aldehydes or under all conditions. As demonstrated here, highly polymerized glyceraldehyde behaves in a manner precisely opposite to that of the monomeric glyceraldehyde.

The chemical details of the apparently involved mechanism will be described later. However, the mechanism appears to involve at least 2 chemical phenomena in a manner quite analogous: (1) to the observed increased fixation of highly aggregated chromium compounds and vegetable tannins by heat-denatured collagen(6,7) due to rupture of hydrogen bridges in adjacent keto imide groups and (2) the intermolecular or interchain cross-linking of globular proteins(8) and polynucleotides by aldehydes(9). Presumably, the first step involves disruption of the stabilizing *intra-molecular* cross links of the molecule (*e.g.*, as in the collagen-gelatin transition) and the second step of an *intermolecular* reaction between the aldehyde group and the protein groups thus freed (2,10).

Summary. The thermal shrinkage of a number of collagenous tissues (skin, aorta, tendon, and decalcified bone) has been studied following incubation with a series of carbohydrate metabolites. The data indicate that only glyceraldehyde among the metabolites studied here, is capable of acting as a tanning or cross-linking agent for collagen under a variety of experimental conditions. These latter roughly approximate the pH, ionic strength and temperature conditions of the extracellular body fluids.

Addendum: Additional details on the relationship between observed tanning properties and the chemical structure of a large number of aldehydes have recently been published (Milch, R. A., *J. Amer. Leather Chems. Assn.*, 1962, v57, 581).

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Studies of Collagen Tissue Aging: Degradation of Glyceraldehyde-Treated Hide Collagen.* (27852)

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In a previous communication(1) it has been indicated that only those intermediary metabolites which are aldehydes are apparently able to act as tanning agents for hide powder collagen. Tanning in this context has been defined primarily by the relative repression at equivalent equilibrium pH's of the swelling phenomena of metabolite-treated as contrasted to untreated hide powders. As such, one, but only one of the several requisites for tannning ability have been fulfilled (2). In this report are recorded additional data indicating that improved stability of collagen preparations follows interaction with such metabolites.

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TABLE I. Apparent Hydrated Swelling Volumes of Untreated and Glyceraldehyde-Treated Hide Powder Preparations in Presence of Tropocollagen Extractants. "Gelation" refers to presence (+) or absence (0) of gel-like solubilization of the collagen phase after 167 hr of interaction with each of the solutions.

Total elapsed time (hr)	Apparent hydrated swelling volume of hide powder collagen (ml)										
	Untreated					Glyceraldehyde-treated					
	NaCl	Citric	Acetic	C'g'ase (40°C)	Urea	Urea (40°C)	NaCl	Citric	Acetic	C'g'ase (40°C)	Urea	Urea (40°C)
1	1.1	4.2	3.1	.25	2.1	2.2	1.4	1.5	1.3	1.7	2.1	2.2
5	1.2	4.8	3.5	.15	2.1	2.3	1.4	1.6	1.3	1.9	2.2	2.3
24	1.5	5.1	4.2	.05	2.2	2.6	1.5	1.7	1.3	2.0	2.3	2.3
167	1.4	5.2	5.1	—	2.5	2.3	1.5	2.0	1.4	1.9	2.3	2.3
"Gelation"	0	+	+	—	+	++	0	0	0	0	0	0
pH (initial)	6.5	1.6	2.6	7.1	7.5	7.5	6.5	1.6	2.6	7.1	7.5	7.5
pH (final)	5.4	1.7	2.9	5.4	7.4	8.8	6.2	1.8	3.4	6.7	7.6	8.8
Hydroxyproline (γ/ml supernate)	72	277	90	2080	<5	289	8	22	11	89	<5	<5

Experimental. Glyceraldehyde - treated American Standard Hide Powder collagen (Frank F. Marshall, Ridgeway, Pa.) was prepared by reacting 15 g aliquots of isoelectric (pH 5.4) hide powder with 500 ml of 0.15M dl-glyceraldehyde in 0.15M phosphate buffers (pH 7.2) at 40°C for one week. (Less than 15% of the hide powder was solubilized by this treatment, whereas virtually all other metabolites resulted in drastic solubilization of the collagen preparation when similarly reacted.)

