commercial xanthine, purified in this laboratory and injected subcutaneously into rats in the same quantities as the crystalline preparation from liver. Rats receiving 100 mg of sodium xanthine 20 hours before acute carbon tetrachloride poisoning by deep anesthesia for one hour and forty minutes, suffered only a slight fatty infiltration of their livers with a very limited round cell infiltration around the central veins. Ninety per cent. of the control rats, subjected to the same poisoning, died within 48 hours following the anesthesia, and after the expiration of that time the surviving rats were killed. Microscopic examinations showed very nearly complete destruction of the liver in practically all the control animals. The protection with mono-sodium-2,6-dioxy-purine was the same as that found with the crystalline preparation from liver.

Sodium guanine, injected into rats in doses of from 50 to 100 mg, exerted the same protective action as did the sodium xanthine and the preparation from liver. Adenine sulfate given in equivalent doses protected the animals to a certain extent but seemed to be definitely toxic itself in the amounts injected. Further experiments using chloroform poisoning showed both sodium guanine and sodium xanthine to protect the livers of rats. Liver sections from animals protected with these substances and subjected to two hours of deep chloroform anesthesia showed no histological changes except for an infiltration of a few fibroblasts intercellularly. There was no fatty infiltration or degeneration. Sections of the livers of control animals exhibited typical central necrosis extending toward the periphery of the lobule involving 50 per cent. of the liver lobule. Many of the control animals died.

Other purine bases and derivatives, both natural and synthetic, are being tested for protective action against carbon tetrachloride, chloroform and other liver poisons. A more complete report will appear in the near future, giving complete chemical identification of the active crystalline substance from liver and showing the effects of purines on the toxicity of the more common poisons.

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## CORTICO-ADRENAL AND NEURAL EFFECTS ON GONADOTROPIC ACTIVITY OF THE PITUITARY[1]

IN the rabbit and cat ovulation usually occurs only after mating and is dependent upon the secretion of the anterior hypophyseal gonadotropic hormones. The data recently published by Brooks[2] and by Haterius and Derbyshire[3] suggest that in the rabbit coitus stimulates the pituitary through nerves in the infundibular stalk. Previously, only one neural pathway to the cells of the anterior hypophysis was recognized, *viz.*, the cervical sympathetic fibers.

In 1935, Friedgood and Pincus[4] and Friedgood and Cannon[5] stimulated electrically the cervical sympathetics of normal estrous rabbits. Such stimulation resulted in ovum maturation in the majority of rabbits, but rarely did it result in ovulation, and then only of a limited number of ova. The simultaneous intravenous injection of adrenalin did not enhance the effect of these electrical stimuli. Quantitatively, therefore, the ovarian response was similar to that evoked by injections of subovulatory doses of FSH and LH. It was concluded that stimulation of the pituitary through its sympathetic innervation increased, to a limited extent only, the normal rate of secretion of its gonadotropic hormones. Collin and Hennequin[6] later reported that stimulation of these nerves resulted in marked cytological changes in the anterior pituitary.

In searching for another factor which might influence the rate of secretion of the gonadotropic pituitary hormones, the possibility of a humoral mechanism was considered. The cat was used as an experimental animal, instead of the rabbit, because its survival period after adrenalectomy is longer. Estrous cats go out of heat and their ovaries atrophy after bilateral adrenalectomy, even if the animals are maintained in apparent good health by daily administration of a potent preparation of cortin[7] containing the life-sustaining hormone. A preliminary unilateral adrenalectomy was therefore carried out on 12 anestrous cats. After objective evidence of the estrous state developed in these animals (4 to 12 weeks later), 9 of them were subjected to a second adrenalectomy from 15 to 55 minutes after mating. This procedure prevented the occurrence of ovulation in every instance. In the remaining 3 cats, adrenalectomy was delayed until 6 hours postcoitum. All 3 animals ovulated. These experiments indicate either that adrenalectomy within one hour after mating interferes with the usual response of the anterior hypophysis to the coital stimulus, or that the ovary is unable to respond normally to

[1] From the Department of Physiology in the Harvard Medical School, Boston. Aided by a grant from the National Research Council Committee for Research in Problems of Sex.

[2] C. McC. Brooks, *Proc. Am. Physiol. Soc.* In press.

[3] H. O. Haterius and A. J. Derbyshire, *ibid.* In press.

[4] H. B. Friedgood and G. Pincus, *Endocrinology*, 19: *Physiol.*, 116: 54, 1936.

[5] H. B. Friedgood and W. B. Cannon, *Am. Jour. Physiol.*, 116: 54, 1936.

[6] R. Collin and L. Hennequin, *Compt. rend. Soc. de biol.*, 121: 84, 1936.

