Citric Acid Cycle Enzymes in Normal and Syphilitic Rabbit Tissues.* (22422)

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Numerous attempts at the *in vitro* cultivation of pathogenic *Treponema pallidum* by various investigators have been unsuccessful (Reviewed in Ref. 1). Therefore, it appears advisable to follow other approaches to this problem. *T. pallidum* like a number of other microorganisms shows a preference for specific tissues. Thus, in the rabbit, regardless of the site of inoculation, a syphilitic orchitis develops. A comparative study of the biochemistry of various tissues, both infected and non-infected, might possibly point to factors required for the growth of the organism. The present investigation is concerned with concentrations in tissues of most enzymes of the citric cycle: α-ketoglutaric oxidase (hereafter referred to as KGO), pyruvic oxidase (PO), fumarase (FU), aconitase (AC), triphosphopyridine nucleotide (TPN) isocitric dehydrogenase (ICD) and succinic dehydrogenase (SD) together with lactic dehydrogenase (LD).

Methods. Preparation of rabbits. Nineteen normal animals were used in 11 experiments. In 6 rabbits of this group trauma was produced by injecting into each testis 1 ml of a mixture containing equal parts of 0.85% NaCl solution and normal rabbit serum. These rabbits were killed 7, 14, 20, 29 and 48 days after trauma was produced. Because no difference was found between these two groups of uninfected animals they were all considered as normal rabbits (Table I). There were 29 rabbits in 14 experiments in the syphilitic group. The Nichols strain of *Treponema pallidum* was employed. 10^7 organisms in 1 ml saline-serum suspension were injected into each testis. The syphilitic rabbits were killed at weekly intervals during a period of 8 to 104 days after infection. *Preparation of tissues for assaying.* Immediately after the death of the animals, the organs were removed, trimmed of fat and connective tissue, then frozen and stored at −10°C until used. For assaying 4 enzymes FU, KGO, PO and LD one extract was prepared as follows: 0.5 g portion of each organ was homogenized in a glass homogenizer with 4.5 ml of sucrose-phosphate solution of pH 6.70 (containing 34.24 g sucrose, 100 ml of 0.1 M Na_2HPO_4, 100 ml 0.1 M KH_2PO_4 and distilled water to make a final volume of 400 ml). The homogenates were centrifuged at 6000 g at 4° for 40 minutes in a Servall angle centrifuge. The supernatants were used for assaying these 4 enzymes. A similar procedure was used for AC assaying, but here the extracting solution contained 0.002 M sodium citrate and 0.002 M citric acid(2). For assaying ICD the extracting solution contained 171 g of sucrose, 500 ml of 0.05 M barbital buffer of pH 7.50 and distilled water to make 2000 ml. Here 0.4 g of each tissue was homogenized with 19.6 ml of the sucrose-barbital solution and the homogenate was filtered through 6 layers of cheese cloth. This homogenate was assayed without centrifuging. In those instances where the supernatants of the specially prepared homogenates have been used, the respective enzymes were largely present in the supernatants of the homogenates. *Assaying procedures.* α-Ketoglutaric oxidase and PO were assayed spectrophotometrically using 2,6-dichlorophenol indophenol as the oxidant(3). One ml of sucrose-phosphate solution of pH 6.70 (described in the preceding paragraph), 300 μg (0.1 ml) of cocarboxylase, 0.1 ml or 0.15 ml of the centrifuged (10%) homogenate and 0.3 ml neutralized KCN (0.1 M) were placed in a test tube and mixed. The mixture was allowed to stand for 1 minute at room temperature, in order to inactivate the enzyme which

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TABLE I. Citric Acid Cycle Enzymes in Testes (Units/mg Dry Basis).

