Action of Growth Hormone and Thyroxine on Aortas of Hypophysectomized Dogs

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SUMMARY

Young dogs were surgically hypophysectomized and maintained for eleven weeks postoperatively with age-matched normal controls. For three weeks prior to sacrifice, four hypophysectomized dogs were given daily injections of bovine growth hormone (GH, 0.2 mg./kg.) and another four were given daily injections of thyroxine (T_4, 5 μg./kg.). Aortas were removed, cleaned of adventitia and divided into three segments: arch, thoracic and abdominal. Each portion was analyzed for collagen, elastin, deoxyribonucleic acid (DNA), calcium, total mucopolysaccharides (MPS), hyaluronic acid (HA), heparan sulfate (HS), dermatan sulfate (DS) and chondroitin sulfate (CS). The arch and thoracic aortas of normal animals were found to contain more DNA, CS and elastin but less collagen than the abdominal aorta. Removal of the hypophysis resulted in an overall increase in elastin and DNA content and caused a decrease in all sulfated MPS. Administration of either GH or T_4 to hypophysectomized dogs had a profound effect on the majority of constituents in all segments of aorta. GH returned the content of elastin, DS and CS toward normal in at least two of the three aortic segments. T_4 returned the content of DNA, DS and CS toward normal in all segments. Moreover, T_4 treatment caused significant reductions in collagen and HA contents of thoracic and abdominal segments. These results indicate that: (1) the composition of normal aorta varies with the segment studied; (2) the composition of the aorta is markedly affected by hypophysectomy, GH and T_4 treatment; and (3) individual aortic segments show differential sensitivity to a given hormone. DIABETES 22:243-50, April, 1973.

It has previously been reported from this laboratory that hypophysectomy in the dog affects the composition of the aortic wall and that replacement therapy with growth hormone (GH) partially corrects the metabolic defects.[1-3] In the present paper we would like to give a more detailed account of the effects of GH than in our preliminary communications, and also to report on the effect of thyroxine (T_4), which was studied in our laboratory under comparable conditions. It was found that the action of T_4 on the aortic wall is different from but in some ways supplementary to that of GH.

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MATERIALS AND METHODS

Mongrel dogs of either sex between the ages of six and eighteen months and weighing between 8 and 12 kg. were hypophysectomized by the transbuccal technic as described.[4] Previous experiments from this laboratory have shown that little difference exists in the composition of aorta between the ages of six and eighteen months in mongrel dogs of either sex.[5] Completeness of the hypophysectomy was checked by histological examination of the removed tissue and by postmortem examination of the sella turcica. After surgery, dogs remained in the animal colony for eight weeks without hormone treatment. Then, four of the hypophysectomized dogs received subcutaneous injections of a highly purified bovine GH preparation (Wilhelmi, Lot no. 12, gift of the Endocrinology Study Section, N.I.H., Bethesda), 0.2 mg./kg./day for three weeks. Another four of the hypophysectomized dogs received subcutaneous injections of T_4 (sodium levothyroxine, "Synthroid," Flint Laboratories, Division of Baxter Laboratories), 5 μg./kg./day for three weeks. In order to exclude the possibility of hypertension, which is known to affect the composition of the vascular wall,[6] aortic blood pressure was recorded prior to sacrifice in anesthetized animals (pentobarbital, Nembutal, 30 mg./kg.) using a Statham strain gauge manometer (courtesy of Dr. J. Grayson of this Department). The average blood pressure which in normal dogs was 155/125 mm. Hg, dropped in hypophysectomized animals to 103/71 mm.

Hg. Systolic and diastolic blood pressures were both partially raised by T_4 treatment and were completely normalized by GH treatment.

