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Brain
epinephrine
growth
growth hormone
insulin
nutrition
pituitary

The Action of Insulin, Growth Hormone, and Epinephrine on Cell Growth in Liver, Muscle, and Brain of the Hypophysectomized Rat[39]

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Extract

This study explores changes in deoxyribonucleic acid (DNA), ribonucleic acid (RNA), protein, and water content of muscle, liver, and cerebrum in hypophysectomized rats and the effects of injecting growth hormone, insulin, or growth hormone with epinephrine conjointly over an eleven-day period.

Male hypophysectomized rats, 26 to 49 days of age, fed an *ad libitum* diet were studied. At 38 days of age they were injected with insulin, 0.4 to 1.8 units per day, with bovine growth hormone (250 μg/day) or with the same amount of growth hormone and epinephrine, 5 to 20 μg/day, concomitantly, or were untreated until the 49th day of age. Control rats of the same age were either pair-fed to untreated hypophysectomized rats or given an *ad libitum* diet.

Untreated hypophysectomized rats showed poor body weight gain per unit food intake and reduced skeletal growth. The nucleic acid and protein content of liver, muscle, and cerebrum was reduced when compared with controls of the same age. The ratio of protein:DNA (cell size) was increased for body size but reduced for age.

Administration of insulin caused hypertrophy of liver cells and increased the protein content of liver, but did not affect muscle and cerebrum. DNA content of liver or cerebrum did not increase, and the gain in DNA content of muscle was not remarkable. There was a definite increase in RNA content of muscle, liver, and cerebrum and in the ratios of RNA:DNA and protein:DNA of liver.

Injections of growth hormone caused an increase in DNA (cell number), RNA, and protein content in liver, muscle, and cerebrum. There was a reduction in the ratio of cytoplasm to nucleus. The protein increment was nullified by the injection of epinephrine in conjunction with growth hormone. DNA content of muscle and liver was increased, but not to the level produced by growth hormone alone. The increase in RNA content of liver, muscle, and cerebrum was again significant; the ratio of RNA:DNA increased only in liver. Caloric intake of untreated hypophysectomized rats and those treated with growth hormone or insulin was comparable. Rats injected with epinephrine showed a significant increase in caloric intake.

The results indicate that insulin is involved with growth in cell size, while growth hormone is active with respect to the increase in cell number. Both hormones are required for optimal growth.

Speculation

The present study indicates that both growth hormone and insulin are required for optimum cell growth. Epinephrine administration retards the increase in cell number that normally occurs in hypophysectomized rats receiving growth hormone. This suggests that overactivity of the sympathetic pathways may retard growth and produce effects that simulate hypopituitarism.

Introduction

The action of growth hormone on somatic growth is the subject of increasing study. Emphasis has been placed on the role of this hormone in protein biosynthesis, but the mechanisms involved are not clearly defined [21]. It is only recently that the effects of growth hormone on DNA replication and on the increase of cell number in children [4, 6] and rats [1, 9, 10] have been studied. Earlier reports found that insulin is important to protein synthesis, although this finding has been difficult to verify by *in vitro* studies [36]. When associated with pancreatectomy, the administration of growth hormone to a hypophysectomized animal is ineffective. Negative nitrogen balance persists in the absence of insulin [15, 16, 22, 31]. SALTER and BEST [29] showed that the administration of protamine zinc insulin to hypophysectomized rats caused increased food intake and growth with large increments in body fat and, to a lesser extent, in body protein. The protein increase was not as significant as that found for the hypophysectomized rat treated with growth hormone alone. Insulin appeared to have the greatest effect on visceral organ weight.

WAGNER and SCOW [35] reported that the small increment in nitrogen retention occurring when hypophysectomized rats were forced fed [30], was similar to that produced by the injection of insulin [29]. They speculated that insulin stimulated an increased food intake in the hypophysectomized rat.

The present study was undertaken in rats hypophysectomized at 21 days of age. The effect of insulin, injected from the 38th to 49th day of age, on nucleic acids and protein content in muscle, liver, and cerebrum was investigated. Carcass protein, collagen, and fat were also studied. Comparisons were made with rats of the same age that were given bovine growth hormone or no treatment at all. An additional group received long-acting epinephrine with growth hormone in an attempt to partly inhibit endogenous insulin release. The findings indicate that insulin, in the absence of the pituitary, produced increased protein content primarily in liver. Gain in muscle and carcass protein was less apparent. The increased liver weight was a result of protein accretion with no increase in DNA content. Growth hormone, in the absence of the pituitary, produced an increase in DNA content and protein. Growth hormone increased the number of new cell units. The increase in protein and DNA content of tissue is retarded by the injection of epinephrine together with the growth hormone.

