A significant increase in the incorporation of [^{3}H]formate into nuclear RNA ($P < 0.001$), ribosomal RNA ($P < 0.01$) and acid-soluble free nucleotides ($P < 0.001$) is observed when compared with vitamin B_{12}-deficient chicks. A smaller but rather significant increase is found in soluble RNA ($P < 0.05$). No significant difference was observed in liver DNA.

The results obtained seem to indicate a further proof of the existence of a close relationship between vitamin B_{12} and orotic acid in the metabolism of nucleic acids. In fact, the increased RNA biosynthesis in subcellular fractions and the increased biosynthesis of total free nucleotides in the liver of chicks, treated with vitamin B_{12} or orotic acid, is in agreement with the results previously reported[1-3].

The variations in the nuclear and ribosomal RNA specific activity that were observed either in the group treated with vitamin B_{12} or with orotic acid, can be regarded as the expression of an increased messenger-RNA biosynthesis.

This finding could possibly lead us to see a common role of orotic acid and vitamin B_{12} at the transcription processes level. The modifications of specific enzymatic activities observed in the same experimental conditions[9-11] may confirm this viewpoint[12].

Riassunto. E' stato studiato l'effetto dell'acido orotico sull'incorporazione di formiato-H^3 nei nucleotidi liberi totali e negli acidi nucleici presenti nelle frazioni subcellulari del fegato di pulcino carente di B_{12}. La maggiore attività specifica dell'RNA nucleare e ribosomiale che si osserva negli animali trattati con acido orotico come in quelli trattati con vitamina B_{12} potrebbe essere considerata l'espressione di una più elevata sintesi di RNA messaggero determinata da queste due sostanze.

C. M. CALDARERA, B. BARBIROLI and M. MARCHETTI

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Glycoside Effect upon Membrane Enzymes of Erythrocytes and Muscle in Duck Myopathy

Myopathies frequently have been associated with muscle-membrane abnormality. The suggestion that these abnormalities are themselves indications of a generalized and probably hereditary defect and might therefore have reflection in non-muscle cells[1] has awaited confirmation. Heretofore, the test applied was relatively insensitive. In an attempt to examine this at higher level of resolution, we have made reference to the pattern of membrane-catalyzed adenosine-triphosphate hydrolysis.

Ducks (*Anas platyrhynchos*) of the white Pekin strain, known to spontaneously developing myopathy[2-4] and Mallards, generally free of the defect, were used in this study. Birds of varying age were used for specimens of blood and of striated muscle from the legs. Individual animals were distinguished as myopathic or as 'normal' (Table). The clinical and pathologic characteristics of the myopathy occurring in the white Pekin duck have been described[2-4].

In each case, five ml of blood was collected in a Vacutainer tube containing 25 mg of Na_2EDTA and stored refrigerated. The blood samples were used within 2 days.

Blood samples were haemolyzed with aqueous *Tris* buffer, 0.002*M*, pH 7.4, with 0.005*M* Na_2EDTA. In each instance, a 1 ml sample was treated with 10 ml of the solution and then refrigerated for 5 min. The preparation was then centrifuged at 20,000 *g* for 15 min. The pellet was washed and recentrifuged 4-5 times in the same buffer and 0.002*M* NaCl. Before each wash the pellet was homogenized. The supernatant was discarded each time. The yellowish-white final pellet was collected and used as the source of ATPase activity.

Membrane fractions from muscle tissues (removed from the leg) were isolated by differential centrifugation.

Summary of experimental results; the effect of ouabain $10^{-4}M$ upon ATPase activity[a]

Bird	Strain	Muscle	Age	Red-blood cell ghosts	100,000 × *g* muscle fraction	80,000 × *g* muscle fraction
3024	Pekin	Normal	Mature	−		
3090	Pekin	Normal	Mature	−		
3041	Mallard	Normal	Mature	−	−	−
3052	Mallard	Normal	Mature	−	−	−
3056	Mallard	Normal	Mature	−	−	−
3192	Pekin	Normal	Mature	−		
3198	Pekin	Normal	Mature	−		
2202	Pekin	Normal	Mature	−		
3238	Pekin	Normal	Mature	−		
3246	Pekin	Normal	Mature	−		
3222	Pekin	Normal	Mature	−		
3221	Pekin	Normal	Mature		−	−
3205	Pekin	Normal	Mature		−	−
3149	Pekin	Myopathic	4 weeks	+	+	
3150	Pekin	Myopathic	4 weeks	+	+	
2928	Pekin	Myopathic	Mature	+	+	+
2945	Pekin	Myopathic	Mature	+	+	+
3102	Pekin	Myopathic	Mature	+	+	+
3140	Pekin	Myopathic	Mature	+	+	+

[a] − = inhibition of ATPase activity; + = stimulation.