Animals were then sacrificed with an overdose of Nembutal. Aortas were immediately removed, cleaned of adventitia and divided into three portions: the arch, from the valves to the left subclavian artery, the thoracic segment, from the left subclavian artery to the celiac artery, and the abdominal segment, from the celiac to the external iliac arteries. The segments were first weighed on an analytical balance and then minced and defatted with acetone-ether 1:1 at 4° C. for seventy-two hours. The acetone-ether was changed twice daily. Tissues were then air-dried and weighed again. The difference between the wet and dry weight was taken as the "water plus lipid" content of the vessel. The dry defatted tissue was stored at —20° C. until used. Total extractable collagen was estimated on 10 mg. of tissue as hydroxyproline by the method of Neuman and Logan[7] with the modification of Woessner.[8] The collagen content was calculated by assuming a hydroxyproline content of 13.4 per cent.[7] Alkali-stable elastin was determined gravimetrically on the residue, after collagen was removed, by the method of Lowry et al.[9] Deoxyribonucleic acid (DNA) was measured according to Burton.[10] Extraction of DNA from 10 mg. of tissue was carried out with 0.5 M perchloric acid at 70° C. for thirty minutes according to Reich et al.[11] A separate set of experiments has shown that this extraction procedure (a) removed over 90 per cent of the DNA from aorta, and (b) caused no measurable destruction of a standard calf thymus DNA preparation.[5] Calcium was measured in a Perkin-Elmer atomic absorption spectrophotometer according to Dawson and Heaton,[12] after extracting 10 mg. of tissue for sixty minutes in 0.5 M perchloric acid at 100° C. Mucopolysaccharides (MPS) were released from 20 mg. of tissue by digestion with papain according to Schiller et al.[13] Proteolytic digestion was carried out at 56° C. for twenty-four hours in a Dubnoff metabolic shaker. The MPS were then isolated by alcohol precipitation according to Kaplan and Meyer[14] and subsequently by precipitation with cetylpylpyridinium chloride according to Scott.[15] Repeat analyses agree within 10 per cent. Total MPS were estimated as uronic acid by the orcinol technic according to Davidson.[16] This fraction was then resolved into individual components by electrophoresis on cellulose acetate strips in 0.2 M $ZnSO_4$ buffer, pH 5.5, 1 ma./cm. for ninety minutes according to Breen et al.[17] This micromethod was chosen in preference to column chromatographic procedures because of its simplicity and good reproducibility. The strips were stained with Alcian blue and scanned in a Beckman RB Analytrol fitted with 600 mμ filters. Aortic MPS were identified by comparing their mobilities with standard preparations kindly supplied by Drs. J. A. Cifonelli and L. Roden of the University of Chicago. Heparan sulfate isolated from calf aorta was kindly supplied by Dr. A. A. Horner of this Department. In accord with earlier work done by Sirek et al.,[18] four distinct fractions were obtained in this system: (1) hyaluronic acid (HA); (2) heparan sulfate (HS); (3) dermatan sulfate (DS); and (4) the isomeric chondroitin sulfates (CS). Separation of the isomeric CS was attempted according to Seno et al.,[19] but in our hands this procedure was not successful with aorta. A standard curve was constructed for each MPS fraction by electrophoresing, staining and scanning known amounts of standard preparations. Each MPS was quantitated by cutting out the appropriate peak on the Beckman Analytrol chart and weighing it on an analytical balance. A linear relationship was obtained for each constituent within the concentration range of 0.5 to 1.5 nM of uronic acid. Repeat analyses of HS, DS and CS from aorta or standard preparations agreed within 3 per cent, those of HA within 7 per cent.[5]

Twenty-four hour urine collections were made from normal and hypophysectomized dogs with and without hormone treatment. Urine was preserved by the addition of a few drops of 8 N HCl. Total volume was measured and aliquots were taken for analysis of free and total urinary hydroxyproline using the colorimetric method of Prockop and Udenfriend.[20] This method was also used for plasma hydroxyproline determinations.

All chemical determinations were routinely done in quadruplicate. Results of analyses of individual aortic segments are expressed as the mean ± standard error of the mean. Differences between means were tested for statistical significance using Student's t test as described by Snedecor.[21] P values of less than 0.05 were taken as significant.

RESULTS

Table 1 gives the results of collagen determinations. It can be seen that in normal "Control" dogs the collagen content was smallest in the arch, higher in the thoracic segment and almost 2.5 times as large in the abdominal segment. Similar values for collagen were obtained from aortas of hypophysectomized animals with or without GH replacement therapy.

