The Isolation and Identification of Methyl Glyoxal

(Pyruvaldehyde or Pyruvic Aldehyde) from Human Milk

Part III

**Identification of acetaldehyde and methyl glyoxal from
human milk by paper partition chromatography**

**265th Report of the Peroxidase Reaction
(212th Human Milk Study)**

By

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INTRODUCTION

The Introduction common to Parts I, II and III of the present treatise was related in the beginning of Part I. The Preliminary Report¹⁾ to this treatise was already published in this Journal of the present treatise. Now the introduction to Part III alone will be stated here.

The separation of amino acids by partition chromatography on paper was first described in 1944 by Consden, Gordon and Martin.¹⁵⁾ A large number of separation of organic compounds have been described since their publication.

By this method, I was able to identify *methyl glyoxal in human milk*, but as to the occurrence of pyruvic acid in human milk, I was not able to prove it.

Thus I succeeded in verifying by using another method of identification—paper partition chromatography—my own results described already in Part I and Part II of the present treatise.

EXPERIMENTAL

A cylinder of Toyo-filter paper No. 2 for qualitative analysis, 20 cm. high and 15 cm. wide was used, and drops of test solution to be analysed were placed 5 cm. from the bottom of the cylinder and marked in pencil. The cylinder was developed by letting it stand in a dish for 1~2 hours

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Fig. 1. Paper chromatogram of 2,4-dinitrophenylhydrazones prepared from human milk and synthetic preparations; solvent; *n*-butanol. color of spot; yellowish.

1. and M.G.: synthetic methyl glyoxal osazone. 2. and P.A.: synthetic pyruvic acid hydrazone. 3. and Aa.: synthetic acetaldehyde hydrazone. 4. and Mixt.: synthetic mixture of acetaldehyde, pyruvic acid and methyl glyoxal hydrazones. 5. and mixt.: mixture hydrazones prepared from human milk. 6. and mixt.-aa.: mixture hydrazones (acetaldehyde hydrazones preliminarily excluded). 7. and aa.: acetaldehyde hydrazone prepared from human milk. 8. and m.g.: methyl glyoxal osazone prepared from milk.

in the case of xylene acetic acid and 5~6 hours in the case of *n*-butanol.

The mixture solution of water saturated xylene and acetic acid (4:1) was suitable for *p*-nitrophenylhydrazones and water saturated *n*-butanol is suitable for 2,4-dinitro-phenylhydrazones.

The separation takes place as is shown in accompanying illustration (Fig 1. & Fig 2.).

Synthetic methyl glyoxal (M.G.) was deposited on the point where the drop was applied ($R_f=0$), by 2,4-dinitrophenylhydrazone and in the case of *p*-nitrophenylhydrazone, the R_f value was 0.2.

Synthetic pyruvic acid hydrazone (P.A.) travels rather slowly ($R_f=0.29$) by 2,4-dinitrophenylhydrazone and ($R_f=0.22$) by *p*-nitrophenylhydrazone. Synthetic acetaldehyde hydrazone (Aa.) develops easily and

Fig. 2. Paper chromatogram of 2,4-dinitrophenylhydrazones extracted from human milk and synthetic preparations; color reactions spraying by caustic ethanolic solution. Numbers and abbreviations signify the same as in Fig. 1.

has R_f value of 0.94–0.95. (Cf. Table I).

The spots of synthetic acetaldehyde and pyruvic acid and methyl glyoxal osazones were at first yellow or orange.

By spraying 25% KOH 50% ethanolic solution, they showed brown, for acetaldehyde (Aa., Fig. 2), portwine-like red for pyruvic acid hydrazones (P.A., Fig. 2) and deep blue color for methyl glyoxal osazones (M.G., Fig. 2) respectively.

The hydrazone (mixt-aa.) prepared from human milk showed two spots for acetaldehyde and methyl glyoxal as 2,4-dinitrophenylosazone as well as *p*-nitrophenylosazone.

The spots for pyruvic acid from human milk was not identified. The method here described has, as is well known, shown the great advantage of separating different substances of minute amounts extracted and prepared from different materials. The material was human milk in my case.

DISCUSSION

The hydrazones of synthetic acetaldehyde, synthetic pyruvic acid

Fig. 3. Paper chromatogram of *p*-nitrophenylhydrazones prepared from human milk and synthetic preparations; solvent; xylene: acetic acid (4:1). Color of spot; yellowish.

1. and Aa: synthetic acetaldehyde hydrazone. 2. and P.A.: synthetic pyruvic acid hydrazone. 3. and M.G.: synthetic methyl glyoxal osazone. 4. and Mixt.: synthetic mixture hydrazones of acetaldehyde, pyruvic acid, and methyl glyoxal. 5. and mixt.-aa.: mixture hydrazones prepared from human milk, (acetaldehyde hydrazone preliminarily excluded). 6. and a.a.: acetaldehyde hydrazone prepared from human milk. 7. and m.g.: methyl glyoxal osazone prepared from human milk. M. P., 277° C. 8. and m.g.: methyl glyoxal osazone prepared from human milk. M. P., 210° C., not recrystallised, not pure.

and synthetic methyl glyoxal showed their proper R_f value.

