

gelding pituitaries into 2 strains of rats. In one a pure follicle stimulating response was noted whereas in the other ovulation occurred with the consequent formation of corpora lutea. When a small amount of pituitary luteinizer was added to the gelding pituitary extract and injected into the first mentioned strain ovulation occurred. Against such a view, however, is the fact that Smith⁴ was able to produce both follicular growth and luteinization in hypophysectomized rats with mare gonadotropic hormone. This, it seems, should be conclusive proof that luteinization following mare gonadotropic hormone treatment is not dependent upon a factor secreted by the pituitary of the recipient. If this is true, then the difference in response must be explained either on the basis of the state of the ovary at the moment of injection or by assuming that a second factor is secreted elsewhere in the body.

The results given in this paper point out clearly the necessity of knowing the stage of sexual development of rats used in studying the qualitative response to gonadotropic substances. As different strains of rats mature at different ages it is obvious that different responses might be obtained in 2 strains of rats even though their ages were the same. This may to some extent explain the divergent results obtained with pituitary extracts in different laboratories.

8428 P

Means of Augmenting the Ovarian Response to Gonadotropic Substances.

F. J. SAUNDERS AND H. H. COLE.

From the College of Agriculture, University of California, Davis.

We presented evidence¹ that the serum of the pregnant mare contains 2 substances, one which produces sexual maturity and fully formed corpora lutea in the immature rat and another which is ineffective by itself but is capable of augmenting the response to pituitary synergist prepared after the method of Evans, *et al.*² We obtained augmentation of this synergist by the use of a fraction prepared from mare serum after the method described by Fevold

⁴ Smith, P. E., *J. Am. Med. Assn.*, 1935, **104**, 553.

¹ Saunders, F. J., and Cole, H. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1476.

² Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **58**, 545.

*et al.*³ for the preparation of luteinizer from the pituitary. Cole and Hart⁴ obtained augmentation of this synergist by serum from non-pregnant mares. Evans⁵ obtained augmentation of his synergist by combining it with sera from pregnant and non-pregnant individuals of various species. From evidence now at hand it appears that the augmentation produced by these sera or their extracts is due not to a specific gonadotropic substance but rather to their protein content. Similar augmentation can be produced by a wide variety of inert materials.

Augmentation by inert materials was first reported by Fevold⁶ in the preparation of his follicle stimulator when he stated that precipitation of the active material on tannic acid and injection of the tannate in colloidal solution accentuates the ovarian response. Maxwell⁶ working with the hypophyseal gonadotropic substance, was able to obtain increased response by the addition of $ZnSO_4$ to the crude pituitary extract. We have been able to confirm Maxwell's work, obtaining an average increase of about 100% in ovarian weight by the addition of $ZnSO_4$ to the crude pituitary extract.* Furthermore using pituitary synergist (Evans' method) we have obtained a 3-fold increase in ovarian weight by the addition of $ZnSO_4$ and also significant increases by the addition of casein or egg albumin.

In making the solutions for these experiments the materials were weighed out and dissolved in an amount of saline such that 6 ml. contained the total dose. The mixtures were allowed to stand for several hours before the first injection. They were then administered in 3 daily subcutaneous injections starting when the rats were 25 days old with necropsy 5 days after the first injection.

From Table I it will be noticed that while significant augmentation is obtained with egg albumin and casein, that obtained with $ZnSO_4$ is much greater. Increasing the amount of egg albumin from 10 to 60 mg. produced no significant change in response. The addition

³ Fevold, H. L., Hisaw, F. L., Hellbaum, A., and Hertz, B., *Am. J. Physiol.*, 1933, **104**, 710.

⁴ Cole, H. H., and Hart, G. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **32**, 370.

⁵ Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **58**, 561.

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

* The crude pituitary extracts were prepared by extraction of the dried gland with 6% butyl alcohol, concentration of the extract under reduced pressure and precipitation of the residue with excess acetone after the method of Meyer and Fevold.⁷

⁷ Meyer, A. E., and Fevold, H. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1934, **31**, 570.

TABLE I.
Effects of Combining Pituitary Synergist with Various Inert Materials.

