leading to increased plasma concentrations of *free* Cpd. B; or actual stimulation of the hypothalamic centers related to ACTH release by development of the hypothalamic lesion.

These results show, at any rate, that the hypothalamic lesion produced in CBA mice by the dose of GTG used in this study does not inhibit release of ACTH normally produced by exposure to stress. Animals with this type of hypothalamic lesion, therefore, cannot be used for an assay of CRF.

1. Marshall, N. B., Barrnett, R. J., Mayer, J., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 240.

2. Liebelt, R. A., Perry, J. H., *ibid.*, 1957, v95, 774.

3. Fukuyama, U., Watanabe, R., *Okajimas fol. anat.*, 1958, v31, 11.

4. Perry, J. H., Liebelt, R. A., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 55.

5. Waxler, S. H., Brecher, G., *Am. J. Physiol.*, 1950, v162, 428.

6. Gray, G. F., Liebelt, R. A., *Tex. Rep. Biol. and Med.*, 1961, v19, 80.

7. Brecher, G., Waxler, S. H., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 498.

8. Mayer, J., Zighera, C. Y., *Science*, 1954, v119, 96.

9. Owen, J. A., Jr., Parson, W., Crispell, K. R., *Metabolism*, 1953, v2, 362.

10. Drachman, R. H., Tepperman, J., *Yale J. Biol. and Med.*, 1954, v26, 394.

11. Liebelt, R. A., Sekiba, K., Liebelt, A. G., Perry, J. H., PROC. SOC. EXP. BIOL. AND MED., 1960, v104, 689.

12. Sayers, G., Redgate, E. S., Royce, P. C., *Ann. Rev. Physiol.*, 1958, v20, 243.

13. Guillemin, R., Clayton, G. W., Lipscomb, H. S., Smith, J. D., *J. Lab. Clin. Med.*, 1959, v53, 830.

14. ———, *Endocrinology*, 1958, v63, 349.

15. Halberg, F., Peterson, R. E., Silber, R. H., *ibid.*, 1959, v64, 222.

16. Guillemin, R., Dear, W. E., Liebelt, R. A., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 394.

17. Sakiz, E., *C. R. Soc. Biol.*, 1960, v154, 1159.

18. Herbst, A. L., Yates, F. E., Glenister, D. W., Urquhart, J., *Endocrinology*, 1960, v67, 222.

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Extension of Fertilizability in the Sand Dollar Egg with Vitamin B_{12}.* (26945)

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Unfertilized eggs of the sand dollar, *Dendraster excentricus*, lose the capacity to be fertilized within 48 hours when allowed to stand uncrowded in fingerbowls at 18°C. Addition of cobaltous chloride to the sea water will increase the fertile life of the eggs 4-8 days. When sulfhydril compounds are added to the proper concentrations of cobalt in sea water the fertile life may be extended to as much as 25 days(1,2). In view of these findings it seemed desirable to determine the effect of cyanocobalamin (Vit. B_{12}) on fertilizability.

Methods. Mature sand dollars were taken from Monterey Bay and maintained in aquaria at the Hopkins Marine Station, Pacific Grove, Calif. Ova were removed from ripe females and washed several times before being placed in various concentrations of the vitamin. Since the crystalline form of the vitamin was not available at the time of experiments a sterile aqueous solution (Squibb's Rubramin–containing 1,000 μg Vit. B_{12} per cc in isotonic NaCl with 1.5% benzyl alcohol as a preservative) was diluted with sea water. As simple dilution with sea water also diluted the benzyl alcohol it was necessary, in some cases, to add benzyl alcohol to the diluent to maintain a constant concentration of the preservative while varying the concentration of the vitamin. All cultures were kept in covered fingerbowls, out of direct sunlight and at 18 $\pm$ 1°C. Fertility was determined at 24-hour intervals by addition of fresh

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TABLE I. Extension of Fertility in Eggs of *Dendraster excentricus* with Vitamin B_{12} and Benzyl Alcohol.

Solution used	24 hr	48 hr	72 hr	96 hr	120 hr
	%				
Sea water (control)	80	—	—	—	—
.25% benzyl alcohol*	20	—	—	—	—
.15% " "	80	50	10	—	—
100 μg vit. B_{12}/cc in .15% benzyl alcohol	90	85	40	35	—
50 μg *idem*	90	75	40	30	30
25 μg "	90	75	40	3	—
12.5 μg "	80	60	30	20	10
6.25 μg "	90	60	40	30	20

* Benzyl alcohol solutions made up with sea water.

sperm suspension to washed samples of approximately 100 eggs. The criterion of fertilization was nuclear division and the number fertilized usually was recorded to the nearest 5.0%.

Results. Vitamin B_{12} clearly extended fertilizability in sand dollar eggs (Table I). None of the control eggs was fertilizable at 48 hours. In 5 different concentrations of B_{12} (100 μg-6.25 μg per cc) in 0.15% benzyl alcohol fertility was extended beyond that of benzyl alcohol alone. It was evident that loss of fertility in these eggs was delayed by the vitamin. Since benzyl alcohol alone was found to preserve fertility it seemed advisable to determine its effects at various strengths. It was found that 0.25% benzyl alcohol (Table I) was toxic enough to decrease while 0.15% (Table I) and 0.075% (Table II) extended (and with 0.075% enhanced) fertilizability in these eggs. With lower concentrations (0.0375%, 0.009375% – Table II) the percentage fertilizable at 24 hours was decreased but there was some extension of the fertile period.