The untreated hypophysectomized rat can be compared with intact rats receiving the same diet per 100 g body weight or with rats receiving an *ad libitum* diet. Data from normal intact rats and pair-fed or calorie-restricted rats have been reported earlier [17].

Methods

Experimental Plan

Intact and hypophysectomized male Sprague Dawley rats [40] were studied. Hypophysectomy was performed in 21-day-old animals prior to shipment. At 23 days of age, the animals were placed in individual metabolic cages and were fed a diet of Purina chow (23 %) protein. The hypophysectomized rats, both those untreated and those that received hormones by subcutaneous injection, were offered 5 % sucrose to drink; normal rats fed an *ad libitum* diet were given tap water. Body weight, food, and fluid intake were monitored daily. The experimental plan of the control and pair-fed rats to the untreated hypophysectomized rats has been given previously [17]. Both investigations were performed simultaneously.

At 38 days of age, the hypophysectomized rats had reached constant weight, and three groups of the hypophysectomized rats were selected at random for hormone treatment. The dosage of insulin for the hypophysectomized rat was the same as that proposed by SALTER and BEST [29], who increased the dose if weight gain was not achieved. A high mortality rate was experienced in the hypophysectomized rats receiving insulin. This was possibly due to hypoglycemia. Data on the experimental plan and dosages are given in table I.

Tissue Preparation and Chemical Methods

Following ether anesthetization, the rats were killed by aortic puncture. The techniques for tissue preparation and the methods of analyses have been described previously [17]. Statistical comparisons were made with the Student 't' test.

Results

Body Weight

Data on the changes in body weight with time are shown in table II. For normal rats, 26 to 38 days of age, weight increased from 72 to 147 g [17], a gain of 74.6 g; from 38 to 49 days of age, the weight increased from 147 to 224 g, a further gain of 76 g. Weight of untreated hypophysectomized rats 26 to 38 days of age increased from 59.2 to 74.1 g, a gain of 14.9 g; from 38 to 49 days of age, the gain was only 3.8 g.

Injection of insulin at 38 to 49 days of age caused the hypophysectomized rat to gain 21 g. The injection of growth hormone increased weight by 32 g. The simultaneous injection of epinephrine with growth hormone caused an increase of 34 g. The starting weights of these three groups on the 38th day were 70.7, 73.2, and 71.6 g, respectively The progressive changes are illustrated in figure 1. The final weight of Groups III and V were the same.

Table I. Experimental plan

Studies of rats from the 38th to 49th day of age (6 to 9 animals per group)

Group I: Diet ad lib.; plain water for drinking from 23rd day of age. Killed on 49th day of age.

Group II: (A) and (B): Hypophysectomized at 21 days of age. Diet ad lib.; 5 % sucrose water for drinking. Killed on 38th and 49th days of age.

Group III: Hypophysectomized at 21 days of age. Given 250 μg bovine growth hormone/day (Batch number, NIH-GH-B12) [41] from the 38th day of age. Diet ad lib.; 5 % sucrose water for drinking. Killed on 49th day of age.

Group IV: Hypophysectomized at 21 days of age. Protamine zinc insulin injected from 38th day of age (dosages on sequential days, 0.4; 0.4; 0.6; 0.84; 0.90; 1.04; 1.2; 1.2; 1.5; 1.8 units). Diet ad lib.; 5 % sucrose water for drinking. Killed on 49th day of age.

Group V: Hypophysectomized at 21 days of age. Agents administered in μg/day: growth hormone, 250, and epinephrine (Susphrine®) dosages on sequential days, 2.5; 2.5; 2.5; 5; 5; 5; 10; 20; 20; 20; 20. Injections from 38th day of age. Diet ad lib.; 5 % sucrose water for drinking. Killed on 49th day of age.

