tryptamine in mast cells, although not in mastocytoma, has been previously reported (3,12). The value 140 μg/g in our mastocytoma is the highest recorded in mammalian tissues apart from carcinoid tumors, in which values up to twenty times greater have been found.

Summary. 1. A transplantable mastocytoma of the mouse is described. The tumor is benign and composed of cells indistinguishable from normal mast cells. 2. It contains greater quantities of histamine and heparin than have previously been found in normal or tumorous mast cells. It also contains large amounts of 5-hydroxytryptamine.

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Received July 3, 1957. P.S.E.B.M., 1957, v95.

Eggs of Floodwater Mosquitoes (*Diptera: culicidae*) VI. Effect of Metabolites on Latent Embryos in Uncapped Eggs.* (23376)

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Latent embryos in intact eggs of floodwater mosquitoes become activated after a series of events called *conditioning*(1). Such embryos hatch quickly and uniformly when subjected to a reduction in oxygen in the medium surrounding the eggs. Reduction in oxygen is effected in nature by metabolism of embryos when eggs are crowded into closely confined sites beneath layers of leaves, or by metabolic activity of microbes dispersed among the plant debris. It has been accomplished in the laboratory by crowding(2), by microbes(3) by reducing agents(1,3,4,5) and by replacing the dissolved oxygen in the medium with nitrogen or other inert gases(3,6).

Activation of conditioned embryos in intact eggs is prompt when oxygen in the medium is reduced to the proper level and at the proper rate. This fact suggests that latency is terminated by a sudden alteration in metabolic activity of the embryo. In preliminary experiments involving the effect of inhibitors of respiration on hatching of the eggs of aedine mosquitoes, the chorion proved to be impervious to solutes. Compounds which were quickly lethal to free larvae did not kill embryos in the intact eggs, even after exposure to these agents for several days. On the other hand, embryos in eggs made permeable by brief exposure to solutions of sodium hypochlorite were killed by the same inhibitors. Because of the barrier effect of the shell, latent embryos deprived of their shells or parts of shells were used in the experiments reported here.

Methods. All experiments cited herein were performed with embryos of *Aedes trivittatus* (Coq.) This species is well suited to experimentation because it (a) is easily conditioned, (b) is readily obtained in large num-

* Research supported by grants from the Division of Research Grants, U. S. Public Health Service.

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bers, (c) is adapted to storage in the laboratory, and (d) is readily uncapped mechanically. Latent embryos used in the experiments were obtained from eggs deposited in the laboratory. Wild-caught females were caged, fed human blood and maintained at 25°C. Eggs deposited on moist cheesecloth bats were removed daily to moist filter paper and incubated in chambers saturated with water vapor until embryos were mature. They were then stored at 4°C. Mechanical uncapping was performed routinely on eggs removed directly from storage at 4°. Caps were removed mechanically from eggs by bending them at the normal circular line of fracture near the anterior end. Essential tools consisted of a pair of finely pointed forceps and a thin-walled, tapered, glass tube (breaking tube) having an inside diameter of such size as to fit snugly over an egg at the fracture line. The breaking tube was made by drawing out a piece of standard, melting-point, capillary tubing (1.5 x 2.0 x 90 mm) in a low flame. It was drawn so that the diameter at the end was smaller than that finally desired. It was shortened to the correct inside, apical diameter by snipping off the end with the forceps. This resulted in a tube with sharp or even jagged edges at the apex which was more effective than a smooth one. The inside diameter of a tube suitable for uncapping eggs of *A. trivittatus* or *A. sollicitans* should be 150-160 μ in diameter while one for *A. stimulans* should be 200-210 μ.

In practice eggs were uncapped while they were submerged in aqueous solutions. A standard, flat-form, porcelain, evaporating dish was a convenient container because it allowed more latitude for movement of tools. Eggs to be uncapped were wedged between the tips of the forceps. The breaking tube was placed over the anterior end of an egg, then was rocked gently until the cap was free along the fracture line. A stereomicroscope providing a magnification of about 10 diameters was ample for the dissection.

The medium (modified Ringer's solution) in which activation of exposed embryos was observed consisted of a nearly isotonic solution of salts. This solution contained 6.790 g of NaCl, 0.153 g of KCl, 0.153 g of $CaCl_2$, and 0.120 g of $NaHCO_3$ per liter of distilled water. Metabolites to be tested for effect on latency were added to this solution and were neutralized with NaOH to pH 7.0.

This procedure for uncapping eggs and activating the embryos has been used in routine rearing of both univoltine and multivoltine species. Many species respond variously to hatching stimuli applied to intact eggs because the degree of conditioning is not uniform. Embryos when removed from their shells and placed in the modified Ringer's solution become activated and develop normally. This procedure has been particularly useful in work with the species having only one generation each year.

