erythrocyte radioiron incorporation. In addition, the increase in erythrocyte radioiron produced by LT_3 and DT_3 in transfused polycythemic rats, when the oxygen carrying capacity of the increased red cell mass would have been expected to obviate an erythropoietic response to oxygen need, further supports the contention that the mechanism of enhanced erythropoiesis is independent of changes in calorigenesis. The rate of response to a single injection of triiodothyronine suggests that the erythropoietic effect is not mediated through erythropoietin elaboration. Furthermore, plasma from T_3 treated animals did not stimulate erythropoiesis in a highly sensitive assay system. Triiodothyronine stimulates erythropoiesis as determined by augmented erythrocyte radioiron incorporation. This stimulation is apparently not due to the calorigenic properties of the hormone alone and is apparently not mediated through augmented erythropoietin production.

Summary. Both dextro-triiodothyronine and levo-triiodothyronine produced similar increases in RBC ^{59}Fe incorporation in polycythemic rats when the increased red blood cell mass would have been expected to obviate the response to oxygen need. Single doses of 25, 100 or 250 μg LT_3 and DT_3 produced similar increases in RBC ^{59}Fe incorporation in normal rats. The RBC ^{59}Fe incorporation was minimally increased at 24 hours and continued to increase 48 and 72 hours following a single dose of LT_3 or DT_3. Plasma obtained from rats following administration of LT_3 or DT_3 failed to demonstrate erythropoietic activity on bioassay in the plethoric mouse, suggesting that increased erythropoietin production is not the mechanism for the augmented erythropoiesis.

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Phosphoglyceric Kinase in Human Vascular Tissue.* (31360)

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The reaction catalyzed by the phosphoglyceric kinase enzyme is an integral part of the glycolytic pathway. Since glycolysis is the main source of biological energy in human aortic tissue(1) it was considered important to determine the phosphoglyceric kinase (PGK) activity in various types of human blood vessels. The PGK measurements were made essentially as described by Axelrod and Bandurski(2) in which procedure the tissue is incubated with 3-phosphoglycerate (3-PGA) and adenosine triphosphate (ATP) in the presence of hydroxylamine. This reverse reaction is especially suitable for PGK activity determinations in crude tissue extracts(3). Colorimetric assay of 1,3-diphosphoglycerate formed was made by the Lipmann-Tuttle(4) ferric hydroxamic method.

Materials and methods. Vascular samples were obtained fresh at autopsy from subjects ranging in age from 0 to 86 years and were immediately frozen. A total of 445 specimens was included in the study. The PGK assays were made separately on normal and arteriosclerotic tissue portions.

Aqueous 2% homogenates of intima-media layers of the vascular samples were prepared at 0°C with a Kontes Duall type tissue

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TABLE I. Mean Phosphoglyceric Kinase Activities of Human Vascular Tissue.*

	Age group, yr	No.	Per g wet tissue	S.E.M.	Per g tissue nitrogen	S.E.M.
Thoracic descending aorta, normal	0- 9	12	.206	.007	4.98	.39
	10-19	4	.321	—	7.66	—
	20-29	10	.276	.031	6.32	.65
	30-39	6	.321	.049	7.73	1.18
	40-49	8	.306	.045	7.88	1.17
	50-59	21	.292	.021	7.96	.60
	60-69	15	.254	.025	6.81	.76
	70-86	14	.292	.023	8.10	.54
Total		90	.277	.011	7.15	.29
Thoracic descending aorta, lipid-arteriosclerotic	18-86	48	.256	.018	7.14	.52
Thoracic descending aorta, fibrous-arteriosclerotic	46-86	20	.288	.017	8.45	.57
Ascending aorta, normal	0-86	32	.276	.019	6.92	.49
Abdominal aorta, normal	0-86	28	.233	.022	6.10	.69
Pulmonary artery, normal	0- 9	11	.208	.036	5.21	.84
	10-19	5	.329	.029	7.94	.55
	20-29	7	.352	.029	9.08	.67
	30-39	7	.310	.021	8.12	.56
	40-49	7	.290	.021	7.75	.82
	50-59	15	.296	.034	8.92	1.06
	60-69	14	.317	.026	9.15	.88
	70-86	12	.317	.021	9.48	.89
Total		78	.298	.011	8.30	.39
Coronary artery, normal	0- 9	5	.184	.043	3.79	1.14
	10-19	5	.254	.042	7.15	1.14
	20-29	7	.271	.044	7.47	1.16
	30-39	7	.311	.037	8.14	.86
	40-49	7	.258	.040	6.61	.82
	50-59	8	.258	.040	7.64	1.21
	60-69	6	.297	.033	9.39	1.47
	70-86	8	.378	.027	11.40	1.13
Total		53	.282	.014	7.89	.49
Coronary artery, lipid-arteriosclerotic	23-86	33	.293	.021	9.00	.61
Vena cava inferior, normal	0- 9	11	.032	.015	.78	.39
	10-19	3	.104	—	2.44	—
	20-29	7	.141	.033	3.52	.87
	30-39	7	.120	.039	2.98	.95
	40-49	6	.201	.015	5.79	.55
	50-59	12	.215	.021	5.79	.59
	60-69	9	.258	.029	6.89	.78
	70-86	8	.212	.026	5.74	.63
Total		63	.164	.013	4.34	.37