Food Intake

In table II, data are presented on dietary intake per 100 g of body weight per day (column 4) and on caloric intake for the period of 38 to 49 days. Untreated hypophysectomized rat received 10 g of food (chow plus sucrose) per day per 100 g of body weight. No significant difference was found in the groups receiving growth hormone with or without additional epinephrine. The injection of insulin, however, increased the food intake to 13 g ($p < 0.005$) or to a level similar to that of a normal control rat. Caloric intake for the groups receiving either insulin or growth hormone was the same as that of the untreated hypophysectomized rat. The group receiving growth hormone and epinephrine conjointly had a higher caloric intake ($p < 0.02$).

The relation of food to growth can also be determined by the weight gain per gram of food per day. Such determinations, however, do not reflect energy expenditure caused by activity. Table II demonstrates that the hypophysectomized rat at 38 days of age had a value of only 0.16, a significant reduction from normal ($p < 0.001$). At 49 days of age, the value was almost zero. Both growth hormone and insulin therapy, however, raised the index ($p < 0.001$) toward normal. The group receiving epinephrine was not evaluated since part of the weight gain was due to water retention.

Carcass Composition

In table III, data are recorded for body weight, fat free carcass (eviscerated rat without fat, skin, or feet), carcass fat, protein, water, and skeletal collagen in each group.

The hypophysectomized rat 38 to 49 days of age showed less body weight, carcass weight, protein, water and skeletal collagen than did controls. Growth hormone induced a significant increase in body weight, protein, water, and skeletal collagen ($p < 0.001$), when compared with untreated rats of the same age. Fat in the carcass decreased ($p < 0.05$). The injection of epinephrine with growth hormone produced similar results. Insulin produced a significant increase in body weight ($p < 0.001$), carcass protein ($p < 0.005$), water ($p < 0.025$), and skeletal collagen ($p < 0.01$). There was no increase in fat-free carcass weight. Carcass fat was particularly high in this group.

Muscle, Liver, and Cerebrum

In table IV, data are recorded for the ratios of water:protein in muscle, liver, and cerebrum for normal and hypophysectomized rats at 49 days of age. When epinephrine was injected with growth hormone, there was an increase in the ratio of water:protein in these tissues ($p < 0.001$).

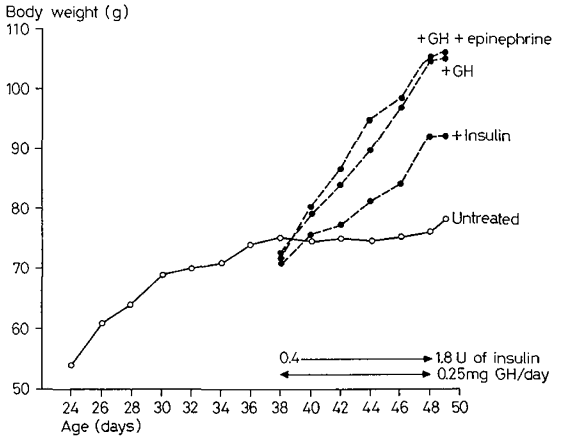

Fig. 1. Changes in body weight as a result of hypophysections and various forms of treatment. Note the time delay for rats to reach a constant weight following hypophysectomy [32].

Table II. Dietary intake and weight increments

	Age Days	Body weight	Weight increase	Dietary intake	Increase of body weight[1]	Purina chow[2]	Sucrose[2]	Caloric intake[2]
		g		g/100 g body weight/day		g		
Hypophysectomized								
Mean	38	74.1	14.9	12.6	0.16	80.1	18.7	304
SD		2.6	2.3	1.1	0.02	0.7	1.9	18
N		8	8	8	8	8	8	8
38 to-49-day period normal								
Mean	49	224.0	75.7	12.4	0.30	283.2	–	745
SD		8.9	4.7	1.3	0.30	18.6	–	4.9
N		7	7	7	7	7	–	7
Hypophysectomized								
Mean	49	77.9	3.8	10.4	0.04	72.9	17.9	264
SD		4.5	3.0	0.8	0.04	1.1	1.5	16
N		9	9	9	9	9	9	9
Hypophysectomized+growth hormone								
Mean	49	105.6	32.4	12.0	0.34	83.8	10.2	268
SD		3.5	4.4	1.8	0.04	14.2	2.9	15
N		8	8	8	8	8	8	8
Hypophysectomized+insulin								
Mean	49	91.9	21.2	13.4	0.21	88.0	10.6	274
SD		4.6	2.8	2.1	0.03	17.9	1.1	20
N		8	8	8	8	8	8	8
Hypophysectomized+growth hormone+adrenaline								
Mean	49	105.6	34.1	10.5	0.33	86.2	16.2	291
SD		2.45	1.9	1.4	0.01	8.2	2.7	16
N		7	7	7	7	8	8	8

[1] g increase/g food/day.

