have investigated the nature of this inhibition to see whether it might have any physiological significance.

The method of Kishi and Folkers was used with a slight modification to determine basal erythrocyte glutamic oxaloacetic transaminase activity and percent B-6 deficiency based on the stimulation of the transaminase by the addition of pyridoxal phosphate. The assay procedure was modified by changing the incubation conditions to 30 min at 37 °C. This enhanced the reproducibility of the assay. Absorption spectra were obtained with a Bausch and Lomb 500 spectrophotometer.

A consideration of the reactions involved in the assay procedure presents a possible explanation for the observed inhibition. The assay is based on the pyridoxal phosphate dependent transaminase reaction converting aspartic acid to oxaloacetic acid. The oxaloacetic acid resulting from this reaction is converted to pyruvic acid which is quantitated spectrophotometrically by measuring the absorbance of the 2,4,dinitrophenylhydrazone of pyruvic acid. This phenylhydrazone is extracted into toluene and subsequently reacted with alcoholic potassium hydroxide to enhance the color reaction. Pyridoxal and pyridoxal phosphate are also known to form phenylhydrazones as evidenced by the changes they effect in the absorption spectrum of 2,4,dinitrophenylhydrazine. Other forms of B-6, viz. pyridoxamine, pyridoxamine phosphate and pyridoxine, do not react.

The effect of adding increasing concentrations of the various forms of B-6, ranging between 0 and 3×10^{-2} mM, to the E-GOT system was studied. There was no stimulation of the reaction by pyridoxine, pyridoxal or pyridoxamine as was to be expected since these are not cofactors of the enzyme. There was slow increasing stimulation with increasing concentrations of pyridoxamine phosphate as the pyridoxamine phosphate is apparently converted to the required pyridoxal phosphate by the system. Increasing concentrations of pyridoxal phosphate between 0 and 1.5×10^{-2} mM stimulated the reaction while a concentration of greater than 1.5×10^{-2} mM appeared to inhibit the overall reaction. The apparent inhibition is not observed until the apoenzyme is fully saturated and excess pyridoxal phosphate is present which competes with pyruvic acid for the available phenylhydrazine. The presumed drop-off in activity occurs because the phenylhydrazone of pyridoxal phosphate is insoluble in toluene hence there is decreased phenylhydrazone chromophore in the toluene layer.

The inhibition, therefore, appears not to involve the enzyme reaction but is rather an artefact due to excess pyridoxal phosphate reacting with the 2,4,dinitrophenylhydrazine which would otherwise be available to the pyruvic acid. The procedure for determining the degree of saturation of the apoenzyme is valid in that interference is noted only when excess amounts of B-6 are present. Taking these factors into account we believe the method of Kishi and Folkers is a valid assay for B-6 status.

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Human albumin synthesis via an albumin precursor in liver tissue slices[1]

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Summary. A precursor of plasma albumin in man was identified in liver obtained from cadaver renal transplant donors. After 30 min incubation with ^{14}C-(U) leucine, most of the labeled immunoreactive albumin was identified as proalbumin, as was evident from its characteristic elution profile on DEAE cellulose 52. After an additional 30 min incubation with unlabeled leucine (60 min total), no precursor form could be identified and the label coeluted with serum albumin. These data strongly suggest that human albumin is initially synthesized in a precursor form and is subsequently transformed into serum albumin.

Recent studies have shown that in human liver a_1-acid glycoprotein, a_1-antitrypsin, and transferrin are produced via precursor forms[3]. For serum albumin a liver specific form has been isolated from rat[4,5], bovine[6] and monkey[7] liver. Rat and bovine proalbumin differ from albumin by a hexapeptide extension at the N-terminal end[4,6]. The propeptide is strongly basic, and proalbumin can therefore be separated from albumin either by anion exchange chromatography[5,7] or by isoelectrofocusing[4,6]. The purpose of this investigation was to demonstrate that human albumin also is initially synthesized in a precursor form.

