(19) found no change in cortisol secretion rate in dogs (oophorectomized 5 to 8 days previously) with isolated adrenal pouches, immediately after a single intra-arterial perfusion with 20 or 100 μg of estradiol-17β. A similar absence of an acute response in the rat has also been observed in this laboratory (unpublished data) consistent with the conclusion that the action of estradiol is not as rapid as that of ACTH.

Summary. Corticosterone production by adrenal tissue *in vitro* from intact female rats weighing 168-181 g was significantly lower than that obtained from animals of higher or lower body weights. A single dose of a depot preparation of estradiol-17β in rats oophorectomized prior to puberty increased adrenal corticosterone production *in vitro* for a period beginning 3 days after injection and lasting 3 weeks. The peak effect was noted 2 weeks after injection. The minimal effective dose was 10 μg/100 g body weight. Rats oophorectomized after puberty demonstrated significant reductions in adrenal corticosterone production at 1 and 2 weeks after operation, but not the same degree as that observed 6 weeks after pre-pubertal oophorectomy. The minimal effective replacement dose of estradiol in mature oophorectomized animals was 40 μg/100 g body weight.

Thanks are due Dr. John B. Jewell, Ayerst Laboratories, for generous supplies of polyestradiol phosphate used in these studies. The author is indebted to Miss Nancy L. Hough and Miss Lydia K. Brown for expert technical assistance.

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Received June 25, 1965. P.S.E.B.M., 1965, v120.

Effect of Cold Shock, Disease, and Mammalian Corticoids on the Spleen of *Lepomis machrochirus.** (30484)

WARREN R. FLEMING AND JAMES N. PASLEY (Introduced by C. W. Turner)
Department of Zoology, University of Missouri, Columbia

Bern(1) has recently shown that the spleen of the cichlid fish, *Tilapia mossambica*, responds differentially to mammalian corticoids, a response not generally shown by teleosts(2, 3). The glucocorticoid, cortisol, caused a marked hypertrophy and hyperplasia of the spleen, accompanied by the proliferation of reticular phagocytes. The mineralocorticoid,

* Supported by a grant from National Science Foundation.

TABLE I. Response of the Spleen of the Blue-Gill to Stress and to Corticoids.

Group No.	No. of fish	Treatment	Duration	Spleen wt, mg/g body wt	Significance of difference From group	P
Ia	6	4°C	14	.46 ± .14		
Ib	5	20°C	14	.88 ± .20	Ia	.02
IIa	6	20°-4°-20°	7	.86 ± .25		
IIb	6	20°	7	.69 ± .14	IIa	n.s.
IIIa	7	DCA	10	1.06 ± .07		
IIIb	7	Control	10	1.34 ± .05	IIIa	.02
IVa	10	CA	10	2.11 ± .25		
IVb	10	Control	10	1.46 ± .14	IVa	.05
Va	7	CA	14	.75 ± .05	Vc	.02
Vb	7	DCA	14	.30 ± .04	Vc	.01
Vc	7	Control	14	.52 ± .03		
VIa	12	Fin-rot	Variable	.68 ± .18	VIb	n.s.
VIb	10	Controls		.72 ± .27		

CA = cortisol acetate; DCA = desoxycorticosterone acetate.

desoxycorticosterone acetate, on the other hand, had no consistent effect on splenic weight or histology, other than stimulation of splenic arterioles.

During the course of other investigations, it was noticed that the spleen of the blue-gill, *Lepomis machrochirus,* was responding to mammalian corticoids in a manner somewhat different from that reported by Bern(1) for *T. mossambica.* This paper reports on the response of the spleen of the blue-gill to a mammalian "glucocorticoid" and "mineralocorticoid," to intermittent and prolonged exposures to low temperatures, and, by chance, to a fungus infection.