In another set of experiments the concentration of B_{12} was kept constant while the strength of the benzyl alcohol was varied. As noted above (Table I) 6.25 μg of the vitamin per cc in 0.15% benzyl alcohol prolonged the fertilizable life of 20% of the eggs for over 120 hours. When this same quantity of B_{12} was used with benzyl alcohol of lower concentrations (0.075%–0.009375%) the preserving effect was not significantly better than benzyl alcohol alone (Table II). It seems clear from these data that low concentrations of B_{12} in sea water require benzyl alcohol (or something similar) in proper concentration to prolong fertilizability in these eggs.

Discussion. The fertile life of various marine eggs has been extended by means other than temperature. Low calcium sea water prolongs the fertile life of *Arbacia* eggs(3) while the fertile life of *Urechis* eggs has been extended with ethyl alcohol and dextrose (4). Various amino acids and proteins have been used to improve fertilization (but not to preserve fertility) in echinoderm eggs(5). It has been suggested that fertility may be preserved by preventing internal clotting, by

TABLE II. Extension of Fertility in Eggs of *Dendraster excentricus* with Vitamin B_{12} and Benzyl Alcohol.

Solution used	24 hr	48 hr	72 hr	96 hr	120 hr
	%				
Sea water (control)	80	—	—	—	—
.075% benzyl alcohol*	95	95	1	—	—
.0375% " "	70	40	—	—	—
.009375% " "	50	5	—	—	—
6.25 μg vit. B_{12}/cc in .075% benzyl alcohol	95	90	5	—	—
Idem .0375% " "	80	50	—	—	—
" .009375% " "	70	—	—	—	—

* Benzyl alcohol solutions made up with sea water.

supplying nutritional substances, and/or by decreasing permeability.

Loeb(6) suggested that the starfish egg normally becomes unfertilizable after a damaging amount of aerobic oxidation takes place, and believed that the slowing down of this action (through use of potassium cyanide and low oxygen) was responsible for prolonging fertilizability in these eggs. The writer(2) has suggested that cobalt prevents deterioration of fertilizability by uniting with (and preventing oxidation of) thiol groups at the egg surface. The action of Vit. B_{12} in preserving fertility may well be through protection of the egg surface from destructive oxidations. It seems not unlikely that B_{12} combines with surface proteins in much the same way that Co^{60} Vit. B_{12} combines with serum proteins (alpha and beta globulins) and the protein of cerebro-spinal fluid(7). The preserving action of benzyl alcohol alone may be in the retardation of the processes (oxidations, increased permeability, etc.) which normally lead to the decay of fertility.

Summary. Vitamin B_{12} (100-6.25 μg/cc) in the presence of 0.15% benzyl alcohol extends fertilizability in the eggs of *Dendraster excentricus*. Benzyl alcohol alone also has some preserving action. It is suggested that both substances prolong fertility by inhibiting destructive processes that occur with aging of the eggs.

1. Rulon, O., *Anat. Rec.*, 1960, v137, 390.
2. Rulon, O., *ibid.*, 1960, v138, 381.
3. Schechter, V., *Biol. Bull.*, 1937, v72, 366.
4. Whitaker, D. M., *J. Exp. Zool.*, 1937, v75, 155.
5. Runnström, J., *Symposia of Society for Experimental Biology*. Cambridge Univ. Press, 1952, v6, 39.
6. Loeb, J., *The Mechanistic Conception of Life*, Univ. of Chicago Press, 1912, 155.
7. Meyer, L. M., Bertcher, R. W., Mulzac, C., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 607.

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Effects of Clotting and Clot Lysis on Platelet Thromboplastic Activity.* (26946)

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During coagulation of shed blood the majority of platelets undergo clumping, "viscous metamorphosis",(1,2) and, eventually, disappear from the liquid phase. Presumably these platelets are either trapped in the fibrin meshwork of the clot or disintegrated. The few platelets which remain in the liquid phase have been called "serum platelets" and their properties described by Alexander(3). Platelets contain a thromboplastic factor (platelet factor 3) which is essential to normal blood coagulation. These studies were undertaken to determine what happens to this factor during and after clotting. To avoid interference from red cells, platelet-rich plasma was used. To free platelets from enmeshing fibrin network, clots were either chopped and ground or lysed.

Materials and methods. Platelet-rich plasma. One unit of ACD blood, drawn in a plastic bag, was centrifuged at 400 g for 25 min.; the plasma was transferred to plastic tubes and recentrifuged at 300 g a number of times until essentially free from red cells. *Streptokinase* (SK), Varidase, was kindly supplied by Dr. Ruegsegger of Lederle Laboratories. *Platelet-thromboplastic factor* was estimated by 2 methods: (a) a standard TGT test(4) in which equal volumes of normal $BaSO_4$ adsorbed plasma (dil. 1-5), normal serum (dil. 1-10), platelets and 0.025 M $CaCl_2$ were mixed and tested for thromboplastic activity every minute by adding 0.1 ml to 0.2 ml recalcified normal substrate plasma. Minimum clotting time of the latter is reported.

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