and citric acid was determined on trichloroacetic acid filtrates of plasma by the method of TAYLOR[4].

The levels of citric acid, total, diffusible and non-diffusible calcium in the plasma after PTH injection were compared statistically with the corresponding levels before injection, using the paired "*t*" test, and the results are shown in Table I. In the control group, PTH exerted a typical effect on the blood calcium, elevating both total and diffusible levels significantly, though the increase in the non-diffusible calcium was not significant. The mean plasma citric acid level was not affected by the PTH and it seems probable that the high level of oestrogen in the circulation prevented the expected rise.

PTH treatment had no effect on the total calcium level of the plasma of the birds deficient in vitamin D. The diffusible calcium and the citric acid levels on the other hand were increased (significantly only in the former), and it would appear, therefore, that vitamin D is not necessary for the action of PTH in the hen. Associated with the rise in diffusible calcium was a significant fall in the non-diffusible calcium level after PTH injection. It seems most unlikely that this can have been due to a reduction in the protein concentration of the plasma since the main bleedings were only 24 h apart and a fall in total calcium would have been expected at the time of the bleeding immediately before the PTH injection if a fall in plasma proteins had occurred. LLOYD AND ROSE[5], as a result of investigations on 12 hyperparathyroid patients before and after operation, and on a normal subject treated with PTH, have produced evidence that PTH reduces the calcium-binding power of the plasma proteins, a conclusion also reached by FANCONI AND ROSE[6]. The results of the present experiments are consistent with this suggestion.

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[1] H. C. HARRISON, H. E. HARRISON AND E. A. PARK, *Am. J. Physiol.*, 192 (1958) 432.
[2] C. MARNAY AND Y. RAOUL, *Comp. rend. soc. biol.*, 153 (1959) 1949.
[3] G. A. ROSE, *Clin. Chim. Acta*, 2 (1957) 227.
[4] T. G. TAYLOR, *Biochem. J.*, 54 (1953) 48.
[5] H. M. LLOYD AND G. A. ROSE, *Lancet*, (1958 II), 1258.
[6] A. FANCONI AND G. A. ROSE, *Quart. J. Med.*, 27 (1958) 463.

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Cytochrome 556 and electron-transport system in snail hepatopancreas

The occurrence of a cytochrome-like haemoprotein called enterochrome 556 in the gut-fluid of garden snails has recently been reported and some of its properties have been described[1]. A cytochrome resembling enterochrome 556 has now been found to occur in the hepatopancreatic cells of garden snails, *Euhadra amaliae* and *E. sandai*.

Abbreviation: DPNH, reduced diphosphopyridine nucleotide.

This cytochrome is similar to cytochrome h[2,3] in certain respects, but definitely differs from it in several characteristics. It has, therefore, been provisionally designated as "Cytochrome 556".

The cytochrome was partially purified from the tissues of snail hepatopancreas as follows. Excised tissues were rinsed with snail Ringer solution[4], and stored at — 15° until needed. The frozen tissues were homogenized with 3 vol. water in a Waring blendor, passed through a thin cloth, and centrifuged. The supernatant collected was treated with a small amount of 0.5 M lead acetate, followed by the procedures applied for the purification of enterochrome 556[1]. The preparation of cytochrome 556 thus obtained was a clear orange-red solution.

The visible absorption maxima of reduced cytochrome lie at 556 mμ (α), 526 mμ (β), and 424 mμ (Soret; 412 mμ, oxidized form). The peaks in the pyridine haemochromogen spectrum are located at 553 mμ (α), 521 mμ (β), and 415 mμ (Soret). The oxidation–reduction potential as measured with the aid of 2,6-dichlorophenol indophenol was found to be approximately + 0.20 V at pH 7.0, 30°. The prosthetic group could be easily split off from the protein moiety by the treatment with acetone containing 1 % HCl, and the acid haematin thus separated was soluble in ether. The cytochrome was found to combine neither with CO nor with HCN at pH 7.0, and to show no peroxidase activity. It migrated toward the cathode when subjected to paper electrophoresis in 0.05 M phosphate buffer, pH 7.0. These properties of cytochrome 556 are almost identical with those of enterochrome 556[1].

