Squirrel testicular MAO activity from animals trapped on April 19, 1972 (Group I) and animals trapped on May 25, 1972 (Group II)

Treatment	No. of animals	Body weight		Testicular weight		Per mg tissue	
		(g)	*P* value	(mg)	*P* value	CPM $\times 10^3$	*P* value
Group I	5	258.2 $\pm$ 13.4		968 $\pm$ 73		26.14 $\pm$ 1.51	
Group II	5	303.9 $\pm$ 5.7	< 0.01	368 $\pm$ 25	< 0.001	7.85 $\pm$ 0.36	< 0.001

Values expressed are mean $\pm$ standard error of mean.

and testicular MAO activity (expressed on per mg of tissue basis), than those captured in April.

Discussion. Of significance is the observation that in house sparrows MAO activity on either a per mg of tissue or total activity basis was highest at the peak of reproductive activity and testicular development (weights), but declined sharply as the breeding season ended and testicular weight declined. It is significant that an increase in MAO activity per mg tissue from December to January preceeded an increase in testicular weight from January to March. This early increase in MAO activity may have been a necessary factor for the subsequent increase in testicular weight and testicular development and is probably a reflection of gonadrotrophin release since FSH stimulates testicular MAO activity and testicular development in the rat[6].

The Uinta ground squirrels also showed seasonal variations with a large decrease in testicular MAO activity from April to June. The rapid decline in enzyme activity in the squirrels can best be explained by a diminished release of FSH from the pituitary. This observation is corroborated by the finding that pineal activity increases in this species during this time interval[7,8]. The low MAO activity observed as the squirrels pass into a non-reproductive period may serve to increase endogenous biogenic amine levels in the testis. This could serve to help insure a rapid decrease in testicular function as the animals enter into a period of reproductive quiescence. Other investigators[9] have shown dramatic alterations in primate testicular weights in as little as 9 days after orbital space flight. These workers suggested that 5-HT might be involved in this rapid decline in testicular weight. 5-HT may be one of the endogenous factors responsible for the cessation of seasonal breeding in house sparrows. This hypothesis is supported by a recent study showing that pargyline, an MAO inhibitor, has a detrimental effect on testicular development and spermatogenesis[10]. Our data show that testicular MAO activity undergoes seasonal variations, and that in conjunction with other factors 5-HT may induce the rapid testicular regression that is exhibited by seasonal breeders as they go out of breeding.

Of importance is the observation that testicular weight and MAO activity reached a peak by April 1, decreased somewhat by June, then rose slightly in July before reaching a low by September (Figure). In a previous study[11], testicular development was initiated in mid January and was completely regressed by late August similar to this study, but maximum gonadal size was delayed until May and June, with a slight delay in development occurring during April and May. These data indicate that the sparrow can adjust testicular development within a rather broad period to match the particular climatic conditions desired for reproduction. In this respect, the first study coincided with a late spring, while an early spring was manifest during the present study.

Résumé. Des moinaux familiers (*Passer domesticus*) et des écureuils (*Spermophilus armatus*) des montagnes Uinta ont été capturés à différentes époques de l'année et l'activité de la MAO a été mesurée dans leurs testicules. Chez les deux espèces l'activité de la MAO était la plus grande lorsque le poids des testicules était le plus élevé et la plus basse avant et après la saison du rut. Chez le moineau, l'augmentation de l'activité de MAO a précédé le développement des testicules.

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Departments of Biology and Wildlife Resources and the Ecology Center, UMC 53, Utah State University, Logan (Utah 84322, USA), 7 October 1974.