	KGO	PO	FU	AC	LD	ICD
	Avg of normal group					
	24	22	10	11	80	5
	Activity ranges of normal groups					
	13–34	11–37	7–13	9–16	62–101	3–8
	Avg of syphilitic groups					
(8– 14)*	14	3	11	5	84	4
(21– 40)	7	6	10	5	91	3
(45– 72)	11	10	14	6	91	4
(73–104)	14	15	16	7	73	5
	Avg of 4 syphilitic groups					
	11	9	13	6	84	4
	Activity ranges of syphilitic groups					
	5–21	2–18	7–17	2–9	60–150	2–7

* Figures in parentheses indicate period of infection in days.

reoxidizes the reduced dye; then 0.4 ml of 0.01% dye solution and 60 μM sodium pyruvate or neutralized α-ketoglutaric acid were added per final volume of 3 ml reaction mixture. The reaction mixture was transferred to a cuvette and the decrease in optical density (OD) at 600 mμ was observed. All enzyme activities were measured at 25.5°. One unit is defined as that amount of enzyme which produces a decrease in OD of 0.001/min. Fumarase and AC were assayed at 250 mμ and 240 mμ respectively at pH 7.40 by the method of Racker(4). One unit of AC and FU activity is defined as the quantity of enzyme causing an increase in OD of 0.001/min. ICD was measured by observing the rate of reduction of TPN in the presence of manganous ions and isocitrate at pH 7.50 at 340 mμ(5). One unit is defined as the quantity of enzyme which causes an increase in OD of 0.01/min. LD was assayed by measuring the rate of reduction of DPN at pH 10.00 at 340 mμ(6). One unit of enzyme activity is defined as the quantity of enzyme causing an increase in OD of 0.001/min. SD assays(7) were done on whole homogenates (20%) measuring tetrazolium reduction at pH 7.40 in 1 hour at 37° in the Klett-Summerson colorimeter. A calibration curve was obtained by reduction of the tetrazolium salt with 0.3 ml of a 10% solution of sodium hydrosulfite per tube (25 to 200 μg triphenyltetrazolium chloride). This method produced maximum and stable color. One unit of enzyme activity is defined as μg of tetrazolium salt reduced per mg of tissue under the condi-

TABLE II. Citric Acid Cycle Enzymes in Liver (Units/mg Dry Basis).

	KGO	PO	FU	AC	LD	ICD	SD
	Avg of normal group						
	18	19	56	14	124	17	2
	Activity ranges of normal groups						
	8–28	8–32	42–70	12–16	72–189	9–28	1–3
	Avg of syphilitic groups						
(8– 14)*	18	16	37	9	89	16	2
(21– 40)	16	12	50	13	109	16	2
(45– 72)	20	18	52	10	147	15	1
(73–104)	22	21	58	9	141	13	1
	Avg of 4 syphilitic groups						
	19	17	51	10	127	15	1
	Activity ranges of syphilitic groups						
	8–33	8–28	34–74	5–15	72–203	11–18	0–3

* Figures in parentheses indicate period of infection in days.

TABLE III. Citric Acid Cycle Enzymes in Heart (Units/mg Dry Basis).

	KGO	PO	FU	LD	ICD	SD
			Avg of normal group			
	12	8	192	725	18	1
			Activity ranges of normal groups			
	7–16	3–11	86–248	426–881	12–27	0–3
			Avg of syphilitic groups			
(8– 14)*	22	8	177	677	27	1
(21– 40)	17	5	218	696	17	2
(45– 72)	7	5	224	682	22	1
(73–104)	9	5	218	652	23	2
			Avg of 4 syphilitic groups			
	12	6	214	677	21	1
			Activity ranges of syphilitic groups			
	5–37	3–11	154–246	518–834	4–34	1–3

* Figures in parentheses indicate period of infection in days.

tions of the assay.