Material	No. of animals	Aver. ovarian wt., mg.	Significance of increase over synergist alone	
			t	P
Synergist alone	12	34.0± 5.8		
'' + ZnSO ₄ · 7H ₂ O (9 mg.)	6	104.7±40.1	5.2	0.01>P
'' + casein (10 mg.)	6	44.7± 6.8	3.3	0.01>P
'' + egg albumin (10-60 mg.)	12	55.4± 7.6	9.2	0.01>P
'' + casein (10 mg.)				
'' + ZnSO ₄ · 7H ₂ O (9 mg.)	6	106.5±33.6	5.0	0.01>P

The same dose of synergist (2 mg.) was used in all cases. Values of P less than 0.05 may be considered significant.

of casein to the synergist-zinc sulfate mixture produced no further augmentation. Similarly addition of ZnSO₄ to untreated mare serum, in doses containing 11 mg. of protein, was without effect. The questions arise, how are these substances acting and are they all acting in the same manner? Fevold explained the augmentation of the follicle stimulator response by tannic acid as being due to the action of the latter in delaying absorption. Maxwell used the same explanation for the effect of ZnSO₄ on the crude pituitary extracts and presented quite conclusive evidence in favor of this view. The same explanation might hold equally well as regards the effect of protein. Cole, Guilbert and Goss⁸ found that mare serum, with its relatively high content of protein, was just as effective when administered in a single dose to 25-day-old rats as when the dose was divided into 8 injections over a period of 4 days. As to the second question, whether or not all these substances are acting in the same manner, we have little conclusive evidence except that mare serum, which contains considerable protein, is not activated by ZnSO₄, casein did not increase the response when added to the synergist-zinc sulfate mixture, and no significant difference in qualitative response is evident when different agents are used as augmenting principles. In all of these cases in which we obtained an augmented response, the ovaries were heavily luteinized. Therefore it seems logical to assume that all these substances are acting in the same manner, possibly by delaying absorption of the active principle. The relationship between the augmenting principles and the pituitary luteinizers is not clear. In our hands, both the augmenting substances herein discussed and the pituitary luteinizer augment the response to certain pituitary fractions and both, in conjunction with these fractions, produce heavily luteinized ovaries. The point is far from being

⁸ Cole, H. H., Guilbert, H. R., and Goss, H., *Am. J. Physiol.*, 1932, **102**, 227.

settled, however, and it should be stated that work in other laboratories points toward a more specific effect of the pituitary luteinizer.

8429 P

Quantitative Interpretation of Biological X-Ray Effects.*

FRED M. UBER. (Introduced by S. C. Brooks.)

From the Department of Botany, University of California, Berkeley.

In dark-field photomicrographs¹ of microincinerated pollen mother cells of the lily, the chromosome ash is most conspicuous against the black background which results from the paucity of ash in the karyolymph or nucleoplasm. Animal cells yield a similar picture (Scott,² Kruszynski³). This marked concentration in the chromosomes of elements of comparatively high atomic weight, as potassium, calcium and iron probably and phosphorus certainly, may be related quantitatively to X-ray phenomena in at least 3 ways.

First, rough calculations based on microincineration evidence and on the composition of nucleic acid indicate that the mass absorption coefficient of chromatin is more than double that of air and, hence, that of nucleoplasm. This relatively greater absorption of radiant energy by the chromosomes may account in large measure for the greater sensitivity of the nucleus compared to the cytoplasm, an experimental fact confirmed by a number of investigators.

Second, the amount of chromatin in a "sensitive volume," a concept associated with the quantum hit theory of radiation injury, would be but a fraction of that computed for it on the ordinary assumptions, *i. e.*, those which underlie the definition of the roentgen as a dosage unit. Were the validity of the theory established and relevant measurements precise enough, this size factor might prove important in assigning the "sensitive volume" to a definite cytological entity.

Third, the localization of nuclear ash in the chromosomes should manifest itself in a biologically detectable wave-length effect at

* Part of investigation supported by grant of the Rockefeller Foundation to Prof. T. H. Goodspeed.

¹ Uber, F. M., and Goodspeed, T. H., *Bot. Gaz.*, 1935, **97**, No. 2, in press.

² Scott, G. H., *Bull. d'Hist.*, 1930, **7**, 251.

³ Kruszynski, J., *Bull. internat. Acad. Polonaise, Cl. Sci. Math. et Nat.*, 1934, **2**, 105.