CYTOCHROME 559 IN THE MICROSOMES OF THE ADRENAL MEDULLA

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It is well known that two cytochromes are present in microsomes of mammalian tissues, i.e. cytochrome b_5(Strittmatter and Ball, 1952) and a CO-binding pigment (named P-450 by Omura and Sato, 1964). On the other hand, Hashimoto et al. (1962) have found a new component of electron transport in rabbit liver microsomes by electron spin resonance spectroscopy, and this species was tentatively defined as " microsomal Fe_x". It is capable of alternate reduction and oxidation, and this species has been found to be closely associated with P-450 (Ichikawa and Yamano, 1965).

Spiro and Ball (1961) and Krisch (1962) observed that the microsomes of beef adrenal medulla show the absorption of the α-band of a hemoprotein at 559 mμ, but they have not reported its separation or its distinction from cytochrome b_5 or the microsomal CO-binding pigment, which also has an α-band at 559 mμ in the solubilized state. However, in the adrenal medulla, the cytochrome has absorption maxima at 559, 530 and 428 mμ in the reduced form in both the microsomal and solubilized state. This cytochrome is of the b-type and has also been found in the adrenal medulla of horse, pig and sheep. The present communication presents evidence that the nature of the cytochrome of adrenal medulla is different from those of other known microsomal cytochromes in certain important properties.

Materials and Methods : Adrenal glands of cow,horse, pig and sheep were obtained at a local slaughter house and immediately brought on ice to the laboratory. The medullary tissues and cortical tissues were homogenized separately with 0.25 M sucrose in a teflon homogenizer. The microsomes used were prepared from these homogenates by a modification of the method of Schneider and Hogeboom (1950). The microsomes used were shown to be free from contamination by hemoglobin and contamination with mitochondria was found to be less than 4 per cent on protein basis, as judged by their succinic oxidase activity.

The cytochrome was extracted from the adrenomedullary microsomes by treatment with unheated 0.1 % cobra venom in 0.1 M Tris-HCl buffer at pH 8.5 for 15 hours under anaerobic conditions. The extract was fractionated by precipitation at between 30 and 60 per cent saturation of ammonium sulfate and adsorption on DEAE cellulose at pH 7.0, from which it was eluted with 0.5 M NaCl.

NADH cytochrome b_5 reductase was purified by the method of Strittmatter and Velick (1956) and NADPH cytochrome c reductase was obtained by the method of Williams and Kamin (1962). The contents of cytochrome b_5 and P-450 were measured by the method of Garfinkel (1958) and Omura and Sato (1964), respectively, and that of cytochrome b-559 was measured spectrophotometrically from the total protoheme content minus the protoheme contents of cytochrome b_5 and P-450. The content of microsomal Fe_x was calculated by electron spin resonance spectroscopy using 2 mM cupric sulfate in 20 mM EDTA solution as standard.

Results and Discussion : The table shows the amounts of cytochromes in the microsomes of the adrenal glands from various sources. Cytochrome b_5 and P-450 were found in adrenal cortical microsomes. The finding that cytochrome b_5 was present in beef cortical micro-

somes is in disagreement with the observations of Krisch (1962), and Spiro and Ball (1961). The presence of cytochrome b_5 was confirmed by their purification and the demonstration of α and β band splitting at the temperature of liquid nitrogen.

However, in beef adrenomedullary microsomes, the cytochrome had an α band at 559 mμ and this type of cytochrome was generally present not only in the adrenomedullary microsomes of various mammals, but also in catecholamine granules, which were prepared by the method of Blaschko et al.(1957). Although cytochrome b_5 was fully reduced and P-450 was scarcely reduced by NADH or NADPH under aerobic conditions. The adrenomedullary cytochrome was partially reduced by these reagents under the same conditions. Fig. 1.(left) shows the difference spectra of the cytochromes of beef adrenomedullary microsomes. Neither the reduced nor oxidized cytochromes combined with carbon monoxide, KCN and ethylisocyanide and were not observed to have the ESR signal of microsomal Fe_x.

The above properties indicate that the adrenomedullary cytochrome differs from cytochrome b_5 and P-450(P-420, microsomal Fe_x). In addition, as shown in Fig.2, the presence of cytochrome b-559 was detected by low temperature spectrophotometry. The α band of the cytochrome was not split at -190° whereas the spectrum of cytochrome b_5 at low temperature possesses two α bands and two β bands, as reported by Lindemeyer and Estabrook (1958) and Bois and Chaix (1964). The shoulder in the α band of the cytochrome in intact microsomes,is not identical with that of purified cytochrome. This fact suggests that it might represent the spectrum of a mixture of two different hemoproteins or a modified native molecule. This can be decided only after further purification of the cytochrome b-559 of the adrenal medulla.

