bjt. Calorimetric investigations around a royal hieroglyph¹

Ingolf Lamprecht

Institut für Biophysik, Freie Universität, Thielallee 63, D-14195 Berlin 33 (Germany) (Received 26 November 1992; accepted 10 June 1993)

Abstract

Honeybees ("bjt") and their products honey and wax played an important role in Ancient Egypt. The bee became the symbol of the Pharaoh of Lower Egypt and a royal hieroglyph. Honey and wax were used for many purposes in daily life, as votive offering or as salary.

Microcalorimetric experiments on bees of various ages or occupations and different castes as well as social effects among them are presented. Calorimetric curves are investigated for temporal structures indicating locomotor activities of the animals. Moreover, adult animals of the domesticated "usual" European honey bee *Apis mellifera carnica* are compared with those of the (modern) Egyptian bee *Apis mellifera lamarckii*.

Differential scanning calorimetry and combustion bomb calorimetry have been applied to pollen, to honeys of various origins (lime-tree, pine, rape) or of intended purposes (royal jelly, hoarded food), to propolis and to different types of beeswax (comb wax from various places in the stock, wax from uncapping, wax for queen cells and commercial waxes).

My son, eat thou honey, because it is good; and the honeycomb, which is sweet to thy taste: So shall the knowledge of wisdom be unto thy soul.

Proverbs of Solomon, Chapter 23, 13,14

INTRODUCTION

Historical

King Solomon was not the first to sing about the importance of honey. The Old Testament is full of hints at the importance of apiculture, of honey and wax. Biblic regions specially suited for agriculture and cattle-breeding, such as the famous Goshen (Genesis 45,10; 46,34) were also predestinated

¹ Dedicated to Hans Georg Wiedemann.

for bee-keeping, and the Fourth Book of Moses tells that certain children of Israel rose up against Moses: "Is it a small thing that thou hast brought us up out of a land that floweth with milk and honey, to kill us in the wilderness, ...". Such "land that floweth with milk and honey" remained a symbol for prosperity, health and a protecting hand of God.

However bee-keeping dates back much further than Solomon or even Moses. Honeybees became a "domestic" animal in the Neolithic period, although our earlier ancestors already knew how to harvest honey and wax after killing the bees. First pictures about honeycombs and the life cycle of bees were found in Catal Hüyük dating back to 6000 B.C. In the fourth millennium B.C. apiculture was generally spread in the Nile delta. At that time the bee became the heraldic sign of Lower Egypt and Egyptians called their country poetically "the land of the bee", while Mesopotamia was that "of the wasp". The middle of the third millennium showed a flourishing of bee-keeping in strong connection with agriculture and cattle-breeding. Wall pictures and reliefs from that period underline the influence of apiculture and explain the techniques used at that time (Fig. 1). Bee amulets prove that people believed in the auspicious and apotropaic force of the bee which belonged to the Egyptian canon of charm and luck symbols [1].

Thus, honeybees ("bjt") played an important role in daily life and mythology in Ancient Egypt. They moved up from the heraldic sign of Lower Egypt to the symbol of its Pharaoh, just as the rush ("sut") was the ideogram for Upper Egypt. The Pharaoh title "That who belongs to the bee" and the corresponding hieroglyph developed to become the most powerful letter, the royal hieroglyph. Rush and bee form the introduction to the proper name of pharaoh [1]. Figure 2 shows a detail from a pillar in Carnac with the rush and the bee above the cartouche with the royal name of King Sesostris I. Figure 3(a) and (b) taken from a papyrus sold nowadays in the streets of Cairo represent a ram god talking with King Ramses II,



Fig. 1. Apicultural scene from the sun temple of Pharoah Ny-user-Ra near Abu Gurab. The flat relief, approximately 2360 B.C., shows from left to right the gathering of honey, cleaning of storage containers and sealing of globular pots [1].



Fig. 2. Essential detail from the upper part of a pillar of the royal jubilee building of King Sesostris I in Carnac. It shows the rush and the bcc as idcograms of both parts of Egypt, the two half circles as symbol of a special form of bread, here used as the phonetic complement "t" and the cartridge with the throne name of Sesostris I. The sun represents the sun god, and scarabeus and the two hands serve as writing signs with 3 and 2 consonants, but without a symbolic meaning [44].

above them the royal cartouche with the bee and the rush (enlarged in Fig. 3(b)). Thus, the bee was the symbol of monarchy, of the highest god, of divinity itself. Goddess Nut could appear as a bee, and a temple "House of Bees" existed in Sais where a local form of Osiris was worshipped. During the Old Kingdom the bee was shown in a peaceful posture, unable to sting. Posture changed with the times; the hieroglyph mutated to an agressive animal with a clearly indicated sting, ready for defence (Fig. 4) [1], but they remained the sign of faithfulness, of the brave fight for legitimate causes and against the foul.

Bees were supposed to be created from the tears of sun-god Ra or to emerge from the dead corpse of the holy Apis bull, giving links to its eternity. It would be nice to connect the (latter) Latin word for bee (Apis) with this myth which was told in many versions by Greek and Roman poets (see, for example, the "bugony" of Ovid) and survived up to the medieval literature and even further [1], but etymologic dictionaries tell us that the origin of the word apis is unknown and the similarity merely fortuitous.



