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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# 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

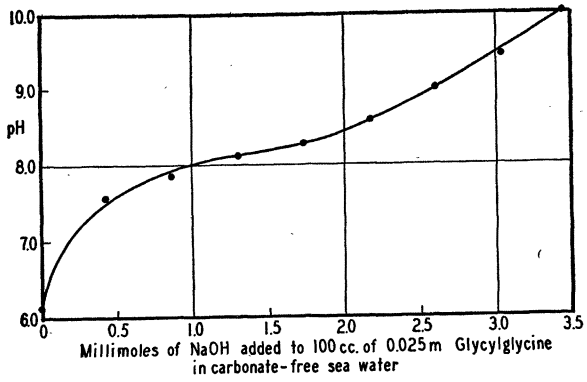


FIG. 1

and were made isosmotic with sea water. Even in the highest concentrations that we employed, namely, 0.25 molar glycylglycine at pH 8.2, cleavage was 100 per cent. Later development is, however, distinctly abnormal in the solutions stronger than 0.10 molar. Between 0.05 and 0.10 molar there is an apparent effect of the glycylglycine in producing thick-walled blastulae and gastrulae. Below 0.05 molar, there is no evidence of any particular effect. For most purposes (*e.g.*, respiration experiments) a 0.005 to 0.02 molar solution provides sufficient buffer action. Veronal[9] which was tried, since it has also a pH of 8.0, causes abnormal development in a concentration of 0.002 molar; although cleavage may proceed in a 0.01 molar solution.

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## UNIVERSAL JOINTS FOR SKELETONS

SEVERAL attempts have been made to produce a mechanical joint for mounted skeletons that would permit motion in all three planes. A joint mechanism was sought that would give movements similar to those observed in a living body, yet without being so large as to be cumbersome nor so flimsy as to be unable to withstand the rigorous handling of freshman medical students.

Fig. 1 shows a joint mechanism that has been successful in three years of service in the medical school laboratory. Twelve of these devices are in good working order on the laboratory skeletons. In the sketch in Fig. 1 is seen a wrist joint assembled in such a fashion as to allow flexion and extension of the hand as well as abduction and adduction. Pronation and supination are obtained by a device modeled after one which has been used by others.

Abduction and adduction are obtained by a sliding joint, which is seen dissembled in Fig. 2. The part A has two rivets which fit into slots in the part B and

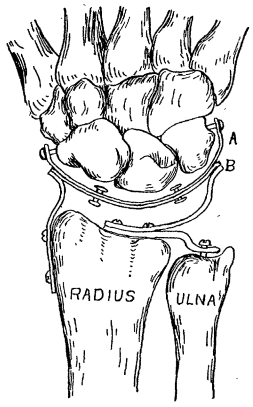


FIG. 1

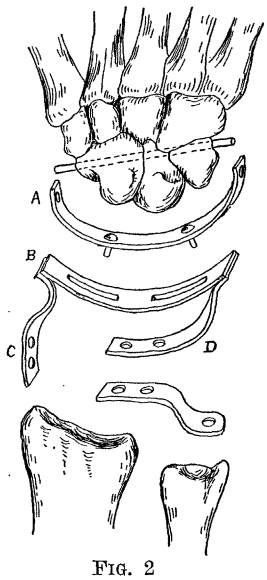


FIG. 2

their ends are headed to hold them in the slots. The length of the two slots can be varied, depending upon the amount of abduction desired. The part B is held stationary to the radius bone by the parts C and D, which are attached to the bone by small brass screws. B is attached to C and D by solder. Such a device allows the part A, which is attached to the hand, to slide back and forth upon the part B and simulate the abduction and adduction of the living hand.

The part A is attached to the carpal bones of the hand by an axle or pin passing through the proximal three carpal bones, as seen by the dotted line in Fig. 2. The ends of the pin are inserted into the holes in the metal piece A, and these ends are flattened to prevent them from pulling out of the holes. A complete flexion or extension of the hand can thus be obtained, as the pin through the carpus acts similarly to an axle.

The same method was utilized in a joint for the shoulder to obtain medial and lateral rotation of the arm as well as flexion and extension, and abduction and adduction.

The parts used in this joint can be made of sheet brass or of any durable iron alloy in bands about one thirty-second of an inch in thickness.

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