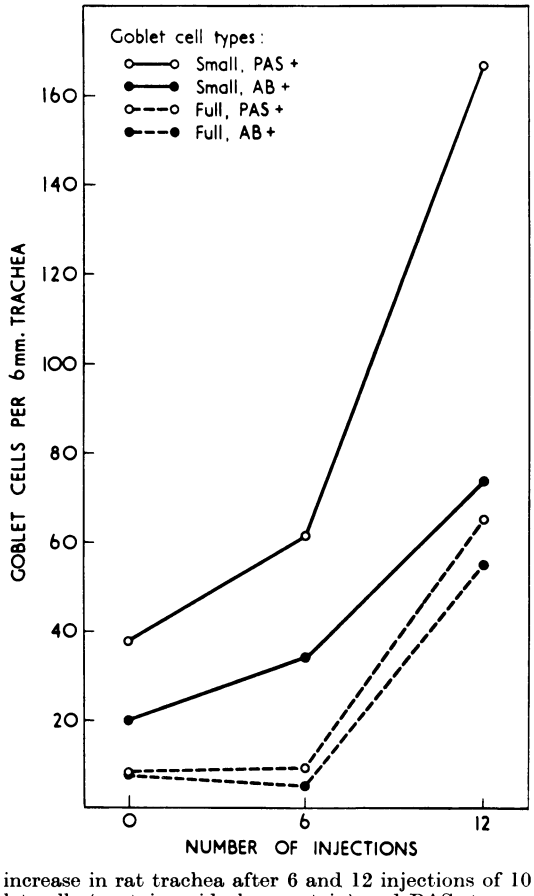

FIG. 5.—Goblet cells increase in rat trachea after 6 and 12 injections of 10 mg of pilocarpine. Both AB +ve goblet cells (contain acid glycoprotein) and PAS +ve goblet cells (contain neutral glyocoprotein) are increased, particularly the small PAS +ve. Each point, mean value of 6 rats.

in organ culture since the cells appeared to be poorly preserved and took up little radioactive tracer. The assessment of SI in these was inconclusive.

B. *Organs other than lung*

Isoprenaline

Pancreas.—IPN caused approximately 25% increase in weight of the pancreas after 12 injections of either 10 mg or 25 mg. Microscopical examination revealed hypertrophy of the acinar cell as well as a larger area of the cell filled with intracellular secretion. With H & E the cell base was more strongly basophilic, the secretory granules more eosinophilic.

Salivary glands.—After IPN the submaxillary and parotid glands increased in volume and in weight, confirming previous findings (as reviewed by Schneyer and Schneyer, 1967). No change was detected in the sublingual gland. The submaxillary gland showed a four-fold increase in weight after 6 injections of

