Blood Xanthine Dehydrogenase and Iodine Value of Blood Fatty Acids

In previous work using paper electrophoresis[1] it was shown that the rat blood serum xanthine dehydrogenase (XD) activity migrates with the globulin fractions, and it has been suggested that the enzyme is bound to lipoproteins. The increase of XD activity on rat blood serum of animals poisoned with colchicine[2] and of carbon tetrachloride[3] seems linked to a derangement of the lipid metabolism.

The increase of blood fatty acids in CCl_4 (refs. 4 and 5) as well as in colchicine-poisoned animals[6] indicates either a pathological change in the liberation of the fatty acids by adipose tissue, or a change in the uptake and decrease of this metabolite utilization due to a modification of the fatty acid desaturation process in the liver or possibly in other organs.

Since the increase of the activity in blood serum of animals poisoned with colchicine or CCl_4 seems to be a consequence of this transport lipid derangement, the purpose of the present communication is to study the correlation of the XD activity and the unsaturated fatty acid in rat blood serum.

Normal rats of different ages are used in this work. CCl_4 (0·1 ml./100 g body weight) was injected into some animals in order to obtain a range of variability in the blood serum enzyme activity. Blood samples were withdrawn by heart puncture and each determination was carried out on pooled non-haemolysed serum from three rats. The coagulated blood was centrifuged, and the serum was separated and analysed immediately.

Xanthine dehydrogenase activity was measured colorimetrically by a method previously described[3] and the results were measured in μg formazan/0·5 ml. serum/30 min at 37° C. Iodine value was determined according to Yasuda[7] and blood lipids determined colorimetrically by Bragdon's method[8]. Twenty-two determinations were made, the volume being 1·0–2·0 for each assay.

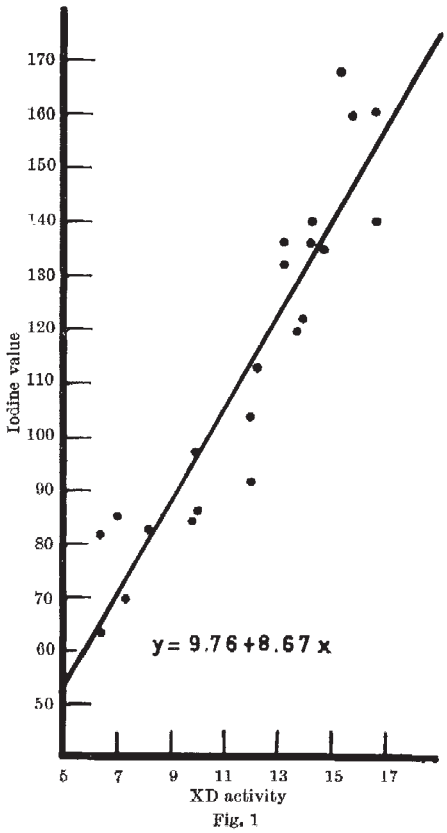

Fig. 1

The results showed a correlation between the XD activity and the iodine value of blood serum lipids. The correlation coefficient was calculated from the data pooled and given the value of +0·903. The regression line is given in Fig. 1; the regression equation is $y = 9{\cdot}76 + 8{\cdot}67x$. The actual determinations have been plotted to illustrate this relationship.

We thank Dr. G. G. Villela for his help and advice.

Emilio Mitidieri
Ottilia R. Affonso

Biochemical Laboratory,
Instituto Oswaldo Cruz,
Rio de Janeiro, Brazil.

[1] Mitidieri, E., Ribeiro, L. P., Affonso, O. R., and Villela, G. G., *Biochim. Biophys. Acta*, **17**, 587 (1955).
[2] Affonso, O. R., Mitidieri, E., and Villela, G. G., *Nature*, **193**, 64 (1962).
[3] Affonso, O. R., Mitidieri, E., Ribeiro, L. P., and Villela, G. G., *Proc. Soc. Exp. Biol. and Med.*, **90**, 527 (1955).
[4] Spitzer, J. J., and Miller, H. I., *Proc. Soc. Exp. Biol. and Med.*, **92**, 124 (1956).
[5] Villela, G. G., *Biochem. Pharmacol.*, **13**, 665 (1964).
[6] Spitzer, J. J., and Spitzer, J. A., *Experientia*, **15**, 26 (1959).
[7] Yasuda, M., *J. Biol. Chem.*, **94**, 401 (1931).
[8] Bragdon, J., *J. Biol. Chem.*, **190**, 513 (1951).

Increased Sensitivity in the Detection of Adenine and Pyridine Nucleotides by Exposure to Ultra-violet Light at Low Temperature

In 1957 Szent-Györgyi described a procedure for the enhancement of fluorescence of compounds spotted on paper by cooling with solid carbon dioxide or liquid nitrogen[1]. Gordon and South have applied this procedure to the action of aromatic acids and other aromatic ring-containing compounds[2]. We have found that by observing absorption, fluorescence and delayed fluorescence on exposure to long and short wave-length ultra-violet light, NAD^+ and NADH can be distinguished from each other and from ADP and ATP.

Heat-resistant thin-layer chromatographic plates coated with 250-μ layers of silica gel (Merck-Darmstadt) or DEAE cellulose ('BIORAD') and Whatman No. 1 filter paper disks were spotted with 2 ml. each of adenosine diphosphate (ADP), adenosine triphosphate (ATP), niacin adenine dinucleotide (NAD^+), and reduced niacin adenine dinucleotide (NADH) solutions. NADH showed a strong light blue fluorescence, while the other compounds showed dark areas indicating absorption of the ultra-violet light when exposed to ultra-violet light at room temperature. On cooling with liquid nitrogen the results reported in Table 1 were observed.

Maximum sensitivity in the detection of ADP and ATP by both absorption and delayed fluorescence at −196° was obtained by exposure 3 in. from the shorter wave-length lamp. For observation of low levels of delayed fluorescence, the eyes were kept closed during the exposure to ultra-violet light to maintain partial dark adaptation. As soon as the exposure was ended the eyes were opened to observe the delayed fluorescence. ADP and ATP spotted on a DEAE cellulose-coated thin-layer plate could be detected by both absorbance and delayed fluorescence with the shorter wave-length lamp at 10^{-11} moles at −196° and 10^{-8} moles by absorbance at 27°. When chromatograms of ADP and ATP were developed in 0·02 N HCl (ref. 3), it was necessary to expose the plate to ammonia fumes in order to observe the delayed fluorescence at −196° C.

NAD^+ was distinguished from the adenine nucleotides through reduction to NADH by application of a weak solution of dithionite to the NAD^+ spot. The reduction should be made following the initial identification of the spots as the dithionite also absorbs ultra-violet light. Both NAD^+ and NADH could be detected at 10^{-11} moles at −196°.

We believe this technique, which offers a thousand-fold increase in sensitivity of detection of adenine and pyridine