[2] Caloric, chow, or sucrose intake over entire period (38 to 49 days) except for hypophysectomized rats killed at 38 days, in which case period equalled 26 to 38 days.

Table III. Carcass and muscle analyses

	Age killed	Body weight	Fat free carcass	Muscle mass	Carcass			Skeletal collagen	Muscle cell pop.	Protein: DNA	RNA: DNA	Total RNA
					Protein	Water	Fat					
	Day	g							$N \times 10^9$			mg
I. E. Normal												
Mean	49	224.0	105.49	79.57	22.34	79.22	4.89	3.23	12.96	215.01	2.26	175.33
SD		8.9	4.56	3.99	2.05	2.69	0.88	0.24	1.93	39.99	0.43	18.57
N		7	7	7	7	7	7	7	7	7	7	7
II. A. Hypophysectomized												
Mean	38	74.1	28.36	21.32	5.46	20.81	0.98	0.95	5.44	132.51	1.20	38.90
SD		2.6	0.82	1.33	0.23	0.60	0.21	0.06	0.94	11.59	0.19	3.21
N		8	8	8	8	8	8	8	8	8	8	8
II. B. Hypophysectomized												
Mean	49	77.9	35.55	25.84	6.73	26.30	1.80	1.01	4.46	184.77	1.53	41.27
SD		4.5	1.50	1.35	0.32	1.18	0.41	0.06	0.47	27.09	0.22	6.23
N		9	9	9	9	9	9	9	9	9	9	9
III. Hypophysectomized +growth hormone												
Mean	49	105.6	48.70	37.80	9.30	36.60	1.43	1.32	8.06	152.73	2.28	111.21
SD		3.5	2.92	1.73	0.49	2.08	0.22	0.07	0.84	10.16	0.26	4.02
N		8	8	8	8	8	8	8	8	8	8	8
IV. Hypophysectomized +insulin												
Mean	49	91.9	37.78	27.46	7.26	27.93	2.67	1.14	5.29	175.03	2.51	80.35
SD		4.6	1.99	1.44	0.32	1.50	0.54	0.08	0.55	16.81	0.20	7.59
N		8	8	8	8	8	8	8	8	8	8	8
V. Hypophysectomized +growth hormone +adrenaline												
Mean	49	105.6	49.60	36.07	8.94	37.39	1.43	1.45	6.96	144.51	2.13	91.29
SD		2.45	2.24	1.85	0.35	1.77	0.23	0.115	0.65	22.99	0.33	12.78
N		7	7	7	7	7	7	7	7	7	7	7

In table III, data are recorded for muscle mass, muscle cell population, and ratios of protein:DNA and RNA:DNA in muscle. Data relating to liver studies and to rat cerebrum are shown in tables V and VI, respectively.

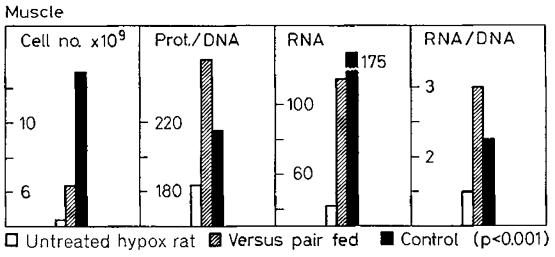

Fig. 2. Composition of muscle from hypophysectomized rats and control or pair-fed animals. Rats 49 days of age. Data from pair-fed or calorie-restricted rats are taken from Reference No. 17.

