More refined studies with gelatins, reconstituted collagens and various synthetic amino acids, polypeptides and collagen analogues which tend to support this same hypothesis will be reported later.

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Succinic Cytochrome C Reductase Activity in Kidney and Liver Tissue from Starved and Fed Rabbits.* (27853)

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Some aspects of the relationship between oxidative metabolism and dietary regimens have been studied by O'dell *et al.*(1) who found that the cytochrome oxidase activity of liver in growing rats is not altered by food restriction. However, Wainio *et al.*(2) found that caloric and protein restriction lowers the succinic dehydrogenase activity in liver homogenates from mature rats. Cambria(3) also found significant decreases for succinic dehydrogenase activity for heart muscle homogenates of starving pigeons. Unpublished studies of kidney and liver enzyme systems from fed and starved rabbits indicated that incubation of tissue homogenates for 24 to 48 hr at 4°C resulted in increased oxidative enzyme activity. It was of interest, therefore, to investigate further the effect of starvation, incubation and analytical methods for some oxidative enzyme systems in rabbit organs. The present study is limited to the succinic cytochrome C reductase system in rabbit liver and kidney homogenates.

Materials and methods. Young adult female white rabbits weighing 2-3 kg were maintained on commercial rabbit chow. Experimental *ad libitum* fed and starving rabbits were maintained in individual cages for 5 or 10 days with water available at all times. Following sacrifice by cervical fracture, the liver and kidneys were quickly removed, rinsed with 0.154 M NaCl and placed on crushed ice. Two gram portions of tissue were placed in 20 ml cold (4°C) M/10 phosphate buffer pH 7.5 and homogenized with a teflon pestle in a glass tube kept in crushed ice. Care was taken in cutting kidneys to use cross sections containing proportionate quantities of cortex and medulla. For liver tissue, representative portions of all lobes were used. Fibrous material was removed by passing the homogenates through 4 layers of cheese cloth.

The assay system was modified from Lehman and Nason(4). The rate of reduction of oxidized horse cytochrome C (Sigma Chemical Company) was determined every 30 seconds for 3.5 minutes in a Beckman DU at 550 mμ at 27°C. The reaction mixture, in a 3 ml cell was allowed to reach temperature and optical equilibrium for 3 minutes before adding the homogenate to initiate the reaction. Homogenate protein concentrations were determined by the turbidimetric method of Heepe *et al.*(5).

Experimental procedure and results. In view of the possibility that changes in physiological state of the animal may alter the extractability or other properties of the enzymic activity, a series of experiments was performed to characterize the enzyme system for

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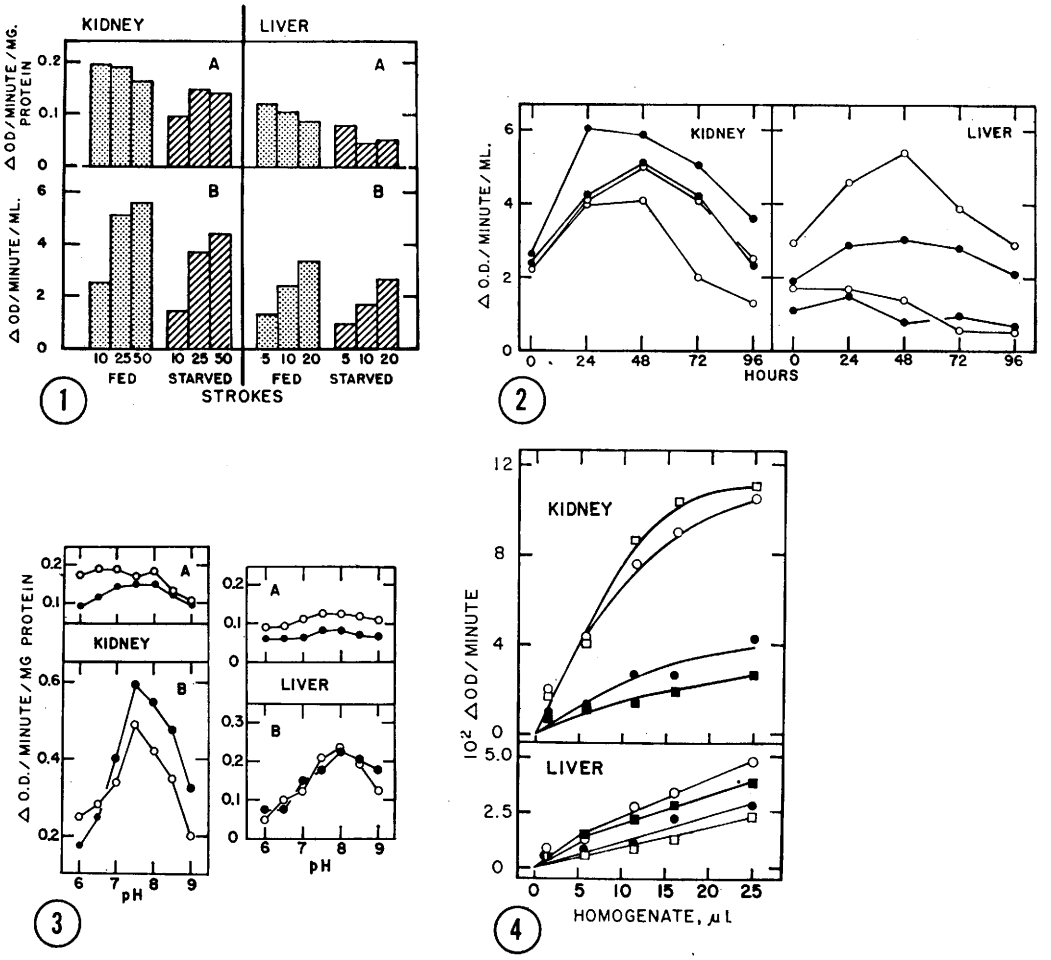

