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%.

† Arcos, J. C., Argus, M. F., Sardesai, V. M., Stacey, R. E., submitted for publication.

1. Schwartz, A., Lee, K. S., *Circulation Research,* 1962, v10, 321.
2. Plaut, G. W. E., Gertler, M. M., *Ann. N. Y. Acad. Sci.,* 1959, v72, 515.
3. Gertler, M. M., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 109.
4. Plaut, G. W. E., Plaut, K. A., *J. Biol. Chem.,* 1952, v199, 141.
5. Arcos, J. C., Gosch, H. H., Zickafoose, D., *J. Biophys. Biochem. Cytol.,* 1961, v10, 23.
6. Argus, M. F., Arcos, J. C., Walder, J. A., *Experientia,* 1962, v18, 519.
7. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.,* 1951, v193, 265.
8. Lowry, O. H., Lopez, J. A., *ibid.,* 1946, v162, 421.

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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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TABLE I. Hematocrit and Total Plasma Protein Values for Normal and Diseased Brook Trout.

No. of fish	Type of fish	Parameter	Mean ± S.E.	Range
		Normal trout		
114	Both sexes	Hematocrit	39 ± 1	19 –51
67	Males	Total plasma protein, g %	4.5 ± .1	3.0– 6.0
52	Females	*Idem*	4.6 ± .1	3.0– 7.4
21	Normal females reabsorbing eggs	"	5.9 ± .4	3.8–11.0
		Diseased trout		
34	Both sexes	Hematocrit	22 ± 1	7 –40
26	Males	Total plasma protein, g %	2.2 ± .2	1.0– 4.2
7	Females	*Idem*	2.2 ± .2	1.4– 3.2
5	Females reabsorbing eggs	"	2.7 ± .6	1.4– 4.4

old brook trout suffering a 3-5% mortality per month. These fish were fed commercial trout pellets twice a day and resided in hard water of known composition(4). Samples were always collected at the same time of day. Following MS-222 (tricane methanesulfonate, Sandoz) anesthesia, 2 cc syringes containing a small amount of Anticlot† were used to collect blood samples from the dorsal aorta *via* the roof of the mouth(5). After removing the needle, the blood was mixed and microhematocrit tubes were filled directly from the syringes. Fish were then autopsied and kidney smears taken.

Total plasma protein was determined with an Aloe-Hitachi hand protein refractometer while further analysis of plasma proteins was carried out with a Spinco model R paper electrophoresis system using B-2 buffer and B-4 dye. Protein separations were made for 18 hours at 5 ± 1°C using a constant current of 2.5 ma per cell. Quantitative analysis of electrophorograms were determined with a Spinco model RB Analytrol.

A positive diagnosis of kidney disease was made if the organism was found on gram-stained smears. Routine cultures from a number of sampled fish showed no other bacteria to be present.

Results. Table I summarizes data on the hematocrit and total plasma protein for normal and diseased brook trout. Early in the study, examination of the electrophorograms indicated that there were definite sex differences in the relative distribution of plasma proteins, therefore, the protein data is given as to sex and condition.

† Heparin preparation, Clinton Laboratories, Los Angeles, Calif.

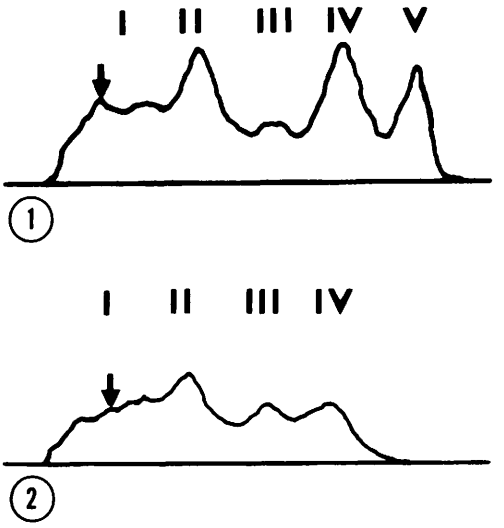

FIG. 1. Electrophorogram of plasma proteins from a normal male brook trout. Arrow indicates point of application.

FIG. 2. Electrophorogram of plasma proteins from a diseased male brook trout. Arrow indicates point of application.

