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A Note on the Color Test for Pentoses.

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With the benzidine color test recently described[1] the following glucuronates* have been tested; borneol glucuronic acid, menthol glucuronic acid, and glucuronic acid monobenzoate. When 20 mg. samples were employed the test was negative, whereas Bial's test was positive with 2 mg. except in the case of borneol glucuronic acid which showed a positive test with Bial's reagent only when large amounts were employed. Glucuronic acid and glucuronic acid lactone gave a positive test with more than 0.2 mg. In this respect the benzidine test resembles Bial's test.

The new test is based on the formation of furfural. This aldehyd gives a red color with the benzidine solution without boiling.

In view of the fact that glucuronic acid does not occur free in urine, but in combination with phenols, etc., the benzidine test may be used to differentiate between pentoses and glucuronates.

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Effect of pH upon Metamorphosing Action of Thyroxine on Tadpoles.

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The basis for the present study was an observation made in experiments performed in the spring of 1935 to investigate the finding of Zondek and Reiter[1] that $CaCl_2$ inhibited and KCl accelerated the metamorphosing action of thyroxine on tadpoles. It was observed that, while $CaCl_2$, Ca gluconate and KCl media, all with the same pH, 6.4-6.8, as our tap water control medium, had no effect upon the metamorphosing action of thyroxine,* alkaline Na phosphate

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[1] Tauber, H., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 600.

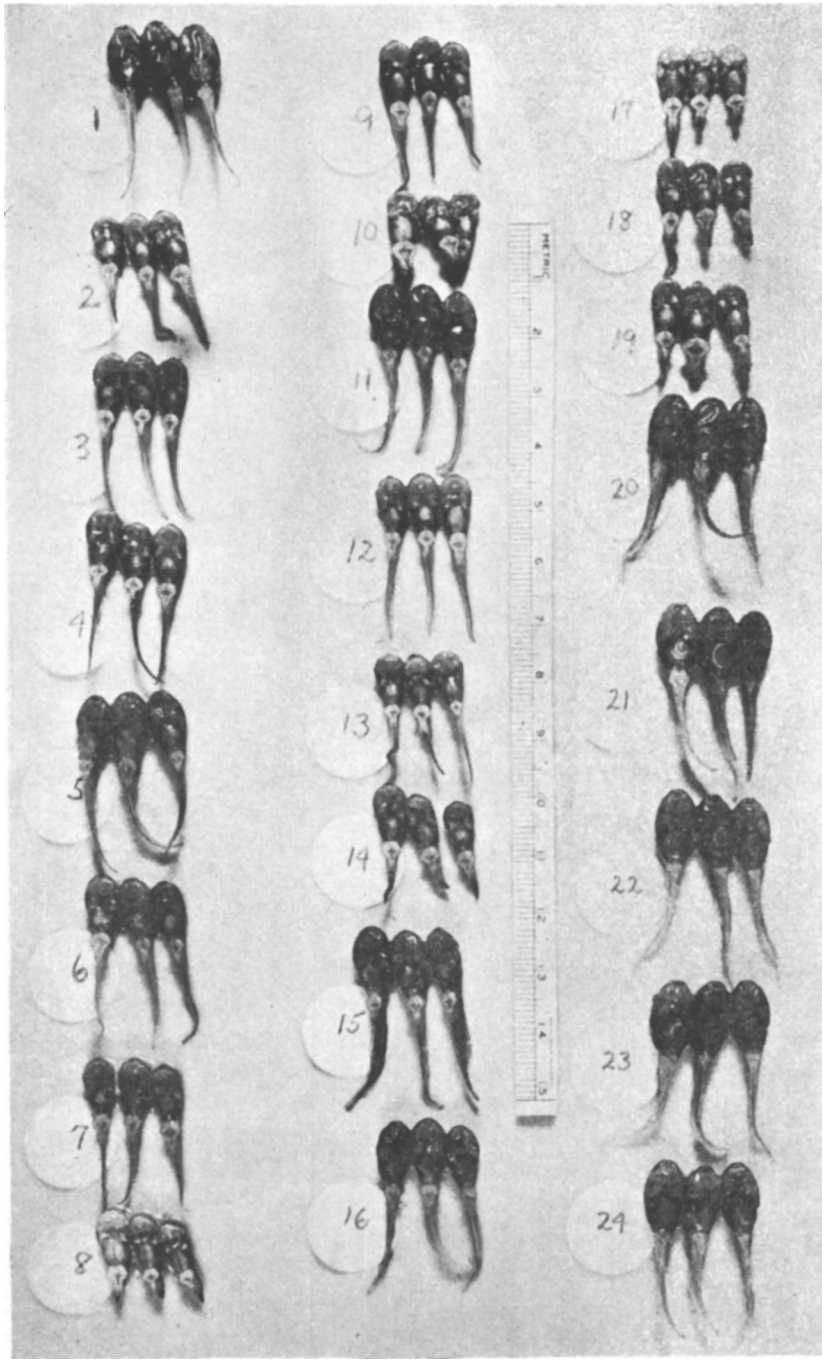
* I am indebted to Dr. A. J. Quick for samples of glucuronates.

