Atti Soc. Tosc. Sci. Nat., Mem., Serie B, **94** (1987)
pagg. 43-52

G.G. FRANCHI (*)

RESEARCHES ON POLLEN DIGESTIBILITY (**)

Riassunto — *Ricerche sulla digeribilità del polline.* Sono stati compiuti studi sulla digeribilità del polline, che da alcuni anni viene raccomandato come integratore alimentare per uso umano, in quanto in letteratura compaiono soltanto ricerche sulla digestione del polline negli insetti che se ne cibano, soprattutto nelle api.

Gli studi del gruppo cui l'Autore appartiene sono ancora in corso; per il momento sono state effettuate prove *in vitro* ed *in vivo* con polline di nocciolo (*Corylus avellana* L.). Le prime sono state eseguite con enzimi digestivi gastrici ed intestinali; le seconde somministrando il polline a topi, ricercato poi nelle feci. In entrambi i casi lo svuotamento è stato stimato con metodi microscopici.

È stato accertato, con le prove *in vitro*, che con gli enzimi gastrici (pepsina, papaina e diastasi a pH = 1,2) vengono digerite solamente le sostanze situate in corrispondenza dei pori, mentre il citoplasma rimane inalterato. Con gli enzimi intestinali (pancreatina e lipasi pancreatica a pH = 7,5 e pH = 8,5) si ottiene invece uno svuotamento, seppure parziale, dei granuli.

Uno svuotamento del tutto simile a quello provocato dagli enzimi intestinali è stato accertato anche mediante le prove *in vivo*.

Gli studi proseguono per stabilire la natura dei prodotti ceduti dai granuli, e verranno estesi anche ad altri tipi di polline, allo scopo di stabilire quali pollini, se ingeriti, possono presentare un effettivo interesse dietetico od alimentare.

Abstract — Studies on pollen digestibility have been carried on because for some years pollen has been recommended as dietetic or integrator food for human consumption, whilst in literature researches are reported only on pollen digestion by insects feeding on it, especially bees.

The studies of the research group to which the Author belongs, are still in progress; up to now tests have been effected both *in vitro* and *in vivo* with hazelnut (*Corylus avellana* L.) pollen. The former have been performed by means of both gastric and intestinal digestive enzymes; the latter, administering pollen to mice, in whose faeces it was then sought. In both cases pollen emptying has been estimated by microscopical methods.

(*) Dipartimento Farmaco Chimico Tecnologico, Sezione Botanica Farmaceutica, Università di Siena, Via P.A. Mattioli, 4, 53100 Siena - Italia.

(**) Research supported by a grant of Ministero della Pubblica Istruzione, Rome (40%).

During *in vitro* tests, it has been checked that gastric enzymes (pepsin, papain and diastase at pH = 1.2) digest only the substances located in correspondence to the pores, while cytoplasm remains unaffected. With intestinal enzymes (pancreatin and pancreatic lipase at pH = 7.5 and pH = 8.5) a certain grain emptying is obtained, although it is always a partial one.

A partial emptying has been checked also by means of the *in vivo* tests, strictly similar to the emptying due to the intestinal enzymes.

Researches will prosecute in order to ascertain the chemical nature of the products released by grains, and will be extended to other kinds of pollens, with the aim to ascertain which pollens, eaten by man, may have a real dietetic or nutritional interest.

Key words — *Corylus avellana* L., dietetic foods, pollen.

The story of the studies on pollen is characterized by many discoveries made by non-botanists. Among these scientists, Giovanni Battista Amici, a physicist mainly interested in optics, who improved the microscopical apparatuses available in the first half of the nineteenth century. These improvements allowed him to be the first to observe that pollen tube enters stigma and style (DUCKER & KNOX, 1985). Amici's life and work, and his wonderful wax-models are described elsewhere in this volume.

Even today pollen interests non-botanists and is studied by them, because of its effective significance in science and human life. The easiest example consists in the studies carried on by physicians (allergologists), being pollen one of main responsible for hay-fevers and related pathologies.

This lecture concerns another peculiar study on pollen, due to the cooperation of botanists and non-botanists: the study of pollen digestibility.

The idea to carry on researches on pollen digestibility arose from the observation that since the Sixties, and more and more in the most recent years, pollen has been frequently utilized in formulations for dietetic or cosmetic purposes, and has been sold alone, just as a dietetic or integrator food.

Most firms commercialize the pollen loads collected by bees (pollen pellets), packed in various ways. It is very easy to get them, by means of a suitable «pollen trap» to be applied to hive entrance. It consists in a close-mesh net, against which bees must rub to enter the hive. This way pollen loads detach from the back legs of bees and fall into a container under the net. On the other hand, other

firms contest the use of pollen as it is collected by bees, and sell pollen extracts, sometimes called with fancy names: hulled pollen, pollen heart, and so on. According to these firms, pollen needs to be pre-treated before human consumption, because of the endurance of its walls to digestion.

