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Received July 6, 1961. P.S.E.B.M., 1962, v109.

Responses of the Snell Dwarf Mouse to Pituitary Tissue from a Bovine Dwarf Mutant.* (27096)

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Numerous distinct dwarf mutants of cattle and mice have been reported. The comparative genetic and biological nature of the mutants in the 2 species are of considerable interest and biological importance, including the problem of genetic homology. This report concerns an attempt to establish possible relationship, or lack of relationship, between a dwarf mutant from each of the 2 species. The study concerns pituitary extract made from a specific type of achondroplastic bovine dwarf mutant injected into a specific type of thyroid-pituitary deficient mouse mutant. The response may also reveal specific information on the physiological nature of both the donor and recipient mutants.

The general morphological characteristics of the bovine mutant that provided the pituitaries have been described(1,2); it is subnormal in size and belongs to an achondroplastic complex. In this laboratory it is called the brachysephalic dwarf(3,4). All components of the complex exhibit lesions of achondroplasia in the axial and/or the appendicular skeleton(5,6). In the brachycephalic dwarf the pituitary-thyroid axis is reported to be normal(7); growth, gonadotropic, and ACTH hormone activity in the pituitary tissue are reported to be normal or nearly normal(8,26); sensitivity to insulin is greater than in normal catle(27); but there is disagreement about whether the pituitary tissue is deficient in thyrotropic hormone activity (7,8,10). Limited work indicates body weight increases(9), but not skeletal growth, in response to hormone therapy such as stilbestrol, testosterone, thyroprotein, and combinations of these. The mouse mutant used is a recessive dwarf(11,12) and suffers from a thyroid-pituitary deficiency(13,14,15,16,17, 18,19,20,21). In being quite sensitive to insulin(28), it is similar to the bovine dwarf (27). Although the pituitary has high concentrations of gonadotropic hormones, its growth hormone activity is difficult to detect (14,15).

Materials and methods. Pituitary glands from normal and dwarf cattle, predominantly of the Hereford breed, were collected within 15 to 20 minutes of slaughter and placed in vacuum bottles containing dry ice. Glands from the 2 stocks were kept separate, and 2 extracts were prepared, one from the dwarfs and one from normal cattle. Before the pituitaries were macerated, the capsules were removed and the glands weighed. They were then sliced into several pieces, placed in a

* This investigation was conducted under California Experiment Station Project 1451 in cooperation with the U.S.D.A. and the State Experiment Stations under Western Regional Project W-1; also supported in part by U.S.P.H.S. grant.

TABLE I. Responses of Dwarf Mice to Pituitary Tissue from Achondroplastic Dwarf and Normal Bovines with Untreated Controls.

Identity of mice	Length of test, days	No. of inj.	Amt of inj., mg	Bovine pituitary treatment	Body wt, g: Day test began	Body wt, g: Day test ended
Dwarf A6	16	16	10	Dwarf	10.0	14.5
" A7	16	16	10	"	9.0	12.5
" A8	16	16	10	"	8.0	12.5
" A9	16	16	10	"	8.0	11.5
" A10	16	16	10	"	7.0	11.5
Dwarf B5	16	16	10	Normal	9.5	14.0
" B6	16	16	10	"	9.0	13.5
" B7	16	16	10	"	9.5	13.0
" B8	16	16	10	"	8.0	11.5
" B9	16	16	10	"	6.0	10.0
Dwarf C5	16	———	No treatment	———	10.0	12.0
" C6	16		*idem*		9.5	10.0
" C7	16		"		7.0	8.5
" C8	16		"		8.5	9.0
" C9	16		"		7.0	8.5

simple hand-operated tissue grinder, and macerated with 10 to 25 ml of sterile saline. The resulting suspension was strained through sterile cheesecloth and diluted with sterile saline so that each ml contained either 50 or 100 mg of equivalent pituitary tissue. Merthiolate (1:1,000), 1 ml per 25 ml of suspension, was used as an antibiotic, and the suspension was stored in brown bottles at 34-36°F.

The dwarf mouse stock used, supplied by Dr. E. C. MacDowell, was from the strain called A dw (Albino-dwarf), originating from a cross between the original English silver dwarf-carrying strain and the Bagg Albino. The parental mice that supplied the test stock were in the 16th generation of inbreeding.

Normal mature non-pregnant females of the mutant stock averaged 28 g (range 22 to 32), but the dwarfs they produced weighed from 5 to 12 g. As the dwarf mice became available and their body weight neared plateau, they were divided into 3 groups: Group A received dwarf bovine pituitary tissue; Group B received normal bovine pituitary tissue; and Group C was untreated. Each group contained both sexes. Subcutaneous injections containing 7 to 20 mg of pituitary tissue were given over periods of 10 to 30 days.

Experimental results. It is evident that pituitary tissue from bovines, both dwarfs and normal control animals, stimulate the growth of dwarf mice (Tables I and II). Mouse groups A and B showed identical weight responses that are consistent for all of the statistics throughout the experimental period. When Groups A and B are compared with the untreated dwarf control (Group C)

TABLE II. Analysis of Total Weight Increase of 3 Groups of Dwarf Mice Employed in the Tests.