TABLE 1

Aortic collagen in hypophysectomized dogs with and without replacement therapy

Specimen	Arch	Thoracic	Abdominal
	mg./gm. dry defatted tissue Mean ± S.E.M. (n)		
Control	168±5(9)	193±6(12)	431± 8(12)
Hypox	177±6(6)	206±4(8)	429± 8(8)
Hypox + GH	163±4(4)	203±8(4)	449±14(4)
Hypox + T_4	184±4(4)	168±2(4)*†	389± 6(4)*†

* Significantly different from control ($P < 0.025$)
† Significantly different from hypox ($P < 0.01$)

TABLE 2

Aortic elastin in hypophysectomized dogs with and without replacement therapy

Specimen	Arch	Thoracic	Abdominal
	mg./gm. dry defatted tissue Mean ± S.E.M. (n)		
Control	357±9(9)	354±10(12)	194±4(12)
Hypox	403±6(6)*	401±11 (8)*	215±4 (8)*
Hypox + GH	381±7(4)†	383±15 (4)	199±7 (4)†
Hypox + T_4	372±7(4)†	399±13 (4)*	207±4 (4)

* Significantly different from control ($P < 0.05$)
† Significantly different from hypox ($P < 0.05$)

Quantitative measurements of total hydroxyproline in urine of these animals maintained on a constant commercial diet revealed that the daily amount excreted by normal dogs was 20 mg. while that of hypophysectomized animals was only 7 mg. Total plasma hydroxyproline (11 μg./ml.) and urinary free hydroxyproline (0.2 mg./24 hr.) were the same in both groups of animals which indicated that neither retention nor dietary intake accounted for the differences in total hydroxyproline excretion. Replacement therapy with GH raised the daily hydroxyproline excretion to an average of 12 mg./day. Assuming that urinary hydroxyproline excretion can indeed be taken as a measure of total body collagen breakdown,[22] these results suggest that the turnover of collagen was decreased in aortas of hypophysectomized animals and could be raised by GH administration without affecting the net collagen content of the aortic wall.

The results of T_4 treatment were different from those obtained with GH (table 1). While T_4 had no marked effect on collagen content of the arch, it lowered the collagen content of the thoracic and abdominal segments. Urinary total hydroxyproline excretion was raised to 14 mg./day. These results are compatible with increased breakdown and diminished synthesis of collagen. Kivirikko et al.[23] have shown that the rate of collagen breakdown in skin and bone of T_4-treated rats is faster than in nontreated animals, and Mikkonen et al.[24] have demonstrated that T_4 can suppress collagen synthesis in granulation tissue.

The results of elastin determinations are presented in table 2. It can be seen that in normal "Control" dogs the elastin content was highest in the arch and thoracic aorta, and lowest in the abdominal segment ($P < 0.025$). Thus, the respective concentrations of elastin in the three segments were reversed in relation to the collagen content. Hypophysectomy caused an increase in elastin content of all three aortic segments. GH lowered the elastin content significantly in the arch and abdominal segment, but only marginally in the thoracic segment. T_4 treatment lowered the collagen content only in the aortic arch.

The results of DNA determinations are given in table 3. It will be noted that in normal "Control" dogs the DNA content was higher in the arch and thoracic aorta than in the abdominal segment ($P < 0.025$). The same relationship was maintained in hypophysectomized animals, except that the actual amounts were significantly increased in all three segments. GH replacement treatment had no effect on the high aortic DNA content of hypophysectomized dogs. On the other hand, T_4 treatment normalized the DNA content in all three segments.

The rise in DNA content was observed in the presence of normal collagen values and increased elastin values. Since these two constituents represent approximately 60 per cent of total dry mass, it is more than likely that the rise in DNA was real and not due to a

TABLE 3

Aortic deoxyribonucleic acid in hypophysectomized dogs with and without replacement therapy

Specimen	Arch	Thoracic	Abdominal
	mg./gm. dry defatted tissue Mean ± S.E.M. (n)		
Control	5.28±0.10(9)	5.05±0.06(12)	4.28±0.11(12)
Hypox	6.04±0.16(6)*	6.22±0.32 (8)*	5.12±0.16 (8)*
Hypox + GH	6.26±0.08(4)*	6.29±0.25 (4)*	5.36±0.15 (4)*
Hypox + T_4	5.40±0.18(4)†	5.08±0.19 (4)†	4.27±0.03 (4)†

* Significantly different from control ($P < 0.01$)
† Significantly different from hypox ($P < 0.05$)

relative increase in cellularity caused by atrophic changes in other parts of the tissue. That the DNA content of a tissue can be used as an index of cellularity was shown by Vendrely and Vendrely[25] and by Mirsky and Ris,[26] who demonstrated that in any given species the DNA content of all diploid cells was constant.

