15. Medical Research Council, *Brit. Med. J.*, 1956, v2, 454.

16. Pittman, M., *Revue d'Immunol.*, 1958, v22, 303.

17. Yoshida, N., Tanaka, K., Takaishi, K., Fukuya, I., Nishino, K., Kakutani, I., Inoi, S., Fukui, K., Kono, A., Hashimoto, T., *Tokushima J. Exp. Med.*, 1955, v2, 11.

18. Maitland, H. B., Guerault, A., *J. Path. Bact.*, 1958, v76, 257.

19. Frappier, A., Guerault, A., *Revue Canad. de Biol.*, 1955, v14, 193.

20. Dolby, J. M., *Immunology*, 1958, v1, 328.

21. Pillemer, L., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 704.

22. Pittman, M., *J. Infect. Dis.*, 1951, v89, 300.

23. Maitland, H. B., Kohn, R., MacDonald, A. D., *J. Hyg. Camb.*, 1955, v63, 196.

24. Joó, I., Pusztai, Z., *Nature*, 1960, v188, 331.

25. Pusztai, Z., Joó, I., Juhász, V. P., *Z. für Immunitätsforsch. u. exp. Ther.*, 1961, v122, 278.

26. Verwey, W. F., Thiele, E. H., Sage, D. N., Schuchardt, L. F., *J. Bact.*, 1949, v58, 127.

27. Munoz, J., Hestekin, B. M., *Nature*, 1962, v196, 1192.

28. Ribi, E., Perrine, T., List, R., Brown, W., Goode, G., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 647.

29. Munoz, J., 1960, unpublished observations.

30. Fishel, C. W., *J. Infect. Dis.*, 1956, v99, 44.

31. Ouchterlony, O., *Progr. Allergy*, 1958, v5, 1.

Received November 27, 1962. P.S.E.B.M., 1963, v112.

Effect of Corticoids on Spleen of the Cichlid Fish, *Tilapia mossambica.** (28174)

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Splenic atrophy is one of the generally observed consequences of glucocorticoid administration to mammals(1). In an experiment designed to determine the effect of short-term treatment with cortisol on interrenal histology of the euryhaline freshwater cichlid teleost, *Tilapia mossambica,* the expected decrease in spleen weight was not observed. Additional experiments were then conducted to examine the response of the spleen in *Tilapia* to the mammalian "glucocorticoid," cortisol, and to the mammalian "mineralocorticoid," deoxycorticosterone.

Materials and methods. Tilapia mossambica of both sexes and weighing less than 30 g, from Oahu, Hawaii, were used. No differences in response relative to sex or size of the animals were noted. In the initial experiment (Table I), 2 groups of 6 fish each were maintained in running tap water. One group received 0.5 mg cortisol acetate (FA) in 0.1 ml aqueous suspension intraperitoneally daily for 7 days; the controls received 0.1 ml distilled water (DW) similarly. Two other groups were injected similarly, but had been transferred to running sea water one month before the beginning of the experiment. When it became apparent that the spleen weight was not decreased by the cortisol treatment, additional groups were set up (Table I), including 3 groups treated as above for 21 days with DW, FA, or deoxycorticosterone acetate (DCA); both steroids were given as a daily intraperitoneal dose of 0.5 mg in 0.1 ml aqueous suspension.

At termination of experiments, spleens were weighed on a 0-500 mg torsion balance, and all spleens except those from the first experimental series were fixed in Bouin's for preparation of hematoxylin and eosin-stained paraffin sections. Final body weights were recorded, and spleen weight/body weight ratios were calculated (Table I). Portions of the hepatopancreas and of the hemopoietic "head kidney" from representatives of Groups III and IV were also fixed and stained.

Results. Short-term treatment with FA had

* Hawaii Marine Laboratory Contribution No. 184. Research conducted during tenure of a National Science Foundation Senior Postdoctoral Fellowship, 1958-9. Author thanks K. B. DeOme for advice on histopathologic findings.

TABLE I. Response of Spleen of *Tilapia mossambica* to Corticoid Administration.