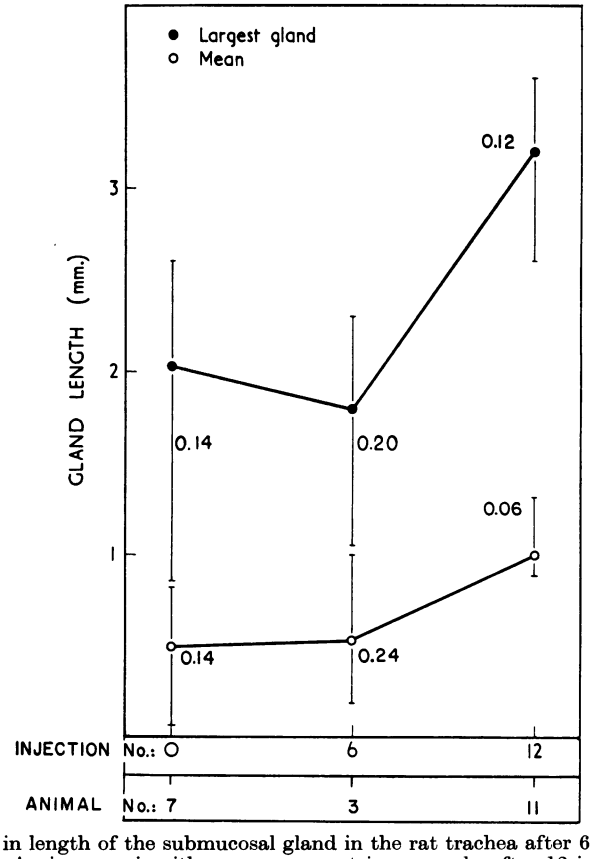

FIG. 6.—Increase in length of the submucosal gland in the rat trachea after 6 or 12 injections of pilocarpine. An increase in either measurement is seen only after 12 injections. Each point, mean gland length; vertical line, the range; the figure, the s.e. mean.

IPN; 12 injections of either 10 or 25 mg was followed by a five-fold increase. The increase in parotid gland weight was even greater—up to six-fold—after 12 injections of either 10 or 25 mg isoprenaline. In both submaxillary and parotid glands the acini were enlarged and individual cells had a larger area of intracellular secretion and were more basophilic. The secretory granules were more obvious, being larger, more conspicuous and staining more intensely blue with AB/PAS.

Heart.—IPN produced an increase in overall heart weight, a relative right ventricular hypertrophy with increase in myocardial fibre size and also inflammatory lesions. All of these features increased with increase in dose and number of injections of IPN. The maximum increase in total heart weight was 55%. In these specimens, the ratio of the weight of the left ventricle and septum to the right fell from 3·3 to 2·3, indicating a relative right ventricular hypertrophy. Microscopical examination of fibres, only from the left ventricle, revealed a two-fold increase in mean fibre diameter of treated animals. In the subendocardial region lesions as described by Rosenblum, Wohl and Stein (1965) were present, save that there was no capillary proliferation or lymphocyte infiltration; the main feature was newly formed collagen.

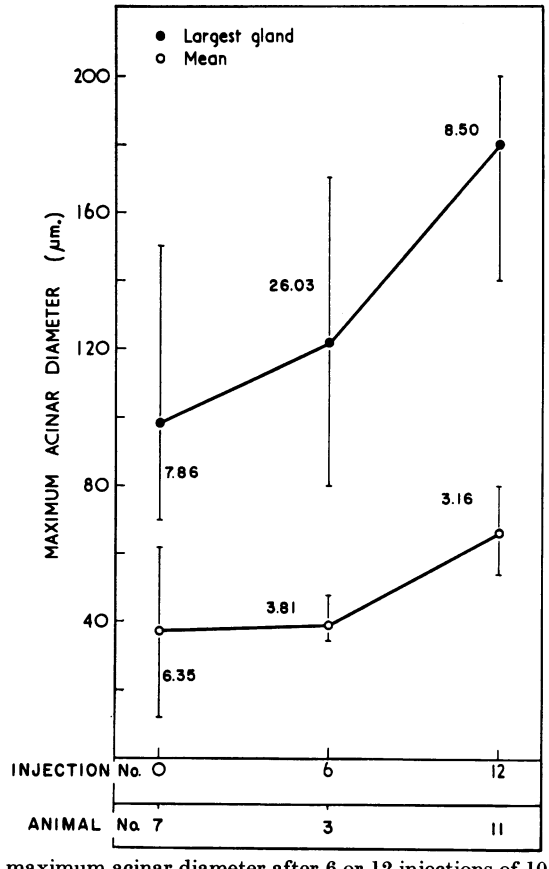

FIG. 7.—Increase in maximum acinar diameter after 6 or 12 injections of 10 mg of pilocarpine. The value for largest gland from each rat has increased after 6 injections, the mean value for each section only after 12.

Other organs.—In the thymus, after 6 injections of 25 mg IPN a dramatic decrease in size and loss of weight was seen. After 12 injections of either dose this was not as great, but there was overlap in the range of values. IPN had no effect on the weight, macroscopical or microscopical appearance of the liver or spleen.

