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Morphological features of subcutaneous tissue of the antebrachial region in human fetus

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Aim – to clarify the morphological features of the structure and topography of adipose tissue in the forearm region of human fetuses at 5–8 months of gestation in order to clarify normal developmental parameters and identify possible variants or abnormalities.

Material and methods. A microscopic study was performed on the material of the antebrachial region of 21 human fetuses measuring 136.0–310.0 mm parietal-coccygeal length (PCL), with subsequent statistical data processing.

Results. In the studied 5-month-old human fetuses, no objects that could be identified as adipocytes were found in the upper, middle, and lower thirds of the antebrachial region. The absence of fatty formations was also observed at the level of the lower third of the forearm in 6-month-old fetuses. In the upper third of the antebrachial region of 6-month-old fetuses, the percentage of multilocular adipocytes is 91.8±0.87%, at the level of the middle third – 72.3±0.85%. In 7-month-old fetuses, multilocular adipocytes in the upper third of the antebrachial region account for 47.8±0.84%, in the middle third – 49.0±0.83%, and in the lower third of the antebrachial region – 61.9±0.86%. In the upper third of the antebrachial region of 8-month-old fetuses, multilocular adipocytes are 39.0±0.85%, in the middle third – 24.4±0.84%, and in the lower third of the antebrachial region – 34.6±0.84%.

Conclusions. The adipose tissue of the forearm area is represented by uni- and multilocular cells. In 6-month-old fetuses at the level of the upper and middle thirds of the antebrachial region, as well as in 7-month-old fetuses at the level of the lower third of the antebrachial region, multilocular cells quantitatively prevailed, at the level of the upper and middle thirds of the antebrachial region of 7-month-old fetuses and in all thirds of the antebrachial region of 8-month-old fetuses – unilocular cells. The largest number of adipocytes was found in 8-month-old fetuses. Between 6 and 7 months of gestation, a leap in the development of adipose tissue is noted.

The study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Local Ethics Committee for all participants.

The authors declare no conflict of interest.

Keywords: multilocular cell, unilocular cell, adipose tissue, antebrachial region, fetus.

Морфологічні особливості підшкірної клітковини ділянки передпліччя у плоду людини

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Мета: встановити морфологічні особливості будови та топографії жирової тканини в ділянці передпліччя плодів людини на терміні 5–8 місяців гестації для уточнення нормальних показників її розвитку, виявлення можливих варіантів чи відхилень.

Матеріал та методи. Мікроскопічне дослідження виконано на матеріалі ділянок передпліччя 21 плода людини розміром 136,0–310,0 мм тім'яно-куприкової довжини (ТКД) з подальшою статистичною обробкою даних.

Результати. У досліджених 5-місячних людських плодах у верхній, середній та нижніх третинах передпліччя не було виявлено об'єктів, які можна ідентифікувати як адипоцити. Відсутність жирових утворень також простежувалась на рівні нижньої третини передпліччя у 6-місячних плодів. У верхній третині передпліччя 6-місячних плодів відсоток багатокамерних адипоцитів становить 91,8±0,87%, на рівні середньої третини – 72,3±0,85%. У 7-місячних плодів у ділянці верхньої третини передпліччя багатокамерні адипоцити становлять 47,8±0,84%, на рівні середньої третини – 49,0±0,83%, на рівні нижньої третини передпліччя 61,9±0,86%. У верхній третині передпліччя 8-місячних плодів багатокамерні адипоцити дорівнюють 39,0±0,85%, у середній третині – 24,4±0,84%, у нижній третині передпліччя – 34,6±0,84%.

Висновки. Жирова тканина ділянки передпліччя представлена одно- та багатокамерними клітинами. У 6-місячних плодів на рівні верхньої та середньої третин передпліччя, а також у 7-місячних плодів на рівні нижньої третини передпліччя кількісно переважали багатокамерні клітини, на рівні верхньої та середньої третин передпліччя 7-місячних плодів та у всіх третинах передпліччя 8-місячних плодів – однокамерні. Найбільша кількість адипоцитів встановлена у 8-місячних плодів. Між 6 і 7 місяцями гестації простежується різке прискорення розвитку жирової тканини.

