

TABLE I.
Antibody Nitrogen Precipitated by 0.20 mg. SI.

Antiserum	SI preparation used—				Deacetylated S12
	S120	S120 heated	S120 alkali treated	S91a ³	
Horse antibody B 77*	mg. .76	mg. .72	mg. .66	mg. .71	mg. .63
Rabbit serum 3.70*	.47	.26	.14	.21	.08†

*Previously absorbed with "C" polysaccharide and Pneumococcus protein "R". N determinations by Dr. D. L. Shrivastava.

† N pptd. by 0.05 and 0.10 mg. S I.

to precipitate antisera from both species. Avery and Goebel³ have shown this to be due to the removal of a labile acetyl group. The effect of heating appears to be related to the length of the chainlike molecules of the polysaccharide and is being studied further.

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Evidence from Dwarf Mice Against the Individuality of Growth Hormone.

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Evans and Long¹ were the first to obtain body growth from anterior pituitary extracts. Later investigators have uniformly considered the growth hormone a single entity, with the exception that recently Riddle and Bates—partly on the basis of data presented here—have sharply questioned this view.²⁻⁵ On a wholly general view it seems to us that since hormones of the anterior lobe act upon or through other endocrine glands (thyroid, suprarenal, pancreas, parathyroid, gonads), most of which (gonads excepted) produce hormones highly important in healthy body maintenance, these latter glands probably take part also in normal body growth. If this is true the repair of the several disabilities caused by hypophysectomy

¹ Evans, H. M., and Long, J. A., *Anat. Rec.*, 1921, **21**, 61.

² Riddle, O., *Endocrinology*, 1935, **19**, 1.

³ Bates, R. W., and Riddle, O., *J. Pharm. and Exp. Therap.*, 1935, **55**, 365.

⁴ Riddle, O., and Bates, R. W., *Carnegie Year Book*, Dec., 1935.

⁵ Riddle, O., Smith, G. C., Bates, R. W., Moran, C. S., and Lahr, E. L., *Endocrinology*, 1936, **20**, 1.

should be accompanied by marked growth; and, it is in fact in hypophysectomized animals that the most striking responses to "growth hormone" preparations have been obtained. Our study shows that gonad-stimulating (F.S.H.) preparations from pregnant mare's serum have no effect on body growth in the dwarf mouse. Prolactin and thyreotropic are well defined anterior lobe hormones which are here shown to be capable of stimulating growth in the dwarf mouse when given separately, and to exhibit synergism of the growth response when given together. The broad bearing of this evidence is attested by other data⁵ which show that in their effects on the B.M.R. of normal and hypophysectomized pigeons these 2 hormones bear a precisely similar relation to each other.

The dwarf mice used (usually 38-150 days old) are from MacDowell's colony of the strain of hereditary dwarfism found by Smith and MacDowell^{6, 7} to have pituitaries wholly deficient in eosinophiles, and to have deficient thyroids and adrenals which could be restored by daily implants of pituitaries of normal mice. Although A.P. implants from normal mice induced fairly rapid body growth in the dwarfs, the one test made with implants of dwarf pituitaries wholly failed to induce growth. Snell,⁸ and MacDowell and Laanes⁹ have shown that moderately good growth (considerable excess of fat) can be obtained from the feeding of desiccated thyroid to the dwarf mouse. Kemp¹⁰ induced some growth in dwarf mice of this strain by injections of an extract ("growth hormone") which, from its method of preparation, must have contained all A.P. hormones.

All of the preparations used here were previously tested or assayed on immature male ring doves; that study either verified the absence or attested the presence of prolactin, F.S.H. and thyreotropic hormone in a particular quantity. For one type of controls a number of available preparations of "growth hormone" were used; these too were subjected to the assays described above, and though F.S.H. was not detectable in some (Collip) preparations we have found both prolactin and thyreotropic in all these "growth hormone" preparations. The prolactin preparations used here were first made to test free of F.S.H. and thyreotropic, and later most of them were heated (at pH 7.5-8.0, 98°C. for 1 hr. or 60°C. for 5

⁶ Smith, P. E., and MacDowell, E. C., *Anat. Rec.*, 1930, **46**, 249.

⁷ Smith, P. E., and MacDowell, E. C., *Anat. Rec.*, 1931, **50**, 85.

⁸ Snell, G. D., *Anat. Rec.*, 1930, **47**, 316.

⁹ MacDowell, E. C., and Laanes, T., *Carnegie Inst. of Wash., Year Book*, 1932, **31**, 47.