Pilocarpine

Pancreas.—After 6 injections of PCP the weight of the pancreas increased by about 10% but after 12 injections a sharp loss occurred so that the average weight of the pancreas was almost 30% less than in the controls.

After 6 injections cells and acini were enlarged and the interacinar spaces had disappeared. Similarly, the lobules had enlarged while the spaces between them had reduced. The volume of the intracellular secretory mass was increased, with secretory granules more intensely eosinophilic and the cytoplasm at the cell base more basophilic.

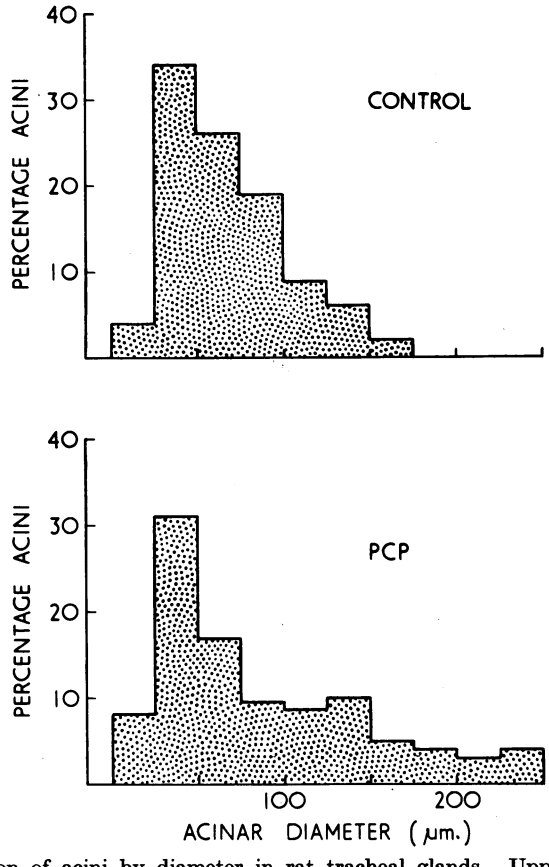

FIG. 8.—Distribution of acini by diameter in rat tracheal glands. Upper histogram, the control gland; lower histogram, change after 12 injections of 10 mg of pilocarpine. This causes an increase in the proportion of larger diameter acini (greater than 150 μm) with no effect on small.

By contrast, after 12 injections the acini were smaller and the space beween them larger; intracellular secretion was reduced. With H & E intense basophilia was still observed in the basal region but the eosinophilia of the secretory granules was reduced. The lobules were more obvious.

Salivary glands.—After 6 injections of PCP the weights of the sublingual, submaxillary and parotid glands were slightly increased; after 12 injections it was normal. In all salivary glands microscopical examination revealed that, after 6 injections, the secretory cells were larger, more basophilic and, with AB/PAS, stained more intensely, particularly with PAS. In the intercalated ducts an increase in number and prominence of PAS +ve granules was observed.

After 12 injections of PCP there was reduction in acinar size; the secretory cells were vacuolated, the granule number was somewhat reduced, giving an appearance of " exhausted " cells; while, with AB/PAS, some blue staining was seen, the predominent staining reaction was red.

Heart.—An increase in heart volume was observed in those rats which had received PCP, but no change in total heart weight, indicating dilatation rather than hypertrophy. No significant variation was found in the ratio of left to right ventricular weight, nor any muscle fibre hypertrophy or inflammatory lesion.

Other organs.—The weight of the thymus was reduced by 50% after 6 injections but had returned to within the normal range after 12 injections. No significant microscopical changes were observed. No macroscopical or microscopical changes were observed in the liver or the spleen after 6 or 12 injections of PCP.

DISCUSSION

The increase in the number of goblet cells after either IPN or PCP administration was not expected since Florey and his colleagues (1932) concluded from their experiments that secretion from the goblet cells was not under nervous control. An increase in the number of bronchial goblet cells has previously been caused only by irritation; this is the first time that such a change has been induced by drugs. That PCP increases the number of all histochemical types of goblet cell whereas IPN increases only those producing acid glycoprotein suggests that the 2 drugs are acting through different pathways.