Group	Treatment of mice	No. of tests N	Mean wt increase during test period	Significance of response: Groups compared	Significance of response: t value	Significance of response: P
A	Pituitary extract from bovine dwarfs	10*	4.14	A - B	.02	.9
B	Pituitary extract from cattle of normal size	9	4.15	A - C	4.26	.01
C	Untreated control	9	.94	B - C	5.99	.01

* Included in this table are 13 mice used in trials similar to the one tabulated in Table I except for slight variations in size of inj. and length of trial.

the difference in respective mean weight increases for the A and B groups for the injection period are 4.14 and 4.15 g, while mean weight increase of group C is 0.94 g.

Discussion. From the studies made of the Snell dwarf mouse(14,15,16,17,23,24,25), it is certain that the thyroid-pituitary relationship is impaired. Reports on the bovine dwarf (1,2,3,4,5,6), however, indicate it belongs to an achondroplastic complex with a normal thyroid-pituitary axis(26).

Pituitary extracts from both bovine dwarfs and normal cattle elicited similar growth response in the dwarf mouse. That both cattle stocks have pituitary tissue equal in quality cannot be presumed from these results or from other reports(7,8). It seems doubtful, however, that the bovine dwarf suffers from a deficiency of the growth hormone. Possibly the hormone of the bovine dwarf fails to reach the target tissue in an effective state. Since the cartilage tissue in achondroplastic animals is defective(29), this deficiency alone seems sufficient to explain the lack of response of achondroplastic animals to the pituitary growth hormone regardless of how it may be administered. Bovine achondroplastic mutants have not been treated with growth hormone, but it is assumed that their response would be similar to that of the achondroplastic dachshund(23). The prolonged injection of the hormone doubled the body weight, but most of the weight increase came from the soft tissues. Even though both length and diameter of the bones were slightly increased, the basic proportions were unchanged and the dachshund retained its inherent achondroplastic pattern.

These results suggest that 2 entirely different types of mutations are implicated. One, a deficiency of the pituitary gland and production of growth hormone, as in the dwarf mouse and rat(22), the other, a deficiency in the target tissues in which mutant animals are unable to respond to normal growth processes, or the effects of the growth hormone produced by their own pituitary, or by normal growth hormone supplied by injection as exemplified by the achondroplastic bovine dwarf and the dachshund. The experimental evidence indicates that the pituitary deficient mouse mutant is neither homologous nor isogenic with the achondroplastic bovine mutant.

Summary. Dwarf mice (Snell) from A dw (Albino-dwarf) stock, in which the thyroid-pituitary axis is abnormal, were injected with pituitary tissue extract from the achondroplastic brachycephalic bovine dwarf mutant, which suffers from achondroplasia but is reported to have a normal thyroid-pituitary axis. Controls were (i) dwarf mice injected with pituitary tissue extract from nonachondroplastic cattle of normal size and (ii) uninjected dwarf mice. The extracts from 2 sources were standardized so that both possessed the same concentration of pituitary tissue. As the weight of dwarf mice neared plateau, they were divided into 3 groups: one received pituitary extract from bovine dwarfs, one received pituitary extract from cattle of normal size, and one was untreated. Body weight response was checked by daily weighing. Injection of pituitary extract from bovine dwarfs stimulated mouse growth significantly, as did extract from normal cattle and the responses were identical. This supports the hypothesis that the bovine dwarf mutant and the mouse dwarf mutant are not homologous.

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Received July 7, 1961. P.S.E.B.M., 1962, v109.

Enzymatic Estimation of Phosphocreatine.* (27097)

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The most widely used method for estimation of phosphocreatine (PC)† in tissues is that of Fiske and Subbarow(1). In this method, inorganic phosphate (Pi) is precipitated from a neutralized trichloracetic or perchloric acid extract by alkalinized calcium chloride, and the PC in the filtrate is determined as Pi after hydrolysis with acid molybdate at room temperature for 30 minutes. The criticisms of this method have been reviewed by Ennor and Morrison(2). In our experience, the main disadvantage of this method is that relatively large tissue samples are required. If small organs are under study, a micro-method is required for a complete analysis of all phosphorus compounds and other metabolites. The method described here meets such a need. It makes use of the enzyme creatine-phosphokinase (CPK), first crystallized by Kuby, Noda and Lardy(3) and now available commercially. Furthermore, the CPK-catalysed reaction is coupled with the enzymatic reactions utilized for estimation of ATP. It is thus possible to determine ATP and PC in the same sample of muscle extract corresponding to as little as 2 to 5 mg of fresh muscle.

Experimental. Principle of method. In the presence of excess 3-phosphoglyceric acid (3-PGA), limiting amounts of ATP can be determined according to the reactions:

1. 3-phosphoglyceric acid + ATP $\xrightarrow{\text{3-phosphoglyceric kinase (PGK)}}$ 1,3-diphosphoglyceric acid + ADP

2. 1,3-diphosphoglyceric acid + DPNH + H^+ $\xrightarrow{\text{glyceraldehyde phosphate dehydrogenase (GAPDH)}}$ glyceraldehyde phosphate + DPN^+ + H_3PO_4

The decrease in DPNH and thus of the optical density (ΔE) at 340 mμ is a measure

* This study was supported by a grant from Am. Heart Assn.

† Abbreviations: Phosphocreatine (PC), inorganic phosphate (Pi), Adenosine-5′, tri- and diphosphate (ATP, ADP), Creatine Phosphokinase (CPK), 3-Phosphoglyceric kinase (PGK), Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), reduced glutathione (GSH), Phosphoglyceric acid (PGA), reduced diphosphopyridine nucleotide (DPNH).