Metabolic Changes Associated with the Germination of Corn I. Changes in Weight and Metabolites and their Redistribution in the Embryo Axis, Scutellum, and Endosperm [1, 2]

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Introduction

There is a paucity of information in the literature concerning the metabolic changes that occur in the endosperm, scutellum, and embryonic axis during the germination of corn. The work of Toole (20) constitutes a major exception, although this work was more concerned with morphological changes during germination than with changes in chemical constituents and metabolism. Although seed germination has been studied extensively (3, 13, 21) much of the work has been concerned with changes in the whole seed, which may obscure changes occurring in the embryo or embryonic parts. In other instances work has been conducted with an isolated organ or tissue, or has been concerned with a single constituent, or with a particular class of enzymes. In recent years major emphasis has been placed on the effect of light, inhibitors, or dormancy on germination.

This investigation was prompted by the lack of data on the time sequence of metabolic changes, and the interrelationship of these changes in the endosperm, scutellum, and embryonic axis of germinating corn. Furthermore it was hoped that the results obtained would serve as a standard for further studies concerned with applied treatments. The changes in fresh and dry weight, nitrogen fractions, sugars, fats, and nucleic acids which occur in the endosperm, scutellum, and embryonic axis during a 5-day period of germination are presented. These changes are discussed in relation to the role of the endosperm and scutellum in supplying metabolic components to the developing embryo axis.

Materials and Methods

Uniform-sized seeds of a hybrid (WF9 × M14) corn (*Zea mays* L.) were soaked in deionized water for 4 hours and then planted (100 seeds per dish), embryo side down, on a double layer of paper towelling in a 3-quart Pyrex dish. After the addition of 100 ml of 10^{-4} M $CaCl_2$, the dish was covered with perforated Saran Wrap (Dow Chemical Company, Midland, Michigan), and placed in a humid, dark, germination cabinet maintained at 25°. An additional 50 ml of $CaCl_2$ solution was added on the third day of germination.

Seedlings were removed at daily intervals and the embryo axis (root plus shoot), scutellum, and endosperm were separated by dissection for subsequent analysis. Duplicate samples of tissue were used for all the determinations, and the whole experiment was repeated.

The Determination of Fresh Weight, Dry Weight, Total Nitrogen, and Fats. Fresh weights were determined immediately after dissection and dry weights were obtained after drying the tissues at 65° for 24 hours.

The total nitrogen content of the dried material was determined by a standard Kjeldahl procedure (19). The insoluble nitrogen (total nitrogen minus the alcohol-soluble nitrogen) has been expressed as protein using the × 6.25 conversion factor.

The fat content of the dried material was determined by petroleum-ether extraction (1).

The Determination of Soluble Nitrogen, Soluble Sugars, and Amino Acids. A sample of the freshly dissected material was dropped into boiling 95% ethanol, and after cooling, homogenized (Omni-Mixer, 2 min, full-speed) in the ethanol. The homogenate was cleared by centrifugation.

The soluble nitrogen content of the ethanol extract was determined as described for total nitrogen. After subtraction of the α-amino nitrogen from the soluble nitrogen, the remaining nitrogen has been multiplied by the 6.25 conversion factor and expressed as soluble protein.

The α-amino nitrogen content of the ethanol extract was determined by the method described by Yemm and Cocking (22). This value has been expressed as amino acid content by using a × 8.9 conversion factor.

The soluble sugar content of the ethanol extract was determined by the anthrone color reaction (23), using glucose as the standard.

The Determination of Soluble Nucleotides and Nucleic Acids. Nucleotides and nucleic acids were determined essentially as described by Ingle (10).

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Table I. *Growth and Changes in Metabolites During the Initial Phase of Germination of Maize*

Germination period (hr)	Axis			Scutellum		
	4	23	48	4	23	48
Dry wt (mg)	3.00	2.90	8.20	19.00	19.60	22.70
Root length (cm)	0.37	0.44	3.48	...	...	...
Shoot length (cm)	0.30	0.32	1.05	...	...	...
Total nitrogen as N (mg)	0.14	0.14	0.45	0.56	0.56	0.70
H_2O (mg)	4.20	9.70	85.60	28.10	34.90	48.30
Insoluble protein (mg)	0.84	0.61	2.04	3.34	3.16	3.85
Soluble protein (mg)	0.04	0.20	0.47	0.02	0.17	0.28
Total amino acids (mg)	0.05	0.13	0.54	0.17	0.27	0.44
Fat (mg)	0.92	0.68	0.64	7.00	7.70	7.80
Nucleic acid (mg)	0.07	0.07	0.16	0.12	0.11	0.13
Sugar as glucose (mg)	0.36	0.63	1.35	1.94	1.49	0.75
Total insoluble (mg)	2.53	1.91	5.74	16.81	17.60	21.12
Total soluble (mg)	0.47	0.99	2.46	2.19	2.00	1.58