Recently, nerve endings have been demonstrated within the rat epithelium of extrapulmonary airways, superficial to the basement membrane (Jeffery and Reid, 1973). The various structural types suggest that some are sensory, some motor, and these may be of either cholinergic or adrenergic type. The PCP effect would be mimicking a cholinergic effect, the IPN a β adrenergic one. The differences between these 2 pathways calls for further study since even the effect of blocking agents has not yet been followed.

While each drug produces gland hypertrophy, here also differences are found between them. Pilocarpine causes increase in gland size because the proportion of large tubules is increased as well as the secretory activity of both serous and mucous cells, although ultimately both cell types appear exhausted. This all points to general overactivity, suggesting that mucus gland secretion in the rat, certainly as measured by discharge from the cell, is under parasympathetic control as in man. It also seems that PCP produces its effect directly on discharge. If cell discharge followed passively the accumulation of cell secretion, the appearance of an empty cell would not be expected. This suggests that synthesis within the cell cannot keep up with an active stimulus to discharge.

After IPN small and large tubules increase, mainly the mucous type; here, synthesis seems to have increased but not discharge, as measured by the Secretory Index. This suggests a selective effect, leading to hypertrophy of cells rather than hypersecretion from them. The IPN may be producing an effect on β adrenergic receptors in the bronchial gland resembling the rat submaxillary gland hypertrophy produced with this drug by Wells (1963) and inhibited by agents blocking β adrenergic receptors.

That IPN produces its effect by modifying intracellular metabolism is suggested by the relative increase in the acid glycoprotein secretion both in surface goblet cells and in the serous and mucous cells within the gland. Furthermore, the volume of secretion within the goblet and mucous cell is greatly increased. Recently the addition to tobacco of phenylmethyloxadiazole (PMO), an anti-inflammatory agent, has been shown to cause a similar increase in volume of

retained secretion within the bronchial goblet cells (Jones *et al.*, 1972, 1973). These 2 effects are currently being compared in more detail. Isoprenaline seems to cause an increase in cell synthesis and thus in gland size, PCP an increase in cell secretion with associated gland hypertrophy.

Although the IPN administered in these experiments is greatly in excess of any clinical dose, the rapid appearance of an increased number of goblet cells has serious clinical implications. Because salbutamol is now often used instead of IPN, these experiments are being repeated with salbutamol (Jones and Reid, 1973*c*). The ultrastructural features of these changes are also being followed.

Systemic changes following administration of isoprenaline and pilocarpine

In the rat both IPN and PCP cause widespread changes in various organs and glands other than the lung; the changes are different for each drug. Some have been reported previously but other features, as in the heart, pancreas and salivary glands, are reported here for the first time.

Heart.—With IPN heart enlargement was accompanied by increase in weight and by muscle fibre hypertrophy. This increased with dose and number of IPN injections. After PCP the heart was dilated but with no weight gain or muscle hypertrophy. After IPN myocardial fibres were hypertrophied and were more eosinophilic suggesting an increase in protein synthesis. In his salivary gland studies, Schneyer (1962) reported only increase in heart volume but the changes were not quantified. Gould (1960) states that " hypertrophy ", as shown by enlargement of the heart, is characteristic of myocarditis. The development of myocardial lesions in the rat heart after IPN has been described by Chappel *et al.* (1959) and Rosenblum *et al.* (1965). In the present study lower doses than theirs have been used and it has been possible to show that lesions appear at lower doses than those used by these authors and that the lesions increase in size and severity as dose and number of injections increase. Preliminary results of further studies suggest that IPN causes an increase in muscle fibre size in both the right and left ventricle, particularly the right, but that this is not due to changes in pulmonary artery muscle (Hislop, personal communication).

