Such an alteration could lead to a pellagra-like syndrome[24], behavioral depression[25], and changes in sleep patterns[26] in the infected host. Moreover, some of the parasite metabolites, such as tryptophol, may have toxic or soporific effects of their own[27–29].

Résumé. La transformation du tryptophane en tryptophol chez le trypanosome consiste en une transamination suivie par une décarboxylation; une réduction subséquente est probable mais n'a pas été prouvée. La transamination requiert l'α-ketoglutarate; la conversion du tryptophane en indole-lactate requiert le NADH. L'indole-acétate est un autre produit du métabolisme du tryptophane.

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Creatine Phosphokinase Activity in Human Polymorphonuclear Leukocytes

It is generally accepted that there are many similarities between the mechanism of muscular contraction and cell motility. HUXLEY[1] has recently pointed out that the molecular structures and mechanisms for cell movement resemble those of muscle fibre contraction. This view is further substantiated by our present finding that human polymorphonuclear leukocytes possess a cytoplasmic creatine phosphokinase, an enzyme that is essential for the normal contraction of the muscular fibre.

Leukocytes, having contractile structures[2], could also be a convenient means to investigate hereditary muscular diseases, as for instance Duchenne muscular dystrophy. This disease, one of the most severe human myopathies, shows sex inheritance characteristics[3] and is generally regarded as a primary myopathic process[4], although some authors have postulated a primary neuronal abnormality[5,6]. We have now studied the properties of leukocyte creatine phosphokinase isolated from both normal and dystrophic subjects: no significant differences have been found, however, between the enzymes from the two sources

Table I. Specific activity of polymorphonuclear leukocyte-creatine phosphokinase from different subjects

		Spec. activity (Units/mg protein)
Healthy volunteers	1	0.55
	2	0.62
	3	0.57
	4	0.50
Dystrophic	L.C.	0.57
	T.C.	0.61
	R.R.	0.50
	P.G.	0.60
Mothers (carriers)	L. and T.C.	0.64
	R.R.	0.54
	P.G.	0.66

The specific activity of creatine phosphokinase from crude extracts of leukocytes from healthy volunteers, 4 patients (two brothers) affected by Duchenne muscular distrophy, and their mothers, was determined as described under material and methods.

Materials and methods. Venous blood samples of 4 normal, 4 dystrophic subjects and 3 healthy carriers of the Duchenne dystrophy (represented by the mothers of the 4 patients) were used. The clinical diagnosis of Duchenne myopathy for the 4 patients (R.R., T.C., L.C., and P.G., which were respectively 7, 9, 10 and 14 years old) was confirmed also by the result of the electromyographic analysis.

Polymorphonuclear leukocytes were obtained from freshly drawn, heparinized blood. Separation of granulocytes from red cells and lymphocytes was carried out by sedimentation in gelatin followed by differential centrifugation according to the method of YAM et al.[7]: 20 ml of blood yielded approximately $11–12 \times 10^6$ of 90–95% pure polymorphonuclear leukocytes, that were stored at −20 °C until handling. The lysis of leukocytes was obtained by freezing at −40 °C and thawing at 37 °C 3 times in the presence of 1 ml of saline solution[7]. The suspension was centrifuged at $20,000 \times g$ for 20 min and to the clear supernatant, containing creatine phosphokinase activity, 4 mg/ml of albumin to increase the protein concentration were added. Protein was then precipitated by addition of 326 mg of ammonium sulfate per ml of solution. The precipitate, collected by centrifugation, was dissolved in 0.5 ml of 0.05 *M tris* acetate containing 1 m*M* EDTA, pH 7.2. The ammonium sulfate fraction was almost free of myokinase, which interfered in the determination of creatine phosphokinase activity. Creatine phosphokinase was assayed spectrophotometrically ac-

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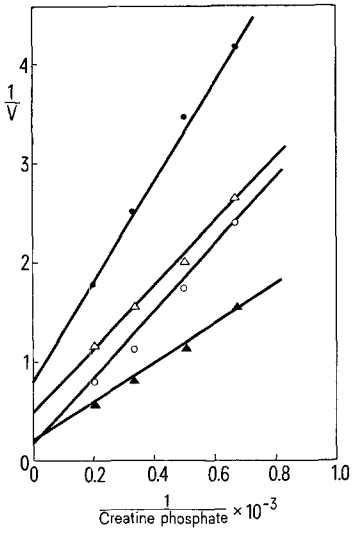

Fig. 1. Reciprocal plot of initial velocity against creatine phosphate concentration at a series of different pH values. The samples (1 ml) contained 2.5 mUnits of creatine phosphokinase (ammonium sulfate fraction) from normal subjects, creatine phosphate as indicated and 0.08 *M* glycylglycine buffer pH 6.0, (●); pH 7.0, (○); pH 7.35, (×) and pH 8.0 (△). Other conditions were as reported under methods. Initial velocity is expressed as μmoles of NADPH formed per min.