Samples of freshly-dissected embryo axis or scutellum material were homogenized in 5 % perchloric acid, 0°, using a power-driven, conical glass homogenizer. Aliquots of this homogenate were used for the estimation of RNA and DNA. Endosperm material was homogenized in an Omni-Mixer (5 min full speed), and the total homogenate was used for either RNA or DNA determination. Homogenates were cleared by centrifugation, and the acid-precipitable material was washed twice with 5 % perchloric acid, 0°. The acid supernatant fraction and the 2 washes were combined and used for the estimation of soluble nucleotides, and the residue was used for the extraction of either RNA or DNA.

For the estimation of soluble nucleotides the acid extract was neutralized to pH 7 to 8 with 5 N KOH, and concentrated at 35° under reduced pressure. In order to remove contaminating pigment from acid extracts of embryo axis material, 3 extractions were made with 5 volumes of diethylether prior to neutralization. After cooling, the $KClO_4$ was removed by centrifugation and the supernatant fluid was run onto a column (10 × 1 cm) of Dowex 1-X4 formate resin. The column was washed with water, and then the nucleotides were eluted in bulk with 70 ml of 4 N formic acid containing 0.8 M ammonium formate. The soluble nucleotide content of the eluate was calculated using an arbitrary 260 mμ millimolar extinction coefficient of 14.

For the determination of RNA the residue from acid precipitation was extracted 3 times with an ethanol-ether-chloroform mixture (2: 2: 1) and then hydrolyzed with 1.0 N KOH for 24 hours at 25°. The resulting RNA mononucleotides were purified by bulk elution from anion exchange resin (10), and the RNA content was estimated on the basis of a theoretical tetranucleotide structure (260 mμ optical density × 31.7). For the determination of DNA the residue from acid precipitation was similarly extracted with the ethanol-ether-chloroform mixture, and then further extracted with 5 % perchloric acid for 2 periods of 20 minutes at 70°. The DNA content of the extract was estimated by the diphenylamine procedure (10). The RNA and DNA were summed to give the total nucleic acid content.

The soluble sugar, soluble protein, amino acid, and nucleotide contents have been summed and expressed as the total soluble content of the material. The subtraction of this value from the dry weight has given the total insoluble content of the material. Since other soluble constituents, undoubtedly present, were not measured, the total soluble and insoluble contents are estimates.