The absorption spectra in the visible region of partially

Table 1. Amounts of microsomal cytochromes of mammalian adrenal glands

	Cyt.b-559	Cyt.b_5	P-450	Mic.Fe_x
Beef adrenal cortex	–	0.13	0.78	0.76
" " medulla	0.82	–	–	–
Pig adrenal cortex	–	0.24	1.14	1.00
" " medulla	0.20	0.10	0.61	0.60
Horse adrenal cortex	–	0.51	0.76	0.74
" " medulla	0.91	–	0.01	0.01
Sheep adrenal cortex	–	0.08	0.80	0.82
" " medulla	0.11	0.12	0.55	0.52

The amounts of cytochromes are expressed as mμmoles per mg microsomal protein. The protein content was determined by the biuret method of Gornall et al. (1949). The abbreviations used are : Cyt.b-559, cytochrome b-559 ; Cyt.b_5, cytochrome b_5 ; Mic.Fe_x, microsomal Fe_x.

purified cytochrome from adrenomedullary microsomes is given in Fig. 1.(right). As can be seen, the spectra of this cytochrome have α, β and Soret peaks at 565, 525 and 414 mμ in the oxidized

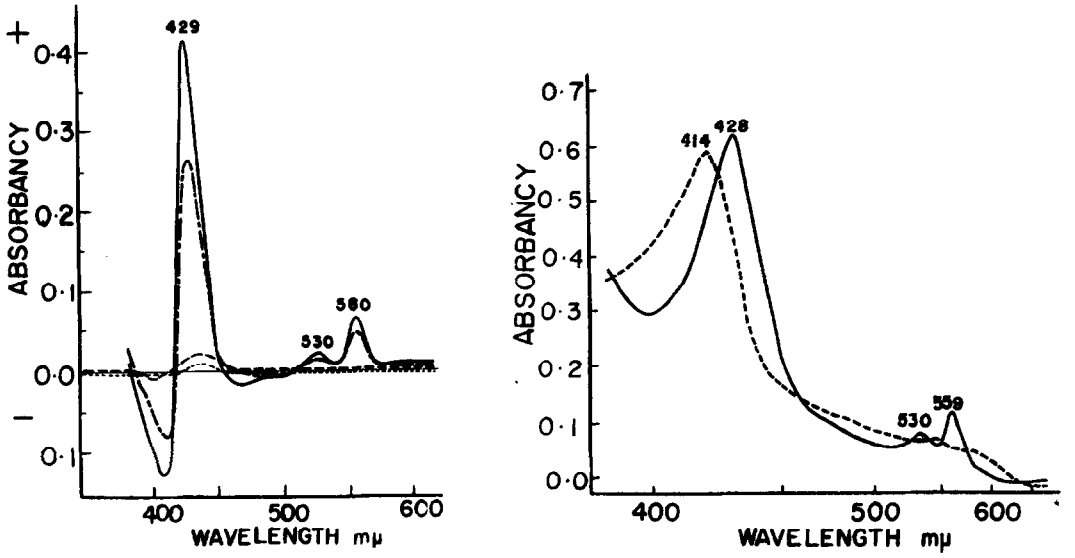

Fig.1. (left) Difference spectra of beef adrenomedullary microsomes, NADPH-reduced minus oxidized. Protein concentration, 2.7 mg/ml, 0.1 M phosphate, pH 7.0. Succinate was used to measure contamination with mitochondria. The final concentration of NADPH was 0.3 mM.
—— Anaerobic conditions, — — Aerobic conditions, ... Base line, ------ Reduced with 5 x 10^{-3}M succinate after addition of antimycin A (0.2γ/ml).
Fig.1. (right) Absolute absorption spectrum of purified cytochrome b-559 from beef adrenomedullary microsomes at 20°. Protein concentration, 0.5 mg/ml, 0.1 M Tris-HCl buffer(pH 7.4).

form, and at 559, 530 and 428 mμ in the reduced form. The purified preparation of cytochrome b-559 could not be reduced with either NADH or NADPH or with these reagents together with liver microsomal

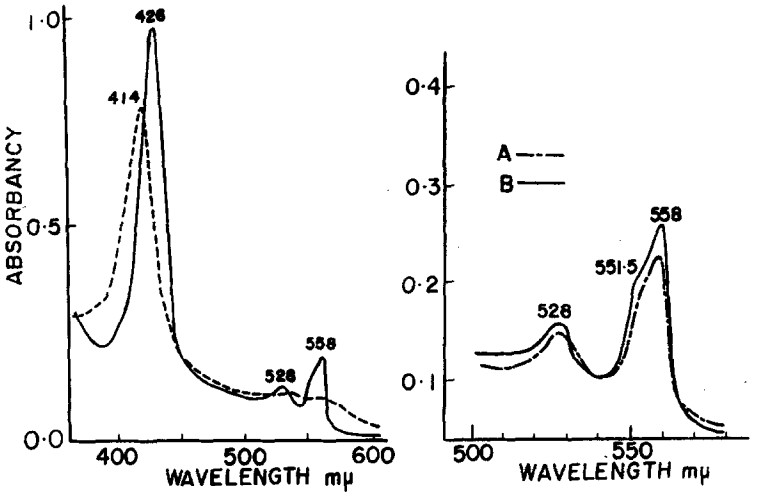

Fig.2. Absolute absorption spectrum of cytochrome b-559 from beef adrenomedullary microsomes at -190° and pH 7.4.
——— Reduced form, ------ Oxidized form.
Curve A. Purified cytochrome b-559. Reduced with dithionite.

Curve B. Untreated cytochrome b-559 reduced with NADPH under aerobic conditions.

NADPH-cytochrome c reductase and NADH-cytochrome b_5 reductase. However, the cytochrome b-559 in the solubilized mixture could be reduced aerobically and anaerobically with NADH and rather faster with NADPH. Peroxidase activity was not detected in the beef adrenomedullary microsome fraction.

A detailed study on the physiological significance of this cytochrome and the nature of the flavoproteins and the other components of electron transport of adrenomedullary microsomes is in progress.

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