IDENTIFICATION OF FRUCTOSE IN MAMMALIAN NERVE*

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Free fructose is an unusual compound in animal tissues. It has been found in some species in the seminal vesicles and fluid (Mann, 1946, and Hers, 1960), fetal blood (Bacon and Bell, 1948), the lens (Green and Solomon, 1959), and the cerebrospinal fluid (Hubbard and Russell, 1937). The function of fructose in these organs has not been determined, but the pathway by which it may originate from glucose via sorbitol has been investigated by a number of workers, (e.g. King and Mann, 1959, and Van Heyningen, 1959). Fructose has not previously been shown to be present in nervous tissue. In this paper we report the presence of substantial levels of fructose in peripheral nerve and lower levels in brain.

Materials. Sciatic nerves were taken either from 3 month old albino rabbits or adult dogs, with the animal under nembutal anesthesia. The nerve was frozen at once in liquid N_2,

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then cleaned of blood and connective tissue at -15°. $HClO_4$ extracts of the tibial branch of the nerve were prepared in the way described by Lowry et al. (1964). In the same way extracts of whole brain were prepared from mice that had been frozen in liquid N_2. The enzymes used were obtained from Boehringer and Sons, except for purified brain hexokinase which was a gift from Dr. R. K. Crane.

Assay Methods. The method of glucose assay used was that described by Lowry et al. (1964), in which the formation of TPNH in the following reactions is followed fluorometrically.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{glucose-6-P} + \text{ADP}$$

$$\text{Glucose-6-P} + \text{TPN} \xrightarrow{+\ \text{G6PDH}} \text{6-P-gluconate} + \text{TPNH} + H^+$$

Results. When glucose in nerve extracts was measured it was found that the curve of TPNH formation had two phases, a rapid one corresponding to the standard glucose curve, followed by a slow rise to a second plateau. A number of compounds which were considered as possible sources of the extra TPNH formation, namely glycogen, fructose-1, 6-diphosphate and 6-phosphogluconate, were tested in the assay system and found not to react. It was also determined that the extra TPNH from the extract was only formed in the presence of the complete reagent. In the absence of ATP and/or hexokinase no TPNH was formed other than that attributable to glucose-6-phosphate in the extract, and when phosphoglucoisomerase was added to reagent lacking ATP and hexokinase no increase in

dianisidine and was clearly γ-tocopherol When synthetic 7-methyl tocol was added to rice unsaponifiable lipid and the mixture chromatographed the 7-methyl tocol appeared as a distinct spot being slightly more polar in both solvent systems than η-tocopherol. Thus η-tocopherol from rice is not 7-methyl tocol but is 7,8-dimethyl tocotrienol.

Palm oil similarly gave five distinct reducing spots on TLC. These appeared to be α-, ζ_1-, η-, ϵ-tocopherols and a δ-tocopherol-like material. In a manner identical as for rice it was shown that the η-tocopherol from palm oil was 7,8-dimethyl tocotrienol and not 7-methyl tocol. Thus there is no evidence at present that 7-methyl tocol appears naturally.

The identity of ζ-tocopherol in rice.

Two ζ-tocopherols are believed to occur in Nature. ζ-Tocopherol was originally found in wheat, barley and rye by Green, Marcinkiewicz and Watt (1955) and later by Green and Marcinkiewicz (1956) in rice. The ζ-tocopherol was identified as 5,7-dimethyl tocol. More recently Green, McHale, Marcinkiewicz, Mamalis and Watt (1959) claimed that the ζ-tocopherol in wheat and palm oil was 5,7,8-trimethyl tocotrienol but the rice ζ-tocopherol was different and was 5,7-dimethyl tocol as originally suggested. Bunyan et al. (1961) refer to these two compounds as ζ_1-(5,7,8-trimethyl tocotrienol) and ζ_2-tocopherol (5,7-dimethyl tocol).

