ingestion of glycerol. While in all of the cases studied this retention of phosphate was accompanied by a rise of the respiratory quotient, indicating increased carbohydrate metabolism, with the ingestion of glycerol the marked retention of phosphate was accompanied by a lowered respiratory quotient.

With diabetic subjects it was frequently observed that the ingestion of glucose or the injection of insulin did not cause a retention of phosphate especially when the blood sugar curves and the respiratory exchange indicated that there was no increased catabolism of carbohydrate. No general relationship was found between the retention of phosphate and the reaction of the urine as measured by the total titratable acidity. The determinations of reducing sugar and of phosphate in blood filtrates before and after hydrolysis with acid failed to reveal the presence in the blood of a carbohydrate-phosphate compound at the height of phosphate retention. If such compounds as hexose phosphate are formed in the process of the oxidation of carbohydrates they are probably to be found in the liver and muscles.

Our data furnish obviously further support to the theory of the close participation of the phosphate ion in the metabolism of carbohydrates.

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The oxygen consumption of thyroid and diiodotyrosine-fed tadpoles.

By O. M. HELFF (by invitation).

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It has been known for some years that thyroid-fed tadpoles undergo precocious metamorphosis, and more recently the fact has been demonstrated that diiodotyrosine has a similar effect upon amphibian larvæ. Students of amphibian metamorphosis have assumed that an increase in metabolic rate occurs during transformation, and the statement is even made that metamorphosis is due to increased metabolism. So far as the writer is

aware, however, no attempt has been made to test the validity of these assumptions. It seemed worth while, therefore, to measure the metabolic rate of precociously metamorphosing tadpoles at various stages of transformation. Since oxygen consumption is considered a rough gauge of metabolic rate, this method was employed.

EXPERIMENTAL

During the months of June, July, August and September, 1923, two hundred *Rana pipiens* tadpoles of approximately the same size and developmental stage were collected. They were fed for two days on Spirogyra and then starved for an equal period, preparatory to running the initial test for oxygen consumption. Dessicated thyroid, containing .20 per cent of iodine, and diiodotyrosine were the agents employed to accelerate metamorphosis. The animals were placed in small jars of water, one tadpole to a container, and kept at a constant temperature of $22 \pm 1°$ C. It was found that continued feedings of thyroid lowered the resistance of the animals to such an extent that death occurred before advanced stages of metamorphosis were reached. Consequently, the method employed was to feed the tadpoles one or two days with thyroid and then keep the animals in solutions of diiodotyrosine which, although it greatly hastens metamorphosis, does not weaken the larvæ to the extent that is true of continuous administrations of thyroid.

Successive stages of metamorphosis were arbitrarily selected: the normal tadpole showing no indications of transformation was designated stage one, the metamorphosed animal whose left fore leg had appeared and been free for at least a day was designated stage 8. The remaining six stages represented intermediate points of development. On the average, a thyroid-diiodotyrosine-fed tadpole passed from stage 1 to stage 8 in a period varying from eight to eleven days. Tadpoles so metamorphosed and whose oxygen consumption had been measured several times, invariably died after reaching stage 8, and consequently, no measurements were made on animals with both fore legs through the operculum.

The Winkler method was used throughout in determining the oxygen consumption, the water used in the experimental flasks being in all cases maintained at 24° C. It was found necessary

to etherize tadpoles of stage 1 (normal animals) during the test in order to eliminate errors arising from the activity of the individuals under operation. Etherization was found unnecessary in all succeeding stages since tadpoles undergoing transformation remain quiescent for considerable periods. All individuals were weighed accurately to the milligram, whenever tested, in order to determine the oxygen consumption per gram weight.

RESULTS

The results obtained show, beyond doubt, that there is a progressive increase in oxygen consumption per gram weight of metamorphosing tadpoles. Between stages 1 and 2 there is an increase of 48 per cent. Stages 3 and 4 show a still further increase of 59 and 76 per cent respectively. The next two stages (5 and 6) show a somewhat lessened increase over stage 1 as compared with stage 4 or 61 and 54 per cent respectively. The last two stages (7 and 8) give a respective increase of 71 and 79 per cent over the normal or stage 1 consumption, stage 8, as was expected owing to the advanced stage of metamorphosis, possessing the greatest increase per gram weight of any stage tested. Table I gives the average actual amount of oxygen in cubic centimeters consumed per gram weight in one hour for the eight stages, while Fig. 1 represents graphically the per cent increase in oxygen consumption of the same individuals.

TABLE I.

Average Oxygen Consumption in cc. per gm. wt.