[1] Zondek, H., and Reiter, T., *Klin. Wschr.*, 1923, **2**, 1344.

* Kosmin and Resnicenko[3] had also obtained negative results with $CaCl_2$ and KCl, and Hellwig[4] subsequently obtained negative results with $CaCl_2$.

[3] Kosmin, N. P., and Resnicenko, M. S., *Trudy laboratorii eksperimentalnoj biologii Moskovskogo zooparka*, 1927, **3**, 9.

[4] Hellwig, C. A., *J. Lab. and Clin. Med.*, 1936, **21**, 1131.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FIGS. 1-24.

Group	Thyroxine	Solutions Tested	%	pH
1	—	—		6.4
2	1:20 million	—		6.4
3	"	$Na_2HPO_4 \cdot 12H_2O$	.036	8.2
4	"	"	.36	8.8
5	"	$Na_3PO_4 \cdot 12H_2O$	.12	11.0
		$NaH_2PO_4 \cdot H_2O$	.035	
6	"	$Na_2HPO_4 \cdot 12H_2O$	.2	8.4-8.6
7	"	"	.08	6.6-6.8
		$NaH_2PO_4 \cdot H_2O$	.046	
8	"	"	.078	5.0-5.2
9	"	$Na_2HPO_4 \cdot 12H_2O$	.2	6.8
		HCl		
10	"	"	.2	4.8-5.0
11	"	$CaCO_3$ saturated sol.		10.0
12	"	" " "		8.0
		HCl		
13	"	$CaCO_2$ saturated sol.		6.4-6.6
		HCl		
14	"	$CaCO_2$ saturated sol.		4.8-5.0
		HCl		
15	"	Na_2CO_3	.012	10.0
		$NaHCO_3$	.062	
16	"	$3MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$ saturated sol.		10.0
17	"	Citric acid		4.8-5.0
18	"	Acetic acid		5.0
19	"	HCl		4.8-5.0
20	—	$Na_3PO_4 \cdot 12H_2O$	.12	11.0
		$NaH_2PO_4 \cdot H_2O$	.035	
21	—	$CaCO_3$ saturated sol.		10.0
22	—	$NaH_2PO_4 \cdot H_2O$	0.1	5.0
23	—	Acetic acid		5.0
24	—	HCl		5.0

media, pH 8.4 and 9.6, inhibited, and acid Na phosphate media, pH 5.4, accelerated the action of thyroxine. Abelin[2] had obtained a similar inhibiting effect, although not consistently, with the alkaline phosphate Na_2HPO_4, but the acid phosphate, NaH_2PO_4, had no effect; and he implied that a control experiment with another alkaline salt, $NaHCO_3$, gave negative results. He, therefore, attributed the inhibiting effect of Na_2HPO_4 to the PO_4 ion. Our findings, however, indicated that the effects of the phosphate media were dependent upon their pH, and suggested that alkalinity and acidity, *per se,* caused inhibition and acceleration of the metamorphosing action of thyroxine irrespective of particular ions. To demonstrate the latter, experiments were performed, in the spring of 1937, in which a number of different alkaline and acid solutions were employed.