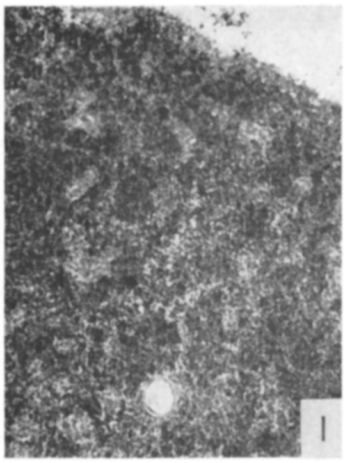
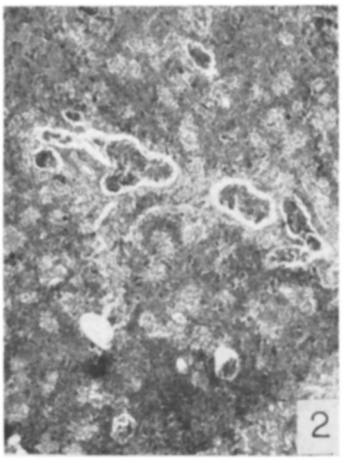
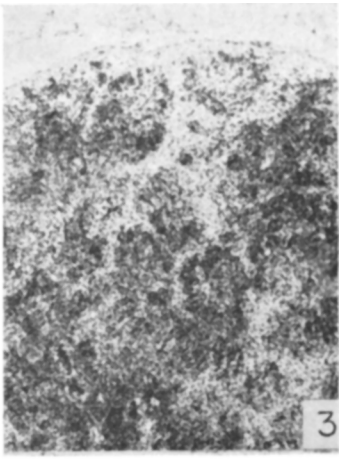
Materials and methods. Blue-gill, *Lepomis machrochirus,* were collected from local farm ponds and moved into concrete holding tanks. These tanks are equipped with stand-pipes, and supplied with running tap water and paddle-type aerators. The water supply at the university is drawn from deep wells and is not chlorinated. The temperature was held at 20 ± 1°C by the use of immersion heaters. Both sexes were used, and fish of approximately the same weight were selected for the steroid injection experiments. The fish were allowed a minimum period of 2 weeks to adjust to the laboratory tanks and were fed a commercial fish chow 3 times a week. With one exception, pointed out below, the fish thrived in the laboratory.

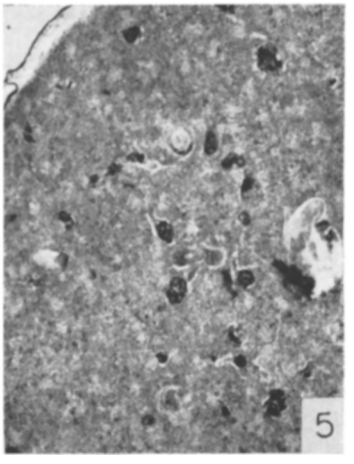
One series of experiments examined the response of the spleen to low temperatures. Animals were transferred from the holding tanks into a cold-room held at 4°C. Their controls were placed into a similar aquarium held at 20°C. Another group of fish was transferred from aquaria held at 20°C into 4°C water for 4 minutes and then returned to 20°C. Their controls were transferred from one 20° aquarium to another for a 4-minute period.

The animals injected with cortical steroids were given daily intraperitoneal injections, 10 in one experiment, 14 in another. Fifty μg of steroid were injected, carried in a volume of 0.1 ml. The steroids were prepared as a water suspension by grinding them onto gum arabic. The controls were treated in the same fashion, except that the injection solution did not contain steroids. The animals used in these experiments were marked by sewing a small colored wooden ball onto the dorsal fin, and all the animals were held in the same tank. The steroids used were cortisol acetate (CA) and desoxycorticosterone acetate (DCA).

A final observation on the response of the spleen to stress was made when a group of fish became infected with fin-rot disease. These animals were isolated and killed when the disease had reached an advanced stage.

At termination of the experiments, the spleens were weighed on a torsion balance and fixed in Bouin's. Body weights were



All tissues fixed in Bouin's, modified Schorr stain, × 83.

FIG. 1. Spleen from a control animal. Normal histology.

FIG. 2. Spleen from fish receiving 10 daily injections of CA. Clear areas are proliferations of phagocytes. Note dilated vessels.

FIG. 3. Spleen from fish held at 4°C for 14 days. Note presence of phagocytic areas.

