Further work along these lines is needed to determine the reasons for the lowered digestibility.

Summary. Ten rats born and maintained in a low pressure, pure oxygen environment did not differ in growth rates from ground level controls. However, overall digestibility was lowered in experimentals, 74.3% *vs* 79.5% for controls ($P<.001$), with even greater differences in protein digestibility, 65.5% *vs* 74.6% ($P<.001$). Although less fat was digested by experimentals, 75.6% *vs* 81.4% for controls, these differences were not significant. Net caloric intake was similar due to a slightly greater food intake by experimentals. Calories given off as heat did not differ, suggesting similar metabolic rates.

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Connective Tissue XV. Amino Acid Composition of Soluble Collagen of Pig Uterus. (32192)

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Variations in amino acid composition of collagen, both from species to species and from tissue to tissue in a given species have been reported by a number of investigators (1-9). The importance of total pyrolidine amino acid, (*i.e.*, proline plus hydroxyproline) to the stability of the collagen structure has been discussed by Harrington *et al* (10). A possible role of lysine in the formation of intra- and inter-molecular cross linkages in collagen was reported by two independent groups(11,12). To understand alterations of collagen in pathological states, the complete elucidation of the normal composition of collagens from the various tissues is necessary. In the past few years, soluble collagen has been prepared from skin, tendon, and fish swim bladder(7,8,9) and the heat denatured products and their amino acid compositions have also been determined. Recently we reported the preparation and amino acid composition of collagen from human uterus(13). The present report describes (a) methods of preparing 1 M NaCl- and 0.5 M acetic acid-soluble collagens from pig uterus; (b) the separation of the α- and β-chains of these collagens after heat denaturation; and (c) the determination of the amino acid composition of the α- and β-chains of these pig uterine collagens.

Methods. Uteri from 6- to 12-month-old pigs, obtained from a local slaughter house, were cut into 1 inch squares and ground with dry ice in a meat grinder. All procedures were performed at 5°C. All extractions were done with a magnetic type stirrer and the extracts were cleared at 30,000 rpm in a Spinco Model L centrifuge for 1 hour before precipitation.

Preparation of 1M NaCl soluble collagen. Uterine tissue was first extracted with 1M NaCl and collagen was precipitated by the addition of solid NaCl to 20% concentration. In one instance this precipitation was

repeated twice. The crude collagen precipitate was reextracted with 1 M NaCl and dialyzed against phosphate buffer pH 7.6, ionic strength = 0.4. Trichloroacetic acid (TCA) was then added dropwise to a pH of 3.5 in order to precipitate non-collagenous protein (NCP). After removing the NCP collagen was precipitated by addition of ETOH to 14%(14).

Preparation of 0.5 M acetic acid-soluble collagen. The residue after 1 M NaCl extraction was extracted with 0.5 M acetic acid. After centrifugation the collagen was precipitated in the supernate by the addition of solid NaCl to 5%. The crude collagen was reextracted with 0.5 M acetic acid and precipitated by dialyzing against 0.02 M Na_2HPO_4. This partially purified collagen was extracted with 1 M NaCl and either reprecipitated with 20% NaCl or purified by removal of NCP with TCA and then precipitated with ETOH as described previously. In some instances, a combination of these two methods was used.

Separation of β- and α-chains on Sephadex G-200. Soluble collagen was dissolved in 0.1 M acetate buffer, pH 4.8 and denatured at 40° for 30 minutes. The resulting solution was chromatographed on a Sephadex G-200 column with recycling as described previously (13). The β- and α-components were collected during the 3rd cycle.

Separation of β_{11} and β_{12} and α1 and α2 chains on carboxymethyl (CM)-cellulose columns. β_{11} and β_{12} and α1 and α2 chains were separated on CM-cellulose columns by the method of Piez *et al*(7). The Beckman DB Spectrophotometer was set at 219 mμ. The absorbency was recorded.

Amino acid analysis. Amino acid analysis was done with the Technicon amino acid analyzer (Chauncey, N.Y.). Corrections for destruction and liberation of amino acids during hydrolysis were made according to the method of Piez *et al*(15).

Chemical analysis. Nitrogen was determined by microkjeldahl digestion and the Conway diffusion techniques(16). Hydroxyproline was determined by the method of Newman and Logan(17).

Results and discussion. The purification of 1 M NaCl-soluble and acetic acid-soluble collagens of pig uterus is shown by the successive increases in the concentration of hydroxyproline with each step of purification (Table I). Increasing the time of extraction did not improve the yield of soluble collagen. Precipitation with 20% NaCl increased the hydroxyproline concentration from 0.1-0.5% to approximately 2-8%. Repeated precipitation with 20% NaCl did not further augment the yield of hydroxyproline. TCA precipitation of noncollagenous protein further raised the hydroxyproline concentration to 4-10%. Precipitation with 14% ETOH raised the hydroxyproline concentration of the preparation to that which would be expected for collagen in a purified state (11.0-13.7%). The total yield obtained represented approximately 0.02% of the weight of wet tissue.

