weight. Insoluble non-collagenous protein decreased with increasing body weight until 270 g. With further increases in age, dermal scleroprotein increased until the percent of total non-collagenous protein which was insoluble was essentially the same as that found in 100g rats. The maximum concentration of ground substance, total collagen and scleroprotein were found in the skin of rats weighing 150, 350 and 100g respectively. Maximum concentrations of insoluble hexosamine and insoluble collagen were found in the skin of rats weighing 500 and 270g respectively. The differing effects of aging upon ground substance, insoluble collagen and scleroproteins indicate that different mechanisms, possibly hormonal, were involved in controlling the concentration of each component, and suggest that these materials may be metabolically independent of each other.

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Melanophore-Dispersing Activity of Reserpine in Rana Frogs.* (26603)

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The effect of reserpine on the endocrine system in mammals and man has been extensively studied(1-10). We wish to report here on the stimulating effect of reserpine on melanophore dispersion in *Rana pipiens* frogs.

Materials and methods. Fifty adult male and female green-adapted Rana frogs, weighing 30-60 g each, were used. Reserpine stock solution was prepared according to the method described in Martindale's Extra Pharmacopoea, 1959, and diluted to the required concentration. *Exp. 1*: Pituitary homogenates of Rana frogs were injected into 5 Rana frogs to establish the course and extent of the melanophore-dispersing activity of normal frog pituitaries. *Exp. 2*: Reserpine was added *in vitro* to Rana frog skins preserved in Ringer solution to determine whether reserpine has a direct effect on frog-skin melanophores. *Exp. 3*: Single doses of 0.05-0.25 mg reserpine were subcutaneously injected into the dorsal lymph sac of 10 frogs, while 10 other frogs were injected with the solvent alone. Each frog was kept in a separate vessel and watched for 4 weeks.

The degree of darkening was evaluated quantitatively by determining the melanophore index (M.I.) according to Hogben's melanophore scale method(11-14). The M.I. of the middle hind webs of the frogs was determined microscopically.

Results. Exp. 1: Injection of Rana frog pituitary homogenates into normal Rana frogs resulted in darkening of the skin within 6-12 hours. This effect passed within 48 hours. *Exp. 2*: No effect was observed when reserpine was added to Ringer solution-pre-

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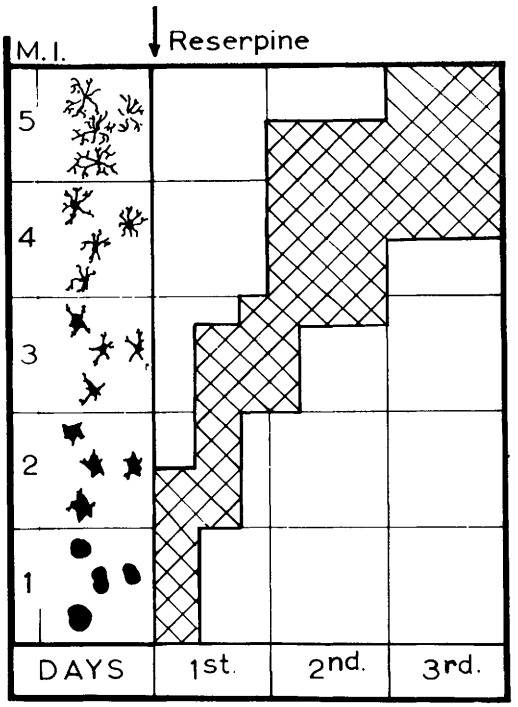

FIG. 1. Melanophore expansion in the large hind web of a Rana frog 3, 6, 12 hours and 2-3 days after injection, respectively. Melanophore index readings (M.I.) according to Landgrebe and Waring(14).

served Rana frog skins. *Exp. 3*: Within 3 hours of injection, the reserpine-treated frogs assumed a darker coloration and became almost black within 24-48 hours, 30% of them retaining their darker skin color for more than 3 weeks. The controls showed no discoloration.

Microscopic examination showed that within a few hours after injection of reserpine, the M.I. rose from the usual 1-2 to a maximum of 5 on the 2nd-3rd day (Fig. 1, 2, 3). Severe ptosis appeared within 1-2 days in all the treated frogs and lasted for 2-3 weeks.

Discussion. In mammals, reserpine was reported to interfere with the normal functioning of the anterior and posterior lobes of the pituitary, which effect was ascribed to its depressing action on the hypothalamus.