Дослідження виконано відповідно до принципів Гельсінської декларації. Протокол дослідження погоджено локальним етичним комітетом для всіх учасників.

Автори заявляють про відсутність конфлікту інтересів.

Ключові слова: багатокамерна клітина, однокамерна клітина, жирова тканина, ділянка передпліччя, плід.

Introduction

The study of adipogenesis is highly relevant in modern conditions due to two global challenges: the obesity epidemic and the growing number of premature births. Obesity, associated with an increased risk of cardiovascular diseases, diabetes, and metabolic syndrome, necessitates new approaches to understanding metabolic mechanisms [10,13].

It should be noted that preterm infants face issues with thermoregulation due to the underdevelopment of adipose tissue, particularly brown fat, which plays a key role in heat production [5,17].

Prematurity has remained the leading cause of neonatal mortality worldwide over the last decade and is now also the primary factor in the mortality of children under five years of age. Annually, about

15 million premature infants are born worldwide, accounting for approximately 11% of all births. Moreover, the frequency of preterm births is increasing in most countries [9].

Adipose tissue is composed of white and brown fat cells with distinct functions. White fat cells store energy in the form of triglycerides and perform an endocrine role by producing hormones that regulate metabolism. In contrast, brown fat cells specialize in thermogenesis due to the UCP1 protein in mitochondria, which generates heat through fat oxidation. In newborns, brown fat is critical for maintaining body temperature, but its development in preterm infants may be insufficient, increasing the risk of hypothermia.

In the context of adipose tissue development, beige fat cells, which represent an intermediate form, also play a significant role [1,3,7,14]. These cells form within white adipose tissue in response to specific stimuli, such as cold or physical activity, and can function similarly to brown fat cells, promoting thermogenesis. Beige cells help the body adapt to temperature changes. However, their development in preterm infants may be impaired, further exacerbating the risks of hypothermia and metabolic complications [18].

Disturbances in the development of adipose tissue can lead to the formation of benign tumors, such as lipomas (tumors arising from white adipose tissue) and hibernomas (rare tumors derived from brown adipose tissue). Lipomas are generally harmless, although they may cause aesthetic or physical discomfort [2,11]. Hibernomas, while rare, attract more attention due to diagnostic challenges and potential functional activity [15].

The study of the developmental mechanisms of white and brown adipose tissues holds significant potential for addressing two key challenges in modern medicine: combating obesity and providing optimal support for premature newborns [12]. Understanding these processes opens avenues for developing new therapeutic approaches, such as stimulating brown adipose tissue to enhance heat production or correcting energy imbalances in critical conditions [6,19].

The aim of this work was to clarify the morphological features of the structure and topography of adipose tissue in the forearm region of human fetuses at 5–8 months of gestation in order to clarify normal developmental parameters and identify possible variants or abnormalities.

Materials and methods of the study

The study was conducted on the antebrachial region of 21 human fetuses with parietal-coccygeal lengths (PCL) ranging from 136.0 to 310.0 mm, all of which were without visible anatomical defects or congenital anomalies of the upper extremities. The research was carried out within the framework of cooperation between the Chernivtsi Regional Pathological Bureau and the Bukovinian State Medical University.

Microscopic analysis was performed using hematoxylin and eosin staining of histological sections, along with histochemical protein studies using the bromophenol blue method (as described by Mikel Calvo). This method, typically used to determine the ratio of amino and carboxyl groups in proteins, proved effective for identifying types of fat cells (unilocular and multilocular). It allowed for precise visualization of cell membranes and intracellular structures forming lipid droplets.

Standard procedures were employed to ensure the study's accuracy: section preparation, fixation, basic hematoxylin and eosin staining for general analysis, and specialized bromophenol blue staining to detail protein components. This approach enabled the acquisition of high-contrast images necessary for detailed examination of the structure of fat cells and their protein membranes [4].

Quantitative analysis was conducted using digital images of histological sections processed with the ImageJ 1.53t software (2022). Based on this data, the percentage of multilocular cells was determined, followed by statistical processing. For comparing quantitative data between groups, the Mann–Whitney U test and t-test were used, providing an assessment of statistically significant differences [16]. Statistical analysis of the results was performed using the PAST software (PAleontological STatistics, Version 4.9, 2022). The average percentage of multilocular cells was calculated, along with the statistical error and confidence intervals at a significance level of $p=0.05$ [8].