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Muscles were macerated in 10 volumes of 0.1 *M* cold *Tris* buffer, pH 7.2 with 0.25 *M* sucrose. The slurry was centrifuged at 600 *g* for 20 min. The pellet was rejected and the supernatant dialyzed against the same *Tris*-sucrose buffer with 5 m*M* Na_2EDTA for 24 h. After dialysis the preparations were centrifuged at 10,000 *g* and 20,000 *g* each for 30 min, and each time the pellets were discarded. The supernatant was then centrifuged in 10 ml tubes at 80,000 *g* for 30 min and again 100,000 *g* for 70 min. The pellets of both 80,000 and 100,000 centrifugations were saved and in both instances resuspended in 2 ml *Tris*-sucrose buffer pH 7.2. The suspensions were stored at 2 °C as the enzymatically active membrane-fractions. Methods for measurement of catalytic activity have been described[5].

Duck 3102 (Table) had contracture of the right leg with fatty streaks in the muscles of each leg. Histologically, fat had replaced many of the muscle fibers. Some of the fibers showed foci of regeneration. Duck 3140 had considerable difficulty in walking and both feet were inverted. Focal areas of necrosis and regeneration were present in the striated muscles from the legs. The legs of Duck 3150 were 'bowed' and he had some difficulty in walking. No lesions were observed in the sections of muscle examined. The feet of Duck 3149 were inverted, resulting in minimal difficulty in walking. Duck 2928 clinically looked normal; however, fatty streaks were present in the muscles of each leg. Duck 2945 was clinically normal; however, a few fatty streaks were present in the muscles in each leg and histologically a few muscle fibers showed focal areas of regeneration.

Results of the enzyme activity studies are summarized in the Table. The characteristic response of membrane systems to the cardiac glycosides is the lowering of the rate of catalytic ATP-hydrolysis[6,7]. ATPase preparations made from normal ducks were inhibited by ouabain. Birds with muscle abnormality, contrary-wise, yielded preparations of both blood cell ghosts and muscle membranes which were stimulated, rather than inhibited by the drug. Figure 1 represents the enzymatic activity of erythrocyte ghosts from a normal animal (3056) in which the catalytic activity was inhibited by 10^{-4} *M* ouabain and a preparation of the blood cell membranes from myopathic duck (3102) which was stimulated by the same concentration of the drug. Figure 2 presents a similar series of curves derived from studies of the muscle membrane fraction (100,000 *g* pellet).

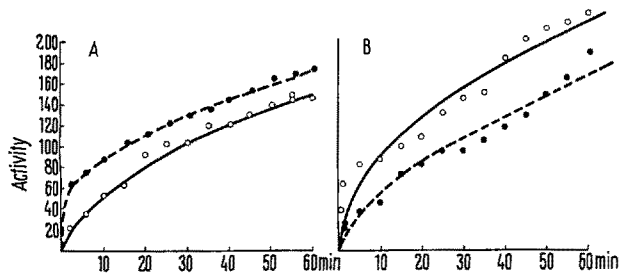

Fig. 1. (A) ATPase activity ghosts from a normal duck (3056) in the presence of ouabain (solid line) and in the absence of ouabain (dashed line). (B) Results of an identical experiment, using ghosts from a myopathic duck (3103).