Methods. Human liver tissue was obtained from cadaver renal transplant donors approximately 30 min after the vena porta had been clamped off. A 15 g section of liver tissue was cut manually with a razor blade into slices, each approximately 1 mm thick. The slices were washed twice in Krebs-Ringer-carbonate buffer (KRC buffer), pH 7.4, containing amino acids other than leucine at the concentrations present in serum, with added streptomycin (100 µg/ml) and potassium penicillin G (100 units/ml). After dividing the slices into 2 fractions, the tissue was incubated in 18 ml of KRC buffer at 37 °C while shaking in a rotary incubator in 95% O_2 and 5% CO_2. After 7 min 0.5 ml of L-^{14}C(U)-leucine (0.05 mCi; 0.027 mg) was added to each sample and the pH was adjusted with an appropriate volume of 1 M bicarbonate to maintain a pH of 7.4. The incorporation was terminated by adding 1 ml of 2% L-^{12}C-leucine after 30 min, which is approximately the secretion time for human serum proteins[8]. One sample was rapidly cooled, while the other was incubated for an additional 30 min (60 min total incubation). Both samples were then minced and extensively homogenized in KRC buffer, in a Potter-Elvehjem apparatus. The homogenate was centrifuged for 20 min at 12,000 rpm and the supernatant was aspirated and saved. The pellet was re-extracted in 18 ml KRC buffer with sodium deoxycholate (final concentration 1.4%). The combined supernatants of each sample were filtered through glass wool and dialyzed against 0.01 M Tris-HCl-buffer, pH 7.6, containing 0.9% NaCl.

Albumin was isolated and purified chemically to constant

specific radioactivity, by a method previously shown to yield radiochemically pure albumin[7,9]. The purification procedure included heat fractionation, ethanol extraction, ammonium sulfate precipitation, gel chromatography on Sephadex G 200 and anion exchange chromatography on DEAE 52. The same result was obtained when an acetone-dried powder was prepared from the liver homogenate, albumin precipitated by specific antiserum, and the dissolved immunoprecipitate submitted to chromatography on Sephadex G 200 and DEAE 52. Total protein was measured by the method of Lowry[10], albumin by electroimmunodiffusion with specific antiserum[11] or by a competitive inhibition radioimmunoassay as described previously[7]. Protein radioactivity was determined by the method of Mans and Novelli[12]. 100 or 200 µl of the samples were counted on filter paper disks in toluene +0.4% PPO and 0.005% POPOP with an efficiency of 78–80%. After DEAE-chromatography the total radioactivity of 0.5- or 1.0-ml samples of the eluted fractions were measured in Aquasol II (New England Nuclear Corp.) with a counting efficiency of 90%. The samples were counted in a Packard liquid scintillation counter Model 3376 and the background (26–30 cpm) was subtracted from all given data.

Results and discussion. Liver slices derived from 4 different donors were incubated for 30 min. The results of all experiments were very similar. ^{14}C-leucine incorporation into total protein ranged from 1685 to 3040 cpm per mg protein. These data represent an incorporation of only 0.5–1.0% of the added radioactive leucine. The conditions under which the tissue was obtained, with a relatively long period of ischemia, may account for the low rate of incorporation. In all experiments protein radioactivity co-eluted with immunoactive albumin during Sephadex G 200 gel filtration (fig. 1a). However, on DEAE-chromatography a clear biphasic elution pattern was always found. The radioactivity peak eluted earlier than the albumin peak, consistent with significant incorporation into proalbumin (fig. 1b). In 2 separate experiments liver slices were incubated for 30 min with ^{14}C-leucine and half of each sample was incubated for another 30 min after addition of ^{12}C-leucine in excess. Again, protein radioactivity coeluted with immunoreactive albumin during Sephadex G 200 gel filtration from slices incubated for both 30 and 60 min (figs 1a, 2a). On DEAE-chromatography the 30-min samples demonstrated a clear biphasic elution pattern (fig. 1b). In contrast, by 60 min, both sample elution profiles showed the radioactivity and the albumin to be nearly superimposable (fig. 2b) indicating that, during the time interval in which incorporation was interrupted, proalbumin was trans-

Purification of radioactive proalbumin and albumin from human liver slices incubated for 30 min with ^{14}C-leucine.

