Yielding and Tomkins[1] suggested that the inhibition of the GDH activity by steroids might be caused by the splitting of the GDH molecule into smaller units, while Hofmann[4] has directed attention to the aromatic character of steroids which are π shell compounds. In this connexion, we note that only DES—the most powerful inhibitor—and clomiphene have as a common part in their structure a —C=C— bond. It represents a special type of reactive π electron bond. This might be the key point of the inhibiting property of these two compounds. Being very reactive, this bond may attack the hydrophobic points of GDH molecule and so split it into smaller units which has been suggested to be the cause of inactivation[1].

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Nucleotide Pyrophosphatase of *Tetrahymena pyriformis*

IN an investigation of the synthesis of nicotinamide adenine dinucleotide (NAD) by *Tetrahymena pyriformis* we examined the enzyme cleavage of NAD by this ciliate. There seems to be a lack of information about the cleavage of this coenzyme by Protozoa[1,2]. In preliminary experiments we incubated cell-free homogenates with NAD and saw that its activity, measured with alcohol dehydrogenase, disappeared rapidly, and detailed investigation to identify the enzyme responsible for the cleavage of NAD seemed to be necessary.

In the case of NADase, nicotinamide is liberated and the complex with cyanide is lost[1]. When the cleavage is in the pyrophosphate bond, the activity of the coenzyme is destroyed, but the capacity to form a complex with cyanide is maintained. NADase is also inhibited by nicotinamide, while pyrophosphatase is not. Phosphorus is released as in the case of sweet almonds and intestinal pyrophosphatases[3].

Tetrahymena pyriformis, variety I, mating type II, cultured in 2 per cent peptone 'Difco' and 0·5 per cent glucose, was used for these experiments. In some cases, 0·1 per cent 'Difco' yeast extract was also added to the medium. After 3 days of incubation at 25° C, without shaking, the cells were collected and washed with distilled water and centrifuged at 2,000 r.p.m.

The cells were suspended in 0·02 molar phosphate buffer, *p*H 7·0, and submitted to ultrasonic treatment in an MSE apparatus for 3 min in an ice bath (until no intact cells could be detected microscopically). The sonicate was centrifuged at 6,000*g* and 4° C and the supernatant was used in the experiments. Coenzyme activity of the extracts prepared with 0·02 molar phosphate buffer, *p*H 7·0, was determined with alcohol dehydrogenase[4]. Crystalline alcohol dehydrogenase diluted to contain 500 U/ml. and NAD (97·5 per cent pure) were used. Phosphorus was estimated by Fiske and Subbarow's method modified by Gomori[5] and proteins by the method of Warburg and Christian, using the formula of Kalckar[6]. The cyanide complex was measured according to Colowick, Ciotti and Kaplan[4]. The extracts for phosphorus were prepared with 0·1 molar *tris* buffer, *p*H 7·0.

The experiments summarized in Table 1 showed that the coenzyme activity decreased rapidly but that the cyanide complex was unchanged even after 120 min of incubation. Acid soluble phosphorus increased with the time of incubation.

Table 1. ACTIVITY OF THE NUCLEOTIDE PYROPHOSPHATASE OF *Tetrahymena pyriformis*

Time of incubation (min)	NAD		Phosphorus split		Potassium cyanide complex. Optical density at 340 mμ ‡
	μ-moles*	(μmoles/mg of protein)	μg†	(μg/mg of protein)	
7	2·12	0·70	—		0·25
10	0·98	0·30	2·2	0·76	—
30	0·11	0·03	6·5	2·1	—
60	0·03	0·01	8·5	2·8	—
120	0·0	0·0	10·5	3·5	0·26
180	0·0	0·0	18·6	6·2	0·25
Heated at 70° C§	0·0	0·0	0·0		—
Incubated for 30 min with 3 mg of nicotinamide	0·12	0·0	0·4		—

* Cell free sonicate (1 ml.) incubated with 7 μmoles of NAD and the activity measured with 0·1 ml. of 10 per cent ethanol, 1·8 ml. of 8·7 per cent sodium pyrophosphate, *p*H 9·5, and 0·1 ml. of alcohol dehydrogenase solution. Optical density measured in a spectrophotometer at 340 mμ.

† Phosphorus (acid soluble) was split by incubating 1 ml. of the sonicate extract, adjusted to contain 3 mg protein and 0·6 mg of NAD. Deproteinization with 12 per cent perchloric acid with 'Celite' as a filter aid. The centrifugate was assayed by Gomori's method and the colour was measured using a red filter in a photocolorimeter[5].

‡ Cell free sonicate (0·2 ml.) was incubated with 0·2 μmoles of NAD and cooled; added with 1 molar potassium cyanide solution to make 3 ml. and the optical density was read at 340 mμ after 1 min[4].

§ The extract (2 ml.) was heated in a water bath at 70° C for 2 min and cooled in an ice bath for the enzyme assay and determination of phosphorus. All these results represent mean values based on five separate experiments.

It is known that nicotinamide inhibits NADase and not pyrophosphatase; the enzyme from *Tetrahymena*, as expected, was not sensitive to nicotinamide (Table 1). Heating at 70° C for 2 min inactivated the enzyme, showing that a protein inhibitor is not present as in the case described for *Proteus vulgaris*[7].

I concluded that the enzyme cleavage of NAD by sonicated extracts of *Tetrahymena pyriformis* is caused by a nucleotide pyrophosphatase and not NADase.

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Electrophoresis of Glucose-6-phosphate Dehydrogenase: a New Technique

WE wish to report a new technique for the investigation of the electrophoretic behaviour of glucose-6-phosphate dehydrogenase (G6PD), which offers, in our opinion, some advantages over the starch gel technique used at present[1,2].

The medium on which the electrophoresis is performed is cellulose acetate in a gel form ('Cellogol', manufactured by Chemetron, via G. Modena 24, Milano, Italy); this has already been used in investigations of the lactic dehydrogenase isozymes[3]. We use strips 4 cm wide, 17 cm long and 0·25 mm thick. It is advisable to make sure that the same batch of strips is used throughout each series of experiments. The electrophoresis buffer consists of 1 l. of 0·075 molar *tris* (containing 0·004 moles EDTA), to which is added 250 ml. of 0·075 molar citric acid solution; this gives a *p*H of 7·5.