It is shown in table 4 that the calcium content of aortic segments from hypophysectomized dogs with or without replacement therapy was similar to control segments. Thus atherosclerosis was most probably not the cause of alterations in composition of the aortic wall.

TABLE 4

Aortic calcium in hypophysectomized dogs with and without replacement therapy

Specimen	Arch	Thoracic	Abdominal
	μg./gm. dry defatted tissue Mean ± S.E.M. (n)		
Control	408± 8(9)	404± 8(12)	392±8(12)
Hypox	396± 8(6)	404±12 (8)	408±8 (8)
Hypox + GH	392±12(4)	400±24 (4)	388±8 (4)
Hypox + T_4	408± 8(4)	392±12 (4)	388±8 (4)

The total MPS content is given in table 5. Measured as uronic acid, the total MPS content was highest in the arch and lower in the thoracic and abdominal segments ($P < 0.025$). The MPS content was markedly decreased in hypophysectomized dogs. Administration of either GH or T_4 to these hypophysectomized dogs returned the uronic acid content to normal.

The values for HA are presented in table 6. The HA content was higher in the arch than in the other two segments ($P < 0.05$). Hypophysectomy with or without GH replacement therapy had no effect on the amount of HA in the three aortic segments. However, T_4 induced a marked decrease in aortic HA in the thoracic and abdominal segments. This would explain, at least in part, the results which we obtained for "water plus lipid" content as shown in table 7. The lowest values were obtained in the thoracic and abdominal segments of hypophysectomized dogs treated with T_4 and this coincided with a reduction in HA. Tissue hydration, however, is not dependent on its HA content only. The "water plus lipid" content was reduced in hypophysectomized animals whether or not they were injected with GH, and this was not accompanied by a reduction in the HA content. It is unlikely that the differences obtained were solely due to a change in lipid content, since the amount of lipid represented only about 1 per cent of the total "water plus lipid" content.[5]

TABLE 5

Aortic total mucopolysaccharides in hypophysectomized dogs with and without replacement therapy

Specimen	Arch	Thoracic	Abdominal
	Uronic acid mg./gm. dry defatted tissue Mean ± S.E.M. (n)		
Control	3.16±0.09(9)	2.86±0.14(12)	2.34±0.07(12)
Hypox	2.29±0.07(6)*	1.92±0.07 (8)*	1.84±0.07 (8)*
Hypox + GH	2.83±0.16(4)†	2.50±0.18 (4)†	2.37±0.14 (4)†
Hypox + T_4	3.14±0.11(4)†	2.66±0.08 (4)†	2.12±0.02 (4)†

* Significantly different from control ($P < 0.001$)
† Significantly different from hypox ($P < 0.025$)

TABLE 6

Aortic hyaluronic acid in hypophysectomized dogs with and without replacement therapy

Specimen	Arch	Thoracic	Abdominal
	Uronic acid mg./gm. dry defatted tissue Mean ± S.E.M. (n)		
Control	0.35±0.02(9)	0.29±0.02(12)	0.29±0.02(12)
Hypox	0.29±0.04(6)	0.33±0.02 (8)	0.29±0.02 (8)
Hypox + GH	0.37±0.04(4)·	0.31±0.02 (4)	0.35±0.04 (4)
Hypox T_4	0.31±0.02(4)	0.17±0.02 (4)*†	0.19±0.02 (4)*†

* Significantly different from control ($P < 0.025$)
† Significantly different from hypox ($P < 0.01$)

TABLE 7

Aortic water plus lipid in hypophysectomized dogs with and without replacement therapy

Specimen	Arch	Thoracic	Abdominal
	gm./gm. dry defatted tissue Mean ± S.E.M. (n)		
Control	2.92±0.04(9)	2.76±0.04(12)	2.69±0.07(12)
Hypox	2.63±0.05(6)*	2.52±0.06 (8)*	2.53±0.04 (8)
Hypox + GH	2.67±0.01(4)*	2.59±0.01 (4)*	2.65±0.07 (4)
Hypox + T_4	2.57±0.04(4)*	2.28±0.05 (4)*†	2.03±0.03 (4)*†

* Significantly different from control ($P < 0.05$)
† Significantly different from hypox ($P < 0.025$)

Thus, it seems justified to assume that the rather large differences were due predominantly to a change in water content.