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Further Studies on the Metabolism of Tryptophan in *Trypanosoma brucei gambiense*: Cofactors, Inhibitors, and End-Products

Trypanosoma brucei gambiense has been previously demonstrated to convert ^{14}C-tryptophan in vitro to two metabolites, indole lactic acid and tryptophol (indole ethanol)[1]. It was suggested that tryptophol produced by parasites located in the central nervous system could be responsible for the behavioral syndrome characteristic of African sleeping sickness. It is the purpose of this report to demonstrate that the metabolism of tryptophan to

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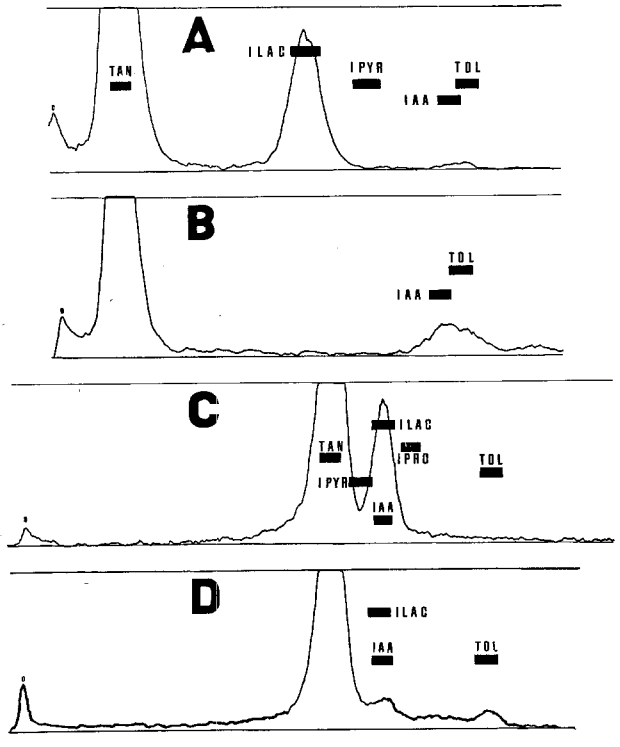

Fig. 1. A) Radioactivity scan (1,000 cpm range) of chromatogram of supernate from incubation of dialyzed enzyme with tryptophan-3-^{14}C for 45 min in the presence of NADH, pyridoxal phosphate, and α-ketoglutarate. Developed in benzene: acetic acid: water (125:72:3). B) Scan (1,000 cpm range) of chromatogram of supernate from incubation as in (A) but with omission of NADH. Same solvent system as in (A). C) Scan (1,000 cpm range) of chromatogram of supernate from incubation as in (A). Developed in 95% ethanol: ammonia: water (16:1:3). D) Scan (1,000 cpm range) of chromatogram of supernate as described in (B). Same solvent system as in (C). Abbreviations: IAA, indole acetic acid; ILAC, indole lactic acid: IPRO, indole propionic acid; IPYR, indole pyruvic acid; TAN, tryptophan; TOL, tryptophol.

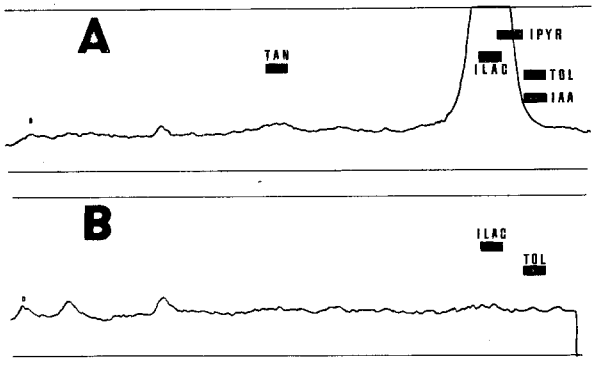

Fig. 2. A) Radioactivity scan (1,000 cpm range) of chromatogram of supernate from incubation of dialyzed enzyme with tryptophan-1-^{14}C for 3 h in the presence of NADH, pyridoxal phosphate, and α-ketoglutarate. Developed in butanol: acetic acid: water (4:1:1). B) Scan (1,000 cpm range) of chromatogram of supernate from incubation as in (A) but with omission of NADH. Same solvent system as in (A). Abbreviations as in Figure 1.

