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Dermal Effects of Unsaponifiable Compounds: The Overlooked Perspective of Vegetable Butters and Oils

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Synopsis

Unsaponifiable compounds are an integral part of vegetable butters and oils. Their composition is typically complex, and includes terpenic and aliphatic compounds, waxes, tocopherols and tocotrienols, phospholipids, and phenolic compounds.

In this article, *in vitro* and *in vivo* studies of the dermal effects of unsaponifiable compounds from avocado (*Persea americana*), canola (*Brassica* sp.), coffee (*Coffea arabica*), hazelnut (*Corylus avellana*), palm (*Elaeis* sp.), and soya (*Glycine max*) oils; perilla (*Perilla frutescens*) seed meal; and shea (*Vitellaria paradoxa*) butter were reviewed.

Unsaponifiable compounds as cosmetically and therapeutically active ingredients were proven to act as antioxidative, anti-inflammatory, antitumor, immunomodulatory, and antimicrobial agents. They express wound healing, anti-acne, and anti-dermatitis activities, as well as hydrating, photoprotective, and anti-wrinkle activities. It is thus also reasonable to recommend the use of unrefined vegetable butters and oils.

However, no systematic studies regarding the dermal use of unsaponifiable compounds have been performed yet, and in-depth clinical research should be encouraged because of their promising dermal activity.

VEGETABLE BUTTERS AND OILS

Vegetable butters and oils are widely used in the pharmaceutical and cosmetic industries as extraction solvents, active ingredients, ingredients of the lipid phase in dermal formulations, and other excipients. Chemically they are a mixture of esters of glycerol and fatty acids (triglycerides), and unsaponifiable compounds (1). In general, the dermal effects of vegetable butters and oils include hydrating, emollient, antimicrobial, antioxidative, and anti-inflammatory activities (2). Although the triglyceride fraction has been subject to

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more research, significantly less scientific work has been performed on unsaponifiable compounds. The focus of this article is on unsaponifiable compounds of vegetable butters and oils, and their action on the skin after dermal application.

UNSAPONIFIABLE COMPOUNDS

By definition, unsaponifiable compounds are components of vegetable butters and oils that are not saponified when treated with alkali such as NaOH and KOH, but are soluble in lipids (3,4). They are typically extracted using organic solvents such as acetone, ether, heptane, hexane, and petroleum ether, and are not volatile at 103°C (4,5). The content of unsaponifiable compounds in vegetable butters and oils usually varies between 0.3 and 2% (6). Composition is complex and includes terpenic and aliphatic compounds, waxes, tocopherols and tocotrienols, phospholipids, and phenolic compounds (1,3), which are reviewed in the sections of this article that follow. Specific chemical families such as alkylfurans, alkylphenols, alkenylphenols, ferulic acid derivatives, lignans, limonoids, and polyprenols are exceptional (3).

The content and composition of unsaponifiable compounds is usually specific to a vegetable butter or oil, but varies depending on the geographical origin, climatic and agronomic conditions, the quality of plant material for extraction, the extraction method, and exposure to refining. Changes in composition may also occur during the storage of a butter or oil, and are mainly due to hydrolysis and oxidation, that is, exposure to air (oxygen and humidity), temperature, and microbial contamination. The identification of unsaponifiable compounds is thus particularly essential for the evaluation of a butter's or oil's quality, including possible falsification. Analytical methods typically used are TLC, HPLC, GC-MS/FID, LC-GC, and SFC (7,8).

DERMAL EFFECTS

Sound scientific evidence about the beneficial effects or effectiveness of dermally applied unsaponifiable compounds in cosmetic and pharmaceutical products is completely lacking because, to the best of our knowledge, there have been no in-depth, systematic studies performed in this area to date.

A detailed search of scientific databases and books was performed for the purpose of this review article. Butters and oils presented in the section on "Unsaponifiable compounds in vegetable butters and oils" were included in Table 1 together with the composition of unsaponifiable compounds. It should be noted that the content of total and individual unsaponifiable compounds is based on the results of different scientific sources listed in the reference section and may vary in different studies. This mainly depends on the geographic origin of a butter or oil, and the extraction procedure.