The study was conducted in compliance with bioethical requirements and the main provisions of the Council of Europe's Convention on Human Rights and Biomedicine (dated 04.04.1997), the World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects (1964–2013), the Order of the Ministry of Health of Ukraine No. 690 dated 23.09.2009, and the methodological recommendations of the

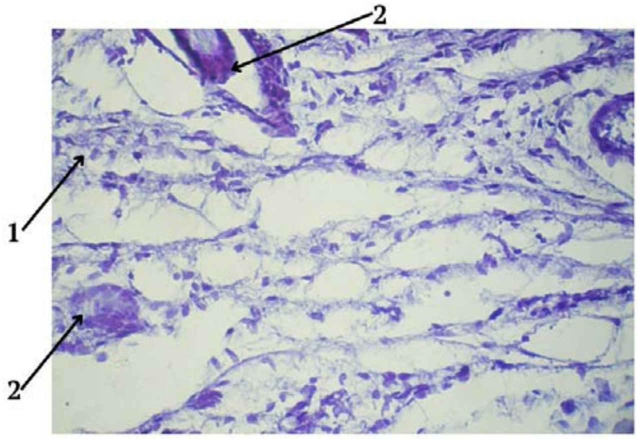

Fig. 1. Structures of the upper third of the antebrachial region in a fetus with a PCL of 160.0 mm: 1 – dermis; 2 – hair follicles. Histological section stained with bromophenol blue using the Mikel Calvo method. Plan achromat 4× objective. Without an eyepiece

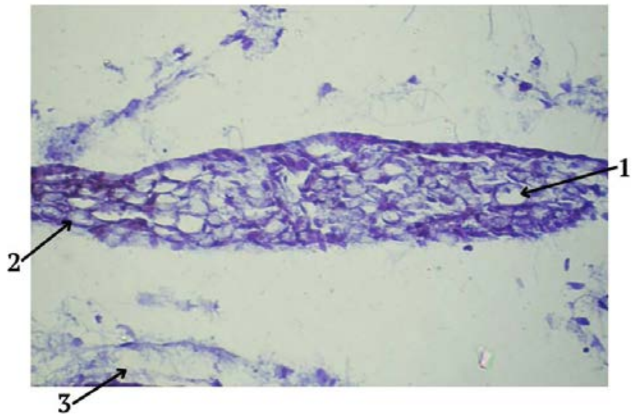

Fig. 2. Structures of the upper third of the antebrachial region in a fetus with a PCL of 195.0 mm: 1 – unilocular adipocytes; 2 – multilocular adipocytes; 3 – loose connective tissue. Histological section stained with bromophenol blue using the Mikel Calvo method. Plan achromat 4× objective. Without an eyepiece

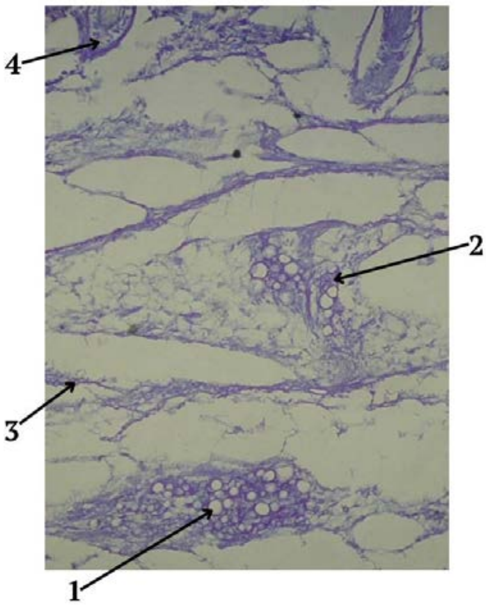

Fig. 3. Structures of the middle third of the antebrachial region in a fetus with a PCL of 205.0 mm: 1 – unilocular adipocytes; 2 – multilocular adipocytes; 3 – loose connective tissue; 4 – hair follicle. Histological section stained with bromophenol blue using the Mikel Calvo method. Plan achromat 4× objective. Without an eyepiece