No. Tested	Stage								Av. Increase
	1	2	3	4	5	6	7	8	
32	.157	.233							.076
19	.160		.254						.094
33	.160			.282					.122
36	.178				.286				.108
16	.171					.264			.093
27	.174						.298		.124
10	.192							.344	.152

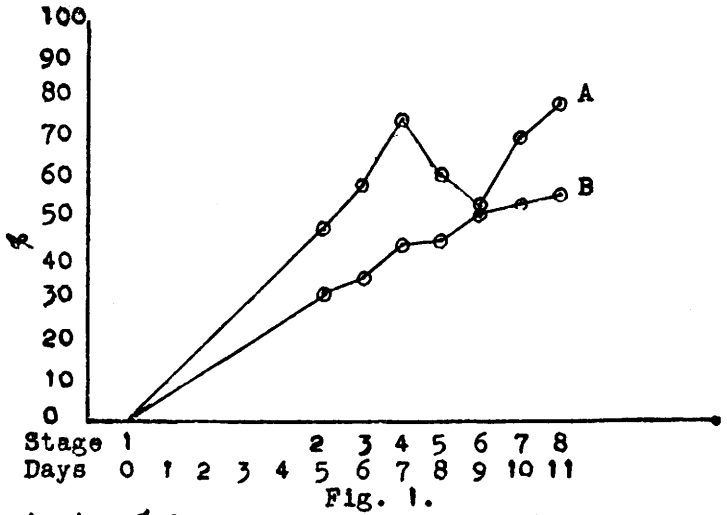

Fig. 1.

A. Av. % increase oxygen consumption per gm. wt.
B. Av. % decrease in total weight.

By referring to Table II, it is at once evident that there is a progressive decrease in total weight as the tadpole attains each successive stage of metamorphosis. Fig. 1 emphasizes this fact by graphically representing the per cent decrease in total weight as compared with the normal or stage 1 weights of the same individuals. If the per cent increase of oxygen consumption per gram weight was of the same percentage as the per cent decrease in total weight at any given stage, the conclusion would be that no increase in total oxygen consumption had occurred. In reality, however, the data show a progressive increase in total oxygen consumption with an increasing stage of metamorphosis.

TABLE II.

Average Weight in gms.

No. Tested	Stage								Av. Decrease
	1	2	3	4	5	6	7	8	
32	1.394	.942							.452
19	1.451		.922						.529
33	1.409			.793					.616
36	1.398				.765				.633
16	1.375					.662			.713
27	1.400						.643		.757
10	1.415							.605	.810

The bulk of the increase per gram weight, however, is accounted for by the large decrease in total weight. The falling off of the per cent increase in oxygen consumption in stages 5 and 6 is in a large measure due to the fact that many of the tadpoles tested at this stage were in a weakened condition and died within five to ten hours after the test and before reaching stage 6 or 7. In other words, the critical period in thyroid-diiodotyrosine metamorphosis seems to be during these stages. Once past these stages, the tadpoles recovered their vigor and hence the increased oxygen consumption in stages 7 and 8.

The effect of etherization showed that, if anything, anesthetization by this agent increased oxygen consumption. In fact, the average increase per gram weight of sixteen individuals so anesthetized, amounted to 19 per cent, which also represents the per cent increase of total oxygen consumed, since the normal and etherized tests were run within an hour of each other during which period no reduction in weight had occurred. This fact, then, adds emphasis to the percentage increases of oxygen consumption recorded in the later stages.

DISCUSSION

The writer was fortunate in obtaining a full series of tadpoles of the same species used in the oxygen tests, which were undergoing normal metamorphosis. Several striking dissimilarities were noticeable when compared to a similar series of thyroid-diiodotyrosin metamorphosed tadpoles. The greatest difference was found in the atrophy of the tail as compared to the growth of the limbs. For example: a normally metamorphosing tadpole, which had succeeded in protruding the left fore limb through the operculum, possessed a tail in the same stage of atrophy as was to be found on a typical stage 4 thyroid-diiodoytrosine metamorphosed tadpole, where the left fore limb would require several days yet to appear. In other words, the fore limb appeared much earlier in relation to the atrophy of the tail in the normal series than in the thyroid-diiodotyrosine series.

Other such differences in correlation were observed, making it clear that the thyroid-diiodotyrosine type of metamorphosis did not permit the atrophy, autolysis or growth of certain parts to proceed in the normal manner. This undoubtedly accounts for the high mortality rate met with in all series of animals so

metamorphosed. It would also account, in a large measure, for the considerable variation in oxygen consumption of individuals of the same metamorphic stage. This variation in oxygen consumption made it necessary to base the consumption at any given stage on the average of a considerable number of animals.

CONCLUSIONS

1. *Rana pipiens* tadpoles, metamorphosed with dessicated thyroid-diiodotyrosine exhibit a progressive rise in metabolic rate as evidenced by oxygen consumption per gram weight. In stage 8 the metabolic rate reaches a maximum increase over that of stage 1 of approximately 79 per cent.

2. Precociously metamorphosed tadpoles increase their total oxygen consumption regardless of decreasing weight.

3. Thyroid-diiodotyrosine-fed tadpoles undergo a gradual decrease in weight amounting to 57 per cent in stage 8.

4. Etherization of stage 1 tadpoles (normal animals) results in approximately 19 per cent increased oxygen consumption.

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The study of hemolytic streptococci associated with scarlet fever.

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The present work was undertaken subsequent to the work of Gordon, Bliss and of Tunnicliff in the study of hemolytic streptococci associated with scarlet fever. The object of the work was to study the agglutination of streptococci from the throats of patients with scarlet fever, and to study the phenomenon of agglutinin absorption with these bacteria. The strains were gathered from numerous sources. The technique was similar to that of Bliss.[1]

[1] Bliss, W. P., *J. Exp. Med.*, 1922, xxxvi, 575.