Group No.	No. of fish	Treatment		Mean body wt, g	Mean spleen wt, mg	Spleen wt, mg / Body wt, g	Significance of difference	
		Hormone	Duration				From group	P
Ia	6*	DW†	7	21.8	19.5	.89 ± .15		
Ib	6*	FA	7	21.5	17.5	.82 ± .06	Ia	n.s.
Ic	6	DW	7	15.0	12.8	.87 ± .06		
Id	6	FA	7	16.7	12.2	.87 ± .06	Ic	n.s.
IIa	5	DCA	7	8.9	6.3	.66 ± .07	Ic	.05
IIb	2	DW	7	14.5	11.3	.78		
III	5	Untreated	—	20.6	11.0	.54 ± .03		
IVa	10	DW	21	10.2	6.5	.65 ± .05	III	n.s.
IVb	8	FA	21	12.8	18.8	1.41 ± .13	IVa	$<<.01$
IVc	8	DCA	21	11.1	8.3	.79 ± .05	IVa	n.s.

* Fish kept in sea water; all others in fresh water.

† DW = distilled water control injections; FA = cortisol acetate; DCA = deoxycorticosterone acetate.

no significant effect upon spleen weight. However, treatment for 21 days resulted in more than doubling of the spleen weight, compared with controls. DCA, on the other hand, had no appreciable effect on spleen weight, other than the suggestion of decrease in weight seen after 7 days of treatment (Group IIa *vs* Ic).

The spleen of *Tilapia* is composed of both lymphoid and myeloid elements. Its architecture is not unlike that of the mammalian spleen. FA treatment for 21 days causes a proliferation of phagocytic elements and formation of numerous islands of these cells. These reticular phagocytes are large vacuolated cells containing variable amounts of yellowish pigment, and their hypertrophy and hyperplasia clearly distinguished the FA-treated from DCA- and DW-treated spleens (Fig. 1 & 2). Only one DW-treated and one DCA-treated fish showed these cells in noticeable numbers.

The "head kidney" is composed largely of lymphoid and myeloid elements. The response to FA was essentially the same as in the spleen, with the appearance of prominent islands of phagocytic cells.

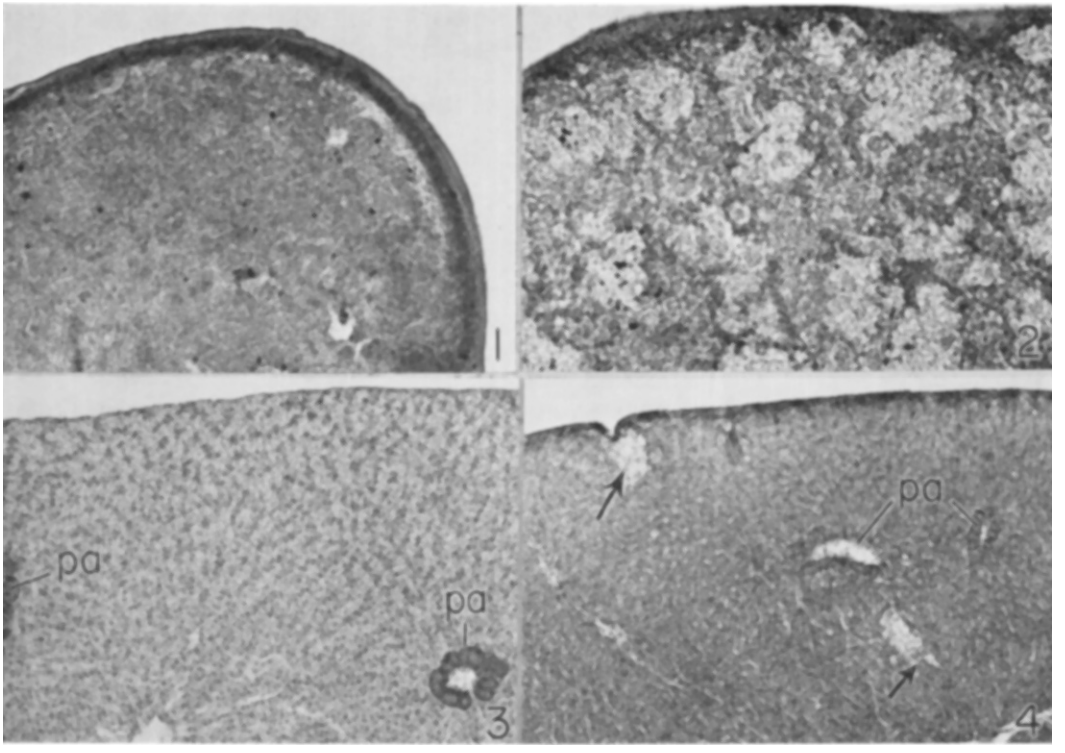
FA treatment also resulted in phagocyte proliferation in the liver, in association with both central veins and portal veins (Fig. 3, 4). In the latter site, the acinar pancreatic tissue was often disrupted by islands of phagocytes. The relation of the pancreatic tissue to hepatic tissue in *Tilapia* is similar to that described in *Fundulus*(2) and in the majority of teleosts examined by Kristal(3). The pancreatic tissue surrounds and follows the path of the portal veins through the liver, making up a true hepatopancreas. As in *Fundulus*, islet tissue was not encountered(2).