DERMAL EFFECTS OF ISOLATED UNSAPONIFIABLE COMPOUNDS

The following section provides a detailed review of the skin or skin-related effects of isolated unsaponifiable compounds grouped according to their general chemical structures (Figure 1).

Table 1
Content and Composition of Unsaponifiable Compounds in the Reviewed Vegetable Butters and Oils [mg/100 g of Oil]. Slash Symbol (/) Is Used When Information Was not Available

		Terpenic compounds [mg/100 g of oil]					Aliphatic compounds [mg/100 g of oil]	
	Unsaponifiable compounds [mg/100 g of oil]	Sterols (β-sitosterol)	4-Methylsterols	Triterpene alcohols	Carotenoids β-carotene	Tocopherols (α-, β-, and Y-tocopherol), tocotrienols, plastochromanol-8)	Hydrocarbons (mainly squalene)	Phenolic compounds [mg/100 g of oil]
Elaeis sp. pulp (1,3)	200–1,000	30–265	9–36	8–32	38–200	10–150	Squalene 42–98	/
Corylus avellana, (1,3,79)	200–300	106–200	/	5–6	/	22–61	Squalene 19–25	291
Persea americana, (1,3,80,81)	400–12,200	250–2,000	90–440	50–170	4–23	0–80	290–390 Squalene 34–37	882
Brassica napus, (1,3,82)	500–5,000	450–1,130	7–27	18–54	/	36–268	Squalene 44 207	/
Butyrospermum parkii/ *Vitellaria paradoxa*, (1,3,21,65,83)	3,000–10,000	150–360	100	800–6,200 3,000	/	3–81	920–4,000	407
Perilla frutescens, (3,69)	1,000–1,800	1,800–4,000	/	/	/	45–67	Squalene 150	/
Glycine max, (1,3,84,85)	500–1,700	180–474	25–66	40–84	7–0.03	60–337	90 Squalene 10–14	42.6
Coffea arabica, (1,3,86)	7,600–15,000	132–5,000	/	/	/	12–61	/	/

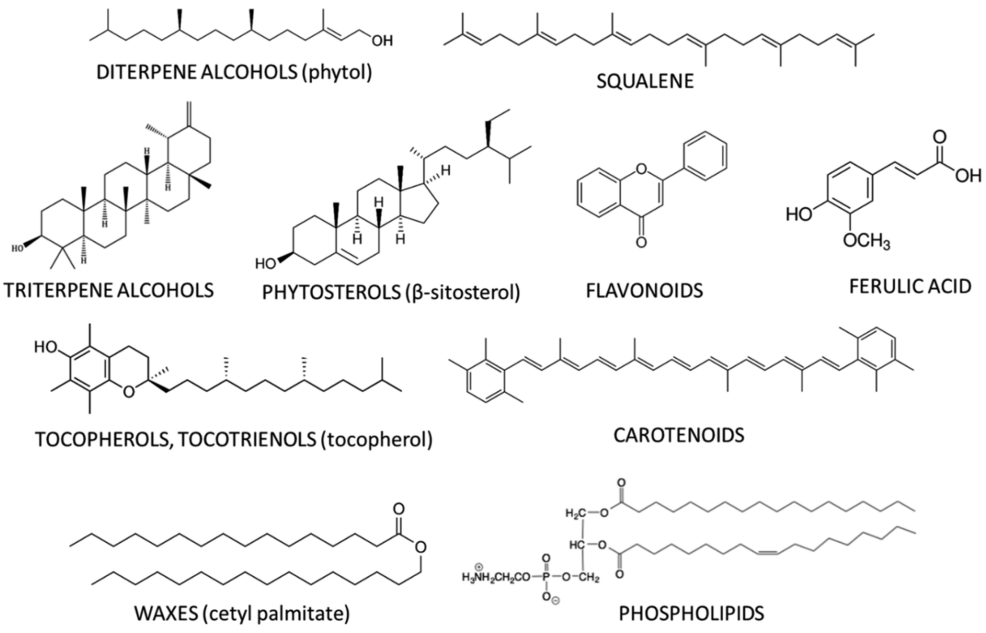

Figure 1. Chemical structures of typical unsaponifiable compounds.