In the preparation of acetic acid-soluble collagens, the 5% NaCl precipitation increased the hydroxyproline concentration by 50 to 100%. Purification of the acid soluble collagen by dialyzing against 0.02 M Na_2HPO_4 or precipitation with 20% NaCl further increased the percentage of hydroxyproline. TCA precipitation of noncollagenous proteins and ETOH precipitation raised the hydroxyproline concentration to 12-13%. The total yield of acetic acid soluble collagen is approximately 0.005-0.01% of the weight of wet tissue. The yield of NaCl soluble and acetic acid soluble collagen from pig uterus was approximately one-half that previously observed with human uterus.

The approximate percentage distribution of the components of denatured salt- and acid-soluble collagen of pig uterus is shown in Table II. The salt-soluble collagen contains approximately 65% α- and 35% β-components. In contrast, acid-soluble collagen contains 55% α- and 45% β-components. In comparison with the component distributions reported by Piez *et al*(7,8), the denatured salt-soluble collagen of pig uterus contains 20% less α-component than that of the rat skin and 10% less than that of human skin. These data agree well with those obtained on human uterine samples(13). Salt-soluble collagen of pig uterus differs from

that of the human uterus by containing a relatively higher percentage of β_{11} and a lower percentage of β_{12}. The $\alpha 1$ component of the acid soluble collagen of pig uterus is slightly greater than that of either human uterus or human skin.

The amino acid composition of pig uterus salt soluble collagen and its components is shown in Table III. The data on the acetic acid soluble collagen are not shown since they were essentially the same as those for salt-soluble collagen. In Table III, the similarity of the amino acid composition of $\alpha 1$ and β_{11} components is apparent. There were differences in amino acid composition between $\alpha 1$ and $\alpha 2$ components in more than half of the amino acids determined. Like the findings in the study of human uterus(13), higher

TABLE I. Purification of NaCl- and Acetic Acid-Soluble Collagen.

Preparation	Tissue wt, g	NaCl-soluble collagen				Acetic acid-soluble collagen			
		Step No.	Method of purification	mg prot.	% OH-Pr.	Step No.	Method of purification	mg prot.	% OH-Pr.
1	525	I	2x24 hr & 1x72 hr extraction	85,590	.11	I	3x96 hr extraction	2,409	2.49
						II	5% NaCl ppt.	569	3.08
		II	1st 20% NaCl ppt.	295	7.11	III	20% NaCl ppt.	77	11.43
			2nd 20% NaCl ppt.	216	8.14	IV	TCA ppt. of NCP	64	11.79
			3rd 20% NaCl ppt.	199	8.04	V	ETOH ppt.	53	13.45
		III	TCA ppt. of NCP*	139	8.88				
		IV	ETOH ppt.	101	11.08				
2	597	I	3x24 hr extraction	25,909	.25	I	1x96 hr & 1x72 hr extraction	1,740	2.96
		II	20% NaCl ppt.	370	6.68				
		III	TCA ppt. of NCP	194	10.45	II	5% NaCl ppt.	596	4.09
		IV	ETOH ppt.	131	13.72	III	.02M Na_2HPO_4 ppt.	443	5.09
						IV	5% NaCl extraction	204	8.28
						V	20% NaCl ppt.	116	10.09
						VI	TCA ppt. of NCP	65	11.76
						VII	ETOH ppt.	50	13.29
3	527	I	2x24 hr extraction	28,368	.47	I	2x24 hr extraction	2,316	.76
		II	20% NaCl ppt.	1,361	1.91	II	5% NaCl ppt.	1,115	1.07
		III	TCA ppt. of NCP	415	4.40	III	.02M Na_2HPO_4 ppt.	79	4.98
		IV	ETOH ppt.	114	12.64	IV	TCA ppt. of NCP	36	8.15
						V	ETOH ppt.	20	12.12

* NCP = Noncollagenous protein.

TABLE II. Percentage Distribution of Components in Denatured Salt- and Acid-Soluble Collagen of Pig Uterus.

Collagen		Components $\alpha 1$	$\alpha 2$	β_{11}	β_{12}
Salt-soluble	1	37	23	15	25
	2	42	21	15	21
	3	43	27	10	19
Avg		41	24	14	22
Acid-soluble	1	43	13	13	31
	2	37	16	15	32
Avg		40	14	14	31

* Calculated from the area under the appropriate peaks on both CM-cellulose and Sephadex G-200 columns. The γ-component is not included.