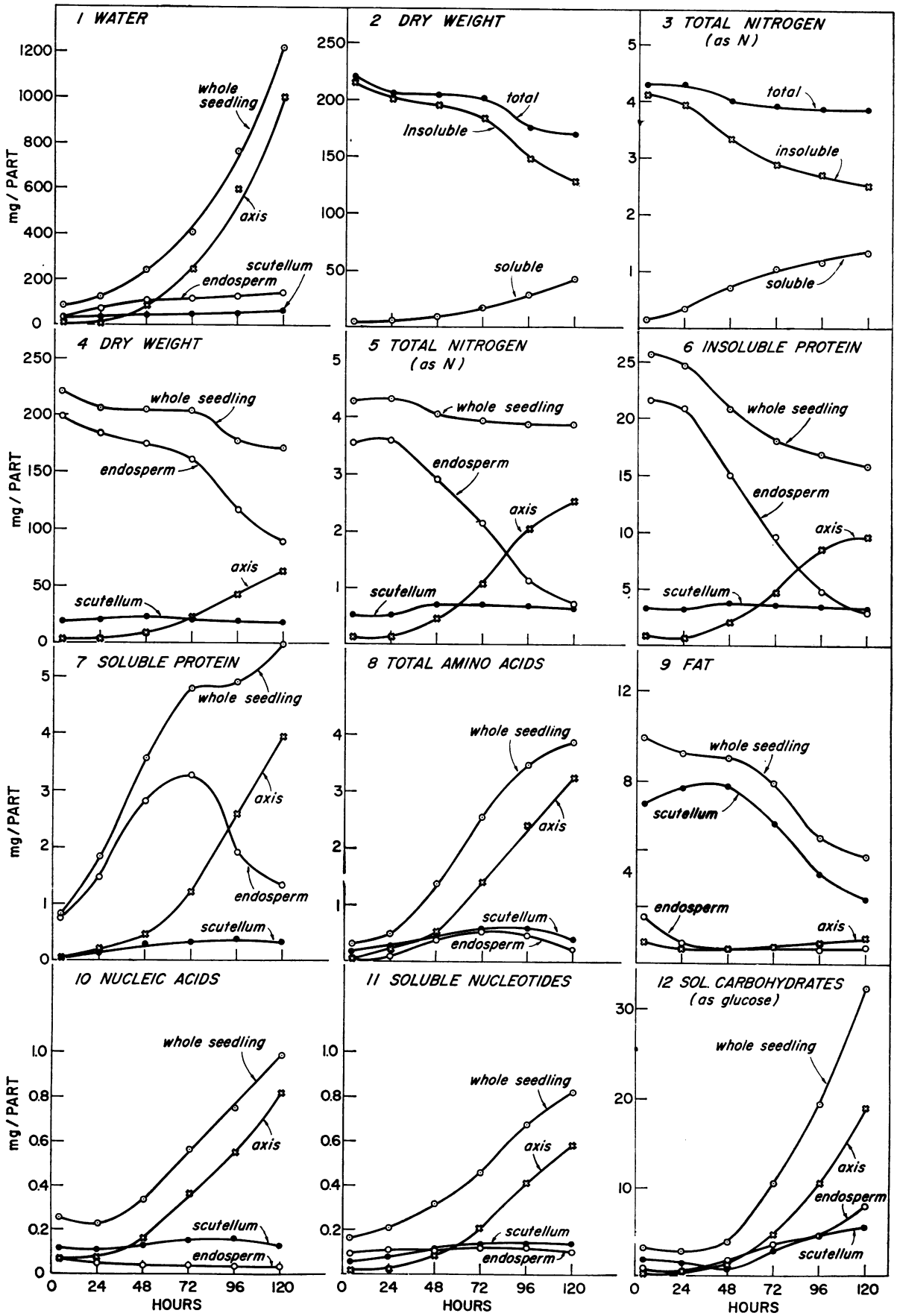
Experimental Results

Changes in the Whole Seedling. The rapid uptake of water was the most obvious change associated with the initiation of germination. Although all parts of the seedling increased in water content over the period of study, the pattern and extent of hydration varied (fig 1). In the first 24 hours the water content of the seedling axis increased 131 % while the scutellum increased 24 % (table I). By the fifth day over 80 % of the water content of the seedling was held by the axis.

With progressive seedling hydration there was an associated change of insoluble to soluble constituents (fig 2). Soluble components, largely carbohydrates, accounted for 2 and 25 % of the total dry weight of the seedling at the initiation and termination of the experiment, respectively. On a whole seedling basis, and with regard to all constituents measured, there was a decrease in insoluble constituents which was accompanied by an increase in soluble material. There was a decrease of about 23 % of the initial dry weight of the seedling over the 5-day period.

The conversion of insoluble to soluble nitrogen

FIG. 1–12. The changes of various components of the embryo axis, scutellum, and endosperm of corn over a 5-day germination period.


1 WATER
mg / PART
whole seedling
axis
scutellum
endosperm
2 DRY WEIGHT
total
Insoluble
soluble
3 TOTAL NITROGEN (as N)
total
insoluble
soluble
4 DRY WEIGHT
whole seedling
endosperm
axis
scutellum
5 TOTAL NITROGEN (as N)
whole seedling
endosperm
axis
scutellum
6 INSOLUBLE PROTEIN
whole seedling
endosperm
axis
scutellum
7 SOLUBLE PROTEIN
mg/PART
whole seedling
axis
endosperm
scutellum
8 TOTAL AMINO ACIDS
whole seedling
axis
scutellum
endosperm
9 FAT
whole seedling
scutellum
endosperm
axis
10 NUCLEIC ACIDS
mg / PART
whole seedling
axis
scutellum
endosperm
11 SOLUBLE NUCLEOTIDES
whole seedling
axis
scutellum
endosperm
12 SOL. CARBOHYDRATES (as glucose)
whole seedling
axis
endosperm
scutellum
0 24 48 72 96 120
HOURS
0 24 48 72 96 120
HOURS
0 24 48 72 96 120
HOURS

components during germination (fig 3) was similar to that observed for the solubilization of total components (fig 2). A small loss (9 %) of the total nitrogen was observed over the germination period, presumably due to a leaching of soluble nitrogenous compounds from the seedling. The rate of formation of the soluble nitrogen compounds was much faster during the first 72 hours than over the remaining portion of the period (fig 3). The decline in rate of appearance of soluble nitrogen compounds after 72 hours does not necessarily indicate a cessation of mobilization of the nitrogen reserves (fig 6). The decline could signify that the rate of synthesis of new protein in the axis and the degradation of protein reserves are approaching a steady state after 72 hours.