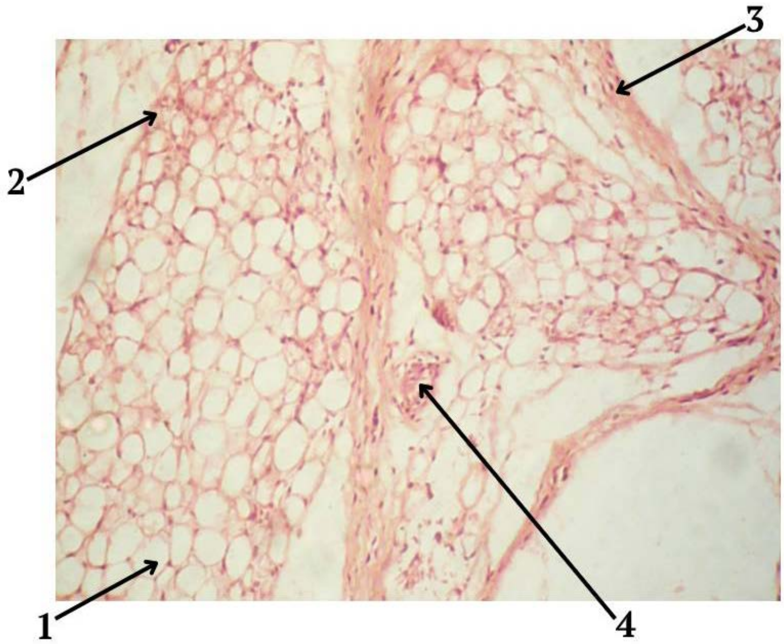

Fig. 4. Structures of the middle third of the antebrachial region in a fetus with a PCL of 245.0 mm: 1 – unilocular adipocytes; 2 – multilocular adipocytes; 3 – loose connective tissue; 4 – blood vessel. Histological section stained with hematoxylin and eosin. Plan achromat 4× objective. Without an eyepiece

Ministry of Health of Ukraine, «Procedure for the Removal of Biological Objects from Deceased Persons Whose Bodies Are Subject to Forensic Examination and Pathological Anatomical Research, for Scientific Purposes» (2018). The Commission on Biomedical Ethics of the Bukovinian State Medical University (Protocol No. 5 dated 16.12.2024) found no violations of moral or legal standards during the conduct of the medical scientific research.

Results of the study and discussion

During the histological examination of the upper, middle, and lower thirds of the antebrachial region of human fetuses aged 5–8 months, specific structural and spatial features of adipose tissue drew our attention.

In the examined human fetuses with a PCL of 136.0–185.0 mm, no identifiable adipocytes were observed in the upper, middle, or lower thirds of the antebrachial region. Among the well-developed dermis, occasional hair follicles were noted (Fig. 1).

In 6-month-old fetuses, fat cells are observed in the upper third of the antebrachial region, forming isolated, small, flat plaques arranged in a single row (Fig. 2). Multilocular adipocytes significantly predominate, accounting for 91.8±0.87% (confidence interval 90.1–93.4%, p=0.05). Other adipocytes were identified as unilocular. Notably, the initial clusters of fat cells appear near blood vessels.

In the middle third of the antebrachial region, the number of plaques increases, their contours become indistinct, and the plaques acquire irregular shapes; occasionally, plaques arranged in two rows are observed (Fig. 3). The ratio of fat cell types in the middle third changes slightly, with multilocular cells comprising 72.3±0.85% (confidence interval 70.6–73.9%, p=0.05).

In this age group, adipocytes are not detected in the lower third of the antebrachial region.

In fetuses with a PCL of 231.0–270.0 mm, intensive development of adipose tissue is observed. In this age group, unilocular adipocytes predominate in plaques located in 2–3 rows in the upper and middle thirds of the antebrachial region.

In the upper third, the plaques are clearly demarcated and do not merge with each other, with multilocular cells comprising 47.8±0.84% (confidence interval 46.1–49.4%, p=0.05).

In the middle third, round-shaped plaques arranged in several rows and different orientations are observed, with the largest number of plaques located around blood vessels (Fig. 4). The percentage of multilocular cells in this region is 49.0±0.83% (confidence interval 47.3–50.6%, p=0.05).

