evaluated. These data indicate the presence of a heat labile spreading factor in the relaxin preparation.

Heat inactivation (60°C for 10 minutes) of the spreading factor failed to diminish the ability of the relaxin preparation to cause pelvic relaxation. Kroc(4) employed even higher temperatures in investigating the heat resistance of relaxin. He found that extracts similar to those employed in these experiments retained all or nearly all their "relaxin" ability after being heated to 90°C-95°C for 10 minutes or autoclaving at 15 lb pressure for 15 minutes.

These data indicate that the mammary spreading factor and relaxin differ both in heat tolerance and biological activity. Further it appears that the spreading factor present in the relaxin preparation plays no part in the relaxation phenomena and that the hormone possessed little or no ability to increase dermal permeability. Therefore, it is probable that the ovarian extract spreading factor was merely present in the relaxin extract and had no connection with the hormone.

It seems possible that the relaxin extract employed by Hamolsky and Sparrow might contain a spreading factor similar to the one observed in this study. There are also possibilities that ovarian extracts of relaxin now in use might contain other biologically active components. Therefore, it is suggested that in future experiments care should be taken to determine whether the synergism of relaxin preparations with estrogen and progestin which produced increased growth of the mammary is due to relaxin, the spreading factor or an undetermined substance.

Summary. 1. It was observed that a relaxin preparation extracted from swine ovaries contained a spreading factor which was inactivated by heating at 50°C for ten minutes. The inactivation of the spreading factor did not diminish the effectiveness of the relaxin preparation to cause pelvic relaxation. 2. The spreading factor extracted from the mammary glands of pregnant rats had no ability to cause pelvic relaxation in the amounts employed.

4. Kroc, R. L., Personal Communication.

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Effect of Administration of Intermedin upon Melanin Content of the Skin of *Rana pipiens.* (18669)

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The relation of humoral factors, especially of the pars intermedia, in controlling the color responses of amphibia have been clearly elucidated by the investigations of Hogben, *et al.*, (1,2). Although the melanophore hormone* is also considered to be involved in melanin synthesis(4), evidence for its role in influencing the actual production of melanin is less direct. Odiorne(5) observed significant decreases in total numbers of melanophores and melanin granules in fundulus maintained for long periods on light backgrounds, as well as a reversal of this effect when the animals were placed on a black background. Sumner (6,7) measured changes in melanin content

* Although some doubt exists as to the identity of intermedin, the name given by Zondek(3) to the erythrophore-expanding substance of the pituitary, with the melanophore hormone, the two terms are used interchangeably.

1. Hogben, L. T., and Slome, D., *Proc. Roy. Soc., B,* 1931, v108, 10.

2. Hogben, L. T., and Slome, D., *Proc. Roy. Soc., B,* 1936, v120, 158.

3. Zondek, B. and Krohn, H., *Klin. Wochschr.,* 1932, v11, 405.

4. Waring, H. and Landgrebe, F. W., in *The Hormones,* p484, Academic Press, New York, 1950.

5. Odiorne, J. M., *J. Exp. Zool.,* 1936, v74, 7.

6. Sumner, F. B., and Doudoroff, P., *Proc. Nat. Acad. Sci. Wash.,* 1938, v24, 463.

7. Sumner, F. B., *Biol. Bull.,* 1943, v84, 195.

of the skins of several species of fish as a function of background albedo, noting that both melanin content and total melanophore number decreased on exposure to light backgrounds. Dawes(8) obtained similar results using *Rana temporaria;* in addition, he showed that prolonged dark adaptation resulted in an actual increase in melanin content. Direct evidence for the implication of intermedin as a factor in melanin formation is limited to a single experiment performed by Dawes. Perfusion of the severed left hind legs of 6 frogs with posterior pituitary extract resulted during 48 hours in a 33% increase in melanin content of these, as compared to control limbs.

The rapidity of the effect observed in these perfusion experiments, especially when contrasted with the slow increases observed with intact animals, suggested the desirability of a reinvestigation of the effect of exogenous intermedin upon melanin synthesis in frogs. It has been found that prolonged administration of intermedin preparations to frogs maintained in surroundings of neutral albedo results in an initial *decrease* in melanin content. This is followed by a slow increase which results, after a total of 8 weeks, in melanin levels approximately 40% above those of control animals.