The hepatic cells of FA-treated fish possess a densely eosinophilic cytoplasm, whereas those of untreated *Tilapia* are generally clear owing to extensive vacuolation (Fig. 3, 4).

No consistent effect of DCA on spleen, head kidney, or hepatopancreas histology was noted, although the splenic arterioles were often particularly prominent after DCA.

Discussion. Short-term treatment of *Tilapia* with FA did not result in the splenic atrophy expected from extrapolation from mammalian studies(1), and continued treatment with FA resulted in significant splenic hypertrophy. The phagocytic tissue apparently responsible for the increase in spleen weight in *Tilapia* also attains prominence in the rat spleen after cortisone treatment of rats, but as a result of the disappearance of lymphatic elements(1). Cortisol causes both lymphopenia and lymphocytosis in *Fundulus*, depending on the breeding conditions of the fish(4,5), but effects on the spleen were not reported.

Teleosts apparently do not distinguish between gluco- and mineralo-corticoids in 2 reported responses: in compensatory interrenal atrophy (negative feedback presumably mediated by the hypophysis)(6; Bern, unpubl.), and in sodium movement across the gill membrane(7). In the present experiment, the tar-



All tissues fixed in Bouin's, stained with H.E., ×115.

FIG. 1. Spleen from fish receiving 21 daily injections of distilled water. Normal histology. Black spots are pigment deposits.

FIG. 2. Spleen from fish receiving 21 daily injections of cortisol acetate. Clear areas are large proliferations of phagocytes.

FIG. 3. Hepatopancreas from fish receiving 21 daily injections of distilled water. pa, pancreatic acini.

FIG. 4. Hepatopancreas from fish receiving 21 daily injections of cortisol acetate. Arrows indicate islands of phagocytic cells.

get organ does react differentially to the 2 "classes" of corticoids. Information on the nature of the corticoids of *Tilapia* is incomplete(8); however, cortisol does not appear to be an endogenous hormone in this teleostean species.

The significance of the hyperplasia of the phagocytic (reticuloendothelial) elements is uncertain. A disease of *Tilapia* was encountered in Hawaii, which involved widespread cyst formation in the viscera, mesenteries, integument, etc. Initially, the causative organism was considered to be a myxosporidian (Noda and Bern, unpubl.), but no final diagnosis has been possible. Only one spleen of the many examined in this series showed cysts characteristic of this disease; however, several infected head kidneys were noted, although not after FA treatment. The phagocytic reaction, in both spleen and liver, accordingly, could be a direct response to the cortisol, or a secondary response to the proliferation of a microorganism, owing to a cortisol-induced reduction of immune responses. In opposition to the second possibility is the fact that the disease, which is capable of natural fulmination and rapid destruction of the individual, did not erupt in this fashion after cortisol administration.

