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Effect of Retinal Extracts on Growth of Blinded Male Rats.*

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It is known that bilateral optic enucleation delays the onset of sexual maturity of both male and female rats.[1,2,3] Bilateral optic enucleation also causes the body weights and organ weights of blinded animals to be significantly less than those of litter-mate normal males, regardless of the environmental light conditions.[2,4] Fiske[5] reported that onset of sexual maturity under conditions of constant dark was delayed in normal animals by approximately 2 weeks, and that normal males in constant dark had lighter testicles, seminal vesicles, and pituitaries than did males in continuous light.

The evidence is quite consistent that total ablation of both eyes retards growth and sexual maturity in the rat. It is possible that the eye exerts its influence on the pituitary which in turn produces growth and gonadotropic substances. The interaction between the eye and the pituitary may be either via direct or indirect nervous connections between the eye and the pituitary, or via a humoral mechanism. Truscott[6] has described nervous pathways which might form the means by which visual stimuli could influence the anterior pituitary. The present investigation, however, is based on the hypothesis that some "principle" or humoral mechanism is elaborated by the eyes of normal mammals which is necessary for normal growth and sexual maturity.

A series of crude extracts of light-adapted beef retinas was prepared and injected into an inbred strain of blinded male rats. Eyes of freshly butchered cattle were removed and immediately placed in chilled sterile physiological saline by a veterinarian or by the author. The retinas were then dissected out under sterile saline and placed in appropriate media. All extracts of beef eyes, other preparations, and injections were carried out by the junior author, while all autopsies of experimental animals were performed by the senior author. The senior author had no

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3 Truscott, B. L., *J. Exp. Zool.*, 1944, **95**, 291.

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5 Fiske, V. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 189.

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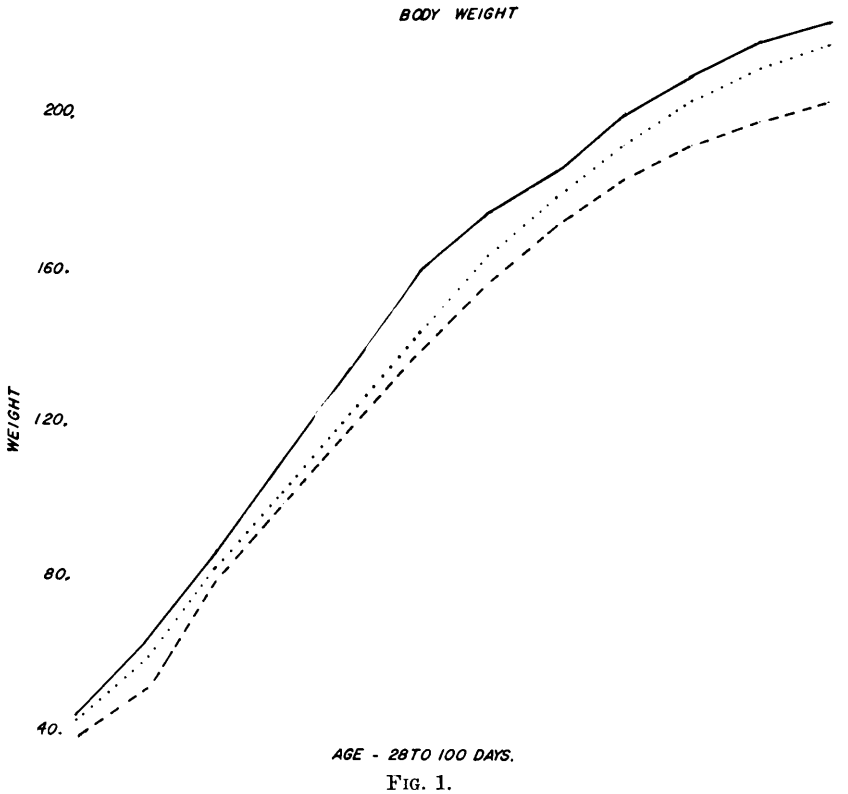

FIG. 1.

Average weekly body weights of
———— 20 normal eyed males.
– – – – – – 20 bilateral optic enucleated males.
. . . . . 10 bilateral optic enucleated males injected with saline extract of beef eyes.

knowledge as to which experimental series any particular animal belonged in until after autopsy.