When examining the tocopherols from rice the ζ-tocopherol ran in the position of ζ_1-tocopherol and on hydrogenation no spot appeared there but the α-tocopherol spot had increased in size. When synthetic 5,7-dimethyl tocol was chromatographed together with rice unsaponifiable lipid a spot distinct from any of the rice tocopherols was observed. 5,7-Dimethyl tocol was a little more polar than ζ-tocopherol of rice in both systems. The ζ-tocopherol of rice behaved in exactly the same manner as ζ-tocopherol from palm oil and the only ζ-tocopherol so far found in Nature is 5,7,8-trimethyl tocotrienol, ζ_1-tocopherol. There is no evidence at the moment for the existence of 5,7-dimethyl tocol.

yeast hexokinase (10 γ per ml) and phosphoglucoisomerase (4 γ per ml) were added and the reaction with fructose was followed. Using this system curves plotted from reactions with standard glucose and fructose mixtures followed the same pattern as curves plotted from the reactions with brain and nerve extracts.

$HClO_4$ extracts of dog tibial nerve or mouse brain were prepared as described above, and neutralized with KOH. The extracts were passed through a mixed bed resin column composed of Amberlite IRA-400 in the CO_3^{--} form and IR-120 in the H^+ form, and then lyophilized. A solution of glucose and fructose in neutralized $HClO_4$ at concentrations similar to those of the extracts was treated in the same way. The lyophilized material was redissolved in a small volume of water and aliquots spotted onto Whatman No. 1 paper. Descending chromatography was carried out with ethyl acetate-pyridine-water (3:1.25:1) as solvent. The papers were developed with $AgNO_3$ saturated acetone and 0.5% NaOH in methanol.

Both brain and nerve extracts showed clearly defined spots at positions corresponding to those produced by known glucose and fructose solutions (treated on resin column or untreated). Both extracts also gave an intense spot with an R_{Glu} of 40, corresponding to that produced by known inositol. Neither inositol nor the unknown developed color when aniline phosphate was used as the developing agent. Since inositol is known to be present in brain in relatively high concentrations (Platt and Glock, 1943), it was felt

that the unknown spot was very probably inositol.

Fructose concentration in peripheral nerve.

Individual tibial nerves from 20 rabbits were analyzed with the following results (values are recorded as mmoles per kg wet weight).

	Range	Mean	S.D.
Glucose	2.57-4.54	3.32	0.47
Fructose	0.65-1.75	0.97	0.27

There was a positive correlation ($r = 0.60$) between glucose and fructose levels. Measurements of fructose in whole mouse brain were made on samples from four animals. The levels in brain were much lower than in nerve, the mean level being 0.1 mmole per kg. No fructose was found in eight rabbit sera and four mouse sera (less than 0.02 mmoles per liter).

Discussion. The presence of fructose in mammalian nervous tissue at concentrations higher than in blood plasma must be due to selective absorption or synthesis in situ and/or slow utilization. Brain hexokinase has a low affinity for fructose (Sols and Crane, 1954), and it is known that fructose alone cannot support respiration in the brain (Harpur and Quastel, 1949). In experiments to be reported elsewhere it has been found that fructose is converted to lactate very slowly in nerve. Possibly the role of fructose in nerve is to mediate transhydrogenation of DPN and TPN, with the aid of aldose reductase and sorbitol dehydrogenase, as has been suggested for lens, placenta and seminal vesicles.

References

Bacon, J.S. and Bell, D.J., Biochem. J. 42, 397 (1948)
Green, H. and Solomon, S.A., A.M.A. Arch. Ophthal. 61, 616 (1959)
Harpur, R.P. and Quastel, J.H., Nature 164, 779 (1949)
Hers, H.G., Biochim. Biophys. Acta 37, 127 (1960)
Hubbard, R.S. and Russell, N.M., J. Biol. Chem. 119, 647 (1937)
King, T.E. and Mann, T., Proc. Roy Soc. B. 151, 226 (1959)
Lowry, O.H., Passonneau, J.V., Hasselberger, F.X. and Schulz, D.W., J. Biol. Chem. 239, 18 (1964)
Mann, T., Biochem. J. 40, 481 (1946)
Platt, B.S. and Glock, G.E., Biochem. J. 37, 709 (1943)
Sols, A. and Crane, R.K., J. Biol. Chem 210, 581 (1954)
Van Heyningen, R., Biochem. J. 73, 197 (1959)