The data for sulfated MPS are presented in tables 8-10. HS was evenly distributed in all three segments of normal aorta, and its concentration was the lowest of all sulfated MPS. The DS content was higher than the HS content in every aortic segment, but the distribution was uneven. The DS content of the thoracic portion was significantly lower ($P < 0.05$) than that of the arch or abdominal segment. The most marked differences were seen in the CS content. The arch and the thoracic portion were particularly rich in CS, but the abdominal segment was poor ($P < 0.01$) and in fact contained less CS than DS. Hypophysectomy caused a marked fall in the content of HS, DS, and CS in all three aortic segments. GH alone or T_4 alone were able to return the content of CS to control levels in all segments of the aorta. The DS content was returned to normal in all segments by T_4 but only in thoracic and abdominal aorta by GH. The concentration of HS was returned to control values in the aortic arch and the thoracic aorta by T_4 and in the abdominal aorta by GH.

TABLE 8

Aortic heparan sulfate in hypophysectomized dogs with and without replacement therapy

Specimen	Arch	Thoracic	Abdominal
	Uronic acid mg./gm. dry defatted tissue Mean ± S.E.M. (n)		
Control	0.54±0.02(9)	0.49±0.02(12)	0.45±0.02(12)
Hypox	0.40±0.02(6)*	0.33±0.02 (8)*	0.35±0.02 (8)*
Hypox + GH	0.48±0.04(4)	0.41±0.04 (4)	0.48±0.04 (4)†
Hypox + T_4	0.50±0.04(4)†	0.52±0.02 (4)†	0.41±0.02 (4)

* Significantly different from control ($P < 0.005$)
† Significantly different from hypox ($P < 0.05$)

DISCUSSION

The results of our experiments have shown that hypophysectomy had a profound effect on every parameter of study with the exception of collagen and HA. This is diagrammatically presented in table 11, in which the direction of change is indicated by arrows. Replacement therapy with GH and T_4 produced a variety of responses in a number of segments and the trends are diagrammatically presented also in table 11.

TABLE 9

Aortic dermatan sulfate in hypophysectomized dogs with and without replacement therapy

Specimen	Arch	Thoracic	Abdominal
	Uronic acid mg./gm. dry defatted tissue Mean ± S.E.M. (n)		
Control	0.80±0.04(9)	0.70±0.04(12)	0.85±0.04(12)
Hypox	0.64±0.02(6)*	0.52±0.02 (8)*	0.72±0.02 (8)*
Hypox + GH	0.68±0.02 (4)	0.65±0.04 (4)†	0.83±0.06 (4)†
Hypox + T_4	0.81±0.02 (4)†	0.71±0.04 (4)†	0.80±0.02 (4)†

* Significantly different from control ($P < 0.025$)
† Significantly different from hypox ($P < 0.05$)

The rise in the elastin content was observed in animals that were hypophysectomized over a period of less than three months. This and the profound effects of GH and T_4 treatment are in contrast with the common belief that the turnover of elastin is extremely slow.[27] Our results are compatible with those of Loeven,[28] who has shown an increase in aortic elastin to occur in rats shortly after hypophysectomy. He assumed that the rise in elastin was due to a decrease in the activity of elastolytic enzymes.

The marked increase in cellularity of all three aortic segments from hypophysectomized dogs seen in our studies is similar to the response observed by Hall[29] in skin and by Eartly et al.[30] in the epidermis of hypophysectomized rats. Since T_4 administration returned the DNA content to normal in all segments of aorta, it

TABLE 10

Aortic chondroitin sulfate in hypophysectomized dogs with and without replacement therapy

Specimen	Arch	Thoracic	Abdominal
	Uronic acid mg./gm. dry defatted tissue Mean ± S.E.M. (n)		
Control	1.47±0.08(9)	1.38±0.10(12)	0.75±0.04(12)
Hypox	0.96±0.06(6)*	0.75±0.04 (8)*	0.48±0.04 (8)*
Hypox + GH	1.30±0.08(4)†	1.13±0.12 (4)†	0.71±0.06 (4)†
Hypox + T_4	1.52±0.06(4)†	1.25±0.04 (4)†	0.72±0.02 (4)†