Pancreas.—In the pancreas also changes were found after IPN or PCP; although these findings are not entirely new, conflicting results in previous reports justify their inclusion here. We have found that IPN produced a slight degree of hypertrophy in the rat pancreas, seen as an increase in intracellular secretion. By contrast, PCP produced marked alteration in the size and structure of the pancreas. Initially, acinar cell hypertrophy was obvious because each cell contained more intracellular secretion and this stained more intensely eosinophilic than normally. Longer administration of PCP caused gland atrophy, as judged by reduced acinar cell size and loss of intracellular secretion.

The empty or " exhausted " look of the pancreatic acinar cells was reminiscent of that change seen in the mucous and serous cells of the tracheal submucosal gland; thus it seems that PCP, while first stimulating secretion, then hypertrophy, leads later to exhaustion of the secretory cell. Either synthesis does not keep up with the discharge caused by PCP or the PCP may have a separate effect on the cell membrane and prevent cell uptake of the necessary metabolic precursors.

Thymus.—After administration of either IPN or PCP to the rat—but particularly the former—the thymus lost weight and volume; this was observed also

in the germ-free rats. No distinctive feature was seen on microscopical examination. The mechanism responsible is not known, nor even whether it is the same for both drugs. Boyd and Jarzylo (1960) reported a similar effect in the puppy after administration of atropine. If in each case the drug caused release of corticosteroid hormones, the decrease in the thymus could be secondary to this (Bloom and Fawcett, 1962).

Cystic fibrosis (or mucoviscidosis)

Some of the changes produced by the administration of IPN and PCP are similar to those seen in cystic fibrosis, supporting the hypothesis that a system " such as the autonomic nervous system may be implicated " in the defect in this inherited disease (Barbero, 1968). Earlier, Roberts (1959) had suggested that it was due to an overactivity of the parasympathetic nervous system.

In the kitten pancreas Farber (1942) by prolonged administration of PCP produced changes that he considered suggested pancreatic achylia, and resembled those seen in cystic fibrosis. Using diisopropyl phosphorofluoridate (DFP)—a potent acetylcholinesterase inhibitor—Wagner and his colleagues (1960) were unable to reproduce the pancreatic changes and disputed Farber's findings although without direct investigation of cholinergic drugs. In puppies, Boyd and Jarzylo (1960) claimed to have reproduced, by chronic atropinization, some of the changes found in cystic fibrosis. The results described here show that administration of pilocarpine causes first " hypertrophy " with increase in intracellular secretion of the pancreatic acinar cells and later results in " exhaustion " of the acinar cells, then degeneration and fibrosis. The pancreatic changes more closely resemble an over-effect of PCP.

Hypersecretion and hypertrophy of mucus cells in the salivary glands have been reported in cystic fibrosis (Shackleford and Bentley, 1964). These changes resemble those reported here after administration of either IPN or PCP. More detailed histochemical change in both children with the disease and in the experimental animal may enable more precise analysis of this effect.

The occurrence of myocardial lesions in cystic fibrosis has been mentioned and discussed by Tayot and his colleagues (1967). While these do not include capillary proliferation and lymphocyte infiltration as are reported here after IPN, they may represent a mild degree of this effect.

Bronchial gland hypertrophy.—Increase in the number of goblet cells and of the size of the submucosal glands, as seen in the rat bronchial tree after injection of either IPN or PCP, are seen also in patients with cystic fibrosis and lung involvement. In a group of animal experiments, not reported here, in rats that received IPN and also had respiratory infection as judged by lymphocyte infiltration, the tracheal gland and epithelial hypertrophy was gross and suggested that the structural changes seen after IPN administration were greatly enhanced by infection. In this respect the changes in cystic fibrosis in the surface epithelium resemble more those seen after IPN than PCP. Furthermore, in the cystic fibrosis children, as in other types of human mucous gland hypertrophy, it is the acid glycoprotein that is particularly increased (Lamb and Reid, 1972). Here an IPN type of overactivity seems to be mimicked by the disease.

Recently it has been reported that the serum of rats in which salivary gland hypertrophy has been induced by IPN contains a factor with a ciliostatic effect similar to that of the serum of children with cystic fibrosis (Mangos *et al.*, 1969).

