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Effectiveness of Blood and Hemin for Augmentation of Pituitary Gonadotropic Extracts in the Male.*

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Various workers have shown that pituitary extracts are less effective in increasing the weights of the gonads of immature male than of immature female rats. The weights of male accessory glands, however, may be greatly increased. Reported investigations have indicated the gonads of male birds to be more responsive than those of female birds and that the testes in some forms are extremely sensitive to pituitary extracts.[1]

Since the augmentive action of red blood cells[2] and of hemin[3] upon the effectiveness of unfractionated pituitary extract has been demonstrated only on the ovaries of immature female rats, it was deemed important to extend the studies to male rats and birds. A comparison then could be made of augmentation in species whose testes differ in sensitivity to anterior pituitary stimulation.

Experiments were performed on immature male rats, pigeons and chicks. The rats were 22 days, chicks 15 days, and pigeons 45 to 53 days old at the beginning of the experiment. Subcutaneous injections of 1 cc were made daily for 5 days in the pigeons, 0.5 cc daily for 10 days in the chicks, and 0.5 cc twice daily for 7 days in the rats. The birds were killed 24 hr and the rats 16 to 18 hr after the last injection. Cow blood was used as the augmenter in the pigeon experiment and crystalline hemin[4] prepared from cow blood in the chick and rat experiments. The hemin was dissolved in dilute sodium carbonate solution and mixed *in vitro* with the gonadotropic extract for injection purposes. The same quantity of alkali was added to the control gonadotropic extract.

Results and Discussion. The various dosages of unfractionated sheep pituitary extract and of augmenter together with the weights of various glands are indicated in Tables I, II and III. Augmentation of gonadotropic activity produced by pituitary extracts in the male rat was best illustrated by the addition of hemin to the dosages of 100 mg-equivalent (Table I). Testes, seminal vesicles and prostate glands were heavier than those from rats receiving pituitary extract alone. The average of the ratios of the weights of the prostates and the seminal vesicles also was markedly decreased. This was interpreted as the result of the prostate being more responsive to hormone stimulation than the seminal vesicles,[5] and thus tending to attain its maximum response on a lower concentration of hormone than does the seminal vesicles.

A comparison of the weights of the testes and accessory glands did not show a statistically significant difference between those rats which received dosages of 100 and 200 mg-equivalent of pituitary extract. A larger number of animals might have made the differences statistically significant but within the limits of the data it is suggested that the testes and accessories were approaching their maximum response from pituitary extract alone even on the lower dosage. Although on the 100 mg dosage the addition of hemin produced augmentation in both testes and accessory glands, the addition of hemin to the 200 mg-equivalent pituitary dosage showed augmentation only in the weights of the prostates and seminal vesicles.

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1 Riddle, O., and Polhemus, I., *Am. J. Physiol.*, 1931, **98**, 121.

2 Casida, L. E., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 570.

3 McShan, W. H., and Meyer, R. K., *Am. J. Physiol.*, 1937, **119**, 574.

4 Drabkin, D. L., and Austin, J. H., *J. Biol. Chem.*, 1935-36, **112**, 91.

5 Moore, C. R., and Gallagher, T. F., *Am. J. Anat.*, 1930, **45**, 39.

TABLE I.
Effect of Pituitary Extract and Hemin on Immature Male Rats.

	Treatment	No. of rats	Avg body wt.-g	Avg testes wt.-mg	Avg sem. ves. wt.-mg	Avg prost.* wt.-mg	Avg prost. sem. ves. ratio
1	Uninj. controls	10	49	359	6.2	39.4	6.7
2	100 mg-equiv. pit. extr.	10	47	589	17.6	86.8	5.1
3	100 mg-equiv. pit. extr. + 10 mg hemin	10	48	649	55.5	135.8	2.5
4	200 mg-equiv. pit. extr.	9	49	624	20.6	104.0	5.1
5	200 mg-equiv. pit. extr. + 10 mg hemin	10	40	604	46.4	127.9	2.8
	Standard error and significance of differences between:						
	2 and 1	—	2.0	45.4‡	1.5‡	7.6‡	0.8
	3 and 2	—	2.2	28.3†	3.1‡	12.0‡	0.5‡
	4 and 2	—	2.5	27.0	1.9	9.6	2.4
	5 and 4	—	4.2†	39.0	3.2‡	10.4†	0.4‡

*Includes weight of coagulating gland.
†Probability of 0.05-0.01.
‡Probability of 0.01.

TABLE II.
Effect of Pituitary Extracts and Hemin on Immature Male Chickens.