¹⁰ Kemp, T., *Klin. Wochenschr.*, 1934, **13**, 1854.

hrs.) before use. This heating, at this pH, should further guard against traces of F.S.H. and thyreotropic; and if a "growth hormone" has the heat lability commonly assigned to it, that hormone should be destroyed by this procedure. All of the thyreotropic preparations used tested free (No. 267) or practically free (No. 243) of prolactin, but all contained F.S.H. which (as noted above) does not affect growth in the dwarf and also has no effect on the B.M.R. of pigeons; most of the thyreotropic preparations were heated (at pH 5.0) before use on the dwarfs.

TABLE I.
Growth Response of Dwarf Mice to Anterior Pituitary Preparations.

Preparation or Hormone	Sex	Dose per day	Days injected	—Weight— Start End		% Initial Wt.
Phyone 41B "Growth H"	♂	0.1 cc.	33	7.0	19.5	280
" 41B "	♀	0.1 "	33	8.2	20.0	245
" 41C "	♂	0.06 "	33	7.0	13.4	192
" 44 "	♂	0.1 "	33	10.0	26.0	260
Collip's Q21 "	♂	0.1 "	33	7.0	12.0	172
" Q21 "	♀	0.1 "	33	7.0	9.5	136
" Q16 "	♂	0.1 "	22	8.5	10.3	121
" Q16 "	♀	0.1 "	22	7.0	9.5	136
" POG "	♂	0.2 "	33	6.6	10.8	164
" POG "	♀	0.05 "	33	8.6	11.5	134
Lee-Schaeffer 720 "	♂	0.15 "	27	8.5	14.6	172
" " "	♀	0.15 "	27	7.8	12.0	154
Thyreotropic 243*	♂	0.8 mg.	33	6.6	8.9	135
" " "	♀	0.8 "	33	7.2	9.6	133
Prolactin 237*	♂	0.8 "	33	5.7	8.2	144
" " "	♀	0.8 "	33	6.0	7.1	118
Pro. 237* + Thyr. 243* (0.8 mg. of each)	♀	1.6 "	33	8.2	19.0	230
" " "	♀	1.6 "	33	8.0	17.0	213
Prolactin 380*	♀	0.7 "	33	9.4	12.0	128
" " "	♀	5.0 "	33	9.5	14.0	147
Desiccated thyroid	♂	2.0 "	33	7.5	13.8	184
" " "	♀	0.4 "	33	8.0	12.2	153
" " "	♀	0.4 "	33	7.8	11.4	146
Des. thyroid + 380*	♀	1.1 "†	33	6.4	10.4	163
" " + 380*	♀	0.5 "†	33	7.3	10.6	146
Prolan-Elberfeld	♀	0.15 "	20	6.7	6.2	93
Mare serum	♀	0.1 cc.	10	7.2	5.3	74
" " "	♀	0.1 "	20	8.6	8.5	99

*Boiled or heated preparation.

†Used 0.7 mg. of 380, 0.4 mg. of thyroid.

‡ " 0.1 " " 380, 0.4 " " " "

Table I records results of tests covering 33 days of injection which are not otherwise shown on Fig. 1. Certain of our results, such as alternate periods of injecting prolactin and thyreotropic, can not be satisfactorily shown in the table. Because the increases in weight are proportional to the initial weight of the mice we express growth in terms of percentage of initial weight, *i. e.*, $100 \times \text{final weight}/\text{initial weight}$ (gm.).

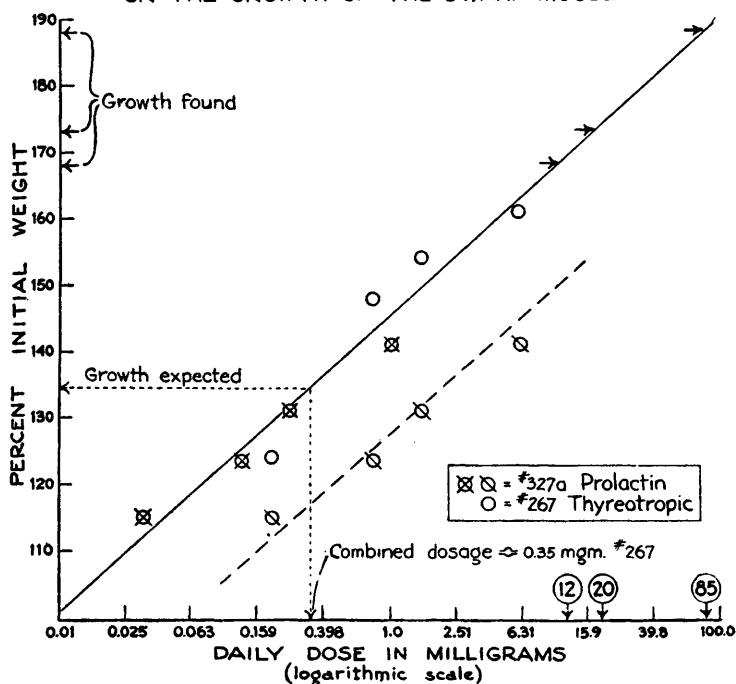
SYNERGISM OF PROLACTIN AND THYREOTROPIC HORMONES
ON THE GROWTH OF THE DWARF MOUSE