It would seem that overactivity of the cholinergic system as well as of the β adrenergic system must each be considered since some of the changes in the disease resemble an IPN, some a PCP, effect. It may be that the " fundamental defect " should be looked for in imbalance between them.

We would like to thank the Medical Research Council who supported this work, and Professor B. Benjamin for his advice with statistical analysis. It is part of the work accepted for the degree of Ph.D. London University (J.S.).

REFERENCES

ANDERSON, D. H. (1938) Cystic Fibrosis of Pancreas and its Relation to Coeliac Disease. Clinical and Pathologic study. *Am. J. Dis. Child.*, **56**, 344.

BARBERO, G. J. (1968) Pathogenesis of Cystic Fibrosis: some Current Views. *Cystic Fibrosis.* Ciba Foundation Study Group 32, p. 2.

BLOOM, W. & FAWCETT, D. W. (1962) *A Textbook on Histology.* Philadelphia/London: W. B. Saunders & Co.

BOYD, E. M. & JARZYLO, S. (1960) Chronic Atropinization and Fibrocystic Disease of the Pancreas. *Can. med. Ass. J.*, **82**, 821.

CHAPPEL, C. I., RONA, G., BALAZS, T. & GAUDRY, R. (1959) Comparison of Cardiotoxic Actions of Certain Sympathomimetic Amines. *Can. J. Biochem. Physiol.*, **37**, 35.

DE HALLER, R. & REID, L. (1965) Adult Chronic Bronchitis. Morphology, Histochemistry and Vascularisation of the Bronchial Mucous Glands. *Med. thorac.*, **22**, 549.

FARBER, S. (1942) The Experimental Production of Achylia Pancreatica. *Am. J. Dis. Child.*, **64**, 953.

FLOREY, H. W., CARLETON, H. M. & WELLS, A. O. (1932) Mucus Secretion in the Trachea. *Br. J. exp. Path.*, **13**, 269.

FREEMAN, G. & HAYDON, G. B. (1964) Emphysema after Low Level Exposure to NO_2. *Archs envir. Hlth*, **8**, 125.

FULTON, R. M., HUTCHINSON, E. C. & JONES, A. M. (1952) Ventricular Weight in Cardiac Hypertrophy. *Br. Heart J.*, **14**, 413.

GOULD, S. E. (1960) *Pathology of the Heart.* Springfield, Illinois: Charles C. Thomas, p. 784.

JEFFERY, P. & REID, L. (1973) Intra-epithelial Nerves in Normal Rat Airways—a Quantitative Electron Microscopic Study. *J. Anat.*, **114**, 35.

JONES, R. & REID, L. (1973*a*) The Effect of pH on Alcian Blue Staining of Epithelial Acid Glycoproteins. I. Sialomucins and Sulphomucins (Singly or in Simple Combinations). *Histochem. J.*, **5**, 9.

JONES, R. & REID, L. (1973*b*) The Effect of pH on Alcian Blue Staining of Epithelial Acid Glycoproteins. II. Human Bronchial Submucosal Gland. *Histochem. J.*, **5**, 19.

JONES, R. & REID, L. (1973*c*) Comparison of Salbutamol with Isoprenaline Effect on Rat Bronchial Epithelium. In preparation.

JONES, R., BOLDUC, P. & REID, L. (1972) Protection of Rat Bronchial Epithelium against Tobacco Smoke. *Br. med. J.*, ii, 142.

JONES, R., BOLDUC, P. & REID, L. (1973) Goblet Cell Glycoprotein and Tracheal Gland Hypertrophy in Rat Airways: the Effect of Tobacco Smoke with or without the Anti-inflammatory Agent Phenylmethyloxadiazole. *Br. J. exp. Path.*, **54**, 229.

LAMB, D. & REID, L. (1968) Mitotic Rates, Goblet Cell Increase and Histochemical Changes in Mucus in Rat Bronchial Epithelium during Exposure to Sulphur Dioxide. *J. Path. Bact.*, **96**, 97.

