dehydrogenase (reviewed by Greville, 1965) indicate that the availability of oxaloacetate to citrate synthase as well as succinate dehydrogenase is governed by the integrity of the energy-conservation mechanisms of oxidative phosphorylation and also the rate of flow through the electron-transport chain. Measurements of the concentration of oxaloacetate in the mitochondrial incubation mixture in the presence of L-malate with or without uncoupling agents showed that the relative rates of conversion of acetyl-CoA into citrate or acetoacetate are independent of the measured oxaloacetate concentration, and 'compartmentation' of oxaloacetate must be proposed.

Other mechanisms for the control of citrate synthase may be considered: (i) inhibition of citrate synthase by palmitoyl-CoA (Tubbs, 1963; Wieland & Weiss, 1963*b*) is rendered non-obligatory by the findings with octanoylcarnitine; (ii) reduction of oxaloacetate to malate by NADH produced during fatty acid oxidation, as suggested by Wieland, Weiss & Eger-Neufeldt (1964), is not excluded by our data but requires modification to take account of the failure of palmitoyl oxidation to alter the oxaloacetate content of the mitochondrial incubation mixture; their suggestion was based on the explicit assumption that 'compartmentation' of oxaloacetate could be disregarded.

Whatever the individual contribution towards the control of malate oxidation and intramitochondrial oxaloacetate concentration made by NAD reduction by 3-hydroxyacyl-CoA dehydrogenase, NAD reduction by acyl-CoA dehydrogenase in an energy-dependent process (Bode & Klingenberg, 1964; D. Shepherd, D. W. Yates & P. B. Garland, unpublished work), energy-dependent oxaloacetate transfer (Tager, 1965) and effects on the hydrogen transfer from NAD available to malate dehydrogenase to NAD available to the electron-transport chain (Gamble & Lehininger, 1956), it is evident that the regulation of acetyl-CoA conversion into citrate or acetoacetate is an energy-controlled process.

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Biochem. J. (1965) **97**, 40c

The Release of Protein from the Stimulated Adrenal Medulla

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Catecholamines are secreted and can be recovered in the perfusate when isolated bovine adrenal glands are perfused with oxygenated Tyrode solution at 37° and stimulated by the injection of carbamoylcholine (Banks, 1965). For the experiments described below the perfusion rate was 20–25ml./min. and during the stimulation periods 0·5ml. volumes of 10mM-carbamoylcholine were injected at 30sec. intervals for 3min.

It was noticed that the perfusates collected during

Table 1. *Effect of stimulation by carbamoylcholine on the release of catecholamines and protein from the perfused bovine adrenal gland*

In each experiment the perfusates were collected during a 3min. control period and during a 3min. period in which 0·5ml. of 10mM-carbamoylcholine was injected at 30sec. intervals. The perfusion rate was 20–25ml./min. and the perfusion medium was Tyrode solution at 37° gassed with O_2+CO_2 (95:5).

	Catecholamines released (μmoles)			Protein released (mg.)		
Expt. no.	Control period	Stimulation period	Difference	Control period	Stimulation period	Difference
1	0·2	7·4	7·2	2·9	4·0	1·1
2	0·4	8·9	8·5	3·0	3·7	0·7
3	0·3	15·4	15·1	3·0	4·9	1·9
4	0·5	9·2	8·7	1·7	4·0	2·3

periods when carbamoylcholine was injected contained more protein than those collected during control periods (Table 1). The haemoglobin contents of the perfusates from control and stimulation periods did not differ significantly, suggesting that the extra protein released during the stimulation periods was not derived from blood expelled from the gland.

In the chromaffin cells of the adrenal medulla the catecholamines are stored, together with ATP, in subcellular particles termed chromaffin granules (Blaschko, Hagen & Hagen, 1957). These granules can be isolated from homogenates of the adrenal medulla by a combination of differential and density-gradient centrifugation and are lysed when transferred to hypo-osmotic media. Lysis of the chromaffin granules is accompanied by the release into solution of the catecholamines, ATP and some 60–70% of the protein of the granules (Eade, 1957). It has been suggested that this soluble protein, together with the ATP, forms a complex which is responsible for binding the amines within the granules. About 5μmoles of catecholamines are associated with 1mg. of soluble protein in the granules. It was found that, on average, 6·6μmoles of catecholamines were secreted from the perfused adrenal gland during the stimulation periods for every milligram of extra protein found in the perfusate. This observation suggested that the extra protein released during the stimulation periods may be derived from the soluble protein of the chromaffin granules. This possibility has been tested by using an immunological technique with the help of Dr H. Winkler and Mr A. D. Smith in Oxford. Perfusates obtained during control and stimulation periods were concentrated by ultrafiltration and any red cells were removed by centrifugation. The concentrated solutions were then examined on Ouchterlony plates by using an antibody prepared against the soluble protein of the chromaffin granules. The protein derived from the stimulation-period perfusates yielded a precipitation band identical with that given by a solution of the soluble protein from a preparation of purified chromaffin granules. No precipitation band was obtained with the protein derived from the control-period perfusates. These observations indicate that the secretion of catecholamines from the adrenal medulla is accompanied by the release of soluble protein from the chromaffin granules.

According to Palade (1959) the secretion of proteins by the acinar cells of the pancreas is effected by the fusion of the membranes of the zymogen granules with the cell membrane, thus allowing the contents of the granules to leave the cells. By analogy with the pancreas, the finding that protein is released from the adrenal medulla, together with catecholamines, suggests that the membranes of the chromaffin granules may fuse with the cell membrane after stimulation. Such a secretory mechanism has been postulated for the adrenal medulla by de Robertis & Sabatini (1960) and by Coupland (1965) on morphological grounds, and by Douglas & Poisner (1965) after their discovery that adenine nucleotides are secreted together with the catecholamines.

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