FIG. 1. Effect of homogenation on enzyme activity. The data represent individual values typical of 3 separate experiments. System: 240 μmoles phosphate, 3 μmoles KCN, 20 μmoles succinate and 8 μmoles cytochrome C in 3.0 ml water at pH 7.5 and 27°C. Reaction started by addition of 50 μl diluted homogenate equivalent to 5 μl of original.

FIG. 2. Effect of aging on enzyme activity. The data represent individual values obtained in 2 experiments for tissues from starved (●) and fed (○) rabbits. System: Same as for Fig. 1 except that 50 μl undiluted homogenate used.

FIG. 3. Effect of pH on enzyme activity. The data represent individual values obtained in freshly prepared liver and kidney homogenates (A) and homogenates aged for 48 hr at 4°C (B) for tissues from starved (●) and fed (○) rabbits. System: Same as for Fig. 1 except for adjustment to appropriate pH.

FIG. 4. Effect of homogenate concentration on activity. The data represent individual values obtained in freshly prepared homogenates from starved (■) and fed (●) rabbits or in homogenates aged for 48 hr at 4°C from starved (□) and fed (○) animals. System: Same as for Fig. 1 except for addition of 50 μl of appropriately diluted homogenate.

kidney and liver tissue from fed and starved rabbits. 1) *Effect of homogenation on enzyme activity.* As shown in Fig. 1B, increasing the number of pestle passes from 10 to 50 strokes for kidney tissue and from 5 to 20 strokes for liver tissue resulted in a 3-fold increase of activity in the homogenates. The specific activity of the enzyme, expressed in terms of protein concentration, is not significantly altered by the number of pestle passes, although slight decreases occur with continued homogenation (Fig. 1A). For this reason, an arbitrary number of strokes of 25 for kidney and 10 for liver was selected for all further studies. *2) Effect of aging on enzyme activity.* As shown in Fig. 2, aging

of the kidney homogenates at 4°C for 48 hours resulted in liberation of maximum amounts of enzyme activity. The enzyme activity does not remain stable but decreases when aging is extended to 96 hours. It may be noted that the activity after 96 hours of aging is approximately the same as the original activity. Similar aging effects occur in some, but not all liver homogenates. The aging effect for kidney and liver preparations from starved animals is essentially similar to that obtained in fed animals. 3) *Effect of pH and ionic strength on enzyme activity.* The pH optimum of liver and kidney homogenates aged for 48 hours at 4°C is quite sharp, approximating pH 7.5-8.0, and is the same for both tissues from either fed or starved rabbits (Fig. 3B). However, in freshly prepared homogenates the pH optimum is broad and the differences in activity between pH 6-9 are of doubtful significance (Fig. 3A). Similar data were obtained for the same pH range with 0.05 M and 0.2 M phosphate buffers. 4) *Effect of homogenate concentration on activity.* Typical data (Fig. 4) show that enzyme activity is essentially linear with concentration up to 10 μl of the original homogenate for tissues from either fed or starved rabbits. The greater activity for kidney homogenate aged for 48 hours is also apparent. 5) *Comparison of enzyme activity for tissues of starved and fed rabbits.* The succinic cytochrome C reductase activity of freshly prepared homogenates of kidney and liver homogenates of animals starved for 5 days is essentially the same as the values obtained in tissues of fed animals (Table I). However, starvation for 10 days resulted in significant decreases of enzyme activity in both kidney and liver homogenates.

