Plasma NADase in Tuberculosis

An increased activity of nicotinamide adenine dinucleotide glycohydrolase (NADase, EC 3.2.2.5.) is found in organs of tuberculous and cord factor treated animals, together with a concomitant decrease in the NAD(P) content in some of the organs[1–10]. It is assumed that there is a causative link between the increased activity of the enzyme and the deficiency of NAD(P)[2,11], which may be supported also by the observation of an inverse relationship between NAD levels and NADase activity in Ehrlich ascites tumor cells after treatment with alkylating agents[12,13].

In experimental tuberculosis there seems to be a correlation between the ability of the animal to develop progressive disease and its ability to react with an increase in the activity of the enzyme[5,14]. The guinea-pig, known to be one of the most sucseptible animals to the disease, shows in experimental tuberculosis a dramatically high increase in NADase activity in the organs. This increase appears in both particulate and soluble fractions of the organs, and a reflection of this can be found in the plasma, where the NADase activity is elevated as well[3]. Leukocytes and erythrocytes, under the same conditions, also show an increased NADase activity but without a decrease in their NAD content[14,10]. A decrease of pyridine nucleotides in the circulation in experimental tuberculosis, which could be abolished by isoniazid treatment, has been reported long ago[15].

In recent years an ever increasing attention has been paid to the passage of soluble enzymes from damaged tissue into blood plasma[16]. In view of the clinical application of plasma NADase assay to medical diagnosis, one should search for newly diagnosed untreated cases of human tuberculosis. The human black race is considered to be very susceptible to tuberculosis, more than the white race. Therefore, plasmas from 10 Ethiopians with newly diagnosed (untreated) tuberculosis were assayed for NADase activity and compared with plasmas from 10 normal Ethiopian controls. (Preliminary experiments with white patients gave inconclusive results.)

Methods. Table I shows 10 new black patients of the 'Tuberculosis Demonstration and Training Centre' in Addis Ababa. All were diagnosed for tuberculosis, radiology positive. Three of the patients showed tubercle bacilli in the sputum. Some blood values are presented. From each of these patients, and from 10 suitable controls, 10 ml of blood were drawn with 0.2 ml of saline containing 50–100 units of heparin. Plasma prepared by centrifugation was stored at –10°C for as long as a month until assayed. Guinea-pigs were treated as previously described[3].

NADase activity was assayed by the method of KAPLAN based on the cyanide reaction of NAD as described previously[1]. The reaction mixture contained: heparinized plasma, 0.2–0.4 ml; 0.2 *M* phosphate buffer pH 5.8, 0.4 ml; 0.003 *M* NAD, 0.2 ml (total volume, 1.0 ml). This was incubated at 37°C for 60 min. and the reaction was stopped by the addition of 3.0 ml of 1.0 *M* KCN. Activities

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Table I. Ten Ethiopian patients with newly diagnosed tuberculosis

Sex/age	Red blood cells count $\times 10^6$/mm^3	White blood cells count $\times 10^3$/mm^3	Erythrocyte sedimentation rate mm/h	Differential count (%)				
				Neutrophils	Lymphocytes	Eosinophils	Monocytes	Basophils
1[a] ♀/19	4.7	9.0	60	64	32	3	1	0
2 ♂/27	5.6	5.8	64	54	38	2	6	0
3 ♂/35	4.3	3.8	68	70	23	3	4	0
4 ♀/20	4.9	4.0	26	58	30	8	2	2
5 ♂/30	6.2	4.6	100	60	33	4	3	0
6 ♂/18	3.9	15.6	10	57	30	9	4	0
7 ♀/25	4.1	4.4	68	61	36	2	1	0
8[a] ♂/26	5.6	5.0	3	54	26	5	15	0
9[a] ♂/55	4.2	6.8	123	64	26	5	5	0
10 ♂/25	4.5	7.6	25	58	33	8	1	0
Average normal values in 50 young Ethiopians[17] ♂5.2, ♀4.9		6.8	not done (usually in white about 10)	50	43	4	3	–

[a] Sputum showed positive staining for acid fast bacilli.

were calculated from slopes of lines obtained by plotting incubation time against NAD cleaved. For calculating the amount of NAD split, 5.9 was used as the millimolar extinction coefficient of NAD-CN complex at 327 nm. (In preliminary experiments for plasma NADase assay, minor negligible differences were found using the pH's of 7.5, 7.0, 6.5, 5.8 and 5.0. The pH of 5.8 was chosen for the assay as a matter of convenience.)