LAMB, D. & REID, L. (1969) Histochemical Types of Acidic Glycoprotein Produced by Mucous Cells of the Tracheobronchial Glands in Man. *J. Path.*, **98**, 213.

LAMB, D. & REID, L. (1972) The Tracheobronchial Submucosal Glands in Cystic Fibrosis: a Qualitative and Quantitative Histochemical Study. *Br. J. Dis. Chest*, **66**, 239.

MANGOS, J. A., MCSHERRY, N. R., BENKE, P. J. & SPOCK, A. (1969) Studies on the Pathogenesis of Cystic Fibrosis: the Isoproterenol Treated Rat as an Experimental Model. 5th International Cystic Fibrosis Conference, Churchill College, Cambridge. Sept. 1969. The Cystic Fibrosis Research Trust, London. p. 25.

MAWDESLEY-THOMAS, L. E., HEALEY, P. & BARRY, D. H. (1971) Experimental Bronchitis in Animals due to Sulphur Dioxide and Cigarette Smoke. An Automated Quantitative Study. In *Inhaled Particles, III.* Vol. 1. Proc. International Symposium, British Occupational Hygiene Soc. Ed. W. H. Walton. London: Unwin.

MEDICAL RESEARCH COUNCIL (1965) Definition and Classification of Chronic Bronchitis. Committee on the Aetiology of Chronic Bronchitis. *Lancet*, i, 775.

REID, L. (1954) Pathology of Chronic Bronchitis. *Lancet*, i, 279.

REID, L. (1960) Measurement of the Bronchial Mucous Gland Layer: a Diagnostic Yardstick in Chronic Bronchitis. *Thorax*, **15**, 132.

REID, L. (1963) An Experimental Study of Hypersecretion of Mucus in the Bronchial Tree. *Br. J. exp. Path.*, **44**, 437.

ROBERTS, G. B. S. (1959) Fundamental Defect in Fibrocystic Disease of the Pancreas. *Lancet*, ii, 964.

ROSENBLUM, I., WOHL, A. & STEIN, A. (1965) Studies on Cardiac Necrosis. I. Production of Cardiac Lesions with Sympathomimetic Amines. *Toxic. appl. Pharmac.*, **7**, 1.

SCHNEYER, C. A. (1962) Salivary Gland Changes after Isoproteronol Enlargement. *Am. J. Physiol.*, **203**, 232.

SHACKLEFORD, J. M. & BENTLEY, H. P. (1964) Changes in the Carbohydrate Histochemistry of the Salivary Glands and Pancreas in Cystic Fibrosis. In *Research on Pathogenesis of Cystic Fibrosis.* Ed. P. A. di Sant Agnese. Bethesda, Maryland: National Institute of Arthritis and Metabolic Disease.

STURGESS, J. (1970) The Control of the Bronchial Mucous Glands and their Secretion. Ph.D. London University.

STURGESS, J. & REID, L. (1972*a*) Secretory Activity of the Human Bronchial Mucous Glands *in vitro*. *Expl & molec. Pathol.*, **16**, 362.

STURGESS, J. & REID, L. (1972*b*) An Organ Culture Study of the Effect of Drugs on the Secretory Activity of the Human Bronchial Submucosal Gland. *Clin. Sci.*, **43**, 533.

TAYOT, J., CHAUVEAU, P., MOUY, A. & BLONDET, P. (1967) Lesions Myocardiques au cours de la Mucoviscidose. *Archs anat. Path.*, Paris, **15**, 25.

WAGNER, B. M., BARBERO, G. J., CHERNICK, W. & KIM, H. S. (1960) Histochemical Studies in Cystic Fibrosis of the Pancreas. *Am. J. Dis. Child.*, **99**, 61.

WELLS, H. (1963) The Neural Regulation of Salivary Gland Growth. *Ann. N.Y. Acad. Sci.*, **106**, 654.