Axis Growth. During the initial period (4–23 hr) there was a negligible elongation of root or shoot (table I), although the solubilization of components (17 % of the total dry weight), interconversion of nitrogenous compounds, decrease in fat content, and increase in soluble carbohydrates indicated that many metabolic processes had been initiated. Subsequently the root elongated rapidly, increasing 8-fold during the second day, while the plumule emerged at 36 hours and elongated more slowly. At the end of 48 hours the axis was well developed and continued to increase in dry weight at a linear rate over the next 3 days. The composition of the axis remained rather constant with respect to moisture, nitrogen, and nitrogen fractions, and fat content per unit of dry weight, throughout. In contrast soluble carbohydrate content increased from 12 to 30 %.

Changes in the Distribution of Components between the Axis, Scutellum, and Endosperm. Another characteristic process of germination is the movement of reserve material to the enlarging axis. This process is dependent on the initial and continued uptake of water and the solubilization and hydrolysis of reserve components. Some insight into the roles of the endosperm, scutellum, and axis in this process can be gained by a time sequence measurement of their metabolic constituents.

The apparent contribution of the scutellum to the growth of the axis is negligible, based on the 5 % decrease in net weight (fig 4). Although the change in net weight was small, significant alterations of components occurred in this organ during the germination period. There was a 13 % increase in total nitrogenous components, a 173 % increase in carbohydrate content and a 59 % decrease in fat content (fig 5, 9, 12).

Data presented in figures 6 and 7 illustrate the rate and extent of solubilization, redistribution, and synthesis of the soluble and insoluble protein fractions within the seedling. The disappearance of insoluble protein from the endosperm was paralleled by an increase of this fraction in the axis (fig 6). The soluble protein content of the whole seedling increased progressively with germination (fig 7). During the first 3 days most of this fraction appeared in the endosperm, but in the later stages of germination (4th and 5th days) the soluble protein content of the endosperm decreased, while that of the axis increased at a rate parallel with its growth.

Although the amino acid content of the endosperm increased to a maximum around the third or fourth day (fig 8) there was no large accumulation analogous to the soluble protein fraction (fig 7). The changes in amino acid content in the scutellum were similar to those observed in the endosperm. The proportion of the total amino acid content present in the axis increased progressively with germination, but at a slightly lower rate than axis growth such that the amino acid content of the axis dropped from 6.7 % to 5.1 % of the dry weight, from day 2 to day 5.

The bulk (80 %) of the fat reserve of the seed was contained in the scutellum (fig 9). This reserve was progressively depleted over the 5-day period without an associated accumulation of fat in the axis.

At the initiation of germination the level of nucleic acids and nucleotides was extremely low in all parts of the seedling (fig 10, 11). Since there was no reserve in the storage organs, the large increase of these components observed in the axis during germination must represent de novo synthesis. In the scutellum there was a slight increase in these components that reached a maximum on the third day and declined slowly during the next 2 days. In the endosperm the nucleic acid content progressively decreased while the nucleotide content remained constant with germination.

Soluble carbohydrates constituted the major portion (50–75 %) of the total soluble fraction of the seedling during the 5-day period. Although the soluble carbohydrate content decreased initially in both endosperm and scutellum (with minima at 24 and 48 hours respectively), there was no corresponding decrease in these constituents in the axis (fig 12). In fact there was a continuous and progressive increase of soluble carbohydrates of the axis (from 12 to 30 % of the dry weight over the germination period). During the last 72 hours of the germination period the soluble carbohydrate content increased in all parts of the seedling.

Discussion

Germination is characterized by a rapid uptake of water which facilitates the mobilization of reserve material and the utilization of these reserves for axis growth. Although this initial uptake of water is a dominant factor in the induction of germination, its mode of entry, and the sequence of events initiated by its entry, are difficult to define (3, 13, 16, 17, 18, 21). In the present work there was a rapid uptake of water and a consistent decrease in dry weight of the axis in the first 23 hours. This loss in dry weight indicated that an initial use of endogenous substrate by the axis preceded the transfer of reserve material from the scutellum or endosperm. The fact that imbibition of water and the associated metabolic changes preceded cell division was implied from the negligible amount

of nucleic acid formed during this initial period, and was confirmed by microscopic examination. The small elongation of the axis during this period has been shown to be due to cellular expansion rather than cell division (20).

