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Effect of Route of Administration on the Bioassay of Prolactin.*

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Our published method¹ for the bioassay of prolactin is based on the growth response of the crop-glands of pigeons after injection into the pectoral muscles. The data of the present communication show that when prolactin is injected otherwise than by the intramuscular route very widely different values are obtained. This point has some immediate practical importance since intramuscular injection of pigeons is being used for this assay in several laboratories, and the results here reported indicate that injections so made as to permit seepage from the muscle into subcutaneous areas would yield erroneously high values. It is also of theoretical interest that mere mode of administration of this hormone may have as much as a 12-fold effect on its potency as a stimulator of the crop-gland.

In previous studies the great influence of age¹ and race of pigeon upon this bioassay was demonstrated. The present study was therefore made on pigeons of the same age and breed. White Carneaux (from the Palmetto Pigeon Plant, Sumter, S. C.) were injected and killed at 6 to 7 weeks after hatching. Five routes of injection were tested; intramuscularly, deep into the breast muscle; intravenously into the wing veins; subcutaneously, and intracutaneously over the breast muscle or on the back and sides behind the leg—the region of the crop-sacs being carefully avoided. The volume injected was 0.4-0.5 cc. daily excepting that 0.08 cc. was used intracutaneously. All birds were injected once daily for 4 days and killed 94-100 hours after the first injection. The same preparation (No. 469) of prolactin was used throughout.

The more essential data—those showing the relative potency of 1 mg. prolactin when administered by 5 different routes—are presented in Table I. These data show that the subcutaneous and intracutaneous routes are most effective; least effective is intravenous injection. The crop-sacs of all birds injected intravenously showed visible thickening when held against a source of light,

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¹ Riddle, O., Bates, R. W., and Dykshorn, S. W., *Am. J. Physiol.*, 1933, **105**, 191.

TABLE I.
Quantitative Response of Crop-glands of Immature Pigeons to Prolactin Adminis-
tered by 5 Different Routes. Dosage = 1 mg. per day for 4 days.

Route of injection	No. birds	Body wt.	Crop-gland weight		Potency* Units/mg.
			Av.	σ	
		gm.	mg.		
Control	30	476	1245 $\pm$ 33		0.0
Intravenous	10	498	1610 $\pm$ 113		1.0
Intraperitoneal	10	465	1890 $\pm$ 91		1.4
Intramuscular	15	457	2355 $\pm$ 188		2.4
Intracutaneous	10	460	3790 $\pm$ 233		10.0
Subcutaneous	15	459	3940 $\pm$ 232		11.5

*Calculated unitage per mg. corresponding to the average crop-gland weight.

although their weights were not all significantly increased; and a similar amount of stimulation was obtained in 10 birds to which 0.1 mg. was administered subcutaneously. This confirms the values of units per mg. as calculated from the average crop-gland weights and shown in the last column of the table.

The conversion of increased crop-gland weights into prolactin units makes possible the expression of the efficiency of different routes of administration in simple terms. Subcutaneous injection is thus found to be about 5 times as efficient as intramuscular and about 11.5 times as efficient as intravenous injection. These differences in response are perhaps associated mainly with unequal rates of absorption from the sites of injection; but it is also possible that unequal rates of enzymatic destruction of hormone at the various sites of administration are involved. Bearing on this point is our earlier reported¹ result showing that dividing the daily (intramuscular) doses of prolactin into three spaced injections gives an increased effect on the crop-gland. Since that increase is of the order of four-fold one observes that the division of an intramuscular dose into more than 3 time-spaced injections should make it as effective as the same amount of prolactin given in a single subcutaneous injection.

We have confirmed the finding of Lyons and Page² that the amount of prolactin that can be detected by single intracutaneous (intradermal) injection directly over the crop-sac (10 tests) is about 1/100th that which can be detected by injection elsewhere in the body. This is not true, however, of single subcutaneous injections made directly over the crop-gland; for, in 23 such tests we found the threshold dose not greatly different from that of daily injections

² Lyons, W. R., and Page, Emery, *PROC. SOC. EXP. BIOL. AND MED.*, 1935, **32**, 1049.

over a 4-day period into subcutaneous areas remote from the crop-sacs. Here again we find another striking example of the importance of route of injection. It is notable also that if subcutaneous instead of intramuscular injection were used in the "threshold" bioassay method of McShan and Turner³ a unit of notably smaller size than that described by them would be expected, unless indeed seepage into the subcutaneous area normally occurs after their injections into very superficial parts of the pectoral muscles of the pigeon.

In our tests with the effect of route of administration on the potency of pituitary F.S.H. we have failed to obtain a significant difference (weights of testes of immature pigeons). And, again unlike prolactin, division of the daily dose of F.S.H. into 3 time-spaced injections failed to change the amount of the response of the testes. In the rat, however, Collip has found subcutaneous injection of gonadotropic hormone much more effective than intraperitoneal injection.

Summary. In young pigeons of the same age and race the response of the crop-glands to prolactin administered by 5 different routes has been studied and found to differ widely. Subcutaneous and intracutaneous injections are about 11 times as efficient as intravenous injections, about 5 times as efficient as intramuscular injections, and about 8 times as efficient as intraperitoneal injections at the dosage level used.

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Type-Specific Anti-M Precipitins in Rheumatic and Non-Rheumatic Patients With Hemolytic Streptococcal Infections.

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The thesis concerning the relationship between streptococcal infections, rheumatic fever, and rheumatoid arthritis, rests in part on clinical observation and in part on a study of antibodies against streptolysins,¹ fibrinolysins,² and various chemical components of

³ McShan, W. H., and Turner, C. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1936, **34**, 50.

¹ Todd, E. W., *Brit. J. Exp. Path.*, 1932, **13**, 248.

² Tillett, W. S., *J. Bacteriol.*, 1935, **29**, 111.