FIG. 1.

It will be noted that 3 different preparations of Phyone (growth hormone), in 0.1 cc. daily dosage, gave weights from 192-280% of the initial weights in 33 days. Either prolactin or thyreotropic causes growth, but a much slower growth. Are our preparations of the 2 latter hormones contaminated with the alleged growth hormone, or do they cause growth—directly or indirectly—by replacing different secretions of the deficient pituitary? In the former case one expects a combination of the 2 fractions to give additive effects upon growth; in the latter case an augmented effect, or synergism, is expected. This point was tested nearly 2 years ago by injecting 2 mice with 0.8 mg. daily of prolactin No. 237 (boiled 1 hr. at pH 7.5), 2 mice with 0.8 mg. daily of thyreotropic (+F.S.H.) No. 243 (boiled 1 hr. at pH 5.5), and lastly 2 mice with 0.8 mg. daily of each of the 2 preparations. The weights after 33 days in percentage of initial weights, for No. 237 were 144 and 118%; for No. 243 these were 135 and 133%; for the combination, 230 and 213%. One of us referred to the significance of this experiment 18 months ago, though no data were then given.²

In order to obtain a measure of the amount of synergism obtained by a combination of the 2 hormone preparations it is necessary to know the relationship between dosage and response (growth). Hence we have carried out the following preliminary experiment: Four dwarf mice 54-106 days old (wt. 6.8-8.6 gm.) were injected daily for 33 days with 0.2, 0.8, 1.6, and 6.4 mg., respectively, of thyreotropic (+F.S.H.) No. 267. Four other dwarf mice 54-83 days old (wt. 7.1-8.6 gm.) were injected daily with similar doses of prolactin No. 327a. When the growth obtained from the 8 mice is plotted against the logarithm of the dose (Fig. 1), the points fall along straight lines which have essentially the same slope. The mice receiving No. 327a (circles with diagonal line) did not grow as much per mg. of injected material as the mice on No. 267 (circles), hence the prolactin preparation is relatively less potent (about one-sixth). If the dosages with No. 327a are divided by 6 and the data again plotted (circles with crosses), one obtains 8 points from which to check the relation between dose and effect—the largest dose being 192 (6×32) times greater than the smallest.

At the same time, 2 dwarf mice, 28 and 45 days old (wt. 6.5 and 7.7 gm.), received daily for 33 days a mixture of 0.3 mg. No. 267 (thyreotropic) and 0.3 mg. No. 327a (Prolactin). Since for promotion of growth 0.3 mg. of No. 327a is equivalent to about 0.05 mg. of No. 267, the combined dosage is equivalent to 0.35 mg. of No. 267. Assuming that a separate entity, growth hormone, contaminates both preparations the expected growth in the 2 mice is 135%. Instead, we obtained growths of 168 and 188%; this is an amount which would require dosages of 12 and 85 mg. respectively of No. 267 alone, and these quantities are 34 and 242 times the dosage actually given. One other dwarf (150 days old, wt. 7.5 gm.) received 0.8 mg. No. 327a + 0.2 mg. No. 267 daily for 33 days (equivalent to 0.33 mg. No. 267). The growth obtained was 174%; this is equivalent to 20 mg. of No. 267, or to 60 times the dosage actually given.

Summary. Prolactin alone, desiccated thyroid alone, or thyreotropic hormone probably unaffected by any contaminating constituent of the preparations used by us, promotes growth in the dwarf mouse. In these mice prolactin and thyreotropic show a synergistic action (32 to 242 $\times$) upon body growth. Though the concept of a growth hormone as an individual entity has been useful it does not seem to be true.