Since only the saline series yielded suggestive results no space will be taken in discussion of the methods, results, etc., concerning ether fraction extracts, posterior lobe powder (melanophore) preparations, beta carotene solutions, and other preparations.

A series of 40 male rats equally divided between normal-eyed and bilateral optic enucleated on day one were autopsied on day 100. Inspection and statistical analysis of the data show that the blind animals had significantly lighter body, testis, seminal vesicle, and pituitary weights than litter-mate normal animals, although the thyroid glands of blinded animals were heavier than those of normal animals (Fig. 1, Table I).

Five beef retinas were macerated in 4 cc of Locke's mammalian saline by shaking vigorously with sand for 5 minutes. This mixture was leached overnight at 2°C and filtered by suction. This extract was prepared and injected 3 times weekly. It was calculated that each rat received a dose equivalent to an extract of 7.3 rat retinas by weight at each injection, or a daily average of 3 rat retinas. These injections began when the rats were 15 days of age and continued for 10 weeks. All animals were autopsied at 100 days of age. Another series of males receiving similar saline extracts was autopsied at intervals of 5 days beginning at 30 days of age, and the last pair in this series was autopsied at 70 days of age.

The number of animals involved in each injection series is not as large as was originally planned because of circumstances beyond our control. The differences between each experimental series are not always sharp and clear cut in magnitude, yet the results are

TABLE I.
Average Weights and Standard Errors at 100 Days of Age.

Injection	Cond. of eye	No. of animals	Body, g	Testes, g	Sem. V., mg	Adr., mg	Thym., mg	Thyr., mg	Pit., mg
None	Nor.	20	224 ± 4	3.0	681 ± 91	34.2	257 ± 9	13.2 ± .3	9.2 ± .2
"	Bl.	20	207 ± 3	2.8	511 ± 78	34.2	261 ± 9	14.8 ± .4	7.4 ± .2
Saline Ext.	Bl.	10	222 ± 5	2.9	735 ± 56	34.0	238 ± 16	12.7 ± .9	7.6 ± .4
B. Carotene	Bl.	10	206 ± 1	2.8	496 ± 24	32.8	269 ± 9	15.0 ± .5	8.2 ± .2
Post. Lobe	Bl.	10	202 ± 1	2.8	504 ± 54	32.2	261 ± 8	14.2 ± .5	7.1 ± .1
Ether Extr.	Bl.	9	190 ± 4	2.5	515 ± 33	29.8	271 ± 17	15.8 ± .5	7.8 ± .1

sufficiently consistent to warrant this report.

Reference to Table I indicates that at 100 days of age the males from the saline extract series more nearly approached the body and organ weights of normal males of the same age than did the animals in any other experimental group. This is especially true of the testis, seminal vesicle, and thyroid weights. Fig. 1 indicates that the males which received saline extracts averaged consistently heavier than bilateral enucleated controls. Injections of sterile saline only into blinded males had no alleviating influence on weight curves.

Five-day Series. One male of each pair autopsied at 5-day intervals received the saline extract beginning day 12, while the blinded litter-mate male received none. Litter-mate pairs were contrasted and revealed: (1) that injected males were heavier in body, testis, seminal vesicle, adrenal, liver, thyroid, and pituitary weights up to age 70 days, (2) that there was no marked difference in thymus weights, (3) that spermatozoa were present in the testes of injected animals by 45 days, and in non-injected males by 50 days. Spermatozoa are present in normal animals of this strain by 35-40 days of age. (4) Although seminal vesicle weights were essentially the same up to day 45, by 65 days, the seminal vesicles of non-injected males were only 10 times, injected animals were 18 times, and normal animals were 20 times the 45-day weight.

Summary. Normal male rats, and bilateral optic enucleated male rats injected with a saline extract of bovine retinas are quite similar in body and most organ weights. Retinal extracts of bovine eyes apparently have an alleviating effect upon the weight retardation and delayed sexual maturity characteristic of optic enucleated male rats.

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Studies on Purification of Poliomyelitis Virus.* 2. pH Stability Range of MVA Strain.

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While it is known that poliomyelitis virus activity is preserved for long periods of time in infected tissues[1] and in sewage,[2] little has been published regarding its stability under conditions where the virus is exposed to the more extreme hydrogen ion concentrations.

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