Experimental. Intermedin preparations. Whole pituitaries of hogs, stored frozen until used, were extracted with dilute (0.25%) acetic acid at 90°C. After removal of the insoluble residue, ethanol was added to the filtrate to a final concentration of 70%. The inactive precipitate was removed, and the ethanol content of the filtrate was raised to 90%, whereupon most of the activity precipitated. The precipitate was redissolved in water and the solution stored in the frozen state. Melanin determinations were made essentially according to the method of Dawes. As a check upon the reproducibility of the method, each skin, after removal from the animal, was divided into 2 approximately equal parts. Each half was weighed, boiled in distilled water for 15 minutes, and finally digested in 2% pepsin at pH 1.2 for 48 hours at 48°C. The undissolved residue was washed twice with water and then extracted with two 50 ml portions of hot 1M NaOH. After diluting the combined alkaline extracts to 100 ml, the transmission of the resulting solution was read in a Klett colorimeter, equipped with a filter having maximum transmission at 540 mμ. The results have been expressed, arbitrarily, by dividing the observed extinction by the weight of skin used. In determinations of the melanin content of the skins of twenty-seven control animals, the average discrepancy between the two values for each animal was 2.1%; the greatest difference encountered was 5.0%. Seventy-two frogs (*Rana pipiens*) were divided into 2 groups containing, respectively, 24 control and 48 experimental animals; the average body weight was the same for each group. They were kept in gray stone aquaria under ordinary laboratory lighting conditions (out of direct sunlight) at 15-20°C. All but 3 survived for the duration of the experiment (8 weeks).

Appropriate preliminary experiments indicated that a single injection of 1500 units† of intermedin was required to effect maximal darkening which would persist for 24 hours. To ensure a sufficiently high level of the hormone, about 9600 units, contained in 0.1 ml of solution, was injected into each experimental animal daily. The controls were injected with an equal volume of normal saline. Groups of control and experimental animals were killed at intervals of two weeks for determination of total melanin.

Results. It was found (Table I) that the injection of relatively large quantities of intermedin results in a decrease in melanin content during the first four weeks of the experiment. The difference between control and experimental values at 2 weeks is of doubtful significance ($p = 0.13$); at 4 weeks, however, there is a real difference ($p < 0.01$) between the means of the 2 sets of determinations. This trend is rather quickly reversed, so that at 6 weeks the 2 groups are practically

8. Dawes, B., *J. Exp. Biol.*, 1941, v18, 26.

† The unit here referred to is defined by Frieden, Fishbein and Hisaw(9), and is approximately equal to the intermedin content of 0.1 microgram of standard posterior lobe powder.

9. Frieden, E. H., Fishbein, J., and Hisaw, F. L., *Arch. Biochem.*, 1948, v17, 183.

TABLE I.

Period of treatment (weeks)	No. of animals		Avg body wt		Melanin contents*			
	control	treated	control, g	treated, g	Control	Treated	Difference	p
2	5	10	36.1	38.0	.176 ± .051	.147 ± .020	—.029	.13
4	6	12	36.2	39.7	.154 ± .020	.125 ± .011	—.029	<.01
6	5	13	40.3	36.9	.140 ± .020	.147 ± .019	+.007	>.50
8	7	11	40.5	36.4	.138 ± .017	.200 ± .023	+.062	<.01

* Melanin contents are expressed in terms of relative color intensity per g of skin. Each value is accompanied by an estimate of its standard error.

identical with respect to melanin content. The value for the treated group then raises rapidly, reaching 41% above the control level at 8 weeks, at which time the experiment was terminated.

The downward trend observed in the pigment content of the skins of control animals during the 8 weeks of the experiment is accompanied by a narrowing of the range of individual values. This may be attributed to the fact that of the heterogeneous group of animals initially selected some were intensely pigmented; these would be likely to lose pigment during the time they were exposed to the experimental environment, which included a gray background and relatively low average illumination intensity. Initially paler animals, on the other hand, would be expected to undergo little change.

Assays of the pituitary glands of control and experimental animals after the eighth week indicated that the hypophyses of animals receiving exogenous intermedin responded by a considerable decrease in total intermedin. Thus, the pituitaries of untreated animals averaged 525 units per gland, whereas the corresponding value after eight weeks of treatment was 140 units per gland.

Discussion. Our results are in decided contrast to the rapid changes obtained in Dawes' perfusion experiments, but are in general qualitative agreement with his background studies.

Perhaps the most surprising result of this investigation was the initial relative decrease in melanin observed. It is conceivable that dispersion of pigment within the melanophores facilitates the normal metabolic processes by which the pigment is degraded; if so, this must occur before the melanin-synthesizing mechanism is significantly accelerated.

It is not yet clear whether the increase in melanin which follows prolonged administration of intermedin is referable directly to the increased concentration of hormone in the tissues, or is an indirect effect resulting from long-continued dispersion of pigment granules within the chromatophores. It is quite likely that oxidation of melanin precursors may be facilitated by an increase of effective surface: volume ratio of pigment. It might be possible to settle this question by varying the amount of hormone injected.

Summary. The administration of intermedin, prepared from hog pituitary glands, results in a decrease, after four weeks, of twenty per cent in the melanin content of the skin of *Rana pipiens*. Subsequently, there is a rapid increase, reaching levels 40% above normal after a total of 8 weeks.

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