It is to be noted here that, as is shown in Fig. 2, in spite of my endeavor to purify the osazone, synthetic pyruvic acid has showed, besides its proper R_f value ($R_f=0.29$), the other R_f value ($R_f=0.5$), (P.A., the upper spot and Mixt., the upper middle spot in Fig. 1) and that methyl glyoxal crystallised from milk extract showed, besides its proper R_f value ($R_f=0$), the other R_f value (mixt. -aa, Fig. 2) though its color reaction was similar to that of the proper spot ($R_f=0$) of methyl glyoxal.

The cause for such a phenomenon may be: hydrazones would form ones of different proportions which might show sometimes a different R_f value other than that of proper hydrazones which would generally

Fig. 4. Paper chromatogram of *p*-nitrophenylhydrazones prepared from human milk and synthetic preparation; color reactions spraying by caustic. ethanolic solution. Numbers and abbreviations signify the same as in Fig. 3.

TABLE I

R_f value and Color Reaction in Paper Chromatogram of Hydrazones, extracted from Human Milk and Synthetic

Substance	R_f value of 2, 4-dinitrophenylhydrazone		R_f value of <i>p</i> -nitrophenylhydrazone		Color reaction of alkali ethanolic solution
	Extracted from human milk	Synthetic	Extracted from human milk	Synthetic	
Acetalde- hyde	a) 0.95	a) 0.94-0.95	a) 0.95	a) 0.94-0.95 b) 0.20	Brownish (dark red)
Pyruvic acid	a) (*) b) (*)	a) 0.29 b) 0.50	a) (*) b) (*) c) (*)	a) 0.22 b) 0.80 c) 0.94-0.95	Red, portwine- like
Methyl glyoxal	a) =0	a) 0 b) 0.94	a) 0.20	a) 0.20 b) 0.79	Blue

a)*, b)*, c)*, Pyruvic acid was never identified.

be expected.

There are a couple of possibilities at least for the solution of such a

problem. Either a better method of the chromatography or a better method of preparation of pure hydrazones in the mixture of hydrazones forming substances may be devised. At any rate, the chemical nature of the above mentioned substances in question will remain to be solved in future.¹⁶⁾

SUMMARY

Hitherto it has been accepted without experimental evidence that in athiaminosis pyruvic acid would occur in human milk, while methyl glyoxal or pyruvaldehyde would never occur in it, especially as this substance has generally been discarded since the Embden-Meyerhof theory came into favor.

But my own actual experiment has shown the following results:

The occurrence of acetaldehyde and methyl glyoxal in human milk was assured by paper chromatography. Pyruvic acid was not identified in human milk. In Parts I and II of this treatise, I stated that I had isolated with success acetaldehyde and methyl glyoxal from human milk. Pyruvic acid was never identified. These results were also ascertained by paper partition chromatography. (Cf. Summary for Parts I & II.)

Remarks. Methyl glyoxal as an intermediate of normal carbohydrate metabolism has, as has been stated in Introduction of Part I, lost in favor. Not only that, but also it has been considered sometimes as secondarily produced or even an "artifact" due to the instability of such substances as glyceraldehyde, dioxycetone, diacetyl, acetol or lactic aldehyde, from which (esp. the first) the glyoxal might be produced. But now I firmly believe, on the basis of many Reports on Human Milk Study from our Laboratory, that there is an abnormal and quite a natural course of production of methyl glyoxal which, in normal metabolism, will not occur in an identifiable form or, if at all, occur in a trace.

CONCLUSIONS

The results obtained in Parts I, II and III of the present treatise will lead to the following conclusions.

Methyl glyoxal was isolated from human milk. It was identified analytically as well as by paper partition chromatography.

Acetaldehyde was also identified in human milk.

Pyruvic acid was neither isolated nor identified from human milk; it was not identified by paper partition chromatography either. (Cf. Conclusions for Parts I & II.)

I am very grateful to Associate Prof. N. Nakamura (Biochemistry) of our University, who is especially interested in carbonyl groups himself, for furnishing

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References and Notes

- 1) Wako, H., Tohoku J. Exp. Med., 1951, **55**, 34.
 - 2) Cf. the originals published on the Reports of the Proxidase Reaction (51-263) this Journal, since 1930 up to date.
 - 3) Sato, A., Jikken Iho, 1936, **244**, 523.
 - 4) Takamatsu, A., and Sato, A., Tohoku J. Exp. Med., 1934, **28**, 506.
 - 5) Sato, Sh., *ibid.*, 1939, **37**, 222.
 - 6) Sato, A., *ibid.*, 1951, **55**, 22.
 - 7) Sato, A., Paediatrica concreta (Jap.) Vol. II, 2nd edition, Tokyo, 1931, 259.
 - 8) Asakura, K., Tohoku J. Exp. Med., 1932, **19**, 269.
 - 9) Asakura, K., and Osako, F., *ibid.*, 1933, **20**, 429.
 - 10) Vogt-møller, Biochem. J., 1931, **25**, 418; Biochem. Z., 1931, **233**, 249.
 - 11) Geiger, A., and Rosenberg, A., Klin. Wochenschr., 1933, **12**, 1259.
 - 12) Johnson, R. E., Biochem. J., 1936, **30**, 31.
 - 13) Neuberg, C and Kobel, M., Biochem. Z., 1928, **203**, 463.
 - 14) Ariyama, T., Journ. Agri. Chem. Soc. Japan, 1931, **7**, 763.
 - 15) Consden, Gordon and Martin, Biochem. J., 1944, **38**, 224.
 - 16) The solvent used for these substances may have been inappropriate, concerning which I expect to be able to report in a coming issue of this Journal.
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