Reserpine in adequate doses caused suppression of anterior pituitary FSH and LH secretion(1,3,9), of TSH secretion(6,7) and of the exophthalmic factor(8). Elevation of prolactin secretion(4,5,9) and transient elevation of ACTH secretion followed by decline were also reported(10). The possibility of a stimulating reserpine effect on the pituitary posterior lobe function through release of ADH was also suggested(1) and confirmed by us. Our present experiments revealed an additional effect of reserpine on the posterior lobe of the pituitary, shown by increased endogenous secretion of MSH. Thus the following picture emerges: reserpine suppresses FSH, LH, TSH and ACTH, but increases LTH, ADH and MSH. This is in conformity with our hypothesis for the mechanism of the endocrine effects obtained with phenothiazine derivatives(15) which probably also holds true for reserpine(9). Following suppression of the hypothalamus, a secondary impairment of pituitary and peripheral glands occurs. With regard to part of the pituitary tropins (FSH, LH,

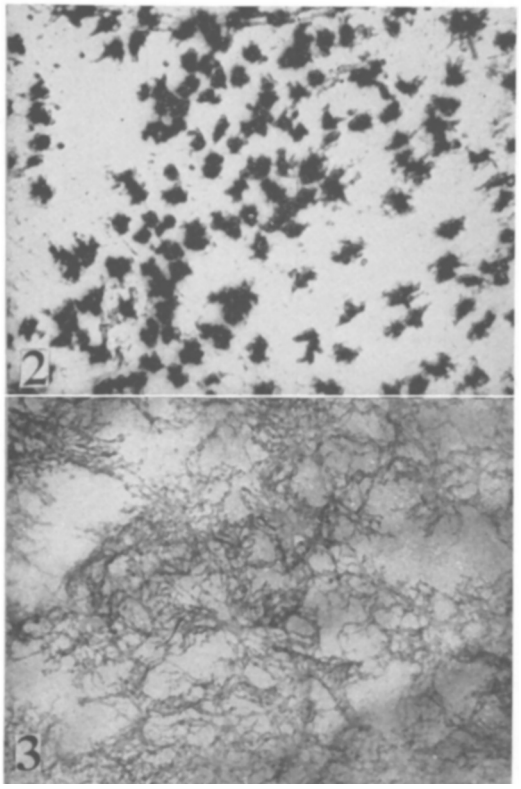

FIG. 2. Microscopic appearance of melanophores in the large hind web of a non-treated Rana frog; M.I.: 1-2 (× 100).

FIG. 3. Microscopic appearance of melanophores in the large hind web of Rana frog 3 days after s.c. injection of 0.1 mg reserpine; M.I.: 5 (× 100).

TSH, ACTH) this suppression becomes balanced according to the push and pull principle, which applies readily to the tropins producing secondary peripheral hormones (balanced tropins). With regard to those pituitary hormones which are not held in abeyance by the feed-back mechanism of their secondary peripheral hormones (LTH, ADH and MSH), an overshoot is to be expected. This would classify LTH, ADH and MSH as unbalanced tropins.

Our hypothesis has been confirmed recently by Scott and Nading(16). They reported that phenothiazine tranquilizers caused release of MSH by counteracting hypothalamic inhibition.

With regard to our experiments it is relevant that the melanophore effect was obtained only in Rana frogs and Bufo. Hyla frogs did not react to reserpine with darkening of the skin. This would indicate that the tree frog has a more stable regulation of MSH secretion than Rana. Hyla melanophores have indeed been considered to react more specifically to MSH and ACTH assays than those of Rana or Bufo(17,18).

Summary. Reserpine injected into the dorsal lymph sac of Rana frogs at dose levels of 0.05-0.25 mg was shown to provoke melanophore-dispersion in the skin. The melanophore index, M.I., in the hind webs of the frog rose from 1 to 5 within 24-72 hours after reserpine injection. Reserpine did not cause any change of skin color *in vitro*. It is suggested that this effect is due to intensified endogenous MSH secretion.

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Distribution and Particle Size of Type A Botulinum Toxin in Body Fluids of Intravenously Injected Rabbits.* (26604)

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The present study reports distribution of type A botulinum toxin in various body fluids after intravenous administration of the toxin to rabbits. The sedimentation coefficient of the toxin in plasma and lymph was determined by partition cell ultracentrifuga-

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