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tryptophol in this protozoan involves transamination, decarboxylation, and possibly reduction; and, furthermore, to identify cofactor requirements, enzyme inhibitors, and possible new end-products. A cofactor requirement and an inhibitor of the pathway from tryptophan to indole lactic acid have also been identified.

Materials and methods. Blood forms of the Wellcome-TS strain of *T. b. gambiense* were harvested from infected rats and purified by DEAE (diethylaminoethyl) cellulose chromatography[2]. Trypanosomes were suspended in 0.2 *M* potassium phosphate buffer, pH 7.3, without glucose, at a concentration of approximately 80 million organisms/ml. This suspension was sonicated for 30 sec using a Branson S-75 sonifier at setting 5. The sonicate was centrifuged at 20,000 *g* for 1 h and the supernatant was dialyzed for 100 h against 5 one-liter changes of buffer at 4 °C. The pellet was washed once with 15 volumes of buffer, again centrifuged, and saved for metabolic studies involving the particulate fraction.

Metabolic studies with the dialyzed soluble fraction were carried out in loosely capped incubation flasks placed in a shaking water bath at 37 °C. The following standard incubation mixture was used: dialyzed enzyme preparation, 3.0 ml; α-ketoglutarate (0.3 *M*), 0.1 ml; pyridoxal phosphate (1.2 m*M*), 0.1 ml; L-tryptophan-3-^{14}C (50.8 mCi/m*M*), or L-tryptophan-1-^{14}C (14.86 mCi/m*M*) (New England Nuclear), 3.0 μCi. When NADH, NADPH, NAD^+, or $NADP^+$ were included, 0.5 mg was added to the incubation mixture. Enzyme inhibitors, when included, were added in 0.1 ml saline to give a final concentration, unless otherwise specified, of 0.001 *M*. Metabolic studies with the particulate fraction were carried out in an identical manner, but the enzyme preparation consisted of the pellet suspended in 10 volumes of buffer. The period of incubation ranged from 45 min to 3 h (see Figures for details).

Two volumes of absolute methanol were added to terminate the incubations. After centrifugation, 100 μl of supernatant were spotted as a 2-inch streak on 57 cm-long strips of Whatman 3MM chromatography paper and one- dimensional chromatography was carried out using *n*-butanol: acetic acid: water (4:1:1), benzene:acetic acid:water (125:72:3), or 95% ethanol:ammonia:water (16:1:3). Purified standards of potential metabolites were also chromatographed simultaneously on the same chromatogram. Chromatograms were scanned for radioactivity using a Packard radiochromatogram scanner.

Results. The major metabolite of ^{14}C-tryptophan in dialyzed enzyme preparations when NADH was included migrated identically with indole lactic acid (Figure 1). Omission of NADH resulted in a total loss of indole lactic acid synthesis; however, the production of other metabolites which lack the C-1 carbon of the tryptophan side-chain was stimulated (Figures 1 and 2). These other metabolites migrated identically with tryptophol and indole acetic acid (Figure 3). The effect of NADH on the metabolic pathway of tryptophan in dialyzed enzyme preparations is illustrated in Figure 4. NADPH could not substitute for NADH as a requirement for the synthesis of indole lactic acid; also, the addition of NAD^+ or $NADP^+$ did not affect this pathway. Indole lactic acid synthesis was inhibited by 65% in the presence of 0.001 *M* *p*-chloromercuribenzoate (Figure 5C), but this compound seemed to have no effect on the production of tryptophol and indole acetic acid.

The transamination of tryptophan by trypanosomes required α-ketoglutarate, the absence of which resulted in an 80% reduction in the rate of tryptophan metabolism (Figure 5B). It did not require pyridoxal phosphate, a cofactor for tyrosine aminotransferase in this organism[3].