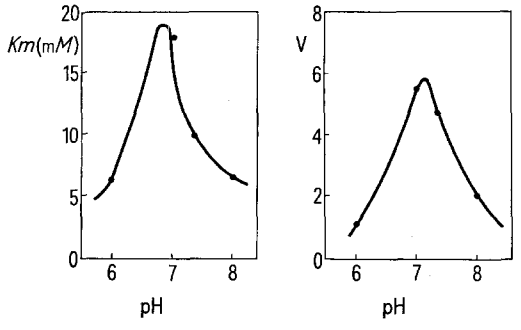

Fig. 2. Effect of pH on the *Km* and V of creatine phosphokinase from normal subjects. Data were taken from Figure 1. Maximal velocity is expressed as nmoles of NADPH formed per min.

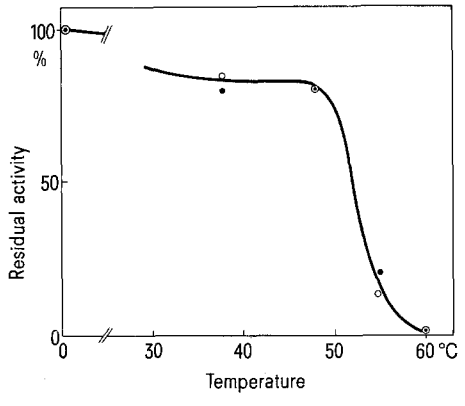

Fig. 3. Heat denaturation of leukocyte creatine phosphokinase from normal and dystrophic subjects. The samples (0.5 ml) contained 0.036 Unit of creatine phosphokinase (ammonium sulfate fraction) from the 2 source, dissolved in 0.05 *M tris*-acetate, containing 1 m*M* EDTA, pH 7.0. Protein concentration was 1 mg/ml. After heating for 10 min at the temperatures indicated, the samples were immediately chilled, and assayed for the catalytic activity as described under methods. Normal (○) and dystropic (●) subjects.

cording to the procedure of NIELSEN and LUDVIGSEN[8], which measured the formation of ATP in the forward reaction by following the reduction of NADP, at 340 nm.

The reaction mixture (1 ml) contained 0.08 *M* glycylglycine buffer, pH 7.4; 0.1 m*M* EDTA; 10 m*M* magnesium acetate; 2.5 m*M* ADP; 5 m*M* glucose; 0.2 m*M* NADP; 2 μg each of glucose 6-phosphate dehydrogenase and hexokinase and 5 m*M* creatine phosphate.

The interference of myokinase was eliminated by substracting the amount of ATP produced in control samples, without creatine phosphate, from that produced in the complete assay system. 1 unit of the enzyme was defined as the amount that will produce 1 μmole of ATP per min under the above conditions. Protein concentration was determined by the method of LOWRY et al.[9].

Results and discussion. Crude extracts of creatine phosphokinase from normal dystrophic subjects and healthy carriers of the Duchene dystrophic (represented by the mothers of the 4 patients), showed an identical specific activity (Table I).

A comparative kinetic study of creatine phosphokinase obtained from the normal and the dystrophic subjects was carried out on the ammonium sulfate fraction of the enzyme. The results with the normal subjects are shown in Figures 1 and 2. pH optimum was at pH 7.2 (Figure 2B). *Km* increased from 6.2 m*M* at pH 6.0 to 20 m*M* at pH 6.8 and decreased again to 6.5 m*M* at pH 8.0 (Figure 2B). Similar values of the *Km* were found for the enzyme from dystrophic subjects. This is shown in Table II.

Unlike creatine phosphokinase from muscle, leukocyte creatine phosphokinase from both normal and dystrophic subjects exhibited a very narrow pH range of stability, from pH 6.8 to 7.3, when stored at 0 °C. Alkaline medium inactivated very rapidly the enzyme from leukocytes, while muscle kinase was completely stable[10]. Low concentrations of EDTA were required to avoid fortuitous loss in activity during storage. Leukocyte creatine phosphokinases from normal and dystrophic subjects were also equally sensitive to heat denaturation at pH 7.0 (Figure 3). Exposure for 10 min to temperature up to 47 °C decreased only slightly the catalytic activity (20%); exposure to higher temperatures was on the contrary much more effective, and complete inactivation was obtained at 60 °C.