The neutral collagen was then washed with three 500-ml aliquots of distilled water at room temperature and dried to constant weight at 40°C. Two hundred milligram samples of this treated material were placed in graduated centrifuge tubes and overlaid with 10 ml of 0.5M aqueous solutions of NaCl, acetic acid, citric acid, 8M urea solutions and with a solution (1 mg/ml in PO_4 buffer) of purified bacterial collagenase (Worthington Biochemical Corp., Freehold, N. J.) at both room temperature and 40°C.

The apparent hydrated swelling volume of the solid (collagen) phase was recorded for each tube with respect to time of interaction, as indicated previously(3). The mean value of a minimum of 5 tubes was noted for each time interval. Initial (pH_i) and final (pH_f) pH values of the supernatant liquid phase were determined using a Beckman Zeromatic pH meter (instrumental error $\pm$ 0.03 pH). The hydroxyproline contents of the supernatants were determined by the Leach(4) modification of the Neuman and Logan method (5).

Results and discussion. Representative data are presented in Table I. It is apparent that, in all circumstances, glyceraldehyde treatment resulted in diminished swelling curves and lesser solubilization of the treated hide powder relative to that observed with the untreated preparation. The data thus confirm the suggestion of the previous investigation(1). They are also in accord with observations reported by Gustavson(6,7) on the tanning effects of glyoxal, pyruvic aldehyde (methylglyoxal) and certain other aldehydes, including formaldehyde. It is suggested accordingly that glyceraldehyde, the one major intermediary through which all known carbohydrate metabolic pathways must pass, can act as a potent tanning agent for collagen under conditions roughly approximating those of the extra-cellular body fluids.

More refined studies with gelatins, reconstituted collagens and various synthetic amino acids, polypeptides and collagen analogues which tend to support this same hypothesis will be reported later.

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Succinic Cytochrome C Reductase Activity in Kidney and Liver Tissue from Starved and Fed Rabbits.* (27853)

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Some aspects of the relationship between oxidative metabolism and dietary regimens have been studied by O'dell *et al.*(1) who found that the cytochrome oxidase activity of liver in growing rats is not altered by food restriction. However, Wainio *et al.*(2) found that caloric and protein restriction lowers the succinic dehydrogenase activity in liver homogenates from mature rats. Cambria(3) also found significant decreases for succinic dehydrogenase activity for heart muscle homogenates of starving pigeons. Unpublished studies of kidney and liver enzyme systems from fed and starved rabbits indicated that incubation of tissue homogenates for 24 to 48 hr at 4°C resulted in increased oxidative enzyme activity. It was of interest, therefore, to investigate further the effect of starvation, incubation and analytical methods for some oxidative enzyme systems in rabbit organs. The present study is limited to the succinic cytochrome C reductase system in rabbit liver and kidney homogenates.

Materials and methods. Young adult female white rabbits weighing 2-3 kg were maintained on commercial rabbit chow. Experimental *ad libitum* fed and starving rabbits were maintained in individual cages for 5 or 10 days with water available at all times. Following sacrifice by cervical fracture, the liver and kidneys were quickly removed, rinsed with 0.154 M NaCl and placed on crushed ice. Two gram portions of tissue were placed in 20 ml cold (4°C) M/10 phosphate buffer pH 7.5 and homogenized with a teflon pestle in a glass tube kept in crushed ice. Care was taken in cutting kidneys to use cross sections containing proportionate quantities of cortex and medulla. For liver tissue, representative portions of all lobes were used. Fibrous material was removed by passing the homogenates through 4 layers of cheese cloth.

The assay system was modified from Lehman and Nason(4). The rate of reduction of oxidized horse cytochrome C (Sigma Chemical Company) was determined every 30 seconds for 3.5 minutes in a Beckman DU at 550 mμ at 27°C. The reaction mixture, in a 3 ml cell was allowed to reach temperature and optical equilibrium for 3 minutes before adding the homogenate to initiate the reaction. Homogenate protein concentrations were determined by the turbidimetric method of Heepe *et al.*(5).

Experimental procedure and results. In view of the possibility that changes in physiological state of the animal may alter the extractability or other properties of the enzymic activity, a series of experiments was performed to characterize the enzyme system for

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