TERPENIC COMPOUNDS

DITERPENE ALCOHOLS: PHYTOL

Phytol is a monounsaturated diterpenic alcohol. It is involved in the synthesis of chlorophyll, tocopherol (vitamin E), phylloquinol (vitamin K), and fatty acid phytyl esters in plants (9). In terms of effects that may be important for dermal activity, research demonstrated its cytotoxic, autophagy- and apoptosis-inducing, anti-inflammatory, immunemodulating, antioxidative, antimicrobial, and antinociceptive effects (10,11). Phytol also showed potential in addressing skin hyperpigmentation, as it decreased melanin production in murine melanoma cells significantly by reducing the expression of tyrosinase and tyrosinase-related protein 1 (12).

SQUALENE

Squalene is a polyunsaturated triterpenic hydrocarbon. It is found in animals (shark liver), humans (sebum), and plants (amaranth and olive oils) (3,13–15). It functions on the human skin primarily as an antioxidant, particularly in ultraviolet (UV)-induced free radical reactions, and contributes to proper skin hydration (16,17). In a macrophage cell line study (18), squalene induced an increase in the synthesis of anti-inflammatory IL-10, IL-13, and IL-4, and a decrease in pro-inflammatory TNF-α and NF-κB. It was also assumed that squalene may significantly contribute to faster wound healing, especially in the last stages of tissue remodelling and wound closure. The inhibition of TPA tumor-promoting action was shown *in vitro* and *in vivo* on mouse skin (19). In addition, squalene was linked to beneficial effects in the care of skin with acne and seborrheic dermatitis (14,15,17). On

37 females, the daily ingestion of 13.5 or 27 g of squalene resulted in significantly decreased facial wrinkles, but was associated with a high incidence of transient loose stool (20).

TRITERPENE ALCOHOLS

Triterpene alcohols are compounds with a general structure of six isoprene units. They exhibit several biological properties, which may be important in terms of their dermal activity: anti-inflammatory, antitumor, chemopreventive, and antimycobacterial activities (21–24).

PHYTOSTEROLS

Phytosterols are sterol compounds chemically similar to cholesterol, which plays a crucial role in ensuring the structural integrity of the skin's stratum corneum (25). About 98% of all phytosterols in plants are represented by β-sitosterol, campesterol, and stigmasterol (26). β-sitosterol has been reported to possess angiogenic activity, which promoted fibroblast multiplication and consequently the wound healing activity (27). β-Sitosterol was also found to increase the effect of vitamin D on the immune function of macrophages (28). In general, *in vitro* studies showed the antioxidative, anti-inflammatory, and antitumor properties of phytosterols (26,29). In a study using skin equivalents and cDNA microarrays, apple seed phytosterols were shown to significantly affect the regulation of genes associated with keratinocyte proliferation and differentiation, stimulate the synthesis of hyaluronic acid, and increase epidermal thickness (30). In addition, phytosterols were proposed for use in hair care because of their hair softening and hair conditioning properties (31).

TETRATERPENOIDS: CAROTENOIDS

Carotenoids are plant pigments of the tetraterpene family, and are responsible for the yellow, orange, or red color of plant tissues such as flowers and fruits. Carotenoids are classified based on their functional groups as xanthophylls, carotenes, and lycopene (32,33). Other compounds such as apocarotenoids are also derived from carotenoids by oxidative cleavage (34). Beneficial biological effects of carotenoids mainly derive from their antioxidative properties, and encompass direct antioxidative effects, and photoprotective, anti-inflammatory, antitumor, and immunostimulative effects (32,35,36).

TOCOPHEROLS AND TOCOTRIENOLS (VITAMIN E)

Vitamin E is a common term for the so-called tocochromanols, which are divided into tocopherols and tocotrienols, each having four forms, that is, alpha, beta, gamma, and delta (33). Vitamin E is a crucial antioxidant in the cell membranes of plants and animals, including the human skin, and in human sebum, with the most biologically active form being α-tocopherol (38). Evidence of its beneficial dermal use due to photoprotective

activity include decreased erythema, decreased tumor incidence and decreased lipid peroxidation, and improvements in wrinkled skin (39).

PHENOLIC COMPOUNDS

FLAVONOIDS

Flavonoids are typical representatives of phenolic compounds that are widely present in the plant world, and give yellow, red, blue and violet color to flowers, leaves, and fruits. They were shown to have significant antioxidative activity both in *in vitro* and *in vivo* systems (40–44). They also act as antibacterial (45,46), antiviral (47,48), and anti-inflammatory agents (47,49).

