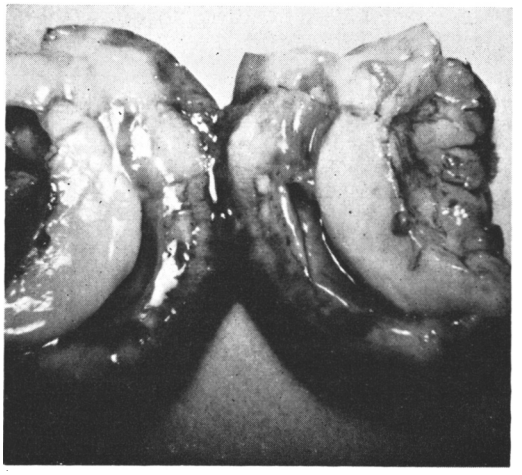

FIG. 3. Cross section of a tumor from site of intradermal fibroma virus inoculation of a suckling cottontail rabbit, showing arrangement as 2 concentric hemispheres.

inactivity. Under these circumstances it would seem likely that there would be less selection for a virus producing as sustained a proliferative response as does fibroma virus. Tumors resulting from mosquito transmitted myxoma virus persist for 10-40 days in the natural host, *Sylvilagus brasiliensis*(2). This period is far shorter than the persistence of fibroma-induced tumors in the eastern cottontail rabbit(11).

Summary. Intradermal injection of 320 domestic rabbit infectious doses of Shope's fibroma virus caused mortality in cottontail rabbits *(Sylvilagus floridanus)* infected at 6 days of age or younger. Cottontails infected at 14 days of age or younger developed immense primary tumors at the site of inoculation. Fatal cases were observed to have foci of tumor cells in the kidneys and occasionally in the liver and spleen. The virus could be isolated from fatally infected animals from kidney, spleen, liver, lung, brain, and tumor tissues. The same dose of virus given by the same route to domestic rabbits *(Oryctolagus cuniculus)* of the same ages produced a more acute, more rapidly fatal and much less proliferative disease. The possible ecological significance of these observations is discussed and contrasted with effects of the closely related myxoma virus.

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Oxidative Rates and Phosphorylation in Sarcosomes from Experimentally-Induced Failing Rat Heart.* (29586)

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Recent studies on the respiratory rates and phosphorylating efficiency of heart mitochondria (sarcosomes) in experimentally induced congestive cardiac failure indicate contradictory results. Schwartz and Lee(1) report that heart sarcosomes, isolated from guinea

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pigs in which chronic heart failure was induced by thoracic aortic constriction, have a decreased oxygen consumption (Qo_2) and depressed oxidative phosphorylation (P/O) with glutamate, succinate or α-ketoglutarate as substrate. On the other hand, with similar sarcosomes, Plaut and Gertler(2) and Gertler(3) found no significant change in these parameters with either glutamate or α-ketoglutarate.

In the present study the technique of abdominal aortic constriction was used to bring about consistent cardiac hypertrophy in male rats. The effect of this condition on the oxidative rates and the P/O of heart sarcosomes was investigated. Except for isocitrate, all those substrates of the citric acid cycle, at the level of which dehydrogenation occurs, were used. Consistent with the findings of others(4) we were not able to establish good normal values for oxidation of isocitrate with heart sarcosomes. Glutamate was not studied since this substrate is oxidized *via* α-ketoglutarate.

Materials and methods. Twenty-seven male, Sprague-Dawley rats (Holtzman Co., Madison, Wisc.), weighing 195-294 g were employed. Experimental rats were housed singly; controls 2 to a cage. A dextrose casein base semi-synthetic diet(5) and water were *ad libitum.*

The abdominal aortic constriction was performed on 12 rats, using pentobarbital sodium as anesthesia (Nembutal®; diluted with 10% ethanol to 5 mg/ml; intraperitoneally, 1.4 ml/220 g body weight). Following depilatory preparation of the abdominal surface with 30% sodium sulfide, a para median incision was made and carried down through the rectus abdominis and peritoneum. The abdominal aorta was identified by putting lateral traction on the left kidney, thus elevating the aorta and inferior vena cava. A narrow thumb forceps was passed between the aforementioned vessels, and the abdominal aorta was ligated, just below the iliolumbar artery, with O silk suture over a No. 20 needle (approximately 1.25 mm diameter) placed longitudinally adjacent to the aorta. The needle was then withdrawn, the peritoneum closed with 2-O running cotton suture, and the skin with O silk.

