Cytochrome oxidase activity is inhibited by chlorpromazine[14, 17] and imipramine[10, 13] (also by laurylamine and phencyclidine, unpublished work), but this could occur in a different manner, e.g. by limiting access of cytochrome *c*, which is cationic around pH 7. In general, the optimal concentration for a stimulatory effect by the four amines on succinoxidase activity has been 0·1 to 0·2 mM for laurylamine and chlorpromazine (depending on the concentration of mitochondria, usually 3–4 mg protein/2·5 ml), and 0·4 mM for imipramine,[3] and about 1 mM for phencyclidine; marked inhibition has been obtained at 0·2 to 0·3 mM with the first two compounds, at 0·8 mM imipramine (unpublished results), and at 7 mM phencyclidine. Thus, in proportion to the amounts that stimulate respiration, a much greater amount of phencyclidine is required for an inhibition of respiration. This suggests, that the sites that influence the stimulatory and inhibitory effects have differing affinities for the amines which would imply that the sites are different.

The various observations discussed here and the previous finding that differences in pH, which influenced respiration, had little or no effect on the amount of amine bound[3] seem to indicate that the amines interact with specific groups on the mitochondria. However, this still does not rule out suggestions that chlorpromazine may act primarily by affecting the mitochondrial membranes (Spirtes and Guth[18]) or structural organization (Løvtrup[12]). Weinbach and Garbus[19] have even suggested that dinitrophenol may bind to an enzyme or structural protein to cause a configurational or structural change rather than at the active site.

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Observations on the release of lysosomal enzymes from the isolated bovine adrenal gland

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STIMULATION of the isolated bovine adrenal gland by acetylcholine or carbachol causes the release of catecholamines,[1–3] of adenine nucleotides and their metabolites,[4, 5] of chromogranin A,[3, 6, 7] the main component of the soluble protein of chromaffin granules,[8] as well as the other soluble proteins

present in these particles.[3] The secretory products are all stored in the chromaffin granules of the adrenal medulla. Another particulate element present in these cells is the lysosomes; they are present in the bovine adrenal medulla[9, 10] and have also been observed in other species.[11, 12] The presence of characteristic lysosomal enzymes in perfusates from adrenal glands after stimulation of the gland with carbachol has now been studied.

Bovine adrenal glands are perfused with Tyrode solution at 37° and stimulated with carbachol (or dimethylphenylpiperazinium iodide, DMPP) as previously described.[3] For analysis of lysosomal enzyme activity, the perfusates were reduced in volume by ultrafiltration and dialyzed against Tris Na-succinate buffer (I 0·015, pH 5·9). Deoxyribonuclease (DNase) and ribonuclease (RNase) were assayed by the procedures of Smith and Winkler,[9] and β-glucuronidase activity was measured by the method of Gianetto and de Duve.[13] One unit of enzyme activity represents the amount of enzyme necessary to utilize 1 μmole substrate in 1 min.

In these experiments the resting secretion of catecholamines from the adrenal gland was generally less than 0·1 μmole/min. In seven experiments the resting value for DNase in the perfusate was 0·0207 ± 0·0041 (±S.E.) units/min and that for RNase was 0·082 ± 0·011 (±S.E.) units/min. Injection of carbachol (3·7 mg in 1 ml over a period of 135 sec) caused an approximately 10-fold increase in the secretion of catecholamines, and also increased the secretion of DNase and RNase (Table 1). The amount of catecholamines released was unrelated to the amounts of either DNase

TABLE 1. RELEASE OF LYSOSOMAL ENZYMES FROM THE BOVINE ADRENAL GLAND IN RESPONSE TO CARBACHOL

Enzyme	*n*	Increase in activity*
DNase	7	3·92 ± 0·92†
RNase	7	2·49 ± 0·47
β-glucuronidase	1	1·94

* Increase in activity is expressed as activity during stimulation period: activity during control period. Perfusates from 2–5 control and stimulation periods, respectively, were combined for analysis in each experiment. The duration of each collection period was 6 min.

† The figures represent the means (±S.E.) of *n* experiments.

or RNase secreted; the ratio micromoles catecholamines:DNase activity varied between 5·8 and 27·8, and the ratio micromoles catecholamines:RNase activity varied between 1·7 and 11·2. The average of the ratio of RNase activity to DNase activity secreted was 2·34; this is similar to the corresponding ratio of 2·64 obtained for these enzymes in lysosomes of bovine adrenal medulla[9] (Table 2).

TABLE 2. RELATIONSHIPS BETWEEN ACTIVITIES OF LYSOSOMAL ENZYMES IN PERFUSATES AND IN LYSOSOMES OF BOVINE ADRENAL MEDULLA

Enzymes	*n*	Perfusates	Adrenal medulla*
RNase/DNase	7	2·34 ± 1·08†	2·64
DNase/β-glucuronidase	1	61·5	26·4
RNase/β-glucuronidase	1	83·7	69·5

* Ratio of enzyme activities for adrenal medulla taken from ref. 9.

† Mean ± S.E.

In one experiment, perfusates from five collection periods were combined and assayed for β-glucuronidase activity as well as for the activities of DNase and RNase respectively. Release of β-glucuronidase was almost doubled after injection of carbachol (Table 1), and the relationship of the activity of this enzyme to DNase activity and to RNase activity in the perfusate was of the same order of magnitude as that found in lysosomes of bovine adrenal medulla[9] (Table 2).