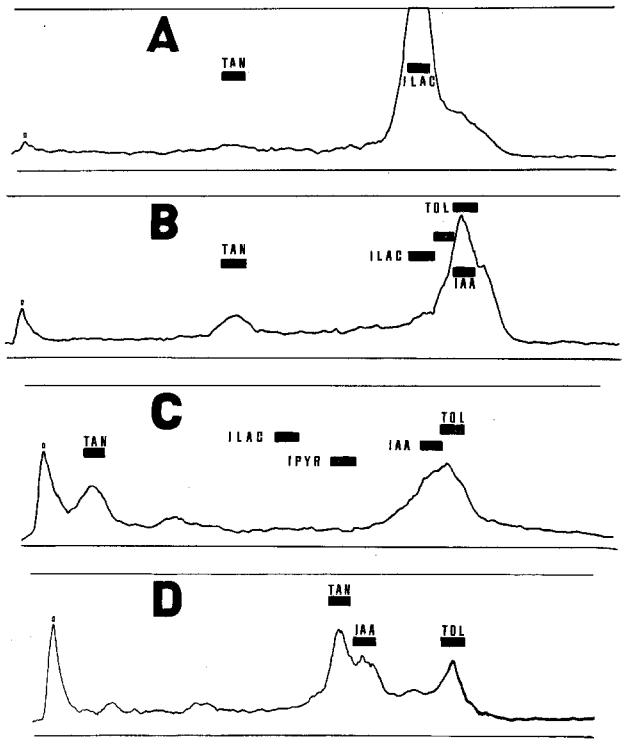
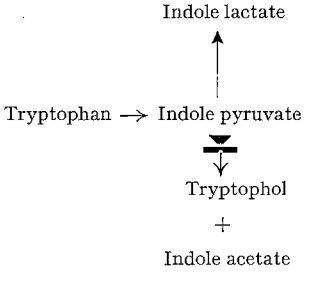


Fig. 3. A) Radioactivity scan (1,000 cpm range) of chromatogram of supernate from incubation of dialyzed enzyme for 2 h with tryptophan-3-^{14}C in the presence of NADH, pyridoxal phosphate, and α-ketoglutarate. Developed in butanol: acetic acid: water (4:1:1). B) Scan (1,000 cpm range) or chromatogram of supernate from incubation as in (A) but with omission of NADH. Same solvent system as in (A). C) Scan (1,000 cpm range) of chromatogram of supernate described in (B). Developed in benzene: acetic acid: water (125:72:3). D) Scan (1,000 cpm range) of chromatogram of supernate described in (B). Developed in 95% ethanol: ammonia: water (16:1:3). Abbreviations as in Figure 1.

I. NADH present



II. NADH absent

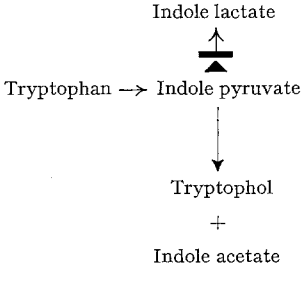


Fig. 4. The effects of NADH on the pathways of tryptophan metabolism in *T. b. gambiense*.

Transamination was inhibited 100% by 0.005 *M* hydroxylamine but not at all by 0.001 *M* hydroxylamine.

^{14}C-labeled tryptophol and indole acetic acid were not detectable in the incubations of dialyzed enzyme with ^{14}C-tryptophan labeled in the C-1 position of the side chain (Figure 2 B). This absence suggests the existence of a decarboxylation in the pathway from tryptophan to tryptophol. Presumably, it is indole pyruvic acid that is decarboxylated. We have also tried trapping $^{14}CO_2$ released from incubations of trypanosome sonicates with tryptophan-1-^{14}C and have found that a considerable amount $^{14}CO_2$ is released. Cocarboxylase (thiamine diphosphate) did not affect tryptophol synthesis, an observation that is somewhat surprizing in light of the reported activation of yeast indole pyruvate decarboxylase by this cofactor[4].