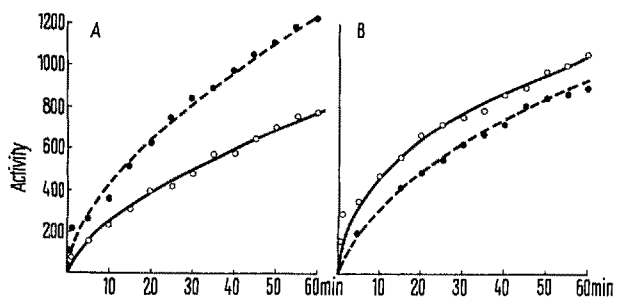

Fig. 2. (A) ATPase activity of muscle membrane fraction from normal duck (3056) in the presence of ouabain (solid line) and in the absence of ouabain (dashed line). (B) Results of an identical experiment, using a preparation from myopathic duck (3102) muscle.

For all animals studied, the relative inhibition of ghost preparation by ouabain, observed characteristically at 30–35 min after inhibition of the reaction, was 35.0% for normals; the average stimulation, 23.0% for abnormals. The equivalent values for the muscle membrane (100,000 *g*) preparations are: normals 41.0% inhibition, abnormals 28.0% stimulation.

We have suggested[8,9] that the ATPase response to ouabain is a reflection of the conformation of the enzyme which it derives in part from its association with the membrane.

Our results support the existence of a correlation, which we had suggested upon theoretical grounds, between the effect of ouabain upon membrane ATPase activity and the muscle abnormality of white Pekin ducks described as spontaneous muscular dystrophy by RIGDON[2-4]. Further, the characteristic response of muscles-membrane enzyme is common also to the erythrocytes ghost enzyme of the same organism. This appears to relate to the conclusions of CORSINI and CACCIARI[1] that myopathy is reflected in the individual's erythrocytes. The present data are in accord with the common supposition that defects of certain muscle diseases involve aberrant ion-transport phenomena. We may therefore interpret the present findings to suggest that a characteristic defect in the muscle abnormality of these experimental birds involves the integrity of the membrane system. This in turn alters characteristics of a transport related adenosine triphosphatase[10].

Zusammenfassung. Skelettmuskelmembrane und Erythrozytenstromata normaler Enten zeigen eine adenosine triphosphatase Aktivität, die durch das herzaktive Glykosid Ouabain gehemmt wird. Das gleiche Material von Enten mit Myopathien zeigte eine durch Ouabain stimulierte ATPase-Aktivität.

H. D. BROWN, S. K. CHATTOPADHYAY, A. PATEL and R. H. RIGDON

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Ischemic Depigmentation[1]

Numerous observations show that, in man, dark skin or hair may lose its pigment in areas exposed to certain types of injury (e.g. by X-rays, trauma, chemical insults). Indeed, according to a recent review of the literature, several verified case reports show that sudden greying of the scalp hair can occur immediately after exposure to severe systemic stress, especially if the latter is associated with intense fear[2]. Here, we should like to describe a simple and reliable experimental model for the induction, by circumscribed cutaneous ischemia, of topical depigmentation in dark-haired rats.

In 10 dark-brown female rats of the ACI-42324 strain (Microbiological Associates, Walkersville, Maryland) with an average body weight of 100 g (range 90–110 g), a transient local ischemia of the skin was produced by the application of special clips. These were prepared from ordinary umbilical clamps ('Hesseltine', Ingram & Bell, Toronto) in which the simple branch (the one not bearing a hook) was covered with rubber tubing. After shaving the back with electric clippers, a skin fold was taken up between the fingers and compressed by the clip for a period of 8 h. Immediately after removal of the clip the previously ischemic skin region remained somewhat pale and, during the following hours, it developed a mild oedema, but otherwise it retained its normal appearance. During the subsequent days, however, the pigmented fur was shed and gradually replaced by completely white hair which grew much more rapidly than the unaffected brown hair in the surroundings (Figure 1).

The previously dark skin of these animals also became white in the region exposed to ischemia. However, in this strain, the dermal colour is primarily determined by the pigmentation of the hair roots and the intracutaneous portions of the hair shafts; even normally, there is no detectable melanin in the epithelial and connective-tissue cells of the skin.

Histological examination of sections (embedded in paraffin and stained with the PAS-technique or with hematoxylin-phloxine) showed that, in the affected region, neither the root nor the shaft of the regenerating hairs contained any detectable melanin. The density of the hair was greatly diminished, the dermal fat cells virtually disappeared and the connective tissue became extremely dense. The epithelium was somewhat thickened and its normally wavy surface became straight. The defect in pigment formation showed no sign of reversibility during a 3 months observation period.