Notably, plaques dominated by unilocular adipocytes are located deeper than those dominated by multilocular adipocytes (Fig. 5).

In the lower third of the antebrachial region, flat plaques arranged in 1–2 rows predominate, with multilocular adipocytes making up 61.9±0.86% (confidence interval 60.2–63.5%, p=0.05). Unilocular adipocytes are located deeper, while multilocular adipocytes are more superficial.

In fetuses with a PCL of 271.0–310.0 mm, intensive adipose tissue development is observed. Fat cells increase in size, and plaques become tightly adjacent, forming continuous structures with thin connective tissue fibers visible between them (Fig. 6). Unilocular cells significantly predominate. The tendency for plaques to form around blood vessels persists (Fig. 7).

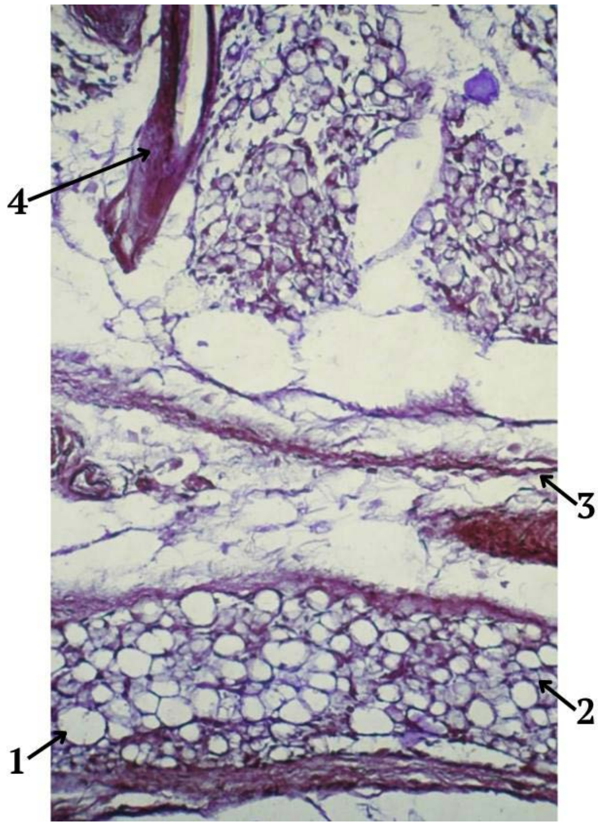

Fig. 5. Structures of the middle third of the antebrachial region in a fetus with a PCL of 220.0 mm: 1 – unilocular adipocytes; 2 – multilocular adipocytes; 3 – loose connective tissue; 4 – hair follicle. Histological section stained with bromphenol blue according to the Mikel Calvo method. Plan achromat 4× objective. Without an eyepiece

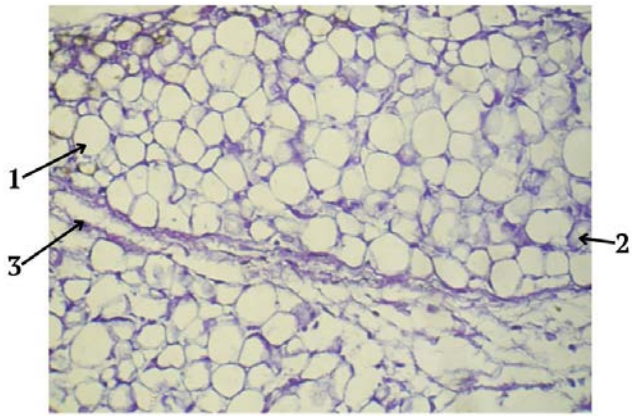

Fig. 6. Structures of the middle third of the antebrachial region in a fetus with a PCL of 275.0 mm: 1 – unilocular adipocytes; 2 – multilocular adipocytes; 3 – loose connective tissue. Histological section stained with bromphenol blue according to the Mikel Calvo method. Plan achromat 4× objective. Without an eyepiece

In fetuses of this age, the percentage of multilocular cells in the upper third of the forearm is 39.0±0.85% (confidence interval 37.3–40.6%, p=0.05), in the middle third is 24.4±0.84% (confidence interval 22.7–26.0%, p=0.05), and in the low-