* Significantly different from control ($P < 0.001$)
† Significantly different from hypox ($P < 0.01$)

TABLE 11

Effects of hypophysectomy (Hx), growth hormone (GH) and thyroxine (T_4) treatment on canine aorta

Constituent	Arch			Thoracic			Abdominal		
	Hx*	GH†	T_4†	Hx*	GH†	T_4†	Hx*	GH†	T_4†
Collagen	→	→	→	→	→	↓	→	→	↓
Elastin	↑	↓	↓	↑	→	→	↑	↓	→
DNA	↑	→	↓	↑	→	↓	↑	→	↓
Water + Lipid	↓	→	→	↓	→	↓	→	→	↓
Total MPS	↓	↑	↑	↓	↑	↑	↓	↑	↑
HA	→	→	→	→	→	↓	→	→	↓
HS	↓	→	↑	↓	→	↑	↓	↑	→
DS	↓	→	↑	↓	↑	↑	↓	↑	↑
CS	↓	↑	↑	↓	↑	↑	↓	↑	↑

* Arrows refer to direction of change relative to controls.
† Arrows refer to direction of change relative to hypox.

appears probable that T_4 exerts a major regulatory influence over aortic cell number. If one assumes that cells from hypophysectomized dogs are metabolically less active than normal cells, an increase in cellularity may serve a useful purpose.

Cell proliferation and a rise in collagen content are the earliest histologically detectable changes in the aorta of atherosclerotic animals.[31] In this connection it is of interest to note that high cholesterol and high fat diets alone are not capable of producing atherosclerotic lesions in the dog. It is necessary at the same time to render the animals hypothyroid, for instance by feeding propylthiouracil.[32] Also, it has been reported that atherosclerosis occurs more frequently in hypothyroid patients than in the general population.[33] Thus a change in concentration of T_4 may cause alterations in the composition of the vessel wall that will make it either prone or resistant, as the case may be, to the development of atherosclerotic and possibly other lesions.

The reduction in total MPS content in aortas of hypophysectomized dogs was caused by a reduction of the sulfated fractions; either one of the two hormones returned the content to normal. GH, but not T_4, exerts its effects on sulfated MPS through the mediation of somatomedin.[34,35] Thus, the mechanism of action of these two hormones on sulfated MPS is not identical. While T_4 and GH raised the content of CS in all segments of aorta, the effects on other MPS constituents did not involve necessarily identical segments. Also, it may be argued that the effect of GH was mediated by insulin: GH administration to our hypophysectomized dogs raised the immunoassayable plasma insulin concentration from 12 ± 1 μU./ml. to the normal range of 21 ± 1 μU./ml. The results of another study from this laboratory (to be published) indicate that insulin has only limited effects on the composition of canine aorta. It is unlikely, therefore, that the relatively small rise in the plasma insulin concentration in our animals could have accounted for the response to GH. It may well be that alterations in plasma GH concentrations are relevant to the problem of diabetic macroangiopathy, because basal GH levels, as well as peak plasma concentrations, have been found to be abnormally high in juvenile diabetic patients.[35]

Sulfated MPS have been mentioned as both hero and villain in the story of atherosclerosis. It has been found that aortic MPS extracts have anticoagulant and lipoprotein lipase activating properties.[37,38] It follows that a decrease in these substances could lead to lipoprotein and fibrin deposition in the aortic wall, which then could be predisposing to, or an actual cause of, atheroma formation. On the other hand, CS has been shown to bind betalipoproteins and fibrinogen in vitro[39] and to complex calcium.[40] Consequently it may be postulated that MPS in the vessel wall could precipitate betalipoproteins and fibrinogen from plasma and could account for their deposition.

In this study we have shown in a quantitative fashion that the chemical composition of normal canine aorta varies from segment to segment and that the major structural components, the fibrous proteins, cells and various MPS, can be markedly affected by the presence or absence of hormones. Differences in composition of aortic segments are known to exist in man and in a variety of animal species.[41,42] Our own study has confirmed this and also has expanded our knowledge by

demonstrating that differences in segmental composition of canine aorta are characteristic not only for scleroproteins[41] but also for DNA and MPS fractions. What appears to have gone unnoticed until now is the difference in sensitiivty of individual aortic segments to hormones. The differences were not always striking, but they were consistently observed; the spread of values within each group was relatively small and, therefore, the statistical significance often was higher than the customary 5 per cent level. It is recognized, however, that the number of animals in the hormone-replaced group was limited to four, hardly constituting a universal population on which to base a statistical study. Thus, the concept of differential sensitivity to hormones has to be further investigated on a larger material before its significance in physiologic and pathologic conditions can be fully appreciated.

