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# Studies on the fructose metabolism in animal tissues

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**Abstract**

An improved chromatographic method for the analysis of sugar phosphates and nucleotides presented in the previous paper was applied on the analysis of the metabolites of fructose produced by the action of hexokinase in some tissues in vitro, and the excellency of this method was demonstrated. The results showed that by this method the metabolites of the sugar in its early stage of catabolism can be analysed very easy and exactly and estimated quantitatively as well, giving no inconsistency with the results presented by the routine method.

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# STUDIES ON THE FRUCTOSE METABOLISM IN ANIMAL TISSUES

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There appears to be some difficulties in the study of fructose metabolism, because rather ambiguous and elaborate methods had to be employed to isolate or estimate each metabolic intermediates.

A chromatographic method for the simultaneous separation and determination of nucleotides and sugar phosphates including fructose-1-phosphate (F-1-P) has been reported in the previous paper[1]. In the present communication, this improved method was applied on the reaction products of some tissue hexokinases in order to get some details on the early stages of fructose metabolism in the animal tissues.

## EXPERIMENTALS

Reaction mixture : 4 ml. of 33 per cent isotonic KCl homogenates of each tissue was mixed with 1 ml. of 0.056 M fructose, 1 ml. of 0.1 M ATP Na, 1 ml. of 0.05 M $MgCl_2$, and 1 ml. of 0.5 M NaF. The mixture was adjusted to pH 7.0 with 0.38 M $KHCO_3$, and the final volume was made up to 10 ml.

Procedure : Aliquot of the above mixture was analysed at once without incubation. Another same mixture was incubated for 20 minutes at 37°C in a constant temperature bath under anaerobic condition. Before incubation, all the operations were carried out at O°C.

Both mixtures, before and after incubation, were deproteinized by addition of 10 ml. of 10 per cent perchloric acid, and neutralized with 5 N KOH. The neutral solution was filled up to 30 ml. with destilled water and filtered.

Two ml. of the filtrate was fixed with each 4 ml. of $ZnSO_4$ and $Ba(OH)_2$. One ml. of $ZnSO_4$-$Ba(OH)_2$ filtrate was analysed for free fructose by cysteine-carbazol-$H_2SO_4$ method[2].

Cation in 25 ml. of the remaining filtrate was deionized with acid form of Dowex 50, and adjusted to pH 8.5 with dilute aqueous ammonia.



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Each deionized solution of both mixtures, before and after incubation, was passed through the chloride form of Dowex 1 column (200—400-mesh, $0.9\,cm^2 \times 10.5\,cm$). The procedures above described are summarized as follows :

*MIXTURE*

| | | |
|---|---|---|
| 0.056 M | Fructose | 1 ml |
| 0.05 M | $MgCl_2$ | 1 ml |
| 0.1 M | ATP Na | 1 ml |
| 33 % isotonic KCl | homogenate | 4 ml |
| adjust to pH 7.0 with 0.38 M $KHCO_3$ | about | 1 ml |
| make up to total volume | 10 ml with $H_2O$ | |

*PROCEDURE*

*MIXTURE* 10 ml

- 5 ml → deproteinize with 10 ml of 10 % perchloric acid → neutralize with 5 N KOH and fill up to 30 ml with $H_2O$ → filtration
  - 2 ml + $ZnSO_4$ 4 ml + $Ba(OH)_2$ 4 ml → filtrate 1 ml (free fructose)
  - 25 ml → treat with Dowex 50 acid form and adjust to pH 8.5 with dilute ammonia → analyse with Dowex 1 Cl form $0.9\,cm^2 \times 10.5\,cm$ column
- 5 ml → incubate 37°C → same as before → 20 min.

Free sugars were removed with 150 ml. of $1 \times 10^{-3}$ M ammonia. A succession of eluting agents (1 liter respectively) was passed through the column in the order described in the previous report[1]. Every 50 ml. of effluent was collected successively with the aid of a fraction collector. The flow rate was 2.0 ml. /min.

Assay of the effluent fraction was conducted by using the phenol-$H_2SO_4$ method[3] for aldose phosphates and cysteine-carbazol-$H_2SO_4$ method for ketose phosphates. Optical densities were measured with Beckman spectrophotometer. Absorption at 490 and 560 $m\mu$ was adopted for aldose and ketose respectively.