NADPH, $NADP^+$, and NAD^+ all had no effect on the synthesis of tryptophol and indole acetic acid by the dialyzed enzyme preparation. While NADH may be a requirement for tryptophol synthesis, its presence is so stimulating to indole lactic acid synthesis that the putative tryptophol-synthesizing enzyme may never encounter the appropriate substrate (indole acetaldehyde?) under these conditions. Pyrazole, sodium sulfide, chlorpromazine, and amobarbital (inhibitors of alcohol dehydrogenase), disulfiram (Antabuse) and its reduced form, diethyldithiocarbamate (inhibitors of aldehyde dehydrogenase), yohimbine (a tryptophan oxygenase inhibitor), and iproniazid (a monamine oxidase inhibitor) all had no effect on production of tryptophol and indole acetic acid.

The particulate fraction of the trypanosome was found to metabolize tryptophan to indole lactic acid and tryptophol (Figure 6).

Discussion. The apparent absence of a requirement for pyridoxal phosphate in the transamination of tryptophan is in agreement with the results of TRUELSEN[5] with bean seedlings but contrasts with a requirement for this cofactor in rat liver[6]. However, inhibition of tryptophan transamination in the trypanosome by hydroxylamine may argue in favor of a pyridoxal phosphate requirement, since this compound is known to release pyridoxal phosphate from monomeric forms of glycogen phosphorylase[7]. Another explanation for this inhibition could be that the active site of the tryptophan-transaminating enzyme may bear a resemblance to that of alcohol dehydrogenase, which is also inhibited by hydroxylamine[8]. The possibility that the tryptophan-transaminating enzyme is also the one which transaminates 5-hydroxytryptophan in this organism[1] is suggested by the work of SANDLER et al.[9] and SPENCER and ZAMCHECK[10] with rat liver.

5-Hydroxyindole lactate has previously been found to be produced from 5-hydroxyindole pyruvate in the presence of conventional hepatic lactate dehydrogenase[10]. However, while the blood forms of the African trypano-

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somes were once believed to contain lactate dehydrogenase, recent studies have indicated that this enzyme may be present only in trypanosome suspensions contaminated with platelets and leukocytes[11,12]. The enzyme responsible for production of indole lactic acid in this organism, therefore, may be one that is specific only for aromatic pyruvates and lactates.

Whether decarboxylation of indole pyruvate occurs enzymatically or spontaneously in this trypanosome is not certain. Decarboxylation is known to take place spontaneously in warm alkaline solutions in higher plants in the synthesis of auxins[13] but it is an enzymatic process in yeasts[4]. Indole pyruvate is unstable and difficult to obtain in pure form[14].

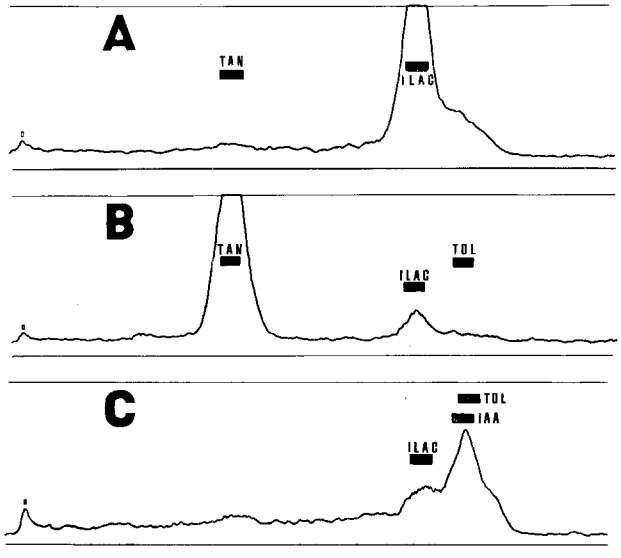
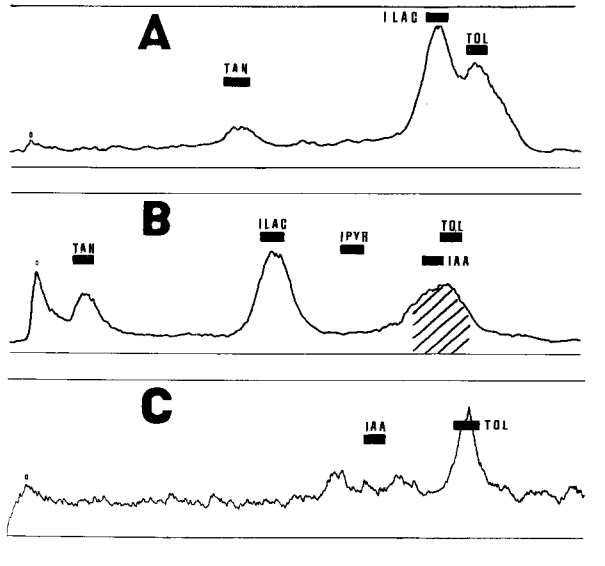


