

average value found in three experiments carried out with a dosage of 60 eV./molecule was 0.4 for the fraction removed at 50°C., pH 7.2, 6 hr.

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Transaminases of *Leishmania donovani*, the Causative Organism of Kala-azar

WHILE making a systematic investigation into the role of individual amino-acids on the nutritional requirements of *Leishmania donovani*, a protozoal parasite causing the disease kala-azar, strong transaminase activities were detected in the cell-free extracts of this organism. *L. donovani*, strain 81, cultured on Ray's medium¹ at 22–24°C. was used for this investigation. After growth for three days on this medium the organism was scraped from the surface of the agar, washed once with isotonic saline, and then ground with sand, with gradual additions of distilled water. The crushed suspension was centrifuged at 2,500 r.p.m. for 10 min. and the supernate was used as the crude enzyme extract.

Transaminase activities were demonstrated by incubating the enzyme extract with either α -ketoglutarate or pyruvate and the corresponding NH_2 -group donors like amino-acids, amino-acid amide, glucosamine, adenine, etc., when formation of glutamate or alanine was noted. For the identification and approximate quantitative determination of the amino-acids in the reaction products, paper chromatography by the ascending method was used. The incubation mixture for the assay of the enzyme activity consisted of 1 ml. of the crude extract (containing nearly 1 mgm. protein N/ml.), 1 ml. 0.13 M amino-acids or other amino donors, 0.5 ml. 0.25 M keto-acid, 0.5 ml. 0.05 M phosphate buffer (pH 7.8) in a total volume of 5 ml. The temperature of incubation was maintained at 32°C. Amino-acids used were all of DL-varieties, unless otherwise mentioned.

The presence of both α -ketoglutaric and pyruvic transaminase activities with a number of amino-acids was demonstrated. With α -ketoglutarate, the highest transaminase activities were observed with aspartic acid, histidine, methionine, lysine, cysteine, both α - and β -alanines, phenylalanine, tyrosine and tryptophan, lower activity with valine, ornithine, arginine, proline, threonine, nor-valine, nor-leucine, taurine, serine and glycine, whereas very feeble or negligible transamination occurred in the presence of hydroxyproline. With pyruvate, on the other hand, the highest transaminase activities were found with arginine, histidine, methionine, ornithine, phenylalanine and tyrosine; lower activities with lysine, β -alanine, typtophan, glutamic acid, serine, *isoleucine*, leucine, proline and γ -amino butyric acid, while very low activities were found with cysteine, aspartic acid, threonine and hydroxyproline. Among the amino-acid amide transaminases both glutamine and asparagine transaminase systems were very strong in the cell-free extracts of this organism. The presence of such active glutamine-keto acid and asparagine-keto acid transaminases, which occur also in rat liver

and kidney², suggests that both glutamine and asparagine might play a significant part in the amino-acid metabolism of this protozoal organism. Using glucosamine, the transaminase activity with either α -ketoglutarate or pyruvate was found to be relatively lower. Among the various purines and pyrimidines tried, only adenine, guanine and cytosine could transaminate with pyruvate but not with α -ketoglutarate in this extract. This suggests a new mechanism of formation and interconversion of purines and pyrimidines in this protozoal organism. A similar observation has also been reported in *E. coli* preparations³.

Repeated dialysis for 96 hr. in ice-cold temperature, charcoal treatment or ageing at room temperature for 2–3 hr., failed to reveal any requirement for pyridoxal phosphate or pyridoxamine phosphate. This failure may also be due to the difficulty of obtaining enzyme preparations free from pyridoxal phosphate-like co-factors.

Among the various inhibitors tried (used at 10⁻³ M concentrations) potassium cyanide, hydroxylamine, 2,4-dinitrophenyl hydrazine, mercuric chloride, silver nitrate, *p*-chloromercuric benzoate were found to have the most striking inhibitory effects on this system. The following inhibitors or compounds were tried and found to be inactive: iodoacetate, azide, isoniazid, urea stibamine, 8-hydroxyquinoline and sulphone.

Detailed studies dealing with the kinetics and other properties of this protozoal enzyme system will be reported in a separate communication elsewhere.

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An Effect of Hexylresorcinol on Bacterial Aerosols

DURING the course of some investigations into the effects of volatile germicides on the viability of bacterial aerosols, we have encountered a phenomenon which appears to have escaped the notice of other workers in this field, and which not only accounts for contradictions in some existing experimental results, but also suggests that in certain circumstances substances such as hexylresorcinol may be of considerably greater value as barriers to cross-infections than has been supposed hitherto.

When spraying bacterial aerosols into atmospheres containing small concentrations of hexylresorcinol vapour (0.1 $\mu\text{gm./l.}$), we observed that the initial recovery of the organisms immediately after spraying was frequently very much less than could be accounted for by the often rather slow killing-rate computed from a series of subsequent samples. Control experiments show that this mortality was not attributable to any factor other than the presence of the bacteri-