[7] We are indebted to Dr. David Klein, of the Wilson Laboratories, who furnished the cortico-adrenal extract for these experiments.

these hormones if the adrenals are removed so early in the experiment. In order to decide this point, another series of observations was carried out with the collaboration of Dr. M. A. Foster.[8]

The ovaries of anestrous cats can be stimulated experimentally[9] by the proper administration of the gonadotropic hormones FSH and LH. Adrenalectomy at various intervals prior to the injection of FSH and LH did not prevent ovulation and subsequent luteinization, although they were retarded beyond the normal period of about 36 hours for as long as 10 to 22 hours; and huge cysts, lined by somewhat atypical lutein cells, were found in the ovaries of those adrenalectomized cats which were autopsied more than 50 hours after the last injection. The injection of FSH and LH into normal control cats has not in our experience resulted in the development of these remarkable cystic structures.

One may conclude, therefore, that the adrenal glands are essential for the proper coital stimulation of the anterior pituitary. Even if coitus in the cat activates the anterior pituitary through nerves in the infundibular stalk (as Brooks suggests for the rabbit), these stimuli are ineffective in the absence of the adrenals. It may be inferred also that it is the cortex of the adrenal which contains the gonadotropic hormone, since the distribution of the splanchnic nerves is limited to the adrenal medulla, and adrenalin, in our experience, has not induced ovulation.

The anterior pituitary of the cat is similar to that of the rabbit[10,11] in that it does not secrete enough gonadotropic hormone during the first hour after mating to induce ovulation. The time which elapses between the coital act and the gonadotropic response of the pituitary is, at least in part, consumed in the secretion (and perhaps elaboration) of an adrenal cortical hormone. This humoral substance is capable of stimulating or of cooperating in the stimulation of the gonadotropic activity of the anterior pituitary. This explanation accounts satisfactorily for the fact that ovulation is not prevented by adrenalectomy if the operation is delayed until 6 hours postcoitum. Sometime within this period, the anterior hypophysis secretes its gonadotropic hormones with the cooperation of the adrenal glands.

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[8] H. B. Friedgood and M. A. Foster, *Proc. Am. Physiol. Soc.* In press.

[9] M. A. Foster and F. L. Hisaw, *Anat. Rec.*, 62: 75, 1935.

[10] A. R. Fee and A. S. Parkes, *Jour. Physiol.*, 67: 383, 1929.

[11] P. E. Smith and W. E. White, *Jour. Am. Med. Asn.*, 97: 1861, 1931.

# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## GLYCYLGLYCINE AS A SEA WATER BUFFER[1]

IT is necessary in many experiments with marine eggs to remove the carbonate components of the sea water, which normally acts as its principal buffer system,[2] and to substitute some suitable buffer. The buffer chosen must, of course, have its dissociation index in about the middle of the pH range in which it is desired to work and, above all, it must have no injurious effects on the living material in the concentrations it is necessary to employ. We find that the dipeptide, glycylglycine, may be used as a satisfactory buffer between pH 7 and 9. Phosphate, which is perhaps the most commonly used buffer, is about the only other agent that has been used[3] in carbonate-free sea water with marine eggs. It is, however, useful only at low pH. At higher pH's it precipitates out the Ca and Mg of the sea water. For example, carbonate-free sea water containing phosphate at a total concentration of 0.01 molar will start to precipitate at pH 6.3, and when the pH is raised to 8.0, more than 95 per cent. of the Ca and the Mg of the sea water is lost. Developing eggs have long been known to be peculiarly affected by Ca or Mg lack.[4] Egg albumen or gelatin, which would buffer over a wide pH range, block cleavage in low concentrations.

Glycylglycine has the appropriate dissociation constant and has sufficient solubility in sea water for buffering around pH 8.0. Recent values of its pK′ (amino) are given as 8.07,[5] 8.80 (0° C.),[6] 8.13 (25° C.),[6] 8.86 (0° C.),[7] 8.17 (25° C.).[7] In sea water the value would be affected by the ionic strength. In Fig. 1, a titration curve (glass electrode) for 0.025 molar glycylglycine in carbonate-free sea water is given. From this we get a pK′ of 8.1 (18.5° C.).

The effect on development was examined by placing freshly fertilized sea-urchin eggs in carbonate-free sea water containing various concentrations of glycylglycine.[8] The solutions were all adjusted to the same pH

[1] From the William G. Kerckhoff Laboratories of the Biological Sciences, California Institute of Technology, Pasadena, Calif.

[2] According to E. Moberg, D. M. Greenberg, R. Revelle and E. C. Allen (*Bull. Scripps Institution of Ocean.*, 3: 231, 1934) the borate in sea water also has a slight buffer action.

[3] H. Smith and G. H. A. Clowes, *Biol. Bull.*, 47: 304, 1924.

[4] C. Herbst, *Roux' Archiv*, 5: 649, 1897.

[5] H. S. Simms, *Jour. Gen. Physiol.*, 11: 629, 1928.

[6] G. E. K. Branch and S. Miyamoto, *Jour. Am. Chem. Soc.*, 52: 863, 1930.

[7] J. P. Greenstein, *Jour. Biol. Chem.*, 101: 603, 1933.

[8] The analytically pure glycylglycine supplied by the