FERULIC ACID

Ferulic acid is widely present in plants, either freely or covalently bound to, for example, mono-, di-, and polysaccharides; glycoproteins; polyamines; and lignin (1). Most important and well researched is its antioxidative activity, which was shown in keratinocytes, fibroblasts, and animals, as well as wound healing, photoprotective, and antimelanogenic activities (50–52). Orally administered to mice, ferulic acid improved psoriasis-like skin lesions (53). In addition, ferulic acid ameliorated symptoms of atopic dermatitis on mice skin (54).

WAXES

In plants, waxes cover surfaces of leaves and fruits, and restrict the loss of water, control the exchange of gases, and protect against pathogens. They typically consist of an ester-bonded long-chain (C18-22) fatty acid to a long-chain (C26-28) alcohol (6). Most known in terms of dermal application is the liquid wax of jojoba seeds also known as jojoba oil, which has been shown to accelerate the wound closure of both the keratinocytes and fibroblasts in scratch wound experiments, and to stimulate collagen I synthesis in fibroblasts, whereas no effect was detected on the secretion of matrix metalloproteinase (MMP)-2 and MMP-9 gelatinases by keratinocytes or fibroblasts (55). In addition, jojoba wax was reported to express hydration, antioxidative, anti-inflammatory, and antibacterial effects on the skin (56).

GAMMA ORYZANOL

γ-Oryzanol is a mixture of esters of ferulic acid and triterpene alcohols (mainly cycloartenol and 24-methylencycloartenol) or sterols (mainly beta-sitosterol and campesterol), extracted from rice bran oil (1). *In vitro* tests confirmed its antioxidative activity, including protection against the oxidation of vegetable oils with a high content of unsaturated fatty acids in triglycerides (57,58). A study on rats showed that intradermally injected γ-oryzanol significantly inhibited dermal allergic reaction due to the inhibition of mast

cell degranulation (59). In another study on rat skin (60), it was observed that a 1% γ-oryzanol ointment, in contrast to the control ointment, caused the stimulation of sebaceous glands, which resulted in an increase in sebum production.

PHOSPHOLIPIDS

Phospholipids are amphiphilic molecules typically composed of hydrophobic fatty acid "tails" and a hydrophilic phosphate "head." Physiologically, they are the dominant lipids in cell membranes (61). Lysophosphatidic acid was reported to be beneficial in wound healing, pruritic skin disease, skin tumors, scleroderma, and skin inflammation reaction (62).

DERMAL EFFECTS OF UNSAPONIFIABLE COMPOUNDS IN VEGETABLE BUTTERS AND OILS

The reviewed studies demonstrate specific mechanisms of action of isolated unsaponifiable compounds such as the antioxidative, anti-inflammatory, antitumor, immunomodulatory, and antimicrobial activities; wound healing; and anti-acne and anti-dermatitis activities, as well as regenerative, hydrating, photoprotective, and anti-wrinkle activities. This provides us basic evidence for the understanding of their general benefits when used dermally, either alone or as ingredients in therapeutic and cosmetic formulations. However, it must be clearly emphasized that the results of *in vitro* and animal *in vivo* research cannot be directly extrapolated as real effects after application on the human skin.

Further, within the concept of this review, special emphasis was placed on studies dealing with total unsaponifiable compounds, particularly as integral structural components of vegetable butters and oils. Presented in the following section is a review of research focused on *in vitro* tests using skin cell cultures of fibroblasts and keratinocytes, and *ex vivo* and *in vivo* tests studying dermal use.

UNSAPONIFIABLE COMPOUNDS OF AVOCADO (*PERSEA AMERICANA*) OIL AND SOYA (*GLYCINE MAX*) OIL

A 5% mixture of unsaponifiable compounds of avocado and soya oils in almond oil, almond oil, and 0.9% NaCl, respectively, were dermally applied on rat skin for 15 d (63). Results indicated the improved composition of the dermis, as the proportion of soluble collagen increased significantly. To explain the aforementioned results, the next study (64) by the same research group was designed using additional methods of differential calorimetry, X-ray diffraction, and extensiometry. The tested unsaponifiable compounds improved the biomechanical properties of animal skin, which was demonstrated by an increase in elasticity.