Fig. 5. A) Radioactivity scan (1,000 cpm range) of chromatogram of supernate from incubation of dialyzed enzyme for 2 h with tryptophan 3-^{14}C in the presence of NADH, pyridoxal phosphate, and α-ketoglutarate. Developed in butanol: acetic acid: water (4:1:1). B) Scan (1,000 cpm range) of chromatogram of supernate from incubation as in (A) but with omission of α-ketoglutarate. Same solvent system as in (A). C) Scan (1,000 cpm range) of chromatogram of supernate from incubation as in (A) but with addition of *p*-chloromercuribenzoate (0.001 *M*). Same solvent system as in (A) and (B). Abbreviations as in Figure 1.



The apparent lack of a requirement for reduced or oxidized pyridine nucleotides in the synthesis of tryptophol and indole acetic acid, and the absence of inhibition by a variety of alcohol and aldehyde dehydrogenase inhibitors, indicate that this protozoan enzyme system differs greatly from analogous mammalian systems[15–21]. The fact that indole pyruvate and indole acetaldehyde were not identified chromatographically as intermediates in the synthesis of tryptophol and indole acetic acid is not surprising, since the intermediacy of these compounds in an almost identical pathway existing in yeast could only be demonstrated indirectly by feeding these intermediates to the yeast and then identifying the tryptophol and indole acetic acid that were produced[22]. Synthesis of indole lactic acid and tryptophol by the particulate fraction of the trypanosome suggests that the enzymes responsible for these conversions may be membrane-bound as well as free in the cytoplasm of the cell.

Tryptophan transamination in *T. b. gambiense* has been suggested to be one method for transferring amino groups to pyruvate via alanine aminotransferase in order to detoxify intracellular concentrations of this keto acid[1]. We would also suggest that alanine, the product of pyruvate transamination, is more valuable to the trypanosome in protein synthesis than are the aromatic amino acids, since it represents one of the most common amino acids in the protein of another strain of this subspecies[23]. Synthesis of indole lactic acid in this organism may serve to replenish intracellular NAD$^+$reduced in glycolysis and possibly elsewhere, thereby helping to preserve the oxidation-reduction balance of the cell.

Metabolism of tryptophan by this parasite in vivo may divert host dietary tryptophan away from more essential pathways such as those leading to niacin and serotonin.

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Fig. 6. A) Radioactivity scan (1,000 cpm range) of chromatogram of supernate from incubation of particulate fraction with tryptophan-3-^{14}C for 90 min in the presence of NADH, pyridoxal phosphate, and α-ketoglutarate. Developed in butanol: acetic acid: water (4:1:1). B) Scan (1,000 cpm range) of chromatogram of supernate described in (A). Developed in benzene: acetic acid: water (125:72:3). Shaded peak was eluted from chromatogram with absolute ethanol and re-chromatographed in (C). C) Scan (300 cpm range) of chromatogram of eluate from shaded peak in (B). Developed in 95% ethanol: ammonia: water (16:1:3). Abbreviations as in Figure 1.

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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