tures were sterilized by 0.0039 units and in the remaining 2 by 0.0019 units. The test is subject to the error of serial dilution methods in general.

It is important to store all solutions containing penicillin in the ice box until the time of testing since high temperatures result in a loss of activity. In one experiment a solution of penicillin in saline containing 100 Florey units per cc lost all activity within 7 days when placed at 37°C, whereas the same solution stored at ice box temperature retained its antibacterial effect. Urines which contain penicillin have been stored in the ice box for 2 weeks without loss of penicillin potency.

For those fluids known to be contaminated, sterilization may be effected by passage through a Berkefeld or Seitz filter. This procedure is not attended by any appreciable loss of penicillin.

The sensitivity of the test is increased when a small number of organisms and a small total volume of culture media are used. In general, an inoculum of between 100 and 100,000 organisms is advisable. Variations within this range usually do not alter the results of the test. The addition of 1% erythrocytes to the culture media aids in reading the test. The cultures which show no hemolysis are usually sterile.

Conclusions. A method for determining the concentration of penicillin in various body fluids and exudates is described. It is possible by this method to determine 0.0039 Florey unit per 0.2 cc of solution.

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Changes in Body Proportions Produced in Frog Embryos by Supra-Normal Temperatures.

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Several investigators have produced changes in body proportions by varying the conditions of temperature in which development occurs. Hoadley[1] has found that constant exposure to slightly supra-maximal temperatures produced gradations of microcephaly in *Rana pipiens* and *Rana sylvatica larvae*. Huxley[2] and his associates[3] and Gilchrist[4] have found that temperature *gradients,* within the ranges of temperatures normal to development, when properly applied to the developing amphibian egg for short intervals have a stimulative effect which is apparent in the development of the larva. Considering the fluid environment of the egg membranes and of the embryo itself, it is improbable that an embryo in nature would be subjected to a temperature gradient. However, short exposures of the whole egg to temperatures higher than the usual maximum for normal development must frequently occur. Possible long range effects on the developnig embryo of abnormal temperatures due to the heating of the spawning ponds of certain amphibia and to fever in the case of the pregnant mammal present problems in this connection. The present work was undertaken to test the effect on body proportions of short exposures of the whole amphibian egg and embryo to temperatures above the normal.

The eggs of *Hyla regilla,* the Pacific tree frog, were used in this work. An effort was made to obtain the larger clusters of 25 or

1 Hoadley, Leigh, *Growth,* 1938, **2**, 25.

2 Huxley, J. S., *Arch. F. Entwmech.,* 1927, **112**, 480.

3 Dean, I. L., Shaw, M. E., and Tazelaar, M. A., *Brit. J. Exp. Biol.,* 1928, **5**, 309.

4 Gilchrist, Francis G., *J. Exp. Zool.,* 1933, **66**, 15.

30 eggs each, for the eggs of a single cluster were the basis for each experimental series in order that all individuals of each series might be as nearly alike in age and genetic constitution as possible. Each cluster was divided into 5 equal groups. When the eggs of Group I reached the late blastula stage, they were exposed to a higher temperature in a thermostatically controlled water bath. After the exposure they were returned to room temperature. Group II was heated for the same length of time at the same higher temperature at the onset of gastrulation. Group III was similarly treated when in the yolk plug stage. In turn, Group IV was exposed at an early tail bud stage. All groups including the unheated controls (Group V) were allowed to develop at room temperature for 4 days at which time hatching usually began. Then all members of the series were removed from their membranes and preserved for study. Exposure periods of 2, 3, 4, 5, and 6 hr were tried at 10 temperature levels between 29° and 38°C. This report will consider the body proportions of 262 individuals representing 9 experimental series in which exposures were made to temperatures below 35°C. Above this temperature marked developmental abnormalities appeared. Careful measurements of the body proportions were made with an ocular micrometer in a dissecting microscope. As a check on the accuracy of the method, Series I consisting of 25 larvæ was remeasured without reference to the original measurements two months after the originals had been made. Differences between the two sets of data thus obtained were so slight as to convince the writer of the accuracy of the method.

TABLE I.
Effect of Heat Treatment on Body Proportions.

	Mean length		
	Heat treated group	Control group	Mean difference (± S.D.)
Head	2.005*	1.880	0.125† (± 0.028)
Body	6.128	6.166	0.038 (± 0.046)
Tail	6.727	6.784	0.057 (± 0.088)

*Ocular micrometer units.
† Highly significant: $P < 0.01$.

Statistical treatment of the data summarized in Table I shows a highly significant difference between the mean head length of control and experimental animals. Body length and tail length were apparently unaffected by the treatment. These results apply to all groups within the experimental series whether treatment was applied for 2 hr or for 6, whether at 29° or at 35°C. Similarly no significant differences among the groups of experimental animals were found to result from treatments applied at different stages of early embryonic development (from the early blastula stage through the tail bud stage). Therefore, in the final summary (Table I) these differences in treatment were disregarded, and all heat-treated embryos were considered as one group.

Since tail length and body length (defined as head length plus trunk length) comprise the total length, it is evident that the changed head length of the heat-treated animals has altered the ratio of body proportions without affecting the total length. This suggests that an age difference is not involved and that the proportions of the embryonic body may be altered by appropriate short exposures to higher temperatures without resorting to the use of temperature gradients.

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