Fig. 3. (a) King Ramses II (right) and a ram god (left). (b) Enlarged upper part with the rush and the bee, besides a goose and a disk indicating "son of the sun god Ra". Picture taken from a modern papyrus bought in Cairo.

In ancient civilisations honey was a substance of magic or religious character as well as a wanted medicine. The word honey was often used for all sweet things, so that the Mosaic destination was "the promised land of milk and date jam" [2]. The real bee-honey (also "bjt") was rare and expensive, but nevertheless so much desired that it had to be imported to



Fig. 4. Bee hieroglyph in the calm, peaceful posture from the 5th dynasty, 2450 till 2290 B.C. (left) and the changed hieroglyph in the aggressive and defensive posture from the 8th dynasty, approximately 2130 B.C. (right) [1].

Ancient Egypt. One pot of honey was as valid as one cow or one donkey. It even became part of the salary of priests or higher officials, was among the funerary gifts and an important votive offering to gods [3]. Together with beeswax it was the most important ingredient of nearly all Egyptian medicaments. Many of these recipes with honey are cited in the famous Papyrus Ebers. For instance, honey was used against the sting of a scorpion or against ptomaine, together with shrub acacia tips and date as contraceptive, was used as medicament in women's diseases, infections of the lung or the intestine, to assure the proper state of vessels or to dress wounds. The nomadic tribes of the Tasa culture (fifth millennium B.C.) already mixed malachite, copper spar, oil, fat and honey for their green eve cosmetic, not only for beauty, but to protect against the Egyptian eye illness [1]. Honey accompanied the Old Egyptians during their campaigns as a special medicament, and it is still used nowadays as an antiphlogistic [4, 5] and wound healing agent [6]. It was shown recently that unprocessed honey inhibited most of the fungi and bacteria causing wound and surgical infection [4].

Wax ("mnh") was intensively used in Ancient Egypt in different fields. Although its application together with honey for mumification appeared only in a later period, natural body holes and surgical dissections were closed by wax during mumification in early times [3]. Beeswax was an appropriate means to cover wounds (a habit known in many parts of the world even nowadays), an essential component of semi-solid pharmaceutical products as pills, ointments and suppositories, again as today, and a demanded sealing material. In encaustic painting, mixing pigments with beeswax intensified their colour impression. Finally, it was the symbol of resurrection and recreation and, together with bees and honey, the supporting force during genesis. Wax and honey were, as the bees themselves, tears of the god [1, 7-9].

Calorimetry

Life is intimately coupled with metabolism and metabolism with heat production. The energy consuming processes of anabolism are negligibly small compared with those of the energy liberating steps of catabolism, so that in all cases a net production of heat results. Daily life usually proceeds under short-term isothermal and isobaric conditions without any volume changes. Thus, the calorimetrically determined positive heat production corresponds to the negative enthalpy change of catabolism. As calorimetry is a non-specific, non-invasive method, it is specially suited to monitor metabolism of living systems in all their existing forms [10].

Thermoregulation and metabolism of socially living insects and especially of honeybees have long since attracted the interest of scientists (see, for example, refs. 11–16), as life in a eusocial community shortens the developmental period of the offspring and guarantees a better adaptation to changing environments. A reasonable temperature constancy in the brood centre is due to metabolic heat or intended shivering of the bee. Moreover, heat production helps the adult members of the hive to survive in cold seasons [12, 16, 17].

Only a few calorimetric experiments are found in the literature [8, 18–23] and most published data on heat are transformed from "indirect" calorimetry, i.e. oxygen consumption and carbon dioxide production, sugar consumption or temperature differences between thorax of the bee and the environment. A compilation of the earlier indirect results is presented in some recent papers [23, 24] and compared with true calorimetric data by assuming a respiratory quotient (RQ) of 1.00 [16, 24–26], a heat production of 21.1 J per ml oxygen and of 15.8 J per mg sugar.

In the present paper, isoperibolic (micro)calorimetry is applied to different questions connected with honeybee colonies (*Apis mellifera carnica* and in few cases *Apis mellifera lamarckii*). The first concerns the heat production rate of worker bees during their "occupational" development from newly emerged bees (1 day) over cell-cleaning (3 days), nursing (6 days), comb-shaping (12 days) to the adult foraging bees (21 days and older). Their mass of 120 ± 15 mg stays nearly constant from emerging until their death after 40 days in summer (see Table 1). Bee life proceeds in a dense community so that isolation of a bee from the stock for calorimetric experiments without the usual social contacts represents a biological artifact. This stress situation produces a noticeable unrest, locomotor activity and a significantly increased metabolic turnover in the animals. The second question, therefore, deals with group effects, social contacts and calming down of individuals by the presence of the queen or a piece of comb with brood.