Purification step	Total protein (mg)	Total albumin (mg)	Specific radioactivity (cpm/mg)
Homogenate	304	12.2	2180
62 °C for 10 min	-	7.6	-
Sephadex G 200	-	4.4	2270
DEAE I eluate (fractions 47–54, fig. 1b)	-	0.31	-
DEAE II eluate (combined proalbumin peak)	0.09	0.07	1670
DEAE II eluate (combined albumin peak)	0.37	0.28	620

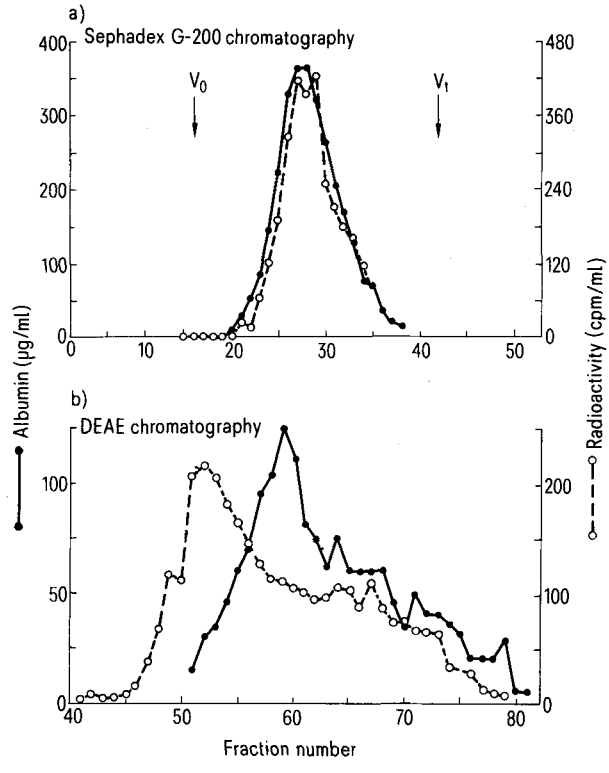

Figure 1. Purification and separation of albumin and proalbumin after 30 min of incubation. The Sephadex column (1.6×90 cm) was eluted with 50 mM ammonium acetate and 3-ml fractions were collected (1a). The DEAE column (1.5×1.6 cm) was eluted with 400 ml of 100–300 mM Tris HCl buffer, pH 7.7, flow rate 40 ml/h. The fraction volumes were 3.2 ml (1b).

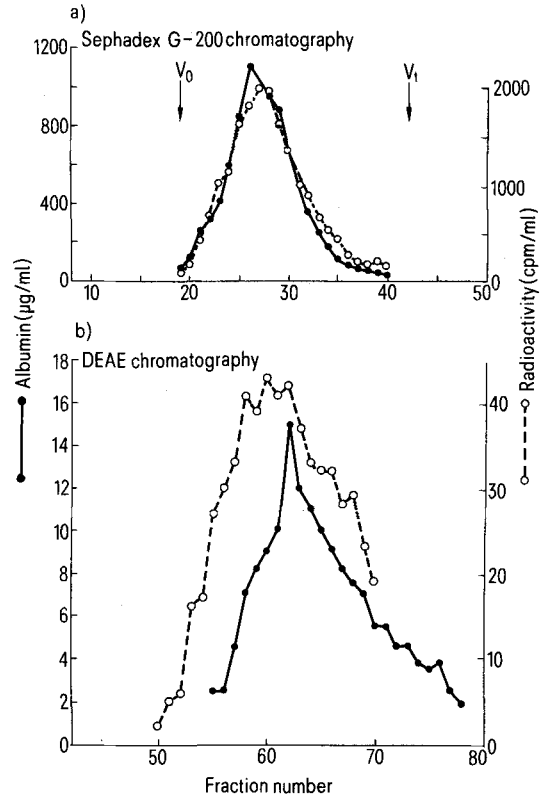

Figure 2. Purification and separation of albumin after 60 min incubation. The Sephadex- (2a) and DEAE-chromatography (2b) were performed as described in legend to figure 1.

formed into albumin. These results are almost identical with the data previously reported for rat liver[14].