Results and discussion. As seen in Table II the plasma NADase activities of all tuberculous Ethiopians were similar to those of normal controls, whereas in the tuberculous guinea-pig an almost 3-fold elevation of plasma NADase activity was noted over controls. Previous results reported an increase in NADase activity of tuberculous guinea-pig plasma of up to 7-fold[3]. In general, the rates for normal human plasma NADase were much lower than those of the normal guinea-pigs, which might be helpful for a detection of any possible increase.

Table II. NADase in plasma of tuberculous Ethiopians and guinea-pigs

	Ethiopians		Guinea-pigs	
	Tuberculous[a]	Control	Tuberculous	Control
1	0.10[b]	0.12	0.41	0.14
2	0.07	0.03	0.27	0.10
3	0.05	0.02	0.58	0.20
4	0.08	0.00	0.41	0.17
5	0.02	0.03		
6	0.09	0.15		
7	0.07	0.12		
8	0.05[b]	0.12		
9	0.13[b]	0.08		
10	0.10	0.09		
Mean ± S.D.	0.08 ± 0.03	0.08 ± 0.04	0.42 ± 0.13	0.15 ± 0.04

NADase unit = micromole NAD cleaved at pH 5.8/1 h at 37 °C/1 ml heparinized plasma. [a] Tuberculous patients sequential numbers as shown in Table I. [b] Positive staining for acid fast bacilli in sputum.

The increase of soluble plasma NADase is known to vary with the degree and duration of the experimental tuberculous infection[18]. It is possible, therefore, that a longer duration of the disease in man may show an elevation in the plasma NADase; but one would not expect to follow a case of tuberculosis for a long duration without treatment. On the other hand, at least 3 of the patients had tubercle bacilli in their sputum (Table I) which indicates a highly progressive disease, and the remainder may also have been tuberculous long before the present diagnosis. At any rate, it seems probable that the disease in man differs from that of the guinea-pig.

The very low activity of normal plasma NADase in man, and the absence of an increased activity during tuberculosis, in contrast to what is found in the tuberculous guinea-pig, rule out any clinical application of plasma NADase for the diagnosis of tuberculosis in man.

Résumé. Des cobayes tuberculeux montrent dans leur plasma une augmentation de l'activité de l'enzyme NADase d'environ 3 fois supérieure à celle du plasma d'animaux normaux. Chez des sujets tuberculeux noirs (Ethiopiens), l'activité de la NADase plasmatique est trés faible et semblable à celle de sujets normaux. Pour le diagnostic de la tuberculose humaine, une application clinique éventuelle du «test NADase» plasmatique est donc exclue.

I. Windman and B. Teoume-Lessane[19]

Institute of Microbiology, Hebrew University, Hadassah Medical School, Jerusalem (Israel); and Tuberculosis Demonstration and Training Centre, Imperial Ethiopian Government, Ministry of Public Health, Addis Ababa (Ethiopia), 15 May 1972.

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Partricin Methyl Ester, a Semisynthetic Polyene Antibiotic

Only a few of the many polyene antifungal antibiotics produced by different strains of *Streptomyces* have found clinical applications. The antifungal antibiotics are usually toxic, almost insoluble in water and unstable, and the efforts made to increase their manageability have been as yet hardly successful[1,2].

A new polyene (partricin, SPA-S-132) produced by a strain of *Streptomyces aureofaciens* (NRRL 3878) has recently been isolated[3], and found to have biological properties similar to those of other known antibiotics. Its structure has not yet been elucidated but it is presumably macrolidic with amphoteric properties. Partricin is very active against fungi and protozoa: the minimum inhibitory concentrations (MIC) on *Candida albicans* were about 0.2 µg/ml and the MIC on *Trichomonas vaginalis* were about 0.25 µg/ml. It is tolerated by oral route (LD_{50} 300 mg/kg), but is very toxic by i.p. administration in mice (LD_{50} 0.5 mg/kg) and shows a high hemolytic activity.

In an attempt to improve the biological properties of partricin, its methyl ester was prepared. Partricin methyl ester (SPA-S-160) was obtained by treating a solution of partricin in dimethylsulfoxide with diazomethane and the product isolated following precipitation with ether was purified by suitable organic solvents.

Partricin methyl ester is a deep yellow crystalline material, almost insoluble in water and in the usual organic solvents, very soluble in dimethylsulfoxide, dimethylformamide, dimethylacetamide and methylcellosolve. In solid form and preserved from light, it is almost stable. Elemental analysis has given the follow-

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