Age/days	Mass/mg w.w.	$p/\mathrm{mW}\mathrm{g}^{-1}$		
		20°C	25°C	30°C
1	110	7 ± 2	56 ± 24	31 ± 6
3	113	113 ± 21	147 ± 18	59 ± 20
6	125	109 ± 16	137 ± 28	80 ± 17
12	125	157 ± 31	203 ± 23	101 ± 15
21	98	280 ± 38	209 ± 21	175 ± 17

TABLE 1

Mass specific mean heat production rates p as function of ambient temperature and age of worker bees; the mean mass (wet weight) of the different bee categories is given additionally

MATERIAL AND METHODS

Bee keeping

Honeybees (*Apis mellifera carnica*) were kept during the summer in a hive located in a laboratory near the calorimeter so that they could be transferred to the instrument without long journeys. Experiments lasted between 2 and 4 h. The age of the insects was exactly known when entering the investigation. The worker bees were caught at the hive entrance or inside the hive. Queens, drones and brood were taken directly from the hive and replaced there to keep the investigation period considerably shorter than with the worker bees. The calorimetric vessels were equipped with a wooden stick for climbing and a small container filled with bee food. All animals were weighed before and after the experiment to the nearest 0.1 mg (mechanical balance type 414; Sauter, Ebingen). Bee masses are given as wet weights because all animals were transferred back to the hive alive and used again later.

Honey, wax, propolis and pollen

The various substances connected with bee-keeping were obtained from the bee-keepers of the Zoological Institute of the Free University, Berlin. Several sorts of honey (lime-tree, rape, stone-pine, heather, acacia and forest as well as aphid honey), beeswax of different origins and propolis were compared as regards their DSC spectra and combustion behaviour. Jelly (or "royal jelly"), the special diet for the brood at the beginning of development and for the queen throughout her life, was directly taken from special queen cells, while pollen was purchased commercially.

Calorimetry

The microcalorimeter (Calvet type; MS 70; SETARAM, Lyon) was equipped with 4 vessels of 100 ml each and had a sensitivity of approximately 53 μ V mW⁻¹ and a time constant of 8 min. The recorder (BD5 + BA5; Kipp & Zonen, Delft) was set to 5 mV full deflection at a paper speed of 12 cm h⁻¹. Due to varying locomotor activities of the bees, the power-time (*P*-*t*) curves of the calorimeter showed strong temporal fluctuations (see, for example, Fig. 6(b)); they were evaluated for the maximum, the minimum and the mean heat production rates.

Figure 5 shows a schematic drawing of the whole instrumental setup as used in the experiments. One of the vessel supports carried an endoscope (rigid Boroskop; Storz, Tuttlingen; length 570 mm; outer diameter 6.5 mm; aperture 67°) to observe the insects during the experiments in the visible and the infrared range [27]. Infrared (IR) is more appropriate for monitoring than visible light as bees are insensitive to the wavelength range



Fig. 5. Schematic drawing of the calorimetric instrumentation. c, Calvet calorimeter (MS 70) with v, vessel of 100 ml and h, heat flow meter; r, recorder; s, container for hoarded food; w, wooden stick for climbing; e, endoscope with f, light filter; l, light source; m, monitor and n, night viewing system; mi, microphone with a, amplifier and t, tape (video) recorder [23].

780–960 nm. It was applied by means of IR filters and an IR sensitive night viewing system (NFP18; AEG, Hamburg). Good observation was possible at a constant, additional optical heat input which amounted to approximately 5% of the heat production rate of the bees. Moreover, the locomotor activities of the bees were registered acoustically by a microphone (20–18 000 Hz) also incorporated in the vessel, taped (PH55, Fisher, Japan) and analysed by means of a sound spectrograph (Nicolet UA 500A) connected to a film camera (Recordine, Tönnies).

The mean heat production rate was determined by electronical integration of the P-t curves over several hours (Digikon; Kontron, Munich) to eliminate individual periods of activity and to obtain the mean metabolism. Signals are "smeared" and maximum heat flows flattened due to the large time constant of the calorimeter. A true picture of the real heat production rates is gained by means of a fast Fourier transform or the Tian-equation [28]. Maximum rates were taken from such "time-resolved" graphs.

A differential scanning calorimeter (type 910, series 99, Du Pont Alzenau, Bad Homburg) with a sensitivity of $234 \,\mu W \,mV^{-1}$ and sample

sizes around 10 mg was applied for measuring bee products. Samples were placed in open or loosely covered pans and pollen used as whole grains or ground to powder in order to increase homogeneity.

Combustion heats were determined with a modified Phillipson bomb calorimeter [29] for sample sizes between 10 and 30 mg, oxygen pressures of 2.5 MPa and with a sensitivity of approximately 86 J mV^{-1} . It was calibrated by burning benzoic acid (26.436 J g^{-1} ; Riedel-deHaen AG, Seelze). The ignition of a sample was performed by discharging a $5000 \,\mu\text{F}$ capacitor via a nickeline fuse wire of 0.1 mm diameter. Samples were pressed to pills of the desired mass or enclosed in gelatine capsules (with a combustion heat of $20.0 \,\text{kJ g}^{-1}$).