Fig. 1 is a typical electrophorogram from a normal male. Electrophorograms of both males and females consistently exhibited five fractions. The percentage of the total protein that each fraction contributed is given in Table II.

Fig. 2 shows an electrophorogram from a diseased male. It will be noted that fraction V, one of the 2 peaks tentatively identified as albumin, has disappeared (see Table II).

Included in the samples in this study were

TABLE II. Percent of Total Plasma Protein in the 5 Fractions as Determined by Paper Electrophoresis.

Type of fish	No. of fish	Protein fractions I	II	III	IV	V
Males						
Normal	35	25.3 ± .6*	28.6 ± .8	9.6 ± .5	24.0 ± .6	12.5 ± .7
Diseased	19	37.9 ± 2.6	38.9 ± .9	14.7 ± 1.8	8.6 ± 1.6	0
Females						
Normal	40	23.6 ± .8	36.1 ± 1.1	6.6 ± .4	23.2 ± .7	10.3 ± .7
Diseased	7	41.6 ± 6.3	37.3 ± 4.9	12.9 ± 2.6	8.1 ± 1.5	0

* Mean ± S.E.

a number of sexually precocious females which had eggs lying free in the body cavity. Reabsorption of these eggs was reflected in distorted electrophorograms occurring in the region of fraction II (tentatively identified as globulin). Other authors(6) and (7) have reported that at spawning time a new lipoprotein fraction appears in the plasma of fish.

Discussion. Normal hematocrit values obtained in this study agree with those of Snieszko(8) for brook trout. Reduced hematocrits in infected fish correlate well with the findings of Wood and Yasutake(3) who showed that the hematopoietic tissue was affected first.

Values for total plasma protein in normal brook trout are higher than those given by Phillips *et al*(9). This is probably due to using the refractometer which has been reported to give, with fish plasma, values 15-20% higher than those obtained using the biuret method(10,11). Reduced plasma protein may have been caused by a loss of protein through open external lesions, in the urine due to kidney damage and by reduced liver function. Reduced albumin (fractions IV and V) content of the blood has long been associated with kidney damage. This condition has also been reported in dropsical carp(12). Further research is necessary to establish which of the above mentioned causes is predominant.

Although the sex ratio of normal fish sampled was unity, twice as many males as females were found to be diseased. This may be correlated with the difference in the quantity of globulins (plasma protein fraction II) which is higher in females.

Summary. The nature of the histopathology of brook trout infected with bacterial kidney disease suggested that significant changes should occur in physiologic parameters associated with hematopoietic, liver and kidney function. Examination of infected fish bears this out as it shows that the hematocrit is reduced by 44% compared to normal while total plasma protein drops 52%. In addition, electrophoretic analysis of the plasma proteins indicates that the faster migrating fractions (albumin) are the most drastically affected. Pathologic findings have been confirmed and the observation made that there was a difference in susceptibility to kidney disease between males and females.

1. Wolf, K., U.S.D.I., F. & W. S., Fishery Leaflet 566, 1964.

2. Ordal, E. J., Earp, B. J., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 85.

3. Wood, E. M., Yasutake, W. T., *Am. J. Path.*, 1956, v32, 845.

4. Warren, J. W., *Prog. Fish-Culturist,* 1963, v25, 121.

5. Schiffman, R. H., *ibid.*, 1959, v21, 151.

6. Vanstone, W. E., Chung-Wai Ho, F., *J. Fish. Res. Bd. Canada,* 1961, v18, 393.

7. Bouck, G. R., M.S. Thesis, School for Advanced Graduate Studies, Mich. State Univ., 1963.

8. Snieszko, S. F., U.S.D.I., F. & W. S., Spec. Sci. Rpt., Fisheries No. 341, 1960.

9. Phillips, A. M., Jr., Podoliak, H. A., Poston, H. A., Livingston, D. L., Booke, H. E., Pyle, E. A., Fish. Res. Bull. No. 26, N. Y., Conservation Dept., 1963, 53.

10. Saito, K., *Bull. Japan. Soc. Sci. Fisheries,* 1957, v22, 752.

11. Snieszko, S. F., personal communication.

12. Liebman, H., Offhaus, K., Riedmuller, S., *Schweiz. Z. Hydrol.,* 1960, v22, 507.

Received May 20, 1964. P.S.E.B.M., 1964, v117.