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REFERENCES

[1] Brosnan, M., Sirek, O. V., and Sirek, A.: Effects of hypophysectomy on the composition of canine aorta. Proc. Can. Fed. Biol. Sci. *14*:26, 1971.

[2] Brosnan, M., Sirek, O. V., and Sirek, A.: Effect of hypophysectomy and growth hormone replacement on the composition of canine aorta. Biochem. J. *125*:116P, 1971.

[3] Sirek, O. V., Brosnan, M., and Sirek, A.: Effect of hypophysectomy and growth hormone treatment on the composition of canine aorta. *In* Second International Symposium on Early Diabetes. Cole, H. S., and Camerini-Davalos, R. A., Eds. New York, Academic Press. In press.

[4] Sirek, A., Schoeffling, K., Webster, M., and Sirek, O. V.: Absence of effect of bovine growth hormone on blood sugar of Houssay dogs. Can. J. Physiol. Pharmacol. *42*:299, 1964.

[5] Brosnan, M. E.: Effects of Hypophysectomy, Pancreatectomy and Hormone Replacement on the Composition of Canine Aorta. Ph.D. Thesis, University of Toronto, 1972.

[6] Manley, G.: Changes in vascular mucopolysaccharides with age and blood pressure. Br. J. Exp. Pathol. *46*:125, 1965.

[7] Neuman, R. E., and Logan, H. A.: The determination of collagen and elastin in tissues. J. Biol. Chem. *186*:549, 1950.

[8] Woessner, J. F., Jr.: The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. Arch. Biochem. Biophys. *93*:440, 1961.

[9] Lowry, D. H., Gilligan, D. R., and Katersky, E. M.: The determination of collagen and elastin in tissues, with results obtained in various normal tissues from different species. J. Biol. Chem. *139*:795, 1941.

[10] Burton, K.: A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. *62*:315, 1956.

[11] Reich, E., Franklin, R. M., Shatkin, A. J., and Tatum, E. L.: Action of actinomycin D on animal cells and viruses. Proc. Natl. Acad. Sci. USA *48*:1238, 1962.

[12] Dawson, J. B., and Heaton, F. W.: The determination of magnesium in biological materials by atomic absorption spectrophotometry. Biochem. J. *80*:99, 1961.

[13] Schiller, S., Slover, G. A., and Dorfman, A.: A method for the separation of acid mucopolysaccharides: Its application to the isolation of heparin from the skin of rats. J. Biol. Chem. *236*:983, 1961.

[14] Kaplan, D., and Meyer, K.: Mucopolysaccharides of aorta at various ages. Proc. Soc. Exp. Biol. Med. *105*:78, 1960.

[15] Scott, J. E.: Aliphatic ammonium salts in the assay of acidic polysaccharides from tissues. *In* Methods of Biochemical Analysis, vol. 8. Glick, D., Ed. New York, Interscience Publishers, 1960, p. 145.

[16] Davidson, E. A.: Analysis of sugar found in mucopolysaccharides. *In* Methods in Enzymology, vol. 8. Neufeld, E. F., and Ginsberg, V., Eds. New York, Academic Press, 1966, p. 52.

[17] Breen, M., Weinstein, H. G., Andersen, M., and Veis, A.: Microanalysis and characterization of acidic glycosaminoglycans in human tissues. Anal. Biochem. *35*:146, 1970.

[18] Sirek, O. V., Schiller, S., and Dorfman, A.: Acid mucopolysaccharides in aortic tissue of the dog. Biochim. Biophys. Acta *83*:148, 1964.

[19] Seno, N., Anno, K., Kondo, K., Nagase, S., and Saito, S.: Improved method for electrophoretic separation and rapid quantitation of isometric chondroitin sulfates on cellulose acetate strips. Anal. Biochem. *37*:197, 1970.

[20] Prockop, D. J., and Udenfriend, S.: A specific method for the analysis of hydroxyproline in tissues and urine. Anal. Biochem. *1*:228, 1960.