Results. Testes. The results given in Table I demonstrate that during the most virulent stage of infection (8 to 14 days) PO dropped to 1/8th of the normal range and later (after 21 to 40 days of infection) the values were 1/4th those of the normal range. A less pronounced but qute significant lowering in KGO, AC and ICD activities took place. Both normal and syphilitic testes contained only a trace of SD. The FU activity range was the same in normal and syphilitic testes. *Liver.* In the livers of two infected rabbits (72 and 104 days after infection) SD was completely absent. The other enzymes of this organ were within the normal range of activity (Table II). *Heart.* Both normal and syphilitic heart tissue showed considerable variations in the ranges of AC activity (2 to 62 units for normal tissue and 2 to 119 units for syphilitic tissue), and because of this the aconitase figures have been omitted from Table III. *Kidneys.* There was a gradual decrease of PO (within a wide range) in syphilitic kidneys. There was a moderate decrease in AC activity. The other enzyme activities of kidneys varied within a wide range so that no significant differences could be observed between normal and infected tissues (Table IV).

DPN isocitric dehydrogenase was not determined since the enzyme was found to be present only in traces in rabbit tissues. It may be seen that of the normal rabbit tissues studied testes contained most KGO and PO, the heart contained most FU and LD, and

TABLE IV. Citric Acid Cycle Enzymes in Kidneys (Units/mg Dry Basis).

	KGO	PO	FU	AC	LD	ICD	SD
			Avg of normal group				
	23	24	173	55	213	23	2
			Activity ranges of normal groups				
	16–31	16–34	125–194	48–62	178–264	18–32	1–4
			Avg of syphilitic groups				
(8– 14)*	31	17	178	43	176	17	1
(21– 40)	26	11	133	40	187	21	1
(45– 72)	15	8	155	35	190	23	2
(73–104)	15	13	146	47	181	23	2
			Avg of 4 syphilitic groups				
	20	12	150	41	185	23	2
			Activity ranges of syphilitic groups				
	8–46	4–28	114–218	22–62	106–247	15–31	1–3

* Figures in parentheses indicate period of infection in days.

kidneys contained most AC, ICD, and SD. These results are reported on dry basis. They appear to be just as significant when evaluated on wet basis.

Summary. 1. Normal and syphilitic rabbit testes, liver, heart and kidneys were assayed for α-ketoglutaric oxidase, pyruvic oxidase, fumarase, aconitase, lactic dehydrogenase, TPN isocitric dehydrogenase and succinic dehydrogenase. 2. In syphilitic testes there is a definite decrease in pyruvic oxidase, α-ketoglutaric oxidase, aconitase and TPN isocitric acid dehydrogenase. 3. Both normal and syphilitic heart tissue showed very considerable variations in aconitase activity. 4. In syphilitic kidneys there was a decrease in pyruvic oxidase activity and a moderate decrease in aconitase activity. 5. The decreased enzyme values found in the testes and kidneys of the syphilitic groups are probably a demonstration of a general reaction to the infection.

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Comparative Biochemical Studies on Normal and Poliomyelitis Infected Tissue Cultures. IV. Enzyme-changes in Host Cells. (22423)

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The successful cultivation of the virus of poliomyelitis by Enders *et al.*(1) provides a unique opportunity to approach host-virus interaction in simple systems by excluding inflammatory reactions. Enzymes connected with nucleic acid metabolism were extensively studied in explants of normal Rhesus kidney with synthetic nutrients and in surviving tissues(2-4) to assure a baseline for the present work. Alterations occurring in similar biocatalysts during *in vitro* growth of poliomyelitis virus are here reported. Since enzymology in normal and in infected tissue culture (TC) is a relatively unexplored field(2,3) a brief synopsis of findings on nine different enzyme systems is presented. Details, together with various secondary problems investigated, will be published separately(5).

Methods. Assay material was obtained from the same source† as previously. In preliminary publications preparation of TC, media and homogenates was described, together with the assay methods for enzymes(2,3). Adherence to these technics makes repetition unnecessary and assures continuity with previous communications. The trypsinized Rhesus kidney epithelial cells were routine material from virus titrations, grown in roller tubes with synthetic medium 199(6) or its modified form, 597(2). All virus work was carried out in the poliomyelitis department. Titres were expressed in negative logarithms calculated by Kärber's method(7). Mahoney, MEFI and Saukett virus types were used. The strains were from TC passages and generally of high dilutions ($10^{-6.5}$ to $10^{-7.5}$). Sin-

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