The tadpoles *(Rana sylvatica)* were taken from a single brook. For the experiments groups of 6 or 7 of uniform size and stage of development (no extremities and large tails) were transferred to small enamel dishes. The following was the daily procedure. From

about 9 A.M. to 1 P.M. the tadpoles were fed grated egg white in fresh tap water. At about 1 P.M. the tap water was again changed, and the thyroxine and solutions to be tested added so that the final volume in each dish was 400 cc. The pH was then determined colorimetrically. The dishes were kept at room temperature exposed to daylight during the experimental period of 5 days. The usual criteria of thyroid action on tadpoles, namely, hastened differentiation (growth of extremities, tail absorption, etc.) and emaciation, were employed. Thyroxine, freshly dissolved in distilled water with the aid of 1 or 2 drops of 10% NaOH at the beginning of the experiments, was used in the tadpole media in final concentration of 1 to 20 million. The possibility that thyroxine might precipitate out of the acid media during the 20-hour daily test period was ruled out by tests in which even more acid solutions, pH 3 to 4, and greater concentrations of thyroxine, 1 mg. in 1 liter or 1 to 1 million, were employed.

Our earlier observations on the effects of alkaline and acid phosphates were confirmed and extended. The alkaline phosphate, Na_2HPO_4, pH 8.2-8.8, retarded the metamorphosing action of thyroxine (Figs. 3, 4, 6). There was no appreciable difference in the degree of retardation dependent upon the concentration of the salt; this is at variance with the finding of Abelin[2] that lower concentrations of Na_2HPO_4 were more effective. A mixture of Na_3PO_4 and NaH_2PO_4, pH 11, (Fig. 5) caused slightly greater retardation of the thyroxine action than did Na_2HPO_4, pH 8.2-8.8. The acid phosphate, NaH_2PO_4, pH 5.0-5.2, on the other hand, definitely accelerated the metamorphosing action of thyroxine (Fig. 8).

Confirmatory evidence that the different effects of the above phosphates were dependent solely upon the pH of the solutions was obtained from the following 2 experiments. The phosphates Na_3PO_4, Na_2HPO_4 and NaH_2PO_4 were employed in such combinations and concentrations as to give the following range of pH: 11, 8.4-8.6, 6.6-6.8, and 5.0-5.2, while containing in every instance the same amount of P, namely 70 mg. (Figs. 5, 6, 7, 8). Comparison of the photographs of these groups with that of the tap water thyroxine control (Fig. 2) reveals a difference, as well as a gradation of effects ranging from retardation of the thyroxine action on the alkaline side to acceleration on the acid side; while in the phosphate combination with the pH 6.6-6.8 the tadpoles show almost the same degree of differentiation and emaciation as in the tap water thyroxine control pH 6.4.

[2] Abelin, I., *Klin. Wschr.*, 1923, **2**, 1650.

The second experiment was the following. Na_2HPO_4 was used in the same final concentration, namely, 0.2% or 70 mg. P, in 3 dishes. One, to which nothing further was added, had a pH of 8.4-8.6. To the second and third, HCl was added to bring the pH to 6.8 and 4.8-5.0 respectively (Figs. 6, 9, 10). The range of effects from retardation of the thyroxine action in the alkaline dish to acceleration in the acid dish, and absence of, or only slight retarding effect in the second dish with a pH only slightly above the tap water-thyroxine control, pH 6.4, is clearly demonstrated.

The effect of hydrogen ion concentration was similarly demonstrated with another salt (Figs. 11, 12, 13, 14). By the addition of increasing amounts of HCl to a fixed amount of saturated $CaCO_3$, a range of pH of 10, 8.0, 6.4-6.6, and 4.8-5.0 was obtained, while maintaining in each instance the same amount of Ca, namely 2.1 mg. As with the phosphate so treated, a range of effects was obtained from retardation of the thyroxine action on the alkaline side to acceleration on the acid side; while at pH 6.4-6.6 there was no effect upon the thyroxine action when compared with the tap water thyroxine control of the same pH.