Table IV. Ratio of water:protein in tissues of 49-day-old rats

	Cerebrum	Liver	Muscle
Intact			
Mean	6.599	3.491	3.674
SD	0.148	0.146	0.091
N	7	7	7
Hypophysectomized			
Mean	6.984	3.468	4.043
SD	0.380	0.215	0.322
N	9	9	9
Hypophysectomized +growth hormone			
Mean	6.933[1]	3.402[1]	3.925[1]
SD	0.368	0.267	0.188
N	8	8	7
Hypophysectomized +insulin			
Mean	6.890	3.437	3.773
SD	0.205	0.437	0.132
N	8	8	8
Hypophysectomized +growth hormone +adrenaline			
Mean	7.766[1]	4.353[1]	4.690[1]
SD	0.311	0.418	0.396
N	7	7	7

[1] Significance of differences: $p < 0.001$

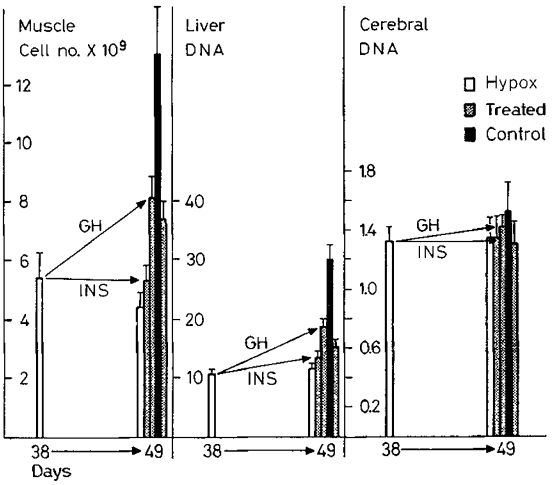

Fig. 3. Changes in DNA content resulting from administration of growth hormone and insulin to hypophysectomized rats. Effects of combined injection of epinephrine and growth hormone shown in the column to the right of the column for control rats. A one-tailed standard deviation is shown.

Hypophysectomized Rats

Untreated hypophysectomized rats, 38 to 49 days of age, showed reduced muscle mass, cell number, RNA content, and RNA per cell when compared with control rats ($p < 0.001$). Similar conclusions were reached if size mates were compared [17]. It was found, however, that hypophysectomized rats had large muscle cells for body size ($p < 0.001$).

Hypophysectomized rats had greatly reduced liver weight, protein, DNA, and RNA content. Ratios of RNA:DNA and protein:DNA were reduced when comparisons were made with age mates on a free diet or with pair-fed controls ($p < 0.001$) [17] (fig. 2).

Hypophysectomized rats, 38 and 49 days of age, showed less DNA in the cerebrum than did control rats of the same age on a free diet, but the difference was borderline ($p < 0.05$). There was a reduction in cerebral weight, protein, water, and RNA ($p < 0.001$).

Hypophysectomized Rats
Given Growth Hormone

Hypophysectomized rats receiving growth hormone injections had increased muscle mass ($p < 0.001$) due primarily to the increase in cell number ($p < 0.001$) (figs. 3 and 5). Protein and DNA content of the liver

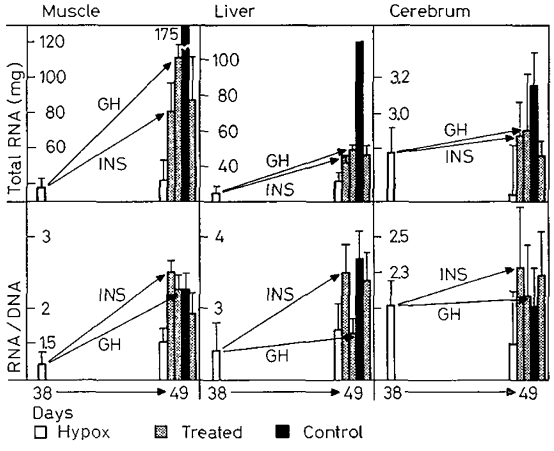

Fig. 4. Changes in RNA content resulting from administration of growth hormone and insulin to hypophysectomized rats. Effects of combined injection of epinephrine and growth hormone are shown in the column to the right of that for control animals. A one-tailed standard deviation is shown.

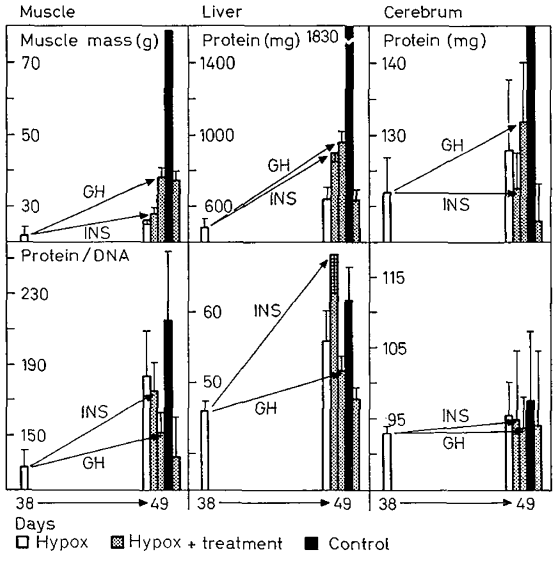

Fig. 5. Changes in protein content resulting from administration of growth hormone and insulin into hypophysectomized rats. Effects of combined injection of epinephrine and growth hormone are shown in the column to the right of that for control animals. A one-tailed standard deviation is shown.