To our knowledge this is the first report on the presence of creatine phosphokinase in motile cells. This

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Table II. Michaelis constant, for creatine phosphate, of leukocyte creatine phosphokinase from normal and dystrophic subjects

pH	*Km* for creatine phosphate	
	Normal	Dystrophic
6.0	6.3 m*M*	6.0 m*M*
7.0	18.0 m*M*	17.0 m*M*
7.35	10.0 m*M*	11.0 m*M*
8.0	6.6 m*M*	6.5 m*M*

Experimental conditions were as described in Figure 1.

observation further emphasizes the functional[1] and structural[2] similarities between motile and muscular cells. Because of these analogies, cells with motile activity could be a suitable system to investigate abnormalities affecting the muscular fibre, such as Duchenne muscular dystrophy.

In our investigations, however, no differences between creatine phosphokinase from leukocytes of normal and dystrophic subjects has been found. The specific activity of the enzyme, the *Km* at different pH, the pH optimum, and the resistance to heat denaturation were similar in both cases, thus indicating that dystrophic leukocytes contain normal amounts of normal creatine phosphokinase molecules.

This last point seems to exclude the leaking of creatine phosphokinase from the leukocytes, at variance with the dystrophic muscle[11–13]. Thus the postulated alteration of membrane permeability to creatine phosphokinase seems to be restricted to the dystrophic muscular fibre and not extended to the leukocyte. Similarly, the recently reported structural alteration of the creatine molecule in human dystrophy[14] seems to be restricted to the muscle fibre. Muscular and leukocyte phosphokinases must therefore be under independent genetic control[15].

Riassunto. Nei leucociti polimorfonucleati umani, cellule capaci di movimenti durante la fagocitosi, è stata osservata la presenza dell'enzima creatina fosfocinasi. Questa è un'altra analogia tra cellule fornite di movimenti e fibre muscolari. Il confronto di alcune caratteristiche dell'enzima isolato da leucociti provenienti da soggetti sani e distrofici non rivela alcuna differenza significativa.

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Copper and Zinc Levels in the Blood Serum and Urine of Bilharzial Hepatic Fibrosis

In our previous work on bilharziasis, the urinary porphyrins, uro and coproporphyrins[1], as well as the urinary amino acids[2], were found to be excreted in large amount, expecially in advanced cases of hepato splenomegaly patients. Moreover, it appears from the work of WATSON and SCHWARTZ[3] that the urinary as well as feacal porphyrins uro- and coproporphyrins are excreted as zinc complexes. While working on rats, VAN CAMPEN et al.[4] found that there is an interrelationship between zinc concentration and copper absorption. Therefore, it appears of interest to study the serum and urinary level of both zinc and copper in bilharzial hepatic fibrosis, and to compare with the results obtained from normal healthy individuals.

Materials and methods. 25 patients, 17 adult males and 8 adult females, in different stages of bilharzial hepatic fibrosis were selected for this study. These cases were subjected to thorough clinical examination, liver function tests, total protein as well as differential blood serum proteins were estimated by electrophoresis. Serum and urinary zinc and copper levels were determined in the patients studied and compared with the results obtained from 10 normal individuals.

Determination of serum and urinary zinc and copper was performed according to the method of WILLIS[5], using the atomic absorption spectrophotometer SP 90 A.

Results. The Table indicates the range and average values of serum and urinary zinc and copper of the studied bilharzial patients, compared with the results obtained from 10 normal healthy individuals. Normal serum zinc ranges from 105 to 208 and the average found was 185 µg/100 ml. In urine the range lies between 0.185 and 0.610 mg/24 h, while the mean urinary zinc recorded was 0.400 mg/24 h. Normal Serum copper, however, ranges from 65–190 µg/100 ml and the mean value 125 µg/100 ml. Urinary copper, however, varied from traces to 0.027 with a mean value of 0.016 mg per day.

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Range and average values of urinary and blood serum zinc and copper of normal controls and patients studied

Cases	Zinc		Copper	
	Serum (µg/100 ml)	Urine (mg/24 h)	Serum (µg/100 ml)	Urine (mg/24 h)
Normal control				
Range	105–208	0.185–0.610	65–190	0.000–0.027
Mean ± S.E.	185 ± 9	0.400 ± 0.05	125 ± 15	0.016 ± 0.002
Bilharzia hepatosplenomegaly				
Range	112–890	0.200–1.800	90–800	0.000–0.070
Mean ± S.E.	341 ± 38	1.113 ± 0.2	338 ± 31	0.021 ± 0.003