Fourteen days following surgery the rats were killed by decapitation, and the hearts rapidly removed and weighed. Using the entire heart, the sarcosomes were isolated as previously described(6). A sarcosome "stock suspension" was prepared by suspending the final sarcosome pellet in 2.8 ml ice cold 0.25 M sucrose. The sarcosome content of the "stock suspension" was determined by the procedure of Lowry *et al*(7), using trichloroacetic acid-precipitated, washed, and acetone-extracted dry liver mitochondria as standard.

Oxygen uptake was measured in a Warburg respirometer. The final reaction mixture contained 50 μmoles pH 7.4 phosphate buffer, 10 μmoles magnesium chloride, 6 μmoles ATP, 40 μmoles sodium fluoride, 0.03 μmole cytochrome c, 20 μmoles α-ketoglutarate or 20 μmoles pyruvate + 2 μmoles fumarate or 40 μmoles succinate or 40 μmoles malate, 2 mg hexokinase, 10 mg glucose, 0.5 ml sarcosome "stock suspension" (0.9 ml when succinate was used as substrate), and 0.25 M sucrose to complete a final volume of 3 ml. All flasks were equilibrated 12 minutes before addition of hexokinase and glucose from the side arm. Oxygen uptake was recorded at 5-minute intervals up to 20 minutes. At termination of each experiment the flask was quickly removed from the bath, placed in an ice bath, and the reaction stopped immediately with 2 ml ice cold 20% trichloroacetic acid. The precipitated proteins were removed by centrifugation, and the orthophosphate content of the protein-free filtrate determined by the method of Lowry and Lopez(8). Initial phosphate level was determined in control flasks in which the protein was precipitated immediately following equilibration.

Results and discussion. During the 2 weeks following surgery, the rat hearts became enlarged and flabby. Average increase in heart weight was 270 mg, while average loss in total body weight was 16 g. The ratio: 10^3 × heart weight/body weight was 3.51 for control animals, 4.71 for experimentals. This increase in ratio was statistically significant

TABLE I. Respiratory Rate and Phosphorylating Efficiency of Heart Sarcosomes from Normal and Aortic Constricted Rats.*

Substrate	Controls		Aortic constricted	
	Qo_2†	P/O	Qo_2†‡	P/O‡
α-Ketoglutarate	73.65 ± 2.70	2.91 ± .11	48.64 ± 4.37 (.001 > p)	2.48 ± .29 (.05 > p >.02)
Pyruvate	65.52 ± 6.60	2.82 ± .13	48.25 ± 6.51 (.02 > p >.01)	2.49 ± .07 (.001 > p)
Succinate	71.13 ± 3.42	1.73 ± .01	48.74 ± .82 (.001 > p)	1.28 ± .05 (.001 > p)
Malate	49.81 ± 1.75	2.81 ± .09	37.50 ± 1.13 (.001 > p)	2.47 ± .08 (.01 > p >.001)

* Control α-ketoglutarate values are the avg from 5 animals; control pyruvate values from 4 animals; all other values from 3 animals. The standard deviation is included for each average. Data for each individual animal were obtained in duplicate.

† μl O_2/mg dry mitochondria/hr.

‡ Value in parentheses is the probability based on the null hypothesis for a true difference between the aortic constricted and the control groups' values. The unpaired "t" test was used, and the "degrees of freedom" were based on the number of animals in each group.

($p<0.001$) and was greater than that previously observed in rats maintained on thiamine deficient diets(6). Similar body weight loss and heart weight increase was reported in guinea pigs with chronic heart failure induced by thoracic aortic constriction(1).

Table I summarizes the results of the metabolic studies. With all 4 substrates there was statistically significant decrease in both Qo_2 and P/O as a result of abdominal aortic constriction in the rats. These indications of impaired respiration and of uncoupling of oxidative phosphorylation in rats parallel the findings of Schwartz and Lee(1) with guinea pigs in chronic congestive heart failure. For one substrate (succinate) where numerical values could be compared the % decrease in Qo_2 was: rat 32%, guinea pig 47%; % decrease in P/O: rat 26%, guinea pig 43%.