RESULTS AND DISCUSSION

Energy metabolism of bees

We determined calorimetrically the heat production rates of workers, drones and queens of the usual European honeybee *Apis mellifera carnica* and, because of this special event, those of the Egyptian honeybee *Apis mellifera lamarckii*. Typical P-t curves with more or less pronounced temporal structures were obtained from which mass specific mean, maximum and minimum heat production rates were calculated. Figure 6 shows two different slopes of heat dissipation for two Egyptian worker bees at 30°C: Fig. 6(a) at a relatively constant high level rendering a total metabolic rate of 12 mW and a mass specific one of 145 mW g⁻¹, whereas Fig. 6(b) presents a bee with periods of strong locomotor activity separated by phases of rest where the metabolism drops to less than 0.5 mW or 7 mW g⁻¹. Such locomotor activities were sometimes further investigated by means of an endoscope or an integrated microphone (see Fig. 5).

Metabolism changes drastically with bee-age, although the mass stays nearly constant, and with ambient temperature (Table 1). Figure 7 shows the mass specific mean heat production rate of isolated worker bees at 25°C. Newly emerged bees exhibit calorimetric curves without significant fluctuations, indicating that locomotor activities are rare or absent; their mean metabolism is close to the resting one which is difficult to determine in older bees. The next category of cell cleaning bees (3 days) show a threefold increase in the mean (Fig. 7) and a maximum factor of $16 \times$ in comparison with the youngest bees. Short term locomotor activities are observed endoscopically and acoustically in this group and the next group, the nursing bees (6 days). The P-t curves become more structured as presented in Fig. 6(b). The last two groups, the comb-shaping bees (12 days) and the foragers (21 days and older), have approximately the same metabolic rate. Frequent flapping of the wings, shivering of the flight muscles or quick movements in the calorimetric vessel lead to pronounced



Fig. 6. (a) Heat production rate of an isolated Egyptian worker bee (*Apis mellifera lamarckii*) of 83.9 mg w.w. at 30°C. The strong decrease in the beginning is presumably due to evaporative processes after defaecation. (b) Heat production rate of an isolated Egyptian worker bee (*Apis mellifera lamarckii*) of 68.1 mg w.w. at 30°C. Periods of rest and of high locomotor activity alternate in this power-time curve.



Fig. 7. Mass specific heat production rate of worker bees (*Apis mellifera carnica*) at 25°C as function of their age.

structures in the heat signals. The influence of the ambient temperature on the mean heat production rate is age dependent: at 25°C highest in the first two categories, 20°C renders maximum values for foragers with a significant drop to 25 and 30°C.

As pointed out above, isolation is a strong artifact for the socially living bees. Adult individuals are usually restless in the calorimetric vessel showing all kinds of locomotor activity. In the extreme case, this behaviour renders nearly constant P-t curves on a high heat output level. It is significantly decreased when three or even more bees are investigated simultaneously. Table 2 demonstrates the strong influence of such group

Number in group	P/mW	<i>p</i> /mW g ⁻¹	Q(p)/%	
1	35.0	280	100	
3	37.5	100	36	
6	25.5	34	12	
12	27.0	18	6	
18	31.5	14	5	
6	25.5	34	100	
6 + queen	9.8	13	38	
6 + brood	16.5	22	65	

Influence of the group size on the mean heat production rate of worker bees at 20°C

TABLE 2

Key: P, mean total heat production rate; p, mass specific mean heat production rate; Q, reduction rate.

effects at 20°C, when the mass specific heat production rates is reduced to 5% for 18 bees compared with one bee. The effects are equally pronounced for 25 and 30°C and were also observed by other authors [12, 19, 20]. Locomotor activities decrease with increasing group size as can be seen in P-t curves, so that, for example, six bees produce only two thirds of the heat of one isolated bee (Table 2). Similar reductions are seen in a group of six worker bees without and with a queen or with uncapped brood. Again, social tasks diminish unrest and locomotor activity (Table 2).

As a further bee caste, juvenile (7 days, 236 mg) and fertile drones (older than 14 days, 205 mg) were included in these investigations. Highest metabolic rates were observed at 25°C with a threefold increase from the juvenile to the fertile insect. A dramatic increase by a factor of $20 \times$ was seen at 20°C. *P*-*t* curves of adult drones exhibit a very pronounced fluctuation, alternate periods of extremely low and of very high activity, so that the ratio between maximum and minimum values amounts to 35 in the mean. Thus, drones behave differently from adult worker bees in their locomotor activities, but they are not as "lazy" as assumed. The mass specific heat production rate of fertile drones at 25°C (184 mW g⁻¹) is equal to that of comb-shaping workers of the same age, although the bee mass is only half.

In contrast to the two other castes, young virgin queens (176 mg, 7 days) and fertile egg laying queens (258 mg, older than 21 days) exhibit a metabolic reduction with age from 117 to 102 mW g⁻¹ at 25°C and a more distinct one from 178 to 96 mW g⁻¹ at 30°C. This results from the development from an insect with an active contribution to body temperature regulation to one which is passively warmed by other members of the society. Young queens show periods of "quasi flight" and walking so that their P-t curves are more structured than those of fertile queens, but in no case can they be compared with those of adult drones.

Brood exhibits very smooth, flat P-t curves on a very low level. With 2.1 mW g⁻¹ at 20°C the metabolic rate of larvae and pupae is only 30% of that of newly emerged workers, and with 4.4 mW g⁻¹ or 7% at 25°C and 5.0 mW g⁻¹ or 16% at 30°C the difference is even larger. Thus, their contribution to thermoregulation of the hive is small or even negligible.

