

# MICROBIOLOGICAL EVALUATION OF HEART PROTEINS USING *CORYNEBACTERIUM CAROTENOGENUM*

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## ABSTRACT

The use of *Corynebacterium carotenogenum*, a species demanding liver extracts for growth, has been suggested for the microbiological evaluation of heart proteins associated with heart extracts increasingly being employed as therapeutics.

## INTRODUCTION

Determination of the nutritive value of proteins has in the past depended largely upon the slow, the more tedious and the expensive biological assays made with chickens, dogs, rabbits, rats and swine or through direct feeding experiments on humans, but during recent years suitable microbiological methods have been designed for the purpose. In fact, within the past decade, several useful procedures employing a wide variety of microorganisms have been proposed (Anderson and Wilhams, 1951; Dunn and Rockland, 1947; Fernell and Rosen, 1956; Halvay and Grossowicz, 1953; Horn *et al.*, 1954; Mertz *et al.*, 1955; Pilcher and Williams, 1954; Terri *et al.*, 1956). The purpose of the present communication is to show that a species from the genus *Corynebacterium*, a genus hitherto not employed for any assay procedures, can be used for the biological evaluation of heart proteins from the heart extracts increasingly being used as therapeutics. A note on the taxonomic characteristics and other aspects of the bacterium, viz., *Corynebacterium carotenogenum* has recently appeared elsewhere (Pradhan and Bhat, 1960). It may be pointed out that this bacterium, by and large, fulfils the requirements of the test organisms for the assay (Gavin, 1956) and a strain thereof has been maintained without any changes for years on liver extract agar.

## MATERIALS, METHOD AND RESULTS

Proteins to be tested for their nutritional value were subjected to enzymatic digestion in a sequence parallel to the human digestion. 5g of the sample, e.g., vitamin-free casein or soyabean, values of which were previously determined by

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biological experiments and were found to be 80 and 50 respectively, were incubated for 24 hours at 37°C in 150 ml. of 0.5% pepsin solution adjusted to pH 1.8 with hydrochloric acid. A layer of toluene was used during all digestions. On completion of peptic digestion, the solution was adjusted to pH 8.4 and buffered with boric acid and potassium chloride buffer. After addition of 30 mg. pancreatin and 100 mg. trypsin the solution was kept in an incubator (37°C) for 72 hours. The solution was shaken frequently to improve contact between enzyme and substrate. The hydrolysate was then adjusted to pH 7.0 and filtered to remove the undigested material. The controls to which no protein was added were also subjected to the same enzymatic treatments. The experimental samples consisted of commercial heart extracts packed at random from three different batches and treated enzymatically in the same way in which the controls were run.

The microbiological assay for the evaluation of the above proteins was developed using *C. carotenogenum* as the test organism whose demands for all the essential amino acids had previously been determined (Pradhan, 1951; 1958). The basal medium employed for the assay had the following composition:

Glucose	1 g
Sodium chloride	0.2 g
Magnesium sulphate	0.1 g
Calcium pantothenate	0.001 g
Pyridoxine HCl	0.001 g
Riboflavin	0.001 g
Folic acid	0.001 g
Adenine	0.001 g
Guanine	0.001 g
Dipotassium hydrogen phosphate	0.4 g
Citric acid	0.2 g
Lactic acid	0.2 g
NiacinamidC	0.001 g
Thiamine H1	0.001 g
Vitamin B <sub>12</sub>	0.000005 g
Biotin	0.00001 g
Uracil	0.001 g
Tween 80	0.04 g

pH adjusted with ammonia to 7 and volume made with distilled water to 100 ml.

The organism was subcultured in liver extract agar slant (Pradhan, 1958) and on the fourth day, a loopful from the culture was suspended in 10 ml. sterile saline to obtain a thin suspension free from clumps. Two drops of this served as the inoculum for each assay tube containing 10 ml. of the above medium. The tubes were incubated at the room temperature (25–28°C) for 10 days and the turbidity was measured on Hilger colorimeter using No 8 (680 m $\mu$ ) filter.

The accuracy of the assay was judged by running a parallel assay using the standard *Streptococcus faecalis* strain. The results recorded are presented in Table I.

TABLE I

Biological values of heart proteins using *C. carotenogenus* and *S. faecalis* as test organisms.

Sl. No.	Proteins	Biological value as determined with	
		<i>S. faecalis</i>	<i>C. carotenogenus</i>
1	Casein	80	80
2	Soyabean	50	35
1	Herzolan B No. 1517	10.89	10.71
2	Herzolan ,, 72/CB	13.08	12.94
3	Herzolan ,, 80/CB	12.41	12.21

## DISCUSSION

Excepting for the low value obtained for soyabean proteins, the results indicate the usefulness of the organism, viz., *C. carotenogenus* in determining the biological values of proteins. In fact, the results compared favourably with those obtained by the use of *S. faecalis* as per the method of Terri *et al.* (1956). Evaluation of other proteins for their biological values remains to be assessed.

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