increased ($p < 0.001$). The ratio of protein:DNA decreased significantly for muscle and liver ($p < 0.001$) (fig. 5). There was increase in the concentration of RNA per gram of muscle ($p < 0.001$) and in total RNA content of muscle ($p < 0.001$), liver ($p < 0.001$), and cerebrum ($p < 0.025$) (fig. 4). The ratio of RNA:DNA increased for muscle ($p < 0.001$) but not for liver or cerebrum.

Hypophysectomized Rats Given Growth Hormone and Epinephrine

In figure 3, it can be noted that the injection of epinephrine, in addition to growth hormone, reduced the effect of growth hormone on the DNA content of muscle ($p < 0.02$) and liver ($p < 0.001$). Total RNA content decreased in muscle ($p < 0.01$) but not in liver. Compared with the effect of growth hormone alone, there was a marked reduction in the protein content of liver ($p < 0.001$) and a decrease in the protein content of cerebrum ($p < 0.01$). The effects on liver resulted in a reduced ratio of protein:DNA ($p < 0.02$) but an increase in the ratio of RNA:DNA ($p < 0.001$). There was no apparent change in muscle mass; however, the ratio of water:protein was increased. The amount of water per unit fat free dry muscle was increased.

Hypophysectomized Rats Receiving Insulin

Muscle mass was unaltered by insulin injections, and muscle cell number did not increase above that of the hypophysectomized rat at 38 days of age (figs. 3 and 5). Insulin increased total RNA content and the ratio of RNA:DNA in muscle. Total RNA content increased from 41 to 80 mg; following the injection of growth hormone, the value was 111 mg. The concentration of RNA per gram of muscle, however, was the same in both groups.

Insulin caused a gain in weight and protein content of liver, which was significant ($p < 0.001$) and comparable with that produced by growth hormone. DNA content of liver did not increase, but the ratios of RNA:DNA and protein:DNA were significantly higher ($p < 0.001$). The increase in the ratio of protein:DNA at 49 days of age exceeded that of control rats fed an *ad libitum* diet. Total RNA content of liver also increased to a level almost equal to that found with growth hormone ($p < 0.025$). The only change in cerebrum was an increased ratio of RNA:DNA ($p < 0.02$).

Table V. Rat liver analyses

	Age killed Day	Liver weight g	DNA mg	RNA mg	Protein mg	Protein: DNA	RNA: DNA	mg DNA: g wet tissue	% H_2O in fresh tissue
I. E. Normal									
Mean	49	9.07	29.88	110.33	1834.68	61.54	3.70	3.31	70.66
SD		1.08	2.62	14.19	171.82	5.01	0.37	0.304	0.530
N		7	7	7	7	7	7	7	7
II. A. Hypophysectomized									
Mean	38	2.50	10.41	24.33	480.83	46.22	2.43	4.21	71.82
SD		0.28	0.73	3.09	33.34	1.86	0.40	0.566	0.485
N		8	8	8	8	8	8	8	8
II. B. Hypophysectomized									
Mean	49	3.12	11.41	30.80	641.43	56.32	2.71	3.68	71.21
SD		0.36	0.81	3.57	44.83	3.96	0.28	0.393	0.882
N		9	9	9	9	9	9	9	9
III. Hypophysectomized+growth hormone									
Mean	49	4.61	18.70	49.66	963.89	51.70	2.66	4.08	71.08
SD		0.40	1.32	1.53	51.28	3.55	0.18	0.437	0.377
N		8	8	8	8	8	8	8	8
IV. Hypophysectomized+insulin									
Mean	49	4.45	13.21	45.99	903.20	68.66	3.50	3.05	70.10
SD		0.76	0.93	3.72	42.12	5.81	0.43	0.593	0.818
N		8	8	8	8	8	8	8	8
V. Hypophysectomized+growth hormone+adrenaline									
Mean	49	3.83	13.65	46.43	636.11	46.61	3.42	3.58	71.86
SD		0.40	1.37	4.77	73.48	3.15	0.38	0.434	0.394
N		7	7	7	7	7	7	7	7