The enzymic activity of kidney homogenates from rabbits starved for 10 days increases more with 48-hour aging than homogenates from fed animals and the values are no longer significantly different (Table II). Liver homogenates from starved or fed animals increase slightly with aging but this change is not significant. It may be noted that the enzyme activity for liver homogenates from fed or starved animals is not significantly different for the small number of animals used in this experiment but becomes significant with larger numbers of animals (Table I).

TABLE I. Succinic Cytochrome C Reductase Activity of Freshly Prepared Kidney and Liver Homogenates from Fed and Starved Rabbits.

	Enzyme activity ± S.E.*	
	Kidney	Liver
Exp 1	(5 days fed or starved)	
Fed	.400 ±.078 (4) †	.240 ±.088 (4)
Starved	.409 ±.074 (4)	.302 ±.079 (4)
Exp 2	(10 days fed or starved)	
Fed	.337 ±.031 (33) †	.235 ±.031 (33)
Starved	.205 ±.034 (18)	.115 ±.023 (17)
"P"	<.01	<.02

* Enzyme activity is expressed as △OD/min/mg protein.

† No. of animals in parentheses.

Discussion. It is apparent from these data that comparisons of enzyme activity of animals of different physiological states depends, to a certain extent, on the ability to extract comparable quantities of enzyme for quantitative analysis. Because aging of the homogenates tends to eliminate much of the difference for enzyme activity between kidney tissue of fed and starved animals suggests the need for caution in interpreting data based on enzyme content of tissue.

It is interesting that rabbit kidney homogenates contain more succinic cytochrome C reductase activity than liver homogenates and that the aging effect is more pronounced in kidney homogenates. It is conceivable that the increased activity of succinic cytochrome

TABLE II. Effect of Aging on Succinic Cytochrome C Reductase Activity of Kidney and Liver Homogenates from Fed and 10-Day Starved Rabbits.

	Enzyme Activity ± S.E.*		
Incubation Hr at 4°C	Kidney Fed (10) †	Starved (9)	"P"
0	.249 ± .036	.171 ± .031	<.01
48	.440 ± .086	.359 ± .056	N.S.
"P"	<.02	.01	
	Liver		
0	.152 ± .036	.082 ± .011	N.S.
48	.176 ± .046	.100 ± .035	N.S.
"P"	N.S.	N.S.	

* Enzyme activity is expressed as △OD/min/mg protein.

† Number of animals in parentheses.

C reductase activity by aged kidney homogenates may be related to changes in permeability of the mitochondrial membrane for the substrate or other intermediates. An essentially similar suggestion has been made by Chance and Hagibara(6) who found that succinate linked pyridine nucleotide reduction in aged mitochondria may be enhanced by additions of ATP. On the other hand, the aging effect is also consistent with the possibility that a second form of the enzyme may be liberated similar to that found in the lactic dehydrogenase family of isozymes(7). Many other hypotheses may be suggested, but it is not possible at this time to offer an explanation for the aging effect, and clarification of this phenomenon must await further study.

Summary. The succinic cytochrome C reductase activity of rabbit kidney and liver homogenates is significantly greater in homogenates aged for 48 hours at 4°C than when freshly prepared. Enzyme activities are greater for freshly prepared kidney and liver homogenates in fed animals than in animals starved for 10 days, but the activity in aged preparations for tissues of fed or starved animals is not significantly different. These data suggest the need for caution in comparison of enzyme activities of tissues from animals in different physiological states.

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Interference with Fecal Excretion of Zn^{65} by Cadmium.* (27854)

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Although cadmium has long been known as a toxic element, recent interest has been aroused in its destructive effects on the testis (1-3). The damaging effects on this organ have been shown to be due to competition with zinc, since testicular destruction by cadmium can be prevented(1), at least temporarily(2), by simultaneous administration of large doses of zinc. Interference with zinc metabolism in this organ was also confirmed by a permanent decrease in the capacity of this organ to take up administered Zn^{65}(4,5). The Zn^{65} concentrating capacity of another organ, the dorsolateral prostate, a gland particularly rich in zinc(6), was also depressed by the presence of cadmium(4). This depression was shown to be by a 2-fold mechanism; one by virtue of cadmium damage to Leydig cells resulting in prostatic atrophy and lowered Zn^{65} uptake, the other by means of a direct competition between cadmium and zinc at the dorsolateral prostate level. That there was no direct morphological damage by cadmium on the dorsolateral prostate was shown by normal appearing cells in cadmium-treated castrates maintained on testosterone. Zn^{65} uptake studies in these animals showed, however, that altered Zn^{65} uptake can occur even in the face of normal histology(4). Although the doses of cadmium which produce acute destruction of testis do not evoke obvious morphologic damage to liver, kidney or pancreas(3), it was of interest to determine whether, nevertheless,

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