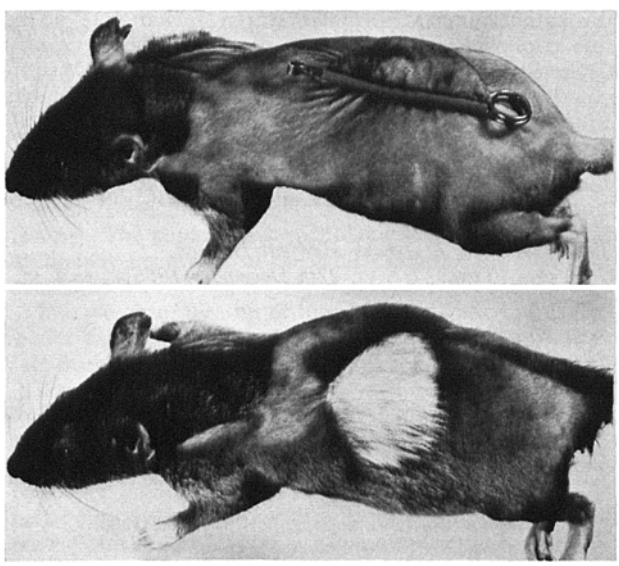

Fig. 1. Top: Clip in position on the shorn back of the rat. Bottom: 1 month after application of clip, the hair in the previously ischemic area is white and longer than in the surroundings where regeneration after shearing is irregular and slow.

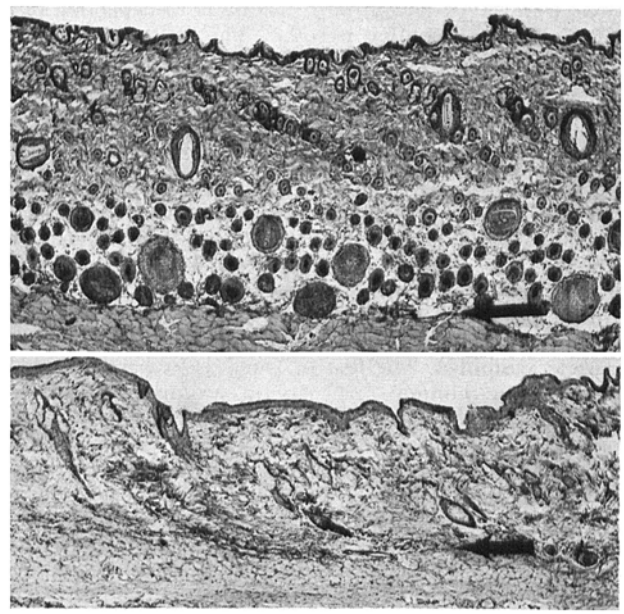

Fig. 2. Top: In the normal skin of the black rat, the medulla of each hair shaft is deeply pigmented. The epithelium is thin and wrinkled. Fat tissue is well developed especially just above the cutaneous muscle (whose outer limit is indicated by an arrow). Bottom: 1 month after application of the clip, hair density is greatly reduced and the medulla of the remaining hair (e.g. that just above the arrow which, again, indicates the outer limit of the cutaneous muscle) is totally devoid of pigment. The epithelium is thick and comparatively flat. The fat tissue has disappeared and the cutaneous muscle layer is atrophic (PAS × 40).

Zusammenfassung. Durch vorübergehende Unterbrechung des Blutkreislaufes mittels einer besonderen Klemme gelingt es, in einem Hautlappen der braunen Ratte selektive Veränderungen des Felles hervorzurufen. In dem behandelten Gebiet fallen die Haare zunächst aus, werden aber dann durch besonders schnell wachsende, weisse Haare ersetzt. Diese Depigmentierung ist anscheinend auf eine permanente Schädigung der Melaninsynthese zurückzuführen.

H. SELYE

Institut de Médecine et de Chirurgie experimentales, Université de Montréal, Montreal (Canada), 10th March 1967.

[1] This work was supported by the U.S. Army Medical Research and Development Command (Contract No. DA-49-193-MD-2039).

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