UNSAPONIFIABLE COMPOUNDS OF CANOLA (*BRASSICA* SP.) OIL AND SHEA (*VITELLARIA PARADOXA* SYN. *BUTYROSPERMUM PARKII*) BUTTER

In a study by Lodén et al. (65), the effects of dermally applied substances (canola oil, unsaponifiable compounds of canola oil, sunflower oil, borage oil, fish oil, petrolatum, water,

and hydrocortisone) were tested on seven men and 14 women (22–75 years), on the volar surface of the forearm, using aluminum chambers filled with tested substances and attached to the skin. First, the skin was exposed to a 14% aqueous solution of sodium lauryl sulfate (SLS) for 7 h, and then the tested substances were applied for 17 h. No pretreatment with sodium dodecyl sulfate was performed on the control arm. Skin areas were examined 24 h later. Shea butter, sunflower oil, petrolatum, and water induced very weak, barely perceptible erythema on the control skin. On the SLS-treated skin, transepidermal water loss was reduced significantly by canola oil, canola oil unsaponifiables, and hydrocortisone, and blood flow was reduced significantly by canola oil unsaponifiables and hydrocortisone, compared with exposure to water; the effects of other substances were insignificant.

UNSAPONIFIABLE COMPOUNDS OF GREEN COFFEE (*COFFEA ARABICA*) OIL

Unsaponifiable compounds of green coffee oil were shown to have moderate antimicrobial and low antioxidative properties (66). In addition, *in vitro* Sun Protection Factor was reported to be 10 times higher than that of the oil (results not shown). However, due to the observed cytotoxicity on keratinocytes and lethality in the brine shrimp assay, the authors stressed that more experiments are needed to evaluate the potential of unsaponifiable compounds in green coffee oil for dermal use.

UNSAPONIFIABLE COMPOUNDS OF HAZELNUT (*CORYLUS AVELLANA*) OIL

Masson et al. (67) designed a study with virgin hazelnut oil, refined hazelnut oil, and refined hazelnut oil enriched with previously extracted phospholipids; the oils contained 286 ppm, only traces and 224 ppm of phospholipids, respectively. Each of the oils was incorporated at 10% in a test emulsion, whereas control emulsion contained no hazelnut oil. A total of 56 women aged 30–45 years, divided into four groups, applied the test emulsions on the volar surface of the forearm twice a day for 28 d. Skin hydration was assessed using corneometry. Results showed a significant hydrating effect for all test emulsions. The effect of the emulsion with virgin oil was statistically significant relative to the emulsion with refined oil, whereas differences between virgin and enriched oil emulsions were not significant. The determined differences in hydration properties were therefore attributed to phospholipids.

UNSAPONIFIABLE COMPOUNDS OF PALM (*ELAEIS* SP.) OIL

Crude palm oil obtained through the direct compression of palm fruit mesocarp and the tocotrienol-rich fraction of palm oil were evaluated as dermal permeation enhancers of 5-fluorouracil, lidocaine, and ibuprofen, respectively, using full-thickness human skin excisions mounted in Franz-type diffusion cells (68). Refined palm oil was used as a negative control, as it did not influence permeation. Permeation enhancement of all three test substances was the highest for tocotrienol fraction, followed by crude oil and then refined oil. However, only the flux of ibuprofen from both the tocotrienol fraction and crude oil was significant.

UNSAPONIFIABLE COMPOUNDS OF PERILLA (*PERILLA FRUTESCENS*) SEED MEAL

A study by Lee et al. (69) focused on unsaponifiable compounds from perilla seed meal that were obtained after the production of perilla oil. Compositional analysis identified 362.6 mg of total tocopherols, 3,761.4 mg of policosanol, 27,860.1 mg of phytosterols, and 1,028.2 mg of squalene in 100 g of unsaponifiable compounds. Tests on human dermal fibroblasts showed, among other effects, decreased UVB-induced cytotoxicity, decreased production of UVB-induced reactive oxygen species, decreased MMP production and c-Jun and c-Fos phosphorylation, and increased synthesis of collagen. These results indicate possible benefits in protection from photoaging processes.