Further evidence that alkalinity retards and acidity accelerates the metamorphosing action of thyroxine, irrespective of the ions employed, was obtained by the use of a number of different salts and acids, namely, Na_2CO_3 and $NaHCO_3$, pH 10 (Fig. 15), saturated $MgCO_3$, pH 10 (Fig. 16), citric acid, pH 4.8-5.0 (Fig. 17) acetic acid, pH 5.0 (Fig. 18), HCl, pH 4.8-5.0 (Fig. 19). In every instance the alkaline salts definitely retarded, and the acids definitely accelerated the action of thyroxine.† Also significant was the observation that groups of tadpoles subjected to the influence of thyroxine at the same, or nearly the same pH, regardless of the composition of the medium, showed similarity in size and stage of metamorphosis.

That acidity and alkalinity, *per se*, had no effect, in the 5-day experimental period, upon the growth or metamorphosis of the tadpoles was demonstrated by the use of some of the alkaline and acid media without thyroxine, namely, Na_3PO_4 and NaH_2PO_4, pH 11

† The observation of Brandt[5] that the water of the Eugenquelle in Bad Kudowa, a German spa, inhibited the metamorphosing action of thyroxine and desiccated thyroid on axolotls, apparently supports this finding with respect to the effect of alkalinity. Although Brandt attributed the inhibiting effect to "the biological action of the combination of all the constituents" of the Eugenquelle water, the fact that this water is rich in alkaline salts, and that its free CO_2 was allowed to escape before it was used, makes it probable that the inhibiting effect was due to alkalinity of the water.

[5] Brandt, W., *Z. f. d. ges. exp. Med.*, 1936, **99**, 478.

(Fig. 20), saturated $CaCO_3$, pH 10 (Fig. 21), NaH_2PO_4, pH 5.0 (Fig. 22), acetic acid, pH 5 (Fig. 23), and HCl, pH 5 (Fig. 24).

Conclusion. Acidity accelerates and alkalinity retards the metamorphosing action of thyroxine on tadpoles.

9782 P

Action of Ultra-violet on Members of the *Pseudomonas fluorescens* Group.

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The early theory of the destructive action of ultra-violet rays on bacteria was that hydrogen peroxide was produced in the medium. Oker-Blom[1] proved that the death of the bacteria was not caused by hydrogen peroxide, but by the direct action of the rays on the protoplasm of the organism. Burge and Neill[2] found fluorescent organisms to be more resistant to ultra-violet than colon organisms. They attributed the superior resistance of the members of the fluorescent group to their power of converting the short waves into long ones, and thereby escaping the coagulative action of the short waves on the protoplasm.

The investigation reported in this paper was undertaken to determine whether the pigment produced by organisms of the genus *Pseudomonas* protects these organisms from radiant energy furnished by a quartz mercury-vapor lamp.

A suitable medium for irradiation was first considered. Both plain broth and lactose broth were found unsuitable because they protected the organism from the effects of the ultra-violet. The synthetic asparagine medium proposed by Georgia and Poe[3] was found to be satisfactory.

In the asparagine medium, 12-, 24-, 36-, and 48-hour cultures of several members of the *Pseudomonas* group were prepared; 10 cc. of each culture were diluted to 100 cc. with sterile water, and 4 cc. of the diluted culture were exposed to the vertical rays of a Gallois mercury-vapor lamp from one to 40 minutes. Undiluted cultures were also tested. Appropriate dilutions of the irradiated cultures

1 Oker-Blom, Max, *Z. f. Hyg.*, 1913, **74**, 242.

2 Burge, W. E., and Neill, A. J., *Am. J. Physiol.*, 1915, **38**, 399.

3 Georgia, F. R., and Poe, C. F., *J. Bact.*, 1931, **22**, 249.