The total immunoreactive albumin was 1.5-4% of the total homogenate protein, greater than the value reported for rat liver[13]. Since the human liver cannot be perfused prior to incubation, incomplete removal of serum from liver slices could account for an increased amount of serum albumin in the homogenate. The radioactive proalbumin (fractions 47-54 in fig. 1b) was completely separated from the albumin peak by a 2nd DEAE-chromatography. The purification procedure of 1 experiment is given in the table. During the purification the radioactivity per mg proalbumin decreased from 2180 to 1670 cpm/mg. In contrast, the peak coeluting with serum albumin contained only 620 cpm/mg (450 cpm/mg for the 2nd experiment). In 4 of the 6 samples, incubated for 30 min, the purification procedure for proalbumin was modified. From the acetone-dried powder the immunoreactive albumin was precipitated with specific antiserum and the dissolved immunoprecipitate submitted to Sephadex- and two subsequent DEAE-chromatographies. The yield of proalbumin was 15-90 µg with the radioactivity ranging from 1378 to 4033 cpm per mg. This small amount, again probably due to the long period of ischemia of the liver, made it impossible to determine the N-terminal sequence. The reproducible elution pattern on DEAE-chromatography, however, strongly suggests that human albumin is initially synthesized in a precursor form and is later transformed into serum albumin. The circulating albumin variant Christchurch found recently in a New Zealand family[15] indicates that the amino acid sequence of the N-terminal of human proalbumin is very similar to that of rat proalbumin.

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An in vitro kinetic study of the mixed inhibition of honeybee hemolymph PNP-α-D-glucosidase by sucrose[1]

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Summary. Sucrose acts in vitro as a mixed inhibitor of (V + K + n) type towards honeybee hemolymph PNP-α-D-glucosidase activity. Between the stages of emergence and foraging, there is little change in the effect of the inhibitor on V_M (f_i = from 1.31 to 1.35 respectively) or n (f_i = from 1.09 to 1.07) but K is more markedly affected (f_i^{-1} = from 1.17 to 1.87). These observations reflect the decrease of K_i from 277 to 98 mM and of I_{50} from 154 to 111 mM, but K_i' scarcely alters during development (from 477 to 425 mM). These inhibitory effects of sucrose are intermediate between those previously reported for trehalose and glucose.

In vertebrates, and in microorganisms, the biosynthesis of some enzymes can be adapted to nutritional or metabolic demand[2,3]. These induction phenomena commonly concern the enzymes of sugar metabolism and particularly those of the glycosidase group[4,5]. Some evidence has been presented previously[6,7] suggesting the induction of honeybee hemolymph α-glucosidase by trehalose. In beekeeping, it is common practice to feed honeybees with sucrose syrups at certain periods of the year. We have therefore investigated the influence of sucrose feeds on the glycemia of honeybees and on the induction of their hemolymph α-glucosidase activity (Bounias and Morgan, unpublished). As an essential preliminary to this work, it was necessary to determine any direct effects of sucrose on the kinetics of the α-glucosidase in order to be able to distinguish these from true induction effects.

Materials and methods. Since the induction experiments were to be conducted over a period of 16 days, the present study encompassed the 2 extreme developmental stages represented by this interval, so as to compare young emerging worker bees, *Apis mellifica mellifica* L., with foraging adults (16-day-old bees will soon commence foraging). The kinetics of α-glucosidase were investigated as previously described[8] using pNP-α-D-glucoside as a specific artificial substrate.

Hemolymph was removed by puncture and centrifuged as previously described[8,9] then diluted by 40 times in the reaction medium. The major α-glucosidase fraction (over 85%) was shown to be cooperative at prenymphal stages[9]; its sp. act. is 0.4 mM · min^{-1}/µg protein. The kinetic contribution of the minor fraction appears as non-significant (less than 1%) when evaluated according to algebraic methods suited for 2 superimposed kinetics[14]. The main parameters (V_M = maximum velocity; K = affinity constant; n = Hill coefficient) were determined according to a non-iterative algebraic method[9] applicable to the general case of the Hill equation, and in Michaelian cases (where n = 1 and K becomes K_m) also according to the Augustinsson/Hofstee plot[10]. The dose/response relationships for sucrose as inhibitor (I) were interpreted according to the Dixon plot[11] and