It was found that secretion of catecholamines and acid nucleases could also be induced by DMPP. In three experiments, injection of 1 mg DMPP (in 1 ml, over a period of 135 sec) caused a 5- to 7-fold increase in the amount of catecholamines in the perfusates, and increased the secretion of DNase 2·19 (range, 1·80 to 2·49) times and of RNase 2·23 (range, 1·81 to 3·15) times. The ratio RNase activity:DNase activity was 2·93 (range, 1·96 to 4·12).

Injection of adrenaline in amounts similar to those found in perfusates after stimulation with carbachol did not cause the release of either DNase or RNase.

The DNase and RNase activities of the perfusates were also examined under conditions which abolish the carbachol-induced secretion of catecholamines. Release of DNase and RNase was expressed as the ratio of units of enzyme activity released during the stimulation period:units of enzyme activity released during the control period. This ratio was reduced to near unity in the presence of 250 μg/ml hexamethonium iodide (DNase, 1·0; RNase, 0·98) and of 30 μg/ml cocaine hydrochloride (DNase, 1·02; RNase, 1·16). In Ca^{2+}-free Tyrode solution, release of both enzymes after carbachol was less than during perfusion without carbachol (DNase, 0·81; RNase, 0·49).

The pH optimum for the secreted DNase was 4·6, the same as that found for DNase of bovine adrenal medulla.[14] With RNase there were two pH optima, one at 5·5 to 6·0, and another around 8 (Fig. 1). Stimulation of the gland with carbachol increased the acid RNase activity in the perfusate, whereas the RNase activity at pH 8·0 was unchanged, as is evident in Fig. 1. A pH optimum of 5·5

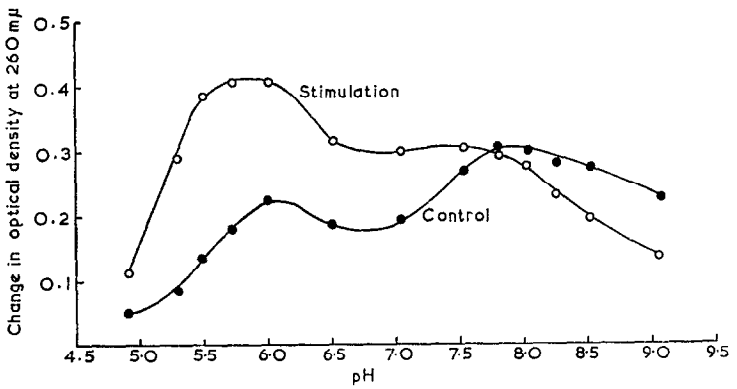

FIG. 1. Effect of stimulation by carbachol on secretion of ribonuclease from the bovine adrenal gland. Ribonuclease activity was determined as described in the text. The units of activity represent the optical density at 260 mμ produced by incubating aliquots of ultrafiltrates from perfusates of control and stimulation periods respectively.

has been reported for RNase of bovine adrenal medulla;[14] the slight shift of the optimum to a less acid value found in these experiments may be due to a contribution of the alkaline RNase also present in the perfusates.

It is possible to conclude from these findings that lysosomal enzymes are released from the isolated adrenal gland upon stimulation with carbachol or DMPP. It appears that the secretion is not due to an effect of catecholamines subsequent to their release from chromaffin cells, since no secretion of DNase or RNase was observed after injection of adrenaline into the gland. However, the possibility that other constituents of the chromaffin granule set free in response to carbachol may in their turn release lysosomal enzymes must also be considered. The similarity between the ratio of RNase activity:DNase activity, and the ratios of the activities of both acid nucleases to β-glucuronidase activity, found in both perfusates and lysosomes of bovine adrenal medulla, respectively, support the idea that it is the lysosomes of chromaffin cells that are involved. The effects of exposure of the gland to hexamethonium

or cocaine as well as to low Ca^{2+} are in agreement with such a suggestion, since these conditions are known to inhibit catecholamine release from chromaffin cells. However, on the basis of the evidence presented, liberation of enzymes from lysosomes of other cells in the adrenal medulla or elsewhere cannot be excluded.

Although it is not possible to interpret these findings in terms of a causal relationship between lysosomal function and secretion of catecholamines and other chromaffin granule constitutents from chromaffin cells, it is of interest that lysosomes have been implicated in various stages of secretory processes in other tissues. For instance, it appears that hormone-containing colloid droplets of the thyroid gland are digested by lysosomes prior to secretion of the hormone,[15] and that lysosomes may remove excess secretory granules of the mammotrophic hormone-producing cells of the rat anterior pituitary.[16]

On the other hand, an extracellular release of lysosomal enzymes from bone cells in response to parathyroid hormone[17] and in response to sucrose[18] has been described. Also, de Duve and Wattiaux[19] have suggested that lysosomes are involved in the secretion of waste products from cells and that this process may give rise to the release of lysosomal enzymes. It is suggested that stimulation of the adrenal gland by carbachol, in addition to causing the secretion of catecholamines from chromaffin granules, may also induce (or increase) cellular excretion.

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