[21] Snedecor, G. W.: Sampling from a normally distributed population. *In* Statistical Methods. Ames, Iowa: Iowa State College Press, 1956, p. 35.

[22] Prockop, D. J., and Kivirikko, K. I.: Relationship of hydroxyproline excretion in urine to collagen metabolism. Biochemistry and clinical applications. Ann. Intern. Med. 66: 1243, 1967.

[23] Kivirikko, K. I., Laitinen, O., Aer, J., and Halme, J.: Metabolism of collagen in experimental hyperthyroidism and hypothyroidism in the rat. Endocrinology *80*:1051, 1967.

[24] Mikkonen, L., Lampiaho, K., and Kulonen, E.: Effect of thyroid hormones, somatotrophin, insulin and corticosteroids on synthesis of collagen in granulation tissue both in vivo and in vitro. Acta Endocrinol. *51*:23, 1966.

[25] Vendrely, R., and Vendrely, C.: La teneur du noyau cellulaire en acide désoxyribonucléique à travers les organes, les individus et les espèces animales. Experientia *5*:327, 1949.

[26] Mirsky, A. E., and Ris, H.: Variable and constant components of chromosomes. Nature (Lond) *163*:666, 1949.

[27] Partridge, S. M.: Elastin. *In* Advances in Protein Chem-

istry, vol. 17. Anfinsen, C. B., Jr., Anson, M. L., Bailey, K., and Edsall, J. T., Eds. New York, Academic Press, 1962, p. 227.

[28] Loeven, W. A.: Effect of hypophysectomy of female rats on the activity of pancreatic elastolytic enzymes and the elastin content of heart, lung and aorta. J. Endocrinol. *48*:401, 1970.

[29] Hall, M. C.: The effect of hypophysectomy in retarding the aging process of the loose connective tissue of the rat. Can. J. Biochem. Physiol. *39*:1525, 1961.

[30] Eartly, H., Grad, B., and Leblond, C. P.: The antagonistic relationship between testosterone and thyroxine in maintaining the epidermis of the male rat. Endocrinology *49*:677, 1951.

[31] Wissler, R. W.: The arterial medial cell, smooth muscle or multifunctional mesenchyme? J. Atheroscler. Res. *8*:201, 1968.

[32] Steiner, A., and Kendall, F. E.: Atherosclerosis and arteriosclerosis in dogs following ingestion of cholesterol and thiouracil. Arch. Pathol. *42*:433, 1946.

[33] Katz, L. N., and Stamler, J.: Experimental Atherosclerosis. Springfield, Ill., Charles C. Thomas, 1953.

[34] Daughaday, W. H., and Kipnis, D. M.: The growth-promoting and anti-insulin actions of somatotropin. Recent Progr. Horm. Res. *22*:49, 1966.

[35] Daughaday, W. H., Hall, K., Raben, M. S., Salmon, W. D., Jr., Van den Brande, J. L., and Van Wyk, J. J.: Somatomedin: Proposed designation for sulphation factor. Nature (Lond) *235*:107, 1972.

[36] Hansen, A. P.: Abnormal serum growth hormone response to exercise in juvenile diabetics. J. Clin. Invest. *49*:1467, 1970.

[37] Kirk, J. E.: Anticoagulant activity of human arterial mucopolysaccharides. Nature (Lond) *184*:369, 1959.

[38] Gore, I., and Larkey, B. J.: Functional activity of aortic mucopolysaccharides. J. Lab. Clin. Med. *56*:839, 1960.

[39] Anderson, A. J.: The formation of chondromucoprotein-fibrinogen and chondromucoprotein-β-lipoprotein complexes. Chemical and fibrinolytic properties. Biochem. J. *88*:460, 1963.

[40] Urist, M. R., Speer, D. P., Ibsen, K. J., and Strates, B. S.: Calcium binding by chondroitin sulfate. Calcif. Tissue Res. *2*:253, 1968.

[41] Cleary, E. G.: A Correlative Study of the Non-Uniform Arterial Wall. M. D. Thesis: National University of Sydney, 1963.

[42] Engel, U. R.: Glycosaminoglycans in the aorta of six animal species. A chemical and morphological comparison of their topographical distribution. Atherosclerosis *13*:45, 1971.