Honey

Honey is one of the most complex mixtures of carbohydrates of natural origin [30]. Around 90% of these carbohydrates are glucose and fructose, and besides them more than 20 di-, tri- and oligosaccharides are responsible for the finger print of honey [30]. Paper chromatography and thin-layer, gas-liquid and high-performance liquid chromatography may be applied to prove the authenticity of honeys of varying origins [30, 31].



Fig. 8. DSC thermogram of 9.0 mg of crystalline rape honey: scan rate, 3°C min⁻¹.

Enzymes, amino and organic acids, aromatic substances, minerals, vitamins and lipids complete the composition [31].

Although a small degree of finger printing of honey is also possible with thermal analysis techniques, they cannot compete with the richness of detail delivered by HPLC. DSC observes some predominant endothermic peaks at lower temperatures. In crystalline samples a melting peak appears between 60 and 70°C (Fig. 8) which vanishes when the honey is first transformed to the liquid state and then investigated (Fig. 9). This peak is weakly indicated in viscous samples. A second endothermic peak is



Fig. 9. DSC thermogram of 4.2 mg of crystalline lime-tree honey fused before investigation: scan rate, 20° C min⁻¹.



Fig. 10. DSC thermograms of three samples of liquid forest honey (a) 7.6 mg, (b) 6.2 mg and (c) 14.6 mg (reduced scale), without correcting the base line drift. Scan rate, 3° C min⁻¹.

apparent at around 135°C, near to the 123°C fructose peak given by Giron [32]. Our own experiments with fructose rendered two endothermic peaks at around 123 and 175°C, while glucose monohydrate was detected at 80, 155 and 180°C. Giron [32] presents 85°C for the monohydrate and 175°C for D-glucose; the corresponding values of Raemy and Schweizer are shifted to higher temperatures [33]. A further typical endothermic peak is seen at around 175°C in many samples, but it is never as sharp and pronounced as in the sugar experiments.

Depending upon the type of honey, several other broad endothermic transitions can be noted at around 200 to 210°C and 230 to 250°C. The latter coincides with that for α -D-glucose [33]. Summing up, one can state that clear differences are seen between crystalline, viscous and liquid honeys, that the reproducibility is acceptable (Fig. 10) although inhomogeneity of the honey may alter the appearance due to the small sample size, but that real finger printing of honey is not possible with DSC.

Royal jelly

Young workerbees ("nurses") produce a special, protein rich secretion in their hypopharyngeal glands which is fed to the queen and to all larvae. This (royal) jelly contains approximately 18% protein, 5.5% fat, 10-15%sugar, and mineral salts as well as vitamins and acetylcholine in high concentrations. Only this special diet enables the queen to produce an egg quantity per day which is double the weight of her own body. New results



Fig. 11. Thermogram of 15.1 mg w.w. jelly in open pans: scan rate, 20°C min⁻¹.

[34] have shown that nurses pass this secretion also to (the older) foragers because pollen as a source of protein is hard to digest for other bees. Because of its valuable constituents, jelly is a wanted substance for creams and other cosmetics as well as in geriatrics.

Figure 11 shows the thermogram for jelly with three distinct endothermic peaks at 129, 205 and 279°C which can be ascribed to the different constituents of the specimen. The total enthalpy change amounts to 1.17 kJ g^{-1} which is significantly higher than the endothermic fusion enthalpies for pure sugars cited in the literature [32].

Pollen

Honey provides the bees with carbohydrates; pollen serves as source of protein, fat and vitamins. The raw protein content varies between 7% and 35% with a mean of 20%. Pollen is collected together with nectar by chance or intentionally by specialized bees. It is stored in so-called "beebread" cells and changes its consistency due to enzymatic digestion during storage in the cells. This beebread is mainly fed to larvae and young bees while the consumption of foragers becomes very low. An adult bee eats 3.4 mg pollen per day [35] while it takes approximately 3.6 mg nitrogen or 112.5 mg pollen to raise a bee [36]. Pollen consumption is especially high in nursing bees (9 days of age) which use the pollen for the production of jelly [35]. Bomb calorimetry renders an energy content of 17.4 ± 0.9 kJ g⁻¹ of ground and mixed pollen and 18.5 ± 1.2 kJ g⁻¹ for complete pollen grains of one sort.

As Mitchell and co-workers [37, 38] have already pointed out, DTA curves of pollen show several distinct exothermic peaks between 200 and 600°C with considerable differences for pollen from various species. Peaks



Fig. 12. DSC curves for two pollen grains of commercial origin with samples of (a) 9.4 mg and (b) 5.6 mg: scan rate, 3° C min⁻¹.

appeared around 300, 430 and 530°C. Figure 12 exhibits the DSC slopes of two grains of a mixed commercial pollen of unknown species used as beebread in apiculture. Both grains, different in size and colour and presumably in origin, show two distinct peaks at low (about 300°C) and high temperatures (about 500°C) while the middle peak (at about 440°C) of curve (b) is hidden in the shoulder. The exothermic peak around 500°C is most pronounced with an energy content of approximately 4 kJ g⁻¹.

