The chromatograms were sprayed with 0.2% ninhydrin reagent and subjected to 80 °C for 15 min. The spots of amino acids were marked and were identified by comparing R_f with that of authentic samples. Later they were eluted in 5 ml of 75% EtOH containing 0.2 mg of $CuSO_4$ 5 H_2O. An Uncoloured area was utilized for the blank. Absorbance was measured at 540 nm using a colorimeter. Quantitative measurement was done by comparing the absorption of an identified spot with the standard curve obtained for the corresponding authentic amino acid treated as above.

Results. Studies on growth of callus under the influence of urea: Agar media containing various concentrations of urea (filter sterilized) ranging from 10 mM to 300 mM were prepared. Nitrates were completely omitted. However, controls received a standard nitrogen supply. Callus pieces weighing 300±30 mg were inoculated in 150 ml Erlenmeyer flasks containing 40 ml of the medium and cultures were incubated at 25±2 °C in continuous light (3000 lx). At the end of 30 days growth of the tissue was measured in terms of fresh and dry weight.

Effect of urea on growth and nicotine biogenesis: It was interesting to find that the callus tissues of *N. tabacum* could be successfully grown on 10 mM urea as sole nitrogen source, but at a slower rate than on MS medium with the standard nitrogen supply of 18.8 mM KNO_3 and 20.6 mM NH_4NO_3. Such a phenomenon has been demonstrated earlier in cell cultures of soybean[14] and tobacco[15]. Of the various levels of urea administered, 10 mM supported maximum growth of the callus and highest yield of nicotine (table 1). Increasing levels of urea reduced growth and nicotine production. The nicotine content of urea-treated tissues was found to be 3.5- and 2.5-fold higher than the control on percent dry weight and per culture basis respectively. Similar increase in nicotine content by foliar application of urea to tobacco plants has been reported[16] from the Beedi Tobacco Research Centre at Anand in Gujarat, India.

Discussion. Possible role of urea in enhancement of nicotine biogenesis: Ornithine is an established precursor of nicotine biogenesis[17] and a key compound in the ornithine-urea cycle. It seemed that feeding of urea increased the endogenous urea level of the tissues, thereby suppressing the activities of enzymes of the urea cycle. Such a situation would result in an increased ornithine level, since its turnover via the urea cycle is restricted. This hypothesis is substantiated by the fact that urea-treated tissues accumulated higher levels of ornithine, citrulline and arginine than the control (table 2). Thus urea seemed to increase nicotine production by suppressing the entry of ornithine into the urea cycle, thereby increasing the availability of ornithine for nicotine biogenesis. The enzymatic evidence for the above statements has been obtained and will be reported elsewhere.

1 Financial support given to one of us (GAR) by the Maharaja Sayajirao University of Baroda is gratefully acknowledged. Reference No. 9 represents part 1 of the series on regulation of nicotine biogenesis.
2 R.F. Dawson, Am. Sci. *48*, 321 (1960).
3 P. Benveniste, L. Hirth and G. Ourisson, Phytochemistry *5*, 31 (1966).
4 T. Furuya, H. Kojima and K. Syono, Chem. Pharm. Bull *15*, 901 (1967).
5 M. Tabata, H. Yamamoto, N. Hiraoka, Y. Morumoto and M. Konoshima, Phytochemistry *10*, 723 (1971).
6 M. Takahashi and Y. Yamada, Agric. Biol. Chem. *37*, 1755 (1973).
7 G.A. Ravishankar and A.R. Mehta, J. Indian bot. Soc. *58S*, 60 (1979).
8 M. Tabata and N. Hiraoka, Physiologia Pl. *38*, 19 (1976).
9 G.A. Ravishankar and A.R. Mehta, in: Proceedings of a National Symposium on Plant Tissue Culture, Genetic Manipulation and Somatic Hybridization of Plant Cells, p. 342. Ed. P.S. Rao, M.R. Heble and M.S. Chadha. Bhabha Atomic Research Centre, Bombay 1980.
10 S.J. Chokshi, Ph.D. Thesis, M.S. University of Baroda, Baroda, India, 1975.
11 T. Murashige and F. Skoog, Physiologia Pl. *15*, 473 (1962).
12 C.O. Willits, M.L. Swain, J.A. Connelly and B.A. Brice, Analyt. Chem. *22*, 430 (1950).
13 M. Brenner, A. Niederwieser and G. Pataki, in: New biochemical separation, p. 121. Ed. A.T. James and L.J. Morris. D-Van Nostrand Co. Ltd, London 1964.
14 J.C. Polacco, Pl. Physiol. *58*, 350 (1976).
15 Y.M. Heimer and P. Filner, Biochim. biophys. Acta *215*, 152 (1970).
16 Silver jubilee celebrations souvenir, vol. II: Research, p. 103. Ed. G.J. Patel. Inst. Agr. Anand, Gujarat, India 1968.
17 E. Leete and K.J. Siegfried, J. Am. chem. Soc. *79*, 4529 (1957).

Apparent inhibition of erythrocyte glutamic oxaloacetic transaminase in subjects on large dosages of B-6[1]

A. Sohler and C.C. Pfeiffer

Princeton Brain Bio Center, 862 Route 518, Skillman (NJ 08558, USA), 29 December 1980

Summary. An apparent inhibition of the erythrocyte glutamic oxaloacetic transaminase E-GOT system of Kishi and Folkers has been observed with erythrocytes from individuals on large amounts of B-6. Evidence is presented that this inhibition is an artefact due to excess pyridoxal phosphate reacting with 2,4,dinitrophenylhydrazine. Excess pyridoxal phosphate competes with pyruvic acid for the available 2,4,dinitrophenylhydrazine which results in a product that is not extractable into toluene, therefore, less chromophore is present as more pyridoxal phosphate is available.

The erythrocyte glutamic oxaloacetic transaminase system (E-GOT) procedure of Kishi and Folkers[2] was used to evaluate the B-6 status of a psychiatric out-patient population who were taking various levels of B-6. In those subjects taking B-6 in amounts greater than 10 mg per diem Sohler and Pfeiffer[3] noted an apparent inhibition of the overall in vitro reaction instead of the maximum reaction rate expected when there is complete saturation of the apoenzyme with cofactor.

Kishi and Folkers have also observed this inhibition with erythrocytes from subjects who were receiving i.v. hyperalimentation with preparations containing about 100 mg B-6 per day and Folkers has reported instances of this inhibition in subjects taking 50–100 mg of oral B-6 daily. We

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.

1 We would like to acknowledge the Tom and Marion Peters' Foundation for their support of this research.

2 H. Kishi and K. Folkers, J. nutr. Sci. Vitam. *22*, 225 (1976).
3 A. Sohler and C.C. Pfeiffer, Fedn Proc. *39*, 558 (1980).

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

K. Weigand and E. Alpert[2]

Department of Clinical Pharmacology, University of Berne, Murtenstrasse 35, CH-3010 Berne (Switzerland), and Department of Medicine, Harvard Medical School and the Medical Service (Gastrointestinal Unit), Massachusetts General Hospital, Boston (Mass. 02114, USA), 13 March 1981

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