Subsequently axis growth commenced and was maintained at the expense of reserve materials transferred from the scutellum and the endosperm.

Although initial increases in scutellum fat were consistently observed, the results are questionable because of difficulty encountered in obtaining complete extraction of the tissue at the first sampling. The data obtained would suggest that scutellum fat was not utilized during the first 2 days, but shows that depletion was most rapid over the next 3 days. Since there was no parallel increase in fat content in the other organs, it may be concluded that the fat was either transformed to sugar (2) or served as respiratory substrate. The RQ values of less than 1 observed during germination of corn seeds (4) and barley (12) support the view that lipids were serving as respiratory substrate. However, the demonstration of a functioning gloxylate cycle in the scutellum from 5-day old corn seedlings (15) indicates that part of the storage fat of the seed may be converted to sugar during germination. The relatively slow and gradual utilization of the fat reserve over the germination period agree with the observations of Toole (20) and Malhotra (14) but contrast with those of Dure (4). Since only 2 mg of lipid material was lost from the whole seedling during the first 3 days of germination (fig 5), the data do not support the contention of Dure (4) that early growth of the axis is dependent upon utilization of the scutellum lipid reserve. These same considerations would also apply to the initial and small loss of fats from the axis.

With the exception of fat, the net changes of other components of the scutellum were of minor magnitude. This is well illustrated by the dry weight data presented in figure 4, which show the scutellum to have identical weights at initiation and termination of the experiment. However, these data provide no clue to the metabolic transformations, synthesis, and transport of material that occurred in this organ. The work of Dure (5) indicated that α-amylase, which represented 90 % of the total amylolytic activity, originated in the scutellum and then moved into the endosperm. James (11) suggested that the hexoses formed by hydrolysis in the endosperm are converted to sucrose in the scutellum prior to transport to the axis. Furthermore the enzymes necessary for this conversion have been observed in the scutellum (7). Energy for synthesis and transport was available as this organ is a rich source of mitochondria (9).

The 2 major constituents of the endosperm, insoluble protein, and carbohydrates (measured indirectly as the residual insoluble matter) decreased drastically with germination. The solubilization of the protein began during the initial phase of germination, however there was essentially no change in total nitrogen (fig 5) during the initial 4 to 23-hour period. This would suggest that hydrolysis and transport of this reserve material was not initiated by the end of the first day of germination. The accumulation of soluble protein in the endosperm during the first 3 days may have been due to the release of zein from protein storage bodies which exist in corn kernels (6). The measurements made did not permit any definite conclusions on the extent of hydrolysis of the protein or the kinds of nitrogenous components transported from the endosperm through the scutellum to the axis. The appearance of a small amount of free amino acids in the endosperm was in agreement with the work of Folkes and Yemm (8), and suggest that the amino acids were transported to the axis at a rate approximately equal to the rate of hydrolytic release in the reserve organ. The site of amino acid interconversion was not established, but this may well be another function of the scutellum.

The minimal sugar content in all parts of the seedling at the 23-hour sampling indicate that carbohydrate hydrolysis is not initiated in this early phase. The hydrolytic breakdown of insoluble carbohydrates in the endosperm exceeded the rate of utilization by the scutellum and axis as sugar content increased in all parts of the seedling with time.

The endosperm is not considered to be active in the elaboration of materials for the axis as all attempts to isolate active mitochondria or certain of the glycolytic enzymes from excised endosperms have been unsuccessful (unpublished data).

Summary

The changes of various chemical components, nitrogen fractions, sugar, fat, and nucleic acid, in the embryo axis, scutellum, and endosperm of corn have been determined over a 5-day germination period. Many changes in these chemical components were observed before any growth of the embryo axis occurred, indicating that these changes were associated with processes responsible for the resumption of growth. The growth of the axis was largely at the expense of the reserves of the endosperm. Extensive loss of protein and insoluble carbohydrates from the endosperm occurred over the 5-day period. Although the growth of the axis may have been partially maintained by the fat supply of the scutellum, there was also a concurrent utilization of carbohydrates as indicated by the changes in sugar constituents and dry weight. The fat content of the scutellum decreased after the second day with a concurrent increase in soluble carbohydrates and soluble nitrogenous components, although the scutellum dry weight remained relatively constant, throughout the experimental period.

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