Spectroscopic examinations have shown that cytochrome 556 is abundantly present both in mitochondrial and microsomal fractions of snail hepatopancreas. Cell particulates were prepared by differential centrifugation from tissue homogenates in 0.2 M sucrose. After removal of nuclei and cell debris at 600 $\times$ g for 10 min, mitochondria were sedimented at 9,000 $\times$ g for 15 min. Microsomes were sedimented at 78,000 $\times$ g for 60 min as firmly packed pellets. Cytochrome contents were estimated from difference spectra by applying μM extinction coefficients recommended by CHANCE[5]. The coefficient for cytochrome 556 was assumed to be equal to that of cytochrome b. The mitochondrial fraction contained, in addition to cytochrome 556 (0.25 μmole/g protein), significant quantities of cytochromes a and b (0.19 and 0.20 μmole/g protein, respectively), but cytochrome c could not be detected even at liquid–air temperature. The microsomal fraction, on the other hand, contained cytochrome 556 (0.24 μmole/g protein) as the only detectable cytochrome component. The fact that cytochrome 556 is richly localized in hepatopancreatic particulate structures and that this organ has a remarkable secreting activity strongly supports the view that enterochrome 556 is a secreted form of cytochrome 556.

Hepatopancreatic mitochondria were able to catalyze the aerobic oxidation of reduced cytochrome 556 as well as of reduced enterochrome 556, whereas these haemoproteins could be rapidly reduced by DPNH under anaerobic conditions in the presence of either mitochondria or microsomes.

As illustrated in Fig. 1, the addition of DPNH to the aerobic suspension of mitochondria caused the immediate reduction of cytochrome 556. On exhaustion of O_2, the peaks of cytochrome 556 were intensified and those of reduced cytochrome a appeared simultaneously. When succinate was added to aerobic mitochondria, only slight peaks due to reduced cytochrome b were observed. This spectrum changed to that observed in the case of DPNH addition after the complete exhaustion of O_2.

The DPNH-cytochrome *c* reductase activity of the mitochondria was not inhibited by 2–10 μg/ml antimycin A, while the succinate-cytochrome *c* reductase activity was completely inhibited by 2 μg/ml antimycin A.

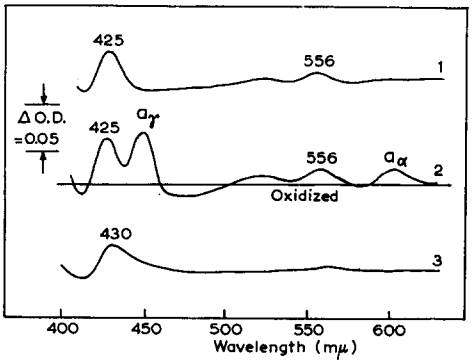
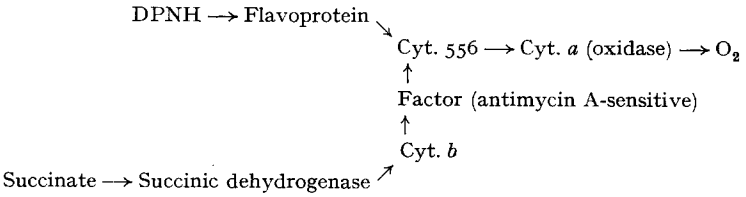


Fig. 1. Difference spectra of the cytochrome components of mitochondria prepared from hepatopancreas, suspended in 0.05 *M* phosphate buffer, pH 7.0. Light path of cuvettes, 1 cm; concn., 5 mg protein/ml. Spectra were recorded with a Cary model 14 recording spectrophotometer. Curve 1: Difference between DPNH-added aerobic mitochondria and aerobic mitochondria. Curve 2: Difference between DPNH-reduced anaerobic mitochondria and aerobic mitochondria. Curve 3: Difference between succinate-added aerobic mitochondria and aerobic mitochondria.

From these experimental results the following scheme may be suggested as a possible pathway of electron transport in the mitochondria of snail hepatopancreas.



As may be seen, cytochrome 556 appears to play a role similar to that of cytochrome *c* in the electron-transport system in mammalian mitochondria.

Full experimental details will be published elsewhere. The author wishes to thank Dr. RYO SATO for his encouragement and advice in this investigation.

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1 K. KAWAI, *Biochim. Biophys. Acta*, 43 (1960) 349.
2 J. KEILIN, *Biochem. J.*, 64 (1956) 663.
3 J. KEILIN, *Nature*, 180 (1957) 427.
4 E. BALDWIN, *Biochem. J.*, 29 (1935) 1538.
5 B. CHANCE, *Nature*, 169 (1952) 215.

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