

persists. The color intensity of the fulminate derivative appears to be about half that of an equimolecular solution of cyanmethemoglobin.

Saturated silver fulminate precipitates methemoglobin from solution, the precipitate being red in color. Where the methemoglobin solution has been prepared by the action of quinhydrone on hemoglobin, mercuric fulminate likewise causes a similar precipitate. This would indicate a difference between the methemoglobins prepared by the action of ferricyanide and that resulting from the action of quinhydrone on hemoglobin. A difference in the behavior of these 2 substances toward hydrogen peroxide has been previously noted.<sup>1</sup>

The fulminates are without effect on the spectroscopic appearance of oxyhemoglobin, carbon monoxide hemoglobin, reduced hemoglobin and of alkaline hematin, whether the last be in alkali carbonate, ammonium hydroxide, triethanolamine or pyridine solution. In the case of the hematin solutions, the fulminates differ from the cyanides in that the latter give rise to cyanhematin.

Solutions of sodium fulminate and of mercuric fulminate which had been oxidized by molecular iodine lost their power of altering the spectroscopic appearance of methemoglobin. Since the fulminate ion and methemoglobin are both essential to the formation of this compound which differs in its reactions from any hitherto described, it is referred to as fulminate methemoglobin.

### 10136

#### Growth and Metamorphosis of Anuran Larvae on Thymus Extracts.\*

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Since the early experiments of Gudernatsch,<sup>1</sup> who produced an acceleration of growth and a delay of metamorphosis of tadpoles fed upon fresh thymus tissue, several investigators have obtained diverse results by feeding thymus glands, either fresh or dried, to amphibian

<sup>1</sup> Barnard, R. D., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 762.

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<sup>1</sup> Gudernatsch, F., *Arch. f. Entw.-Mech.*, 1912, **35**, 457.

larvæ. Uhlenhuth<sup>2, 3</sup> found that when only thymus tissue was given to newly hatched urodeles, their growth was retarded. This was attributed to the particular food value of the thymus since the animals grew at a normal rate when other food was added to the diet. Contrary to the above observations, Kahn<sup>4</sup> and Romeis<sup>5</sup> found that thymus accelerated the growth of amphibian larvae. Swingle,<sup>6</sup> however, fed fresh and powdered thymus to *Rana* larvae and observed no retardation or acceleration of growth or metamorphosis. The rather extensive literature has been covered in a brief review by Gudernatsch.<sup>7</sup>

Asher and Ratti<sup>8</sup> prepared a thymus extract, thymocrescin, which in their hands has produced marked acceleration of growth as well as a gonadotropic effect upon rats on deficient diets. More recently, Rowntree, *et al.*,<sup>9</sup> have obtained accelerated growth and differentiation in rats through the use of thymus extract (Hanson) in successive generations.

The writers have thought that if a growth-promoting factor is present in such extracts, it may have some effect, beyond that of its food value, upon amphibian larval development.

A clutch of *Rana pipiens* eggs was divided into 3 parts, each of which was placed in one of 3 aquaria containing 2200 cc of water. After hatching, the larvae were fed dried boiled beef liver and boiled lettuce. On alternate days when the water was changed, 10 mg of thymocrescin† was added to one aquarium, 1 cc of Hanson's extract‡ to another, and the third aquarium served as a control. The same number of animals was kept in each of the 3 aquaria at all times, although this number was reduced as necessary to prevent crowding. Linear measurements were made at weekly intervals starting 5/14/38 (Table I) and were continued until 6/18/38, at

<sup>2</sup> Uhlenhuth, E., *Proc. Soc. Exp. Biol. and Med.*, 1917, **15**, 37.

<sup>3</sup> Uhlenhuth, E., *Endocrinol.*, 1919, **3**, 285.

<sup>4</sup> Kahn, R. H., *Arch. f. d. ges. Physiol.* (Bonn), 1916, **163**, 384.

<sup>5</sup> Romeis, B., *Münchener Med. Wochen.*, 1921, **68**, 420.

<sup>6</sup> Swingle, W. W., *Biol. Bull.*, 1917, **33**, 116.

<sup>7</sup> Gudernatsch, F., *Med. Record*, 1937, **146**, 101.

<sup>8</sup> Asher, L., and Ratti, P., *Klin. Woch.*, 1929, **8**, 2051.

<sup>9</sup> Rowntree, L. G., Clark, J. H., Hanson, A. M., and Steinberg, A., *Arch. Inter. Med.*, 1935, **56**, 1.

† The thymocrescin was prepared in this laboratory by the method of Asher.<sup>10</sup> One mg was equivalent to 0.97 g of raw calf thymus. The authors gratefully acknowledge the assistance of Parke, Davis & Co. in performing the preliminary extraction.

<sup>10</sup> Asher, L., *Abderhalden's Handbuch der biologischen Arbeitsmethoden*, 1936, **5**, 929.

‡ Dr. A. M. Hanson has generously supplied us with fresh calf thymus extract every 2 weeks.

TABLE I.  
Average measurements in mm of *Rana pipiens* larvæ.

Date:	5/14	5/21	5/28	6/4	6/11	6/18
Control diet	41	48	53	59	63	70
Plus Hanson's extract	41	47	52	58	62	72
'' Thymocrescin	39	48	52	58	62	70

which time most of the animals were undergoing metamorphosis.

Animals exhibiting one fore limb were considered certain to complete metamorphosis. The first appearance of such animals was on 6/11/38, at which time one was found in each of the aquaria. Observations were made daily until 6/29/38 (Table II) when the majority of the animals had metamorphosed.

TABLE II.  
Daily numbers of *R. pipiens* larvæ noted, newly showing one fore limb.

Date:	6/11	6/15	6/16	6/17	6/18	6/20	6/21	6/22	6/23	6/24	6/25	6/29
Control diet	1	3	2	4	0	2	2	2	2	3	0	0
Plus Hanson's extract	1	4	0	0	1	2	1	3	3	2	1	3
Plus Thymocrescin	1	3	1	5	1	4	0	2	3	2	2	2

From the data presented it appears that under the conditions of the experiment there was no specific effect of either thymus extract upon growth or metamorphosis of *Rana pipiens* larvae. The possibility exists, of course, that some effect might have been obtained if larger quantities of the extracts had been used, or if the material had been administered by injection.

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#### The Jacobson Method for Assay of Liver Extracts.

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In relation to the study of tropical anemias, it was the intention of the authors to follow the method advocated by Jacobson.<sup>1-5</sup> This

<sup>1</sup> Jacobson, B. M., *Science*, 1934, **80**, 211.

<sup>2</sup> Jacobson, B. M., *J. Clin. Invest. (Proc.)*, 1934, **13**, 714.

<sup>3</sup> Jacobson, B. M., *J. Clin. Invest.*, 1935, **14**, 665.

<sup>4</sup> Jacobson, B. M., *J. Clin. Invest.*, 1935, **14**, 679.

<sup>5</sup> Jacobson, B. M., *Brit. J. Exp. Path.*, 1936, **17**, 307.