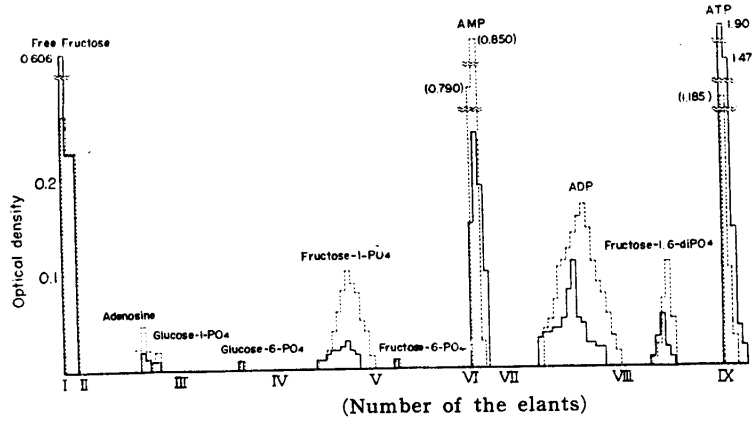
The amount of AMP, ADP, and ATP were estimated by measuring the absorption at 260 $m\mu$.

## RESULT AND DISCUSSION

Early stages of fructose metabolism in rat liver :

C-1-fructokinase which phosphorylates carbon 1 in the presence of ATP and $Mg^{++}$ has been reported to be present in the liver and muscles of some animals[4,5,6].

As shown in Fig. 1, 9.67$\mu$M of free fructose disappeared, and 8.43$\mu$M of F-1-P and 1.76$\mu$M of fructose-1, 6-diphosphate (FDP) were increased after ten-minutes incubation. After twenty minutes, 12.18$\mu$M of free fructose disappeared, and 7.94$\mu$M of F-1-P and 3.83$\mu$M of FDP were increased (Fig. 2). The amount of G-6-P and F-6-P showed no difference before and after incubation, and G-1-P was reduced in a small amount after incubation.



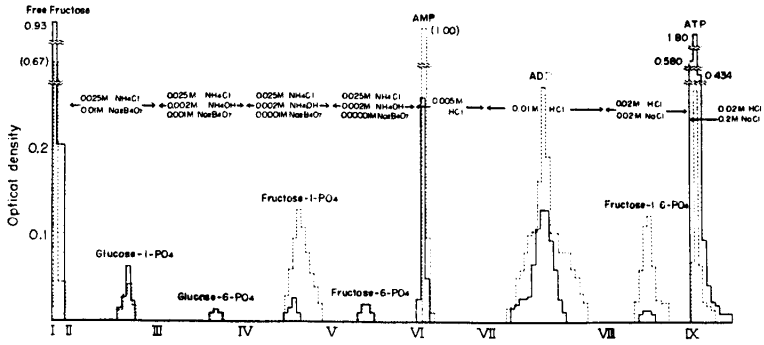

| | Fructose | G-1-P | G-6-P | F-1-P | F-6-P | F-1,6-diP | ATP | ADP | AMP | Adenosine |
|---|---|---|---|---|---|---|---|---|---|---|
| Initial (μM) | 28.57 | 0.69 | 0.28 | 1.20 | 0.12 | 2.06 | 27.52 | 4.03 | 5.38 | 0.23 |
| Final (μM) | 18.90 | 0.48 | 0.28 | 9.63 | 0.12 | 3.82 | 10.18 | 10.94 | 12.78 | 0.66 |

Fig. 1. Chromatogram of the metabolites of fructose incubated with rat liver homogenate, 37°C for 10 minutes.

These results will indicate that the first product of fructose metabolism in the liver is F-1-P which is then transformed into FDP. An explanation for the transformation of F-1-P to FDP has been presented by HERS *et al*[7,8]. But the F-1-P cleavage mechanism presented by HERS seems not to give a full explanation[9]. Although 1-phosphofructokinase which phosphorylates F-1-P to give FDP are not detected in the liver[10], the results obtained in this experiments rather appear to indicate the presence of this enzyme.

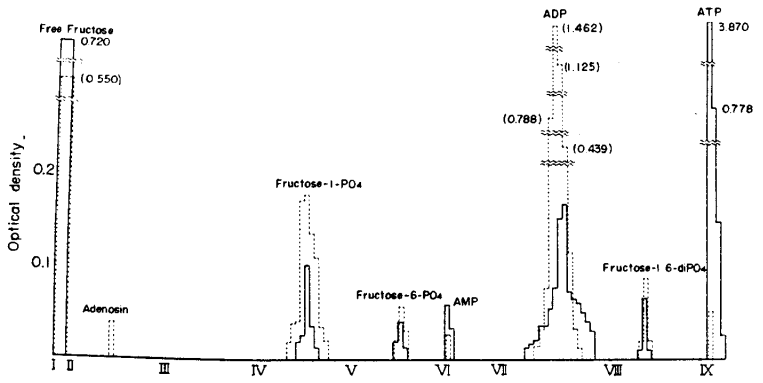
Early stages of fructose metabolism in rat muscle :