FIG. 4. Spleen from fish receiving 10 daily injections of DCA. Note deposition of dark-staining pigment granules.

FIG. 5. Spleen from fish suffering from fin-rot disease. Note marked deposition of pigment granules.

taken and spleen wt/body wt ratios calculated. Spleen sections were stained with a modified Schorr stain, or with prussian blue, which was used to demonstrate the iron content of pigment nodules.

Results. Spleen weights. As shown in Table I, a marked reduction in spleen weight was seen in those fish held at 4°C (Group I) or when given DCA (Groups III and V). Spleens were enlarged when injected with CA (Groups IV and V). No change was noted in those animals exposed to 4°C and returned to 20°C (Group II) or in the animals infected with fin-rot disease (Group VI).

Histology. The spleen of teleosts is composed of 3 basic elements: lymphoid tissue, pulp, and ellipsoids(4) and is not unlike that of mammals(1). Treatment with CA caused a marked proliferation of reticular phagocytes and dilation of splenic vessels (Fig. 2). These large vacuolated cells were also prominent in the spleens of the animals that had undergone prolonged exposure to 4°C (Fig. 3). The spleens of fish treated with DCA were immediately distinguished from both the controls and other experimental groups by

the marked deposition of numerous masses of non-cellular deeply staining pigment granules (Fig. 4). These granules were even more prominent in the spleens of fish infected with fin-rot disease (Fig. 5).

Discussion. In these experiments the target organ did respond differentially to CA and DCA. In agreement with the results of Bern(1) for *T. mossambica,* CA caused hypertrophy of the spleen and the proliferation of reticular elements. These results would not be expected on the basis of mammalian studies(5).

L. machrochirus differs from *T. mossambica* in that administration of DCA caused atrophy of the spleen and a marked deposition of pigment granules. Thus, a differential response is seen, but the meaning of the response is difficult to evaluate. The nature of the corticoids produced by *L. machrochirus* is not known; but it is probable on the basis of studies carried out on other teleosts(6) that DCA would not be a natural steroid for this animal. It also appears that the dosage given was close to a minimum one required to evoke a response, for a pilot experiment in which DCA was given on alternate days for a 10-day period failed to evoke a response. Further, neither prolonged cold stress nor fin-rot disease duplicated the type of response obtained by administration of cortical steroids.

The blue-gill also differs from *T. mossambica* in that a consistent response to CA was a marked dilation of splenic vessels. This response, along with a proliferation of reticular elements, may account for the enlargement of the spleen. Dilation of splenic vessels is considered to be a direct basis for hypertrophy of mammalian spleens(7). As shown by the Group I animals, phagocytic proliferation may occur during the course of splenic atrophy.

Summary. Administration of CA for 10 or 14 days caused splenic hypertrophy, proliferation of phagocytic elements, and dilation of splenic vessels. Treatment with DCA for the same period caused splenic atrophy and deposition of pigment granules. These granules were also prominent in spleens of animals infected by a fungus disease. Holding the animals at 4°C for 14 days caused splenic atrophy and the appearance of phagocytic elements. A 4-minute exposure to 4°C for 7 days had no effect on spleen weight or histology.

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Received June 25, 1965. P.S.E.B.M., 1965, v120.

Effects of Autologous and Homologous Blood Replacement in Hypovolemic Dogs.* (30485)

E. L. SMITH, S. DEAVERS AND R. A. HUGGINS
Departments of Physiology, Baylor University College of Medicine and University of Texas Dental Branch, Houston

A number of different effects have been reported in the dog if homologous, rather than autologous, blood or plasma transfusions were given, but the only effect that has been substantiated in our laboratory was the development of urticaria(1-4). The incidence of urticaria in dogs receiving a homologous overtransfusion was 10% with fresh whole blood, 70-90% with fresh plasma or with previously

* This study was supported, in part, by a grant from Nat. Heart Inst., USPHS HE 08793 and, in part, by the Medical Research and Development Board, Dept. of the Army, Contract DA-49-193-MD-2357.