Although the procedure for isolation of the sarcosomes and the respiratory reaction mixture used in the present studies are practically identical to those used by Gertler(3), the results of the two investigations are not in agreement. In addition to the species difference, one factor contributing to this discrepancy may be that Gertler produced congestive heart failure by thoracic aortic constriction, while we used abdominal aortic constriction below the ilio-lumbar artery. Although it should be noted that both techniques result in considerable cardiac enlargement and increase of the heart weight-body weight ratio, it is not impossible that hemodynamic differences (such as alteration of the flow rate in the carotid sinus and the aortic arch, as well as changes in blood volume) due to the difference in the position of ligation could play a role in the modification of the biochemical response. Furthermore, while in our experiments the sarcosomes were isolated from whole hearts, Gertler possibly used only heart ventricles; this was the procedure in the original work(4) referred to for the isolation of guinea pig heart mitochondria, and the fact that Gertler found it necessary to pool "6-8 guinea pig hearts" for each experiment(3) suggests that not whole hearts were used. On the other hand, while Schwartz and Lee(1) refer to the same original paper(4) for the isolation technique, they probably used the entire heart since one animal was sufficient for each experiment. Further complexity is introduced by the fact that the guinea pigs used by Gertler weighed about 400 g whereas those used by Schwartz and Lee weighed about 900 g.

Although chronic congestive heart failure (produced experimentally by abdominal aortic constriction) and prolonged thiamine deficiency(6) both lead to an increased heart weight-body weight ratio in rats, the two conditions result in a different alteration of the ATP synthesizing ability of rat heart sarcosomes. Congestive heart failure pro-

duces a decrease of the Qo_2 with pyruvate, α-ketoglutarate, and malate. In thiamine deficiency, however, the Qo_2 decreases only with pyruvate and α-ketoglutarate (50% decrease with either substrate in rats maintained 4 weeks on thiamine devoid, semisynthetic diet); when malate is used as substrate, there is no decrease of the Qo_2 in thiamine deficiency.† Decreased phosphorylating efficiency is found only in the sarcosomes isolated from the hearts of rats in which congestive failure was surgically produced. The phosphorylating efficiency (with all 3 substrates) of sarcosomes isolated from the hearts of rats maintained on the thiamine devoid diet is not different from the controls throughout the entire course of progressive thiamine deficiency.† In agreement with the results of Schwartz and Lee(1) the data suggest impairment of sarcosomal electron transport and partial uncoupling of oxidative phosphorylation in sarcosomes derived from the rat hearts in congestive failure. In contrast, neither of these functions appear to be impaired even in extreme thiamine deficiency prior to morbidity; the great decrease of the Qo_2 observed with pyruvate and α-ketoglutarate appears to reflect simply the considerable depletion of cocarboxylase due to thiamine deficiency, since the Qo_2 is not decreased with malate which does not require cocarboxylase for oxidation.

Summary. Abdominal aortic constriction was used to induce cardiac hypertrophy in the rat. Sarcosomes isolated from these hearts had significantly lower respiratory rates. The % decrease in Qo_2 was 34%, 26%, 32% and 25% with α-ketoglutarate, pyruvate, succinate and malate respectively. With these substrates in the same order the efficiency of phosphorylation was also significantly decreased by 15%, 12%, 26% and 12%.

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Some Patho-Physiologic Effects of Bacterial Kidney Disease in Brook Trout. (29587)

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Bacterial kidney disease is a chronic problem in salmonid fish culture in North America(1). Identification and cultivation of the organism responsible was first reported by Ordal and Earp(2) while Wood and Yasutake(3) reported on the pathology in brook trout *(Salvelinus fontinalis),* coho salmon *(Oncorhynchus kisutch)* and chinook salmon *(Oncorhynchus tshawytscha)* from hatcheries having an active epizootic. Pathologic findings suggested possible physiologic responses should occur in the fish but no measurements were made. A recent outbreak of kidney disease at this station has afforded an opportunity to make observations on various physiologic parameters of brook trout infected with corynebacteria.

Materials and methods. Samples of fish were taken from a lot of 12- and 13-month-

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