When rat muscle homogenate was incubated with fructose for twenty




| | Fructose | G-1-P | G-6-P | F-1-P | F-6-P | F-1.6-diP | ATP | ADP | AMP |
|---|---|---|---|---|---|---|---|---|---|
| Initial (μM) | 26.98 | 0.99 | 0.25 | 0.86 | 0.32 | 0.57 | 25.75 | 7.60 | 3.26 |
| Final (μM) | 14.80 | 0.82 | 0.25 | 8.80 | 0.32 | 4.40 | 5.47 | 16.21 | 11.45 |

Fig. 2. Chromatogram of the metabolites of fructose incubated with rat liver homogenate. 37°C for 20 minutes.




| | Fructose | F-1-P | F-6-P | F-1.6-diP | ATP | ADP | AMP | Adenosine | Unidentified (260mμ) Opticaldensity (150ml) |
|---|---|---|---|---|---|---|---|---|---|
| Initial (μM) | 26.29 | 2.70 | 0.37 | 0.85 | 16.65 | 2.99 | 0.29 | 0 | 0.254 |
| Final (μM) | 18.09 | 8.75 | 1.31 | 1.28 | 0.18 | 13.85 | 0.08 | 0.12 | 0.903 |

Fig. 3. Chromatogram of the metabolites of fructose incubated with rat muscle homogenate, 37°C for 20 minutes.

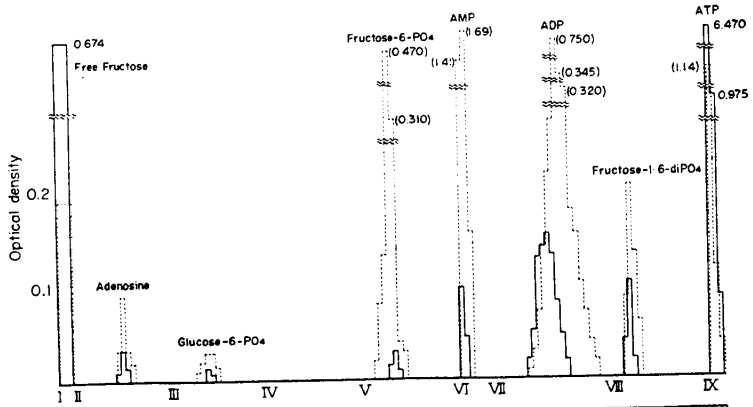
minutes, 8.2 μM of free fructose disappeared and 6.05 μM of F-1-P was increased (Fig. 3). FDP was also increased, but the extent of increment was much lower than that in the case of liver. The amount of F-6-P, which showed no change in the case of liver, was clearly increased in these case of muscle. Glucose phosphates were not detected before and after incubation.

This result will indicate that the first product of fructose catabolism

in the rat muscle is F-1-P, but will not offer any information about the product appeared in the second step whether it is F-6-P or FDP.

Early stages of fructose metabolism in dog brain:

It is generally accepted that the brain hexokinase is of the yeast type and it converts fructose into F-6-P. In my experiment, too, the result, shown in Fig. 4, confirms this well-known fact, i. e. in the present experiments, 17.59 $\mu$M of free fructose disappeared 20 minutes after incubation, and 12.32 $\mu$M of F-6-P, 3.82 $\mu$M of FDP and 1.36 $\mu$M of G-6-P were increased.




| | Fructose | G-6-P | F-6-P | F-1.6-diP | ATP | ADP | AMP | Adenosine | Unidentified (260mμ) Opticaldensity (150ml) |
|---|---|---|---|---|---|---|---|---|---|
| Initial (μM) | 24.48 | 0.58 | 0.66 | 1.94 | 25.20 | 2.17 | 0.44 | 0.22 | 0.068 |
| Final (μM) | 6.89 | 1.94 | 12.98 | 5.76 | 5.82 | 9.24 | 10.13 | 0.58 | 0.181 |

Fig. 4. Chromatogram of the metabolites of fructose incubated with dog brain homogenate, 37°C for 20 minutes.

The fate of ATP added were quite different in each tissue. Although these data presented in this paper may be interpreted by the EMBDEN-MEYERHOF scheme, there are some tissues giving a quite puzzling pattern in the early stages of sugar metabolism, which will be described in the next paper precisely. In any event, the datas presented in this paper just shows the excellency of this improved method for the investigation of sugar metabolism.

## SUMMARY

An improved chromatographic method for the analysis of sugar phosphates and nucleotides presented in the previous paper was applied on the analysis of the metabolites of fructose produced by the action of hexokinase in some tissues in vitro, and the excellency of this method was demonstrated.

The results showed that by this method the metabolites of the sugar in its early stage of catabolism can be analysed very easy and exactly and estimated quantitatively as well, giving no inconsistency with the results presented by the routine method.

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