* Values expressed in millimoles of 1,3-diphosphoglyceric acid formed per g of wet tissue and per g of tissue nitrogen per hr.

grinder. The homogenates were subsequently centrifuged at 20,000 r.p.m. and the supernatants immediately used for enzyme determination. Nitrogen measurements were performed by the Kjeldahl procedure(5) on portions of the same tissue specimens from which the homogenates were prepared.

The final millimolar concentrations employed were: 3-PGA, 7.75; ATP, 4.0; $MgSO_4$, 7.75; cysteine HCl, .4; hydroxylamine, 425.0; and Tris buffer, pH 7.5, 20.0. The hydroxylamine and cysteine reagents were prepared fresh each day. The buffered substrate solution was preheated for 5 minutes at 37°C in a 25 ml Erlenmeyer flask after which 1.0 ml 2% homogenate supernatant was added to the tissue test and an equivalent volume of water to the reagent blank (final volume,

TABLE II. Coefficients of Correlation Between Age and Enzyme Activity.

	Age group, yr	No.	Wet tissue r	Wet tissue t	Tissue nitrogen r	Tissue nitrogen t
Thoracic descending aorta, normal	20-86	74	—.03	.25	+.12	1.02
Pulmonary artery	20-86	62	.00	.00	+.11	.86
Coronary artery, normal	20-86	43	+.25	1.70	+.43	3.06
Vena cava inferior	20-86	49	+.36	2.65	+.40	3.00

7.5 ml). Incubation was then performed over a 10-minute period in a shaking water bath. Two ml aliquots were removed at 0, 5 and 10 minutes; each aliquot was immediately added to a test tube containing 2.0 ml $FeCl_3$-TCA-HCl color reagent followed by 1.0 ml 95% ethyl alcohol. The sample was mixed and allowed to stand at room temperature for 10 minutes. It was then centrifuged to remove precipitated protein and the supernatant was used for spectrophotometric color determination. Readings were made in silica cuvettes at 490 mμ in a Beckman DU spectrophotometer. The recorded values were corrected for minor color changes displayed by the reagent blanks. In accordance with Lipmann and Tuttle(4) a purified acetyl phosphate compound (Boehringer Mannheim Corp., New York City) was used as color standard; the observed molar extinction coefficient of this reagent was 845 at 490 mμ. In the employed enzymic procedure, zero order kinetics were obtained over the utilized brief 10-minute test period and high proportionality was found between amount of tissue added and recorded values.

Results. The average PGK activities for various types of blood vessels are listed in Table I; these values indicate notable concentrations of the enzyme in human arterial tissue. A comparison of activities exhibited by inferior vena cava and aortic specimens from the same subjects revealed distinctly lower values for the venous samples, the mean PGK of the fresh venous tissue being only 61% (t of difference = 7.18) of that recorded for the thoracic descending aorta. When calculated on the basis of tissue nitrogen content, the corresponding percentage value was 63.4 (t of difference = 6.58). For all the species of blood vessels studied, significantly lower PGK activities were found in samples from 0- to 10-year-old children than from adults. The mean activity of fresh aortic tissue derived from children was 71.2% of that observed for 20- to 35-year-old subjects (t of difference = 3.18); the corresponding percentage values for the pulmonary artery, coronary artery, and vena cava were, respectively, 62.2 (t of difference = 2.88), 67.5 (t of difference = 4.44), and 25.2 (t of difference = 3.31). For the 20- to 86-year group a conspicuous increase in PGK activity with age was observed for the vena cava, whereas statistically significant variations were not found for the thoracic descending aorta or the pulmonary artery (Table II).