Summary. Cortisol treatment of *Tilapia mossambica* (Teleostei Cichlidae) for 7 days resulted in no splenic atrophy; similar treatment for 21 days resulted in significant hypertrophy. A notable proliferation of phagocytic cells occurred in the spleen, head kidney, and hepatopancreas. Such changes did not occur after deoxycorticosterone administration.

1. Selye, H., *Stress,* Acta Publishing Co., Montreal, 1950, p476.

2. Boldyreff, E. B., *Copeia,* 1935, 23.

3. Kristal, J., *S. Afr. J. Med. Sci.*, 1946, v11 (Biol. Suppl.), 81.

4. Slicher, A. M., *Bull. Bingham Ocean. Coll.*, 1961, v17, 1.

5. Slicher, A. M., Ball, J. N., *Am. Zool.*, 1962, v2, 449.

6. Krauter, D., *Roux' Arch. Entw.-Mech.*, 1958, v150, 607.

7. Holmes, W. N., *Acta endo.*, 1959, v31, 587.

8. Nandi, J., Bern, H. A., *Endocrinol.*, 1960, v66, 295.

Received December 26, 1962. P.S.E.B.M., 1963, v112.

Combined Gas and Heat Exchange in Extracorporeal Circulation.* (28175)

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In extracorporeal circulation for heart surgery the heat exchanger is now considered an essential adjunct for maintenance of normothermia and induction of rapidly reversible hypothermia.

There would seem to be obvious advantages in combining the functions of gas and heat exchange in one unit, since each requires the dispersion of blood over a large surface area. Priming volume of the extracorporeal system would be reduced, and also the length of tubing and the number of connections used.

We selected the vertical screen oxygenator for modification, first, because of its efficiency (1); and second, because its absence of moving parts made the task theoretically easier. Since beginning this work we have become aware of the successful application of this principle of combined gas and heat exchange to the bubble(2,3), and the disc oxygenator (4,5).

In the proposed modification the wire screens of the oxygenator would be replaced by thin metal plates, through the center of which the heat exchange fluid would circulate, and on the surface of which the blood would be filmed for gas and heat exchange.

This report is concerned with the preliminary results obtained with a single plate.

Methods. The test plate was made from 2 pieces of No. 304 stainless steel measuring 12 in. in height × 16 in. in width × 64/1,000 of an inch in thickness. On one side of each plate mirror image halves of the central duct system were chemically milled to a depth of 15/1,000 of an inch. The plates were then welded together to form a single plate 1/8 of an inch thick containing a central duct system with a cross-sectional area of 30/1,000 of an inch × 3/4 inch. On each side of this plate a pattern was chemically milled to form a "weir" 10/1,000 of an inch deep, and 7/8 of an inch high across the top of the plate, and a 3 mm × 1 mm diamond pattern 5/1,000 of an inch deep on the rest of the plate. The finished plate was the same size as the Kay-Gaertner screen(1), and its surface pattern resembled the standard Tyler #538 type 304 stainless steel Ton-Cap Screen (1,6). Inlet and outlet manifolds were then welded to the bottom of the plate using standard hose threads for connection to the heat exchange fluid system (Fig. 1). The plate was housed in the Lucite Box of a Kay-Gaertner Screen Oxygenator,† modified to take the single plate.‡ Temperature change was affected by a Thermo-Rite hypothermia unit.§ Blood and heat exchange fluid inflow and outflow temperatures were recorded electrically.‖

Oxygenation was studied by partial bypass from an anesthetized dog. The venous blood, obtained from the external jugular vein was circulated across the plate and returned to the femoral artery. Humidified oxygen was passed through the oxygenator casing at a

* Supported in part by U.S.P.H.S. grant.

† The Mark Company, Randolph, Mass.

‡ International Medical Instrument Co., Stoneham, Mass.

§ Therm-O-Rite Products Corp., Buffalo, N. Y.

‖ Yellow Springs Instrument Co., Yellow Springs, Ohio.