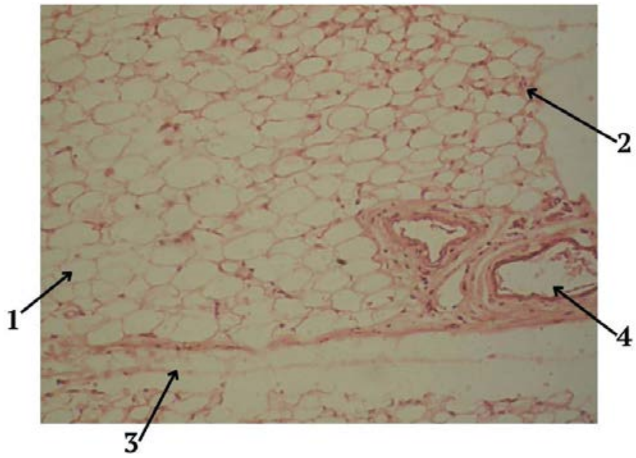

Fig. 7. Structures of the middle third of the antebrachial region in a fetus with a PCL of 290.0 mm: 1 – unilocular adipocytes; 2 – multilocular adipocytes; 3 – loose connective tissue; 4 – blood vessel. Histological section stained with hematoxylin and eosin. Plan achromat 4× objective. Without an eyepiece

er third of the forearm is 34.6±0.84% (confidence interval 32.9–36.2%, p=0.05).

The adipose tissue of the forearm in the studied human fetuses aged 5–8 months is represented by unilocular cells with a large lipid droplet and a nucleus displaced to the periphery, which we identified as white adipose tissue, as well as smaller multilocular cells with several lipid vacuoles, which were identified as brown adipose tissue.

The results of our study demonstrate that the formation of adipose tissue in the human forearm during 5–8 months of gestation is accompanied by pronounced morphological changes, particularly an increase in the proportion of multilocular adipocytes. These findings support the current understanding of adipose tissue as a highly dynamic metabolic organ that begins to develop as early as the embryonic period [1]. Our observations regarding the gradual decrease in the percentage of multilocular adipocytes in later gestational stages are consistent with the conclusions of A.M. Cypess (2022) [3], who emphasizes the variability of adipocyte types and their functional roles in adipose tissue development.

The morphological characteristics of subcutaneous adipose tissue described in our previous work [12] also confirm our current findings and demonstrate the sequential development of subcutaneous adipose tissue in human fetuses. This indicates the consistency of the anatomical and morphological features of adipose tissue across different fetal body regions.

At the same time, some studies [2,7] focus on other aspects of adipose tissue, such as cellular regeneration and functional diversity (white, brown, and beige adipose tissue), which were not the focus of our current research. Considering this, future studies may further expand our understanding of embryonic adipose tissue development by exploring its cellular biology and metabolic functions.

Conclusions

1. In the study of the distribution of adipose tissue in the upper, middle, and lower thirds of the forearm region in human fetuses aged 5–8 months, there is an unevenness in the quantitative and qualitative composition of adipocytes.

2. The adipose tissue of the forearm region is represented by both unilocular and multilocular cells. In 6-month-old fetuses, multilocular cells predominated in the upper and middle thirds of the forearm, as well as in the lower third of 7-month-old fetuses. In the upper and middle thirds of the forearm in 7-month-old fetuses and in all thirds of the forearm in 8-month-old fetuses, unilocular cells predominated.

3. In the study of the adipose tissue of the upper, middle, and lower thirds of the forearm in 5-month-old fetuses, no cells could be identified as adipocytes. The absence of adipocytes was also observed in the lower third of the forearm in 6-month-old fetuses. The highest number of adipocytes was found in 8-month-old fetuses. Intensive adipose tissue development occurs between the 6th and 7th months of gestation.

4. The first clusters of adipocytes appear around blood vessels, and this trend is also observed when forming adipocyte layers in older fetuses.

5. In all studied human fetuses aged 5–8 months, adipocyte clusters form one or several rows of plaques of varying shapes and spatial orientations, clearly separated from adjacent structures by loose connective tissue.

Thus, our study supports the concept of adipose tissue as a dynamic organ starting from the embryonic period and expands the understanding of its morphofunctional development in humans.

The authors declare no conflict of interest.

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