Hoarded food

When honey is harvested from the combs, bees have to be fed artificially to survive in unfavourable times in summer and especially in winter. Commercial hoarded food changes from distributor to distributor but is mainly a mixture of sugar, honey and pollen. Its energy content was determined by combustion calorimetry to be 15.4 ± 0.6 kJ g⁻¹ w.w. Figure 13 shows two DSC curves of hoarded food in the lower temperature range. Two endothermic peaks around 160 and 200°C are predominant in the slopes which may be traced back to fusion processes of the mono- and disaccharides contained in the hoarded food [33].

Beeswax

Initially, beeswax is of white appearance (virgin wax) and takes on the typical wax colour of honey or pollen only later. Its main components are myricin, an ester between palmitic acid and myricin alcohol, free cerin acid, melissic acid, some higher alcohols and carbohydrates. Beeswax shows one



Fig. 13. DSC curves of hoarded food of (a) 8.6 mg and (b) 5.8 mg w.w. in open pans: scan rate, $3^{\circ}C \min^{-1}$

or more endothermic melting transitions between 50 and 70°C which change with the origin of the wax. Because of the pharmaceutical interest in wax and the application as dental impression compounds, various thermal analytical methods have been used in wax analysis [39, 40]. Often, these techniques enabled quality controls in the sense of "finger printing" (e.g. search for adulteration of waxes) to be carried out rather than quantitative evaluations. In general, the melting behaviour of waxes is similar to that of animal and vegetable fats.

It is not the first time with this paper that the Ancient Empire is connected with modern calorimetry. Wiedemann and Bayer [41] investigating the bust of Nefertiti, applied DTA and TG to the inorganic material forming the Egyptian Blue as well as to the black coloured beeswax used as binder for eyelash on the bust of Nefertiti. They observed two endothermic peaks with a maximum at around 65°C and peak onset temperatures at around 49 and 53°C.

Figure 14 presents thermograms of seven waxes of different origin. Their main peaks centre around 65°C with some additional endothermic structures at lower temperatures. Minor details in the thermogram structure are lost in this figure, but "finger printing" capacity of DSC for waxes and reproducibility of the traces is high as is shown in Fig. 15 for three specimens of the same wax. Three endothermic peaks appear around 45, 52 and 64°C with a total melting enthalpy of 196 J g⁻¹. This value is equal to the 197 J g⁻¹ for carnauba wax reported in the literature [39]. In general, the heats of melting vary between 148 and 203 J g⁻¹, in good agreement with data from the literature [39]. Combustion calorimetry of these waxes render enthalpies between 42.4 and 44.8 kJ g⁻¹.



Fig. 14. Thermograms of seven wax specimens of different origin: scan rate, $2^{\circ}C \min^{-1}$. (a) 6.9 mg purified white wax; (b) 7.5 mg wax from uncapping; (c) 8.5 mg light comb wax; (d) 7.2 mg of queen cell wax; (e) 8.9 mg of comb wax of the Egyptian honeybee; (f) 10.9 mg wax coloured by forest honey; (g) 8.4 mg commercial, bleached wax.



Fig. 15. "Finger printing" in three specimens ((a) 7.7, (b) 8.5, (c) 9.4 mg) of yellow comb wax in open pans: scan rate, $2^{\circ}C \min^{-1}$.

Propolis

Propolis (its older name was "bee-glue") is a special bee product approximately composed of 55% resin, 30% wax, 10% volatile oils and 5% pollen. The resin in propolis is collected by specially chosen bees at the bud squamae of various trees, among them birches, robinias, willows and firs. Propolis is used by bees to flatten surfaces, to paste over smaller clefts, to embalm dead intruders and to protect the entrance of their hive. Its strong antimicrobial and germicidal actions have made it an interesting medicine since the time of the Pharaohs.

Figure 16 shows a thermogram of propolis in the range from room temperature to 100°C with a maximum endothermic peak around 61°C and a smaller one at 51°C. These peaks are around melting temperatures of waxes as well as of resins so that discrimination between the two components is not possible. The melting enthalpy amounts to 43 J g⁻¹ in the range of curing enthalpies of resins, while the combustion heat of propolis is 33.6 ± 0.9 kJ g⁻¹ (SD).



Fig. 16. Thermograms of (a) 10.7 mg and (b) 9.4 mg propolis run in open pans under air with a heating rate of 2° C min⁻¹. The two endothermic peaks appear at around 51 and 61°C.

Some energetic considerations concerning bees

Beekeepers have an old rule of thumb that a bee has to fly six times around the earth to gather one kilogram of honey. With a mean flight speed of 30 km h⁻¹ these 6 times 40 000 km consume 8000 h (or 29 × 10⁶ s) of flight. Wind-tunnel measurements on energy consumption during flight render figures of 500 mW g⁻¹ or 50 mW per bee [26]. Six times around the world corresponds to 50 mW × 29 × 10⁶ s = 1450 kJ of fuel for flying. However the energy content of 1 kg of honey with approximately 80% sugar amounts to $16\,000$ kJ so that the efficiency is around 9%, a highly economic result.