Table VI. Rat cerebrum analyses

	Age killed Day	Cerebrum weight g	Protein	DNA	RNA	RNA: DNA	Protein: DNA	mg DNA: g wet tissue	% H_2O in fresh tissue
			mg						
I. E. Normal									
Mean	49	1.21	145.99	1.53	3.16	2.11	96.70	1.25	79.41
SD		0.055	7.72	0.199	0.213	0.233	11.39	0.154	0.154
N		7	7	6	7	6	6	6	7
II. A. Hypophysectomized									
Mean	38	1.06	122.70	1.32	2.78	2.11	93.19	1.25	79.83
SD		0.050	5.50	0.089	0.168	0.127	3.55	0.065	0.207
N		8	8	8	8	8	8	8	8
II. B. Hypophysectomized									
Mean	49	1.12	127.72	1.34	2.54	1.89	95.60	1.20	79.61
SD		0.043	10.71	0.157	0.298	0.285	5.71	0.105	0.402
N		9	9	9	9	9	9	9	9
III. Hypophysectomized + growth hormone									
Mean	49	1.14	131.79	1.41	2.91	2.17	93.72	1.23	79.95
SD		0.044	7.88	0.066	0.349	0.326	4.18	0.071	0.251
N		8	8	8	8	8	8	8	8
IV. Hypophysectomized + insulin									
mean	49	1.08	125.49	1.34	2.88	2.33	94.77	1.24	79.90
SD		0.042	5.16	0.171	0.209	0.386	10.39	0.137	0.150
N		8	8	8	8	8	8	8	8
V. Hypophysectomized + growth hormone + adrenaline									
Mean	49	1.14	116.84	1.28	2.85	2.30	92.93	1.12	79.81
SD		0.026	6.44	0.184	0.096	0.264	12.36	0.148	0.235
N		7	7	7	7	7	7	7	7

Discussion

The analytical data from this study have been related mainly to DNA. There is evidence that for mammalian cells, DNA content is constant and polyploidy does not occur in muscle [6, 9]. In liver, it has been shown that increments in cytoplasm are commensurate with the degree of polyploidy [14] and with the volume of the nucleus. A tetraploid cell, therefore, behaves as two diploid cells in terms of mass composition. Under conditions of pituitary insufficiency, polyploidy fails to occur in liver [11, 18].

Hypophysectomized rats showed growth retardation with no gain in cell number. Tissue mass increased solely by enlargement of the cell. There is a limit, however, to the ratio of cytoplasm:nucleus [3], since delivery of nutrients to the nucleus is related to volume and surface area of the cell. Hypophysectomized rats had reduced levels of RNA per unit DNA and a reduction of total RNA content in muscle, cerebrum and liver. Other studies in rat liver only suggest similar results [12, 19].

Caloric restriction will also retard cell multiplication with a concomitant increase in muscle cell size that exceeds that of a normal age mate [17]. The RNA content per cell is high in comparison with that of the hypophysectomized rat.

Forced feeding produces minimal growth in the hypophysectomized rodent [30]. Thus, failure of growth in the hypophysectomized rat is not a function of caloric intake alone. In this study, the administration of insulin or growth hormone did not change the caloric intake which suggests that these hormones influence the efficiency of caloric utilization. The changes in cerebrum caused by hypophysectomy were not as clearly defined as were those in muscle and liver.

The effect of growth hormone on the hypophysectomized rat indicated that this hormone increases cell number [4, 6, 9], and thereby increases tissue mass. DNA and RNA content increased in liver, muscle, and cerebrum. According to BEACH and KOSTOYO [1] growth hormone increases nuclear number in muscle of the hypophysectomized rat after 24 hours. Other investigators claim that the injection of large doses of growth hormone (3,000 μg) into pregnant rats causes a significant increase in the cerebral neurons of the offspring [38].