All experiments were done with the exposed embryos which had been stored for 24 hours in a test solution of each metabolite. By this means variation in permeability was eliminated. Each group of embryos which was stored in its own test solution was then divided into a control group and an experimental one. The control group was placed in Ringer's solution, and the experimental one remained in the solution of the metabolite dissolved in Ringer's solution. Both groups were kept at room temperature and observations were made of the number of active embryos at periodic intervals.

Results. The objective in developing this procedure for uncapping eggs was to place the embryos in more intimate contact with test media. It was hoped that procedures such as lowering of the oxygen tension or interference with glycolysis or respiration might cause termination of latency. If such were the case, it might be possible to ascertain the mechanism whereby the lowered oxygen tension of the medium stimulates hatching of intact eggs.

When embryos were exposed as described above and placed in an isotonic solution, they became active, normal larvae without further treatment. This behavior prompted a change from a study of the activation of embryos to a study of the factors concerned in maintaining latency. Addition of glucose or one of several other recognized sources of energy to the freshly exposed embryos prevented activation at 4°C. After a suitable period of time at this low temperature, embryos could

TABLE I. Effect of Metabolites on Latency of Conditioned Embryos of *Aedes trivittatus* 5 Months Old in Uncapped Eggs in the Laboratory, 1956.

Metabolite in Ringer's solution		No. embryos treated	Embryos activated in 48 hr	
Name	Conc.		No.	%
Glucose	.3M*	60	3	5
Fructose	.3M	30	5	16
Mannose	.3M	30	8	24
Inositol	.3M	30	14	46
Mannitol	.3M	30	20	66
Sorbitol	.3M	30	23	76
Galactose	.3M	30	26	80
Pyruvate	.3M	30	0	0
Glutamate	.1M	30	2	6
Acetate	.3M*	30	3	10
Citrate	.3M	30	3	10
Fumarate	.1M†	30	3	10
Malate	.1M†	30	10	33
Succinate	.1M	30	lethal	
Gelatin	.5%	30	23	76
Bovine albumin	2.5%	30	27	90
None	—	60	55	92

* Media containing 0.1M permitted activation of 80% of embryos.

† Media containing 0.01M permitted activation of 100% of embryos. (Alpha keto glutarate at 0.01M permitted activation of 83% of embryos.)

be maintained latent at room temperature for at least 48 hours in solutions of glucose and other substrates. Embryos from eggs less than 5 months old required no more than 24 hours at 4° while those 8 months of age required much longer storage at low temperature before use in experiments at room temperature. Bucklin(7) found that latency was terminated and normal morphogenesis of eggs of grasshoppers took place in Ringer's solution.

TABLE II. Effect of Increasing Concentrations of Salts in Ringer's Solution on Activation of Conditioned, Latent Embryos of *Aedes trivittatus* in the Laboratory, 1956.

Concentration of salts	No. of embryos treated	Embryos activated in 48 hr	
		No.	%
Ringer's solution	60	55	92
2× Ringer's solution	60	56	93
3× " "	60	52	88
4× " "	60	46	82
5× " "	60	47	78
10× " "	60	44	73*

* Lethal within 24 hr.

Metabolites dissolved in Ringer's solution varied widely in their effect on latency (Table I). Among the sugars, glucose and fructose caused continuation of latency; galactose permitted activation to the same extent as Ringer's solution alone; the 3 sugar alcohols used had little effect. Salts of several organic acids which are recognized substrates in oxidative metabolism all maintained latency approximately as well as glucose at comparable molar concentrations.

Hypertonic solutions of the inorganic salts used permitted the termination of latency in the same manner as isotonic Ringer's solution. Concentrations as high as 5 times the isotonic level were not harmful, but at 10 times the isotonic concentration activated embryos died (Table II). None of the solutions containing metabolites shown in Table I was used at concentrations which would give an osmotic pressure as great as 5 times the isotonic level.

Cold embryos exposed in distilled water became elongated and died after activation. Embryos from eggs stored at room temperature for a few days prior to uncapping became active and survived in distilled water.

Summary. Latency in both intact and manually uncapped eggs of floodwater mosquitoes may be continued or terminated at will. *Conditioned* embryos in uncapped eggs become active in a modified Ringer's solution. Latency may be continued in such embryos by adding metabolites such as glucose, fructose and salts of organic acids which are recognized substrates in oxidative metabolism to the basic Ringer's solution.

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Received July 3, 1957. P.S.E.B.M., 1957, v95.