The same calculation may be performed in a more direct way. During one foraging flight of one hour a bee visits up to 100 blossoms and collects between 50 and 100 mg nectar, its own body weight! Nectar has a sugar content of between 20% and 40% so that in the worst case only 10 mg sugar (200 J) are brought back to the hive expending 100 J or 50% as fuel. In the best case four times more sugar is harvested and the efficiency rises to 12.5%. Calculations of Southwick and Pimentel [42] are even more optimistic: they come to a ratio of return of 29:1 for honey and of 20:1 for proteins collected as pollen. Looking for the commercial production of honey for human consumption and taking into account all forms of energy for equipment, packaging, supplies and direct fossil fuel (but except human labour) 0.89 kJ input render 1 kJ of honey [43]. Cane sugar by comparison requires 1.7 kJ input and beet sugar 2.1 kJ for each kilojoule produced. Thus, bees are 2 to 2.5 times more efficient in their production than man with cane and beet [43].

We have known since the fundamental discoveries of von Frisch that returning forager bees inform their hive mates about direction, distance, abundance, smell and nectar taste of a special forage. Distance tells foragers at the same time how much honey they have to imbibe to fly to that specific source. Also here, some energetical considerations are possible: if the distance to the source is less than 100 m homecoming bees perform a round dance, if larger a waggling dance. When bees are trained to walk instead of fly, this transition from round to waggling dance happens at approximately 4 m source distance. Flying 100 m at a speed of 30 km h⁻¹ and a power of 500 mW g⁻¹ requires about 0.7 J, the same amount of energy as walking 4 m at a speed of 0.3 km h⁻¹ and 120 mW g⁻¹ for walking.



The Old Egyptian form of salutation: "in life welfare and health".

ACKNOWLEDGEMENT

Several colleagues helped to prepare this manuscript. I am indebted to the beekeepers B. Pollaczek and U. Wolf, to E. Schmolz for calorimetric experiments with the Egyptian honey bee, to Dr. Karin Drong and P. Holzner for support with the figures, to Professor B. Schricker for helpful discussions and mostly to Mrs. G. Welge for continuous support in all investigations reported here. Dr. R. Kraus of the Egyptian Museum in Berlin-Charlottenburg gave important hints regarding the archeological aspects.

REFERENCES

- 1 Botinnen der Götter-Natur- und Kulturgeschichte der Honigbiene, Rheinland-Verlag, Köln, 1988, p. 103.
- 2 R.J. Israel, The promised land of milk and date jam, Nat. Jewish Mon., 87 (1972) 26.
- 3 W. Helck and E. Otto (Eds.), Lexikon der Ägyptologie, O. Harrassowitz, Wiesbaden, 1975 ff.
- 4 S.E.E. Efem, K.T.Udoh and C.I. Iwara, The antimicrobial spectrum of honey and its clinical significance, Infection, 20(4) (1992) 227-229.
- 5 G. Ndayisaba, L. Bazira and E. Habonimana, Treatment of wounds with honey—40 cases, Presse Med., 21(32) (1922) 1516–1518.
- 6 L. Suguna, G. Chandrakasan and K.T. Joseph, Influence of honey on collagen metabolism during wound healing in rats, J. Clin. Biochem. Nutr., 13(1) (1992) 7-12.
- 7 W. Rüdiger, Ihr Name ist Apis Kulturgeschichte der Biene, Ehrenwirth, Munich, 1977, p. 117.
- 8 R. Chauvin (Ed.), Traité de Biologie de l'Abeille, Vol. 5, Masson et Cie, Paris, 1968, p. 152.
- 9 J. Leclant, L'abeille et le miel dans l'Egypte pharaonique, in R. Chauvin (Ed.), Traité de Biologie de l'Abeille, Vol. 5, Masson et Cie, Paris, 1968, pp. 51-60.
- 10 I. Lamprecht, W. Hemminger and G. Höhne, Special Issue: Calorimetry in the Biological Sciences, Thermochim. Acta, 193 (1991) 452.
- 11 L. Armbruster, Über den Wärmehaushalt im Bienenvolk. Arch. Bienenkd., 4 (1922) 268-270.
- 12 J.B. Free and Y. Spencer-Booth, Observations on the temperature regulation and food consumption of honeybees (*Apis mellifera*), J. Exp. Biol., 35 (1958) 930–937.
- 13 H. Esch, Über die Körpertemperaturen und den Wärmehaushalt von Apis mellifica, Z. Vgl. Physiol., 43 (1960) 305–335.
- 14 B. Heinrich, The regulation of temperature in the honeybee swarm, Sci. Am., 244 (1981) 147–160.
- 15 E.E. Southwick, Metabolic energy of intact honey bee colonies, Comp. Biochem. Physiol. A, 71 (1982) 277-281.
- 16 E.E. Southwick, Allometric relations, metabolism and heat conductance in clusters of honey bees at cool temperatures, J. Comp. Physiol. B, 156 (1985) 143-149.
- 17 K.A. Nagy and J.N.Stallone, Temperature maintenance and CO₂ concentration in a swarm cluster of honey bees, *Apis mellifera*, Comp. Biochem. Physiol. A, 55 (1976) 169-171.
- 18 A. Heusner and M. Roth, Consommation d'oxygène de l'abeille à différentes températures, C.R. Acad. Sci., 256 (1963) 284-285.
- 19 M. Roth, Adaptation de la thermogénèse à la température ambiante et effet d'économie thermique du group chex l'abeille (*Apis mellifica* L.), C.R. Acad. Sci., 258 (1964) 5534-5537.
- 20 M. Roth, La production de chaleur chez Apis mellifica L., Ann. Abeille, 8 (1965) 5-77.
- 21 L. Fahrenholz, I. Lamprecht and B. Schricker, Thermal investigations of a honey bee colony: thermoregulation of the hive during summer and winter and heat production of members of different bee castes, J. Comp. Physiol. B, 159 (1989) 551-560.
- 22 L. Fahrenholz, I. Lamprecht and B. Schricker, Microcalorimetric investigations of the energy metabolism of honey bee workers, *Apis mellifera carnica*, Thermochim. Acta, 151 (1989) 13-21.