Treatment	No. of chicks	Avg body wt.-g	Avg testes wt.-mg
Uninj. controls	6	153	27
100 mg-equiv. pit. extr.	6	146	39
100 mg-equiv. pit. extr. + 10 mg hemin	6	131	36
200 mg-equiv. pit. extr.	6	154	49
200 mg-equiv. pit. extr.+ 10 mg hemin	6	127	45

TABLE III.
Effect of Pituitary Extracts and Blood on Immature Male Pigeons.

Treatment	No. of birds	Avg age-days	Avg body wt.-g	Avg testes wt.-g
Uninj. controls	11	44	331	32
15 mg.-equiv. pit. extr.	3	47	360	56
15 mg.-equiv. pit. extr. + 1.2 cc blood	3	46	350	56
30 mg.-equiv. pit. extr.	7	52	385	103
30 mg.-equiv. pit. extr. + 1.2 cc blood	4	52	380	102
30 mg.-equiv. pit. extr. + 2.0 cc blood	3	49	380	80

Furthermore, just as there was no difference in response between the 2 dosages of pituitary extract alone, neither were there any differences in the gland weights between the 2 pituitary dosages when the same quantity of hemin was added to each.

The weights of the testes in both immature chickens and pigeons were not further increased when hemin or blood was combined with the pituitary extracts (Tables II and III). This is in contrast to the augmentation of the rat testes on the lower dosage of pituitary extract noted above. The pigeon testes react to much smaller dosages of pituitary extract than do rats and chickens. Inasmuch as augmentation was observed in the rat but not in the pigeon and chicken, there does not appear to be any correlation between augmentation and the sensitivity of the gonad to pituitary extracts so far as species are concerned. The material at hand, however, does not permit a good analysis of the nature of this difference in species demonstrability of augmentation by blood or hemin. Any explanation of the cause of augmentation by these substances should take into consideration this species difference. If the suggestion is true that augmentation is due

to a delay in the rate of hormone absorption,[6] it would appear that absorption rates are not similarly affected by these substances in different species.

Summary. Augmentation of the effect of pituitary gonadotropic extracts by the addition of hemin to the injection mixture has been demonstrated in immature male rats. Testes, seminal vesicles and prostates were significantly heavier in rats receiving hemin with the pituitary extract than in rats receiving the same dosage of pituitary extract alone.

No augmentation was demonstrable in immature male pigeons or immature male chickens. Further increase in weight of the testes was lacking when hemin was added to the pituitary extract in the case of chickens or whole blood in the case of pigeons.

[6] Maxwell, L. C., *Am. J. Physiol.*, 1934, **110**, 458.

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Ultra-Violet Photomicrography of Muscle.

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A simplified quartz microscope with the 2537 Å line of mercury as the light source has recently been described.[1] Since it is known that nucleic acid, nucleoprotein and proteins absorb in this region, photomicrographs of tissue taken with an ultra-violet light source can be expected to show selective absorption. Also, since these substances do not absorb with the same intensity, it is to be expected that the photomicrographs will show more detail than those obtained with visible light and with the usual staining methods. This technic, then, should be of particular advantage in the cytological study of muscle when it is desired to correlate closely certain morphological and physiological changes. In ordinary light the appearance of muscle is misleading, since the dark and light portions which are seen in unstained material are due to inhomogeneity of the material of the fibers and not to the presence of material with selective absorption.[2]

Microphotographs of muscle made with the electron microscope have been published recently and a new technic was described whereby sections of muscle can be obtained in thicknesses of 0.2 to 0.5 μ.[3] The present report shows that ultra-violet photomicrographs can be taken of sections of tissue embedded in the routine manner, and cut at thicknesses of 5 μ, and that they depict with great clarity structures similar to those revealed by the electron microscope. Moreover, sections of tissue prepared and studied in this way are free from the distortion which results when they are desiccated as in preparation for study with the electron microscope. No report has been found of an attempt to obtain ultra-violet photomicrographs of muscle since that given by Meigs.[4] The photomicrograms which he obtained were made from sections of muscle taken from the frog and from the wings of insects; no studies of human muscle were described.

The apparatus used in this study was that described by Lavin and Pollister[1] except that the focusing screen was not attached to the under side of the ground glass. Instead, the screen was placed in a holder, the image brought in focus, and a photographic plate substituted for the screen in order to secure a permanent record. The screen was made

[1] Lavin, G. I., and Pollister, A. W., *Biol. Bull.*, 1942, **83**, 299.

[2] Bernal, J. D., in *Perspectives in Biochemistry*, Cambridge University Press, 1937, pp. 45-65.

[3] Richards, A. G., Jr., Anderson, T. F., and Hance, R. T., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 148.

[4] Meigs, E. B., *Z. f. allg, Physiol.*, 1908, **8**, 81.