DISCUSSION

Unsaponifiable compounds are an integral part of vegetable butters and oils in terms of their native chemical composition. Based on the reviewed *in vitro* and *in vivo* studies, we conclude that these substances contribute significantly to the overall dermal effects of vegetable butters and oils.

Recent experiments evaluating the antioxidative activity of total unsaponifiable compounds, i.e., not of individual components of unsaponifiable compounds, were published by Tavakoli's group (70,71). Unsaponifiable matter of wild pistachio (*Pistacia* sp.), sesame, and rice bran oils was shown to have significant antioxidative properties, which were stronger than that of the respective oils alone. To summarize, although direct evidence is scarce, it is reasonable to expect that antioxidative effects of unsaponifiable compounds may be expressed directly on the skin's surface and in the epidermis. Similar conclusion can be drawn for the antimicrobial effects, as the human skin hosts an enormous world of microorganisms (72).

Moreover, dermally applied unsaponifiable compounds were found to beneficially affect the function of surfactant-irritated skin (65). Detailed mechanisms of action were not investigated. However, phytosterols were suggested to play an important function by influencing the structure of epidermal lipids and regulating the skin's barrier function. Authors assumed that phytosterols may have supplied SLS-damaged skin with depleted lipids. Some insight into this topic was highlighted in research performed by Menon et al. (73) who showed that SLS provoked a statistically significant burst of synthesis of epidermal sterols. Dermally applied phytosterols or unsaponifiable compounds may therefore represent the additional supplementation of those compounds and help the skin's own mechanisms to normalize barrier disruption more rapidly. However, Man et al. (74) showed that the dermal use of individual lipids (i.e., cholesterol, fatty acid, and ceramides) or incomplete mixtures of those lipids delay barrier recovery in acetone-treated mouse skin, in contrast to complete mixtures comprising all three lipids. This was assumed to originate at the level of lamellar bodies, which gave rise to abnormal intercellular membrane structures in the stratum corneum; such abnormalities did not occur when a complete lipid mixture was provided. In addition to lipid-based structural improvements of the epidermis, effects of unsaponifiable compounds were also observed in the dermis, at the level of protein composition and the improved elasticity of animal skin (64), and at the level of *in vitro* collagen synthesis (69). Finally, in terms of the skin's overall functioning, hydration was shown to be significantly improved by unsaponifiable compounds in a human clinical study (67).

Dermal use of unsaponifiable compounds in the treatment of skin disorders is largely unexplored but has been recognized as promising according to two patents (75,76). Despite the lack of direct evidence, it has been clearly emphasized that unsaponifiable compounds might contribute significantly to an effective wound-healing action (2,27,77). It is, however, crucial to understand that in the treatment of wounds, fatty acids of triglycerides also play an important role (2). It is therefore reasonable to recommend the use of unrefined vegetable butters and oils because unsaponifiable compounds are removed during the refining process.

Unsaponifiable compounds are also not very common in cosmetics. In contrast to a large number of vegetable butters and oils, only a few unsaponifiable compounds have been reported to be used as cosmetic ingredients in dermal formulations: unsaponifiable compounds from shea (*Vitellaria paradoxa* syn. *Butyrospermum parkii*) butter, canola (*Brassica* sp.) oil, soybean (*Glycine max* syn. *Glycine soja*) oil, sunflower (*Helianthus annuus*) oil, olive (*Olea europaea*) oil and hydrogenated olive oil, avocado (*Persea americana* syn. *Persea gratissima*) oil, sesame (*Sesamum indicum*) oil, and corn (*Zea mays*) oil (78). In the context of cosmetic use, the potential of permeation enhancement (68) and a corresponding safety profile may have to be taken into account, as cosmetic products are typically used daily and over an extended period. However, more research is needed to draw reliable conclusions. Considering the current body of evidence, no critical concerns should be highlighted, and unsaponifiable compounds have been assessed to be safe in typical concentrations and practices of use in cosmetics (78).

CONCLUSION

Studies prove that unsaponifiable compounds express beneficial cosmetic and therapeutic effects after dermal application. It is therefore reasonable to recommend the use of unrefined vegetable butters and oils, as well as unsaponifiable compounds alone, for the treatment and care of skin disorders. However, research in the area of dermal application of unsaponifiable compounds is very rare, and a call for in-depth studies seems to be of great interest.

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