TABLE III. Amino Acid Composition of 1 M NaCl-Soluble Collagen and Its Components of Pig Uterus (Residue/1000 Residues).*

	Collagen	$\alpha 1$	β_{11}	β_{12}	$\alpha 2$
Cysteic acid	1	1	2	1	1
4 Hydroxyproline	102	101	96	94	86
Aspartic acid	43	42	43	46	47
Threonine	16	16	16	15	16
Serine	35	36	39	34	31
Glutamic acid	74	75	79	75	71
Proline	121	130	125	122	116
Glycine	334	331	328	332	338
Alanine	113	119	121	114	110
Valine	25	21	22	30	37
Methionine†	4	3	1	2	2
Isoleucine	10	7	8	11	14
Leucine	23	19	19	24	30
Tyrosine	3	1	Trace	1	2
Phenylalanine	12	13	14	13	13
Hydroxylysine	10	8	9	9	11
Lysine	25	28	28	23	19
Histidine	4	2	3	5	8
Arginine	51	52	54	54	55

* Mean value from 3 sets of experiments.
† Methionine sulfoxide is included.

amounts of hydroxyproline, proline, alanine, phenylalanine, lysine and lower amounts of aspartic acid, valine, leucine, isoleucine, hydroxylysine and histidine were present in the $\alpha 1$ chain as compared with the $\alpha 2$ chain. In addition, larger amounts of serine, glutamic acid, methionine and lower amounts of tyrosine were found in the $\alpha 1$ chain than in the $\alpha 2$ chain. The amino acid composition of the β_{12} chain is consistent with the concept that this is a 1:1 mixture of $\alpha 1$ and $\alpha 2$ chains.

The present data are in good accord with previous data on human uterus. Pig uterine collagen contains higher concentrations of hydroxyproline and hydroxylysine than the collagen of the skin of rat or the human (7,8,13). These findings also agree well with those of Harding(6). Pig uterine collagen contains more hydroxylysine than human uterine collagen(13). It also contains less valine and more proline than the human uterine collagen; and less methionine than human skin. Between 0.7-1.5 residue/1000 residues of cysteic acid was found consistently in pig uterine collagen. This was also observed in human uterine collagen(13).

The total collagen content of 36 pig uterine specimens was found to be 26.5 ± 0.7% of the total protein. The solubility of pig uterine collagen is even less than that of human uterine collagen.

Summary. 1. 1 M NaCl-soluble and 0.5 M acetic acid-soluble collagen were purified from pig uterus. The total yield was approximately 0.03% of the weight of the wet tissue. 2. After heat denaturation, the α- and β- components were separated on Sephadex G-200 and CM-cellulose column. Like the finding in human uterine collagen, less α- and more β- components were found in the salt soluble collagen of pig uterus than in the skin of the rat and human being. However, more α- and less β-component were found in the uterine collagen of pig than in that from the human being. 3. Pig uterine collagen contained a somewhat higher level of hydroxyproline and hydroxylysine than dermal collagen of rat and human being.

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Effect of Acute Exercise on Cerebral Blood Flow in Man.* (32193)

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Little is known concerning the effects of exercise on cerebral blood flow. This is perhaps due to the difficulties involved in the application of the methods available in the past (inert gas, indicator injection) which require blood samples from the jugular sinus and an artery(1). Only one report could be found in the literature—that of Kleinerman and Sancetta(2) who observed that mild steady-state exercise produced a slight decrease in cerebral blood flow, which they attributed to decreased carbon dioxide in blood(3).

The development of the impedance rheograph offered an opportunity for quantitative but relative measurement with continuous recording of cerebral blood flow before and after a short period of exercise(4-7).

Methods. The experiments were carried out on a group of 11 volunteers (4 males and 7 females). Two of the females were laboratory technicians, the other 9 subjects were fourth year university students enrolled in Physical and Health Education.

Cerebral blood flow was recorded continuously using an E. & M. Impedance Rheograph and scalp plate electrodes 2 cm in diameter, attached to the frontal and parieto-temporal regions. This instrument measures the changes in impedance to an A.C. current of 75 Kc/sec occurring in the tissue between the electrodes, during each systole, these changes being proportional to the instantaneous blood flow(4-7). Thus it provides a flow pulse that reflects any changes in blood flow. To have a better appreciation of the changes in blood flow, the pulses were electronically integrated and the total "pulse area per unit time" was recorded in a second channel of the polygraph (Grass 5A). The electrocardiogram (E.K.G.) in the II standard derivation and the skin temperature between 2 fingers were also recorded simultaneously.

The subjects sat quietly for 5 minutes, during which a basal recording was taken. The E.K.G. in all the standard derivations was also taken in order to obtain information about possible abnormalities that might interfere with the results. All except one of the hearts were electrocardiographically normal and the only abnormality found in one of the females was a right branch block; data on this subject were not included in the general averages.

After basal recordings, the subjects performed a modified Harvard Step-Test (step up and down to a 45 cm block) at a frequency of 20/min for 5 minutes. No recordings could be done during the exercise because of movement artifacts. Immediately after the exercise they sat down again, and recordings were made for at least 10 minutes.

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