23 L. Fahrenholz, I. Lamprecht and B. Schricker, Calorimetric investigations of the different castes of honey bees, *Apis mellifera carnica*, J. Comp. Physiol. B, 162 (1992) 119–130.

- 24 U. Rothe and W. Nachtigall, Flight of the honey bee. IV. Respiratory quotients and metabolic rates during sitting, walking and flying, J. Comp. Physiol. B, 158 (1989) 739-749.
- 25 W. Ritter, Experimenteller Beitrag zur Thermoregulation des Bienenvolkes (Apis mellifera L.). Apidologie, 13 (1982) 169–195.
- 26 W. Nachtigall, U. Rothe, P. Feller and R. Jungmann, Flight of the honey bee. III. Flight metabolic power calculated from gas analysis, thermoregulation and fuel consumption, J. Comp. Physiol. B, 158 (1989) 729-737.
- 27 I. Lamprecht and W. Becker, Combination of calorimetry and endoscopy for monitoring locomotor activities of small animals, Thermochim. Acta, 130 (1988) 87-93.
- 28 W. Hemminger and G. Höhne, Calorimetry Fundamentals and Practice, Verlag Chemie, Weinheim, 1984.
- 29 J. Phillipson, A miniature bomb calorimeter for small biological samples, Oikos 15 (1964) 130-139.
- 30 K.W. Swallow and N.H. Low, Analysis and quantitation of the carbohydrates in honey using high-performance liquid chromatography, J. Agric. Food Chem., 38(9) (1990) 1828–1832.
- 31 J.J.M. Lipp, Zur Zusammensetzung des Honigs und Verfahren zum Nachweis von Honigverfälschungen, Thesis, Munich, 1989.
- 32 D. Giron, Thermal analysis in pharmaceutical routine analysis, Acta Pharm. Jugosl., 40 (1990) 95-157.
- 33 A. Raemy and T.F. Schweizer, Thermal behaviour of carbohydrates studied by heat flow calorimetry, J. Therm. Anal., 28 (1983) 95–108.
- 34 K. Crailsheim, Interadult feeding of jelly in honeybee (*Apis mellifera* L.) colonies, J. Comp. Physiol. B, 161 (1991) 55-60.
- 35 K. Crailsheim, L.H.W. Schneider, N. Hrassnigg, G. Bühlmann, U. Brosch, R. Gmeinbauer and B. Schöffmann, Pollen consumption and utilization in worker honeybees (*Apis mellifera carnica*): Dependence on individual age and function, J. Insect Physiol., 38(6) (1992) 409-419.
- 36 J.O. Schmidt and S.L. Buchmann, Pollen digestion and nitrogen utilization by Apis mellifera L. (Hymenoptera: Apidae), Comp. Biochem. Physiol. A, 82 (1985) 499-503.
- 37 B.D. Mitchell and A.H. Knight, The application of differential thermal analysis to plant materials, J. Exp. Bot., 16 (1965) 1–15.
- 38 B.D. Mitchell and A.C. Birnie, Biological materials, in R.C. Mackenzie (Ed.), Differential Thermal Analysis, Vol. 1, Academic Press, London, 1970, Chapt. 24, pp. 673-704.
- 39 H.J. Ferrari and M. Inoue, Pharmaceuticals, in R.C. Mackenzie (Ed.), Differential Thermal Analysis, Vol. 2, Academic Press, London, 1972, Chapt. 42, pp. 453–472.
- 40 J.L. Ford and P. Timmins, Pharmaceutical Thermal Analysis, Ellis Horwood, Chichester, 1989.
- 41 H.G. Wiedemann and G. Bayer, The bust of Nefertiti, Anal. Chem., 54 (1982) 619A.
- 42 E.E. Southwick and D. Pimentel, Energy efficiency of honey production by bees, BioScience, 31(10) (1981) 730-732.
- 43 E.E. Southwick, Energy efficiency in commercial honey production, Am. Bee J., 120 (1980) 633-635.
- 44 K. Lang and M. Hirmer, Ägypten-Architektur, Plastik, Malerei in drei Jahrtausenden, Hirmer-Verlag, Munich, 1978.