Assays performed on normal and lipid-arteriosclerotic aortic tissue samples (N = 45) showed moderately lower activities for the pathological specimens both when expressed per gram of wet tissue (86.2%, t of difference = 2.48) and per gram of tissue nitrogen (88.3%, t of difference = 2.06). The corresponding values observed for fibrous-arteriosclerotic aortic tissue (N = 20) were 85.2% (t of difference = 1.77) and 94.0% (t of difference = 0.90). No significant differences were found between PGK activities displayed by normal and lipid-arteriosclerotic coronary artery tissue portions (N = 25).

Summary. Determinations were made of the phosphoglyceric kinase activities exhibited by intima-media layers of various types of human blood vessels. A total of 445 vascular specimens was included in the study. The average activity of the thoracic descending aorta expressed in millimoles of 1,3-diphosphoglycerate formed per gram fresh tissue per hour was .277; the corresponding values recorded for the ascending aorta, abdominal aorta, pulmonary artery, coronary artery, and inferior vena cava were, respectively, .276, .233, .298, .282, and .164. The mean enzyme activity exhibited by lipid-arteriosclerotic

aortic tissue samples was 86.2% of that observed for normal tissue of the same arterial specimens.

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Enhancement of Glucose Absorption by Oleic Acid.* (31361)

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Recent *in vitro* studies have indicated that bile salts inhibit intestinal absorption of glucose(1,2,3). The present experiments were designed to establish the validity of this observation *in vivo* and to study the reciprocal effect of glucose and oleic acid upon their intestinal absorption in the presence of bile salts.

Methods. Adult, male, mongrel dogs were prepared with 6 to 8 inches long Thiry-Vella fistulae constructed from the jejunum distal to the ligament of Treitz. Steel Gregory cannulae were placed in both ends of the loop, wrapped with omentum and brought through the abdominal wall. Each dog was allowed 3 weeks' convalescence before experiments were begun. Before each experiment, the loop was perfused with 100 to 200 ml isotonic saline at 38°C to evacuate cellular debris. An attempt was made to recover all this solution by gentle suction through a polyvinyl tube inserted into the loop. The omental adhesions surrounding the cannulae prevented loss of fluid from the loop.

Test mixtures were made in Krebs-Ringer bicarbonate solution, pH 7.4, with 0.5% polyethylene glycol (PEG). Concentrations of substances contained in the various test mixtures were as follows: glucose 168 mEq/l, sodium taurocholate (NaTC) 8 mEq/l, oleic acid either 20 mEq/l or 5 mEq/l, and 3-0-methylglucose (3-0-MG) 156 mEq/l. In each experiment, 25 ml of a test solution was introduced into the loop for 30 minutes. The fluid recovered at the end of that period was centrifuged at 2000 rpm for 15 minutes, and the supernate analyzed. After each experiment the loop was again rinsed with 100 to 200 ml of warm saline solution. Analysis of this rinse showed no measurable PEG and less than 1% of the total unabsorbed glucose.

Glucose and 3-0-MG were measured by the anthrone method described by Seifter *et al*(4). Oleic acid determinations were done by a modification of the method of Itaya and Michio(5). Cholate was determined by the method of Irvin *et al*(6). Sodium was measured by flame photometry. PEG was determined by a modification of the method of Hyden(7). Maximum turbidity was read 16 minutes after addition of trichloroacetic acid, rather than in 5 minutes noted in the original method. The estimated volume of fluid unabsorbed from the loops was calculated from the concentration of PEG in the fluid recovered at the end of each experiment. The volume of fluid recovered from the loop was usually 1 to 5 ml less than the estimated final volume calculated on the basis of PEG concentration. This discrepancy was greatest in experiments utilizing NaTC, for in these experiments residual fluid was nearly twice as great as when glucose alone was used. The total amount of each solute unabsorbed by the loops after 30 minutes was estimated from

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