Insulin affected primarily liver mass and skeletal collagen. The effect on the skeleton in hypophysectomized rats was emphasized by SALTER and BEST [29]. The increments of protein and RNA content in liver were comparable with either an injection of growth hormone or of insulin; with insulin, however, the ratio of protein:DNA was very high. It could be predicted, therefore, that a substantial alteration occurs in the ratio of cytoplasm:nucleus ratio and that polyploidy probably fails to occur in the liver cell. Commensurate increments in nuclear volume would not be anticipated.

The protein accretion that occurs in liver as a result of insulin injections may be due to an increased protein synthesis or to decreased proteolysis. According to MORTIMERE and MONDON [23], the latter is more likely. It would appear that insulin is capable of causing protein accretion in liver in the absence of growth hormone. Since, in the present study, this finding was not present in rats that received growth hormone and epinephrine, it is possible that insulin *per se* is responsible for protein synthesis. It is thought that epinephrine blocks insulin release [24]. It is apparent, however, that growth hormone and insulin act together [34], the former to permit the formation of new cells or DNA units and the latter to ensure protein accretion [37].

The mechanisms by which these hormones achieve protein biosynthesis are not clarified by *in vitro* studies [21, 37]. KORNER [21] has shown that all types of RNA, not only messenger RNA, increase in liver cells following injection of growth hormone. Stimulation of protein synthesis by growth hormone, assayed in the ribosome, could still be observed when rats were given actinomycin, an inhibitor of RNA synthesis. Thus, KORNER suggests that RNA synthesis is not required for the stimulation of protein synthesis by growth hormone. Other evidence suggests that growth hormone exerts its action on protein biosynthesis at the level of translation rather than at the level of messenger RNA [21].

In vitro experiments may show that there is a lack of a specific factor or that the apparent effect of one hormone may be caused by a hormone other than the one under study. It may be difficult, therefore, to distinguish primary from secondary responses. SAKURAI and KIPNIS [28] found that growth hormone increased DNA-dependent RNA polymerase activity in isolated nuclei from rat liver. Insulin had the same effect. Antiserum to insulin inhibited this action of both hormones. It has been suggested [27, 28] that growth hormone acts on liver RNA by stimulating the release of insulin. In the present study, the ratio of RNA:DNA was high in liver of rats receiving epinephrine and growth hormone concomitantly. Yet, the ratio of protein:DNA was reduced, a finding that suggests that RNA content and protein accretion were unrelated.

The sympathetic nervous system is important to growth. Other investigators have stated that sympathetic over activity is antagonistic to normal growth [8]. Children who exhibit growth retardation associated with emotional deprivation and simulate hypopituitarism [25, 33] may have hyperactivity of the sym-

pathetic nervous system. BLACKARD and HEIDINGSFELDER [2] have concluded that α-receptors stimulate growth hormone release and β-receptors, insulin release. In the present study, it was shown that epinephrine, through its insulin-inhibiting effect, nullifies the action of growth hormone and, as a result, cell multiplication and protein accretion in liver are retarded.

The present findings in hypophysectomized rats can be compared somewhat with findings obtained from the study of pituitary insufficiency in humans [4, 6]. Hypopituitary dwarfs have a predilection for protein foods [5] and a reduced muscle cell population, but they do not have large muscle cells for body size. Cell size is usually smaller. Human growth hormone reverses these changes, but complete absence of pituitary function is rare in the human. In contrast, certain primordial dwarfs that may not respond to growth hormone have large cells for body size and reduced cell population [6]. Future studies should determine whether the different responses to growth hormone in hypopituitarism are related to gradations of insulin release.

Summary

Nucleic acid and protein content was studied in liver, muscle, and cerebrum of hypophysectomized rats between 26 and 49 days of age. Skeletal collagen, carcass protein, fat, water, and muscle mass were also determined and departures from the normal defined by comparing these rats with normal age-mates or pair-fed rats. Food, caloric intake, and body weight changes were monitored daily.

The effect of injecting insulin, growth hormone, or growth hormone and epinephrine concomitantly from 38 to 49 days of age was also reported. The changes in cell size and number and the protein accretion resulting from the administration of these hormones were documented. It was shown that growth hormone is involved with DNA replication and insulin with cytoplasmic growth, particularly in the liver. Epinephrine appears to nullify the action of both hormones by blocking insulin release.

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40. Hormone Assay Laboratories, Chicago, Illinois.
41. This hormone was kindly supplied by the endocrine study section of NIH, and the potency of the growth hormone was found to be 0.97 USP units/mg.
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