As for our knowledge, a specific study does not really exist up-to-date to ascertain whether pollen, eaten intact by man, is digested, or its walls intine and exine are an insuperable barrier to digestive enzymes. In effect exine, surely indigestible because of its chemical structure, shows apertures, the pores, in most kinds of pollen grains, or however has some cracks, which might let enzymes pass through. On the contrary intine is continuous, even if its thickness may widely vary; furthermore the constituent polysaccaride is very similar to cellulose and should be indigestible to man. Nevertheless intine fibrillar structure might allow limited amounts of molecules pass through. The problem is still open, and the use of pollen as food is sceptically viewed by many men of science. I would like to quote ROBERT G. STANLEY and HANS F. LINSKENS (1974) from their book *Pollen-Biology, biochemistry, management*: «Our opinion of... pollen additives in the human diet is that they are interesting, dry in taste, and something we and others consuming a balanced diet can perfectly well do without» (p. 44), and more over: «Pollen probably is truly beneficial, although the benefits, we suspect, can be equalled by many other less expensive, more readily available foods» (p. 115).

A large number of studies exists, but concerning two collateral aspects of pollen, in respect to the argument: the consumption of pollen as food, which is naturally made by some animals — often a reward for pollination — and the chemical analysis of pollen.

The natural consumption of pollen as food has been studied particularly in bees because of the economic importance of these honey-producing insects. It is remarkable that pollen is essential to honey-bees, being the only protein-containing food in their diet, and it has been well-known for centuries that bees need pollen: yet Virgil in *Georgica IV* recommended the establishment of pollen producing plants near the hive. Centuries-old is the observation too, that bees collect both nectar and pollen from flowers. More recent studies demonstrated that pollen is the only protein-containing, and also the main lipid-containing food in bee diet. When pollen is lacking the average lifetime is shorter, and some disadvantages may happen, for instance difficulties in producing wax, venom, or royal jelly. The

use of pollen substitutes, like flours or dried yeast, for bee nourishment, is generally unsatisfactory; pollen is so important that beekeepers, when applying pollen traps to hives, always leave a little space free from the net: thus some bees may pass through bringing the smallest necessary of pollen to the hive (STANLEY and LISKENS, 1974; RICCIARDELLI D'ALBORE and PERSANO ODDO, 1978).

Finally it is to be reminded that bees do really choose which pollen they collect, preferring some ones (generally the richest in nitrogen when analysed) and rejecting some others. It is remarkable that a certain amount of collected pollens belongs to anemophilous plants, especially in particular periods of the year, or during special environmental conditions. On the other hand, some entomophilous plants exist, whose pollens are rejected: among these some Ericaceae as certain species of *Rhododendron*, having poisonous pollen (STANLEY and LISKENS, 1974; RICCIARDELLI D'ALBORE and PERSANO ODDO, 1978).

Some papers (summarized by STANLEY and LISKENS, 1974) describe the various stages of pollen passage through bee digestive tract. Pollen is swallowed intact and cumulated in the honey stomach. After not more than twenty minutes it passes to ventriculus: till now grains are practically unaffected, only a little swallen in correspondence to the pores. This could remind what happens before germination, but nothing similar can take place, because the substances cementing pollen grains together in the pellets contain some inhibitors of germination. The real digestion occurs in the ventriculus, where proteolitic enzymes and lipases were found. Here pollen is rapidly emptied, with the formation inside grains of a structure which has been described as «a transparent vacuole». When reaching the hind intestine, pollen grains are emptied, and often collapsed. The residues of digestion, which is completed in about three hours, will be discharged in flight later on; exine and intine have not been digested, and remain unchanged.

Many other Hymenoptera, and some Lepidoptera, ingest intact pollen, as bees do.

Coleptera on the contrary, when eating pollen, chew it. Obviously a crushed pollen is not difficult to be digested.

The only other insects for which pollen is usual food, are Collembola. Among these, it is to remind for instance the glacier-flea, *Isotoma saltans* (Nicolet) [= *Desoria glacialis* Nicolet], which feeds only the pollen brought to glaciers by wind. Collembola swallow intact pollen, but digestion is not a trouble because they have enzymes

able to degrade both intine and — a singular case indeed in the Animal Kingdom — exine (CELLETTI *et al.*, 1960; GISIN, 1960; STANLEY and LINSKENS, 1974).

In other animals, may they be insects, or other pollinators such as bats or hummingbirds, pollen ingestion has always been considered incidental, during the consumption of nectar, the standard food. On this basis, the problem of pollen digestion in such animals has never been considered.

In the same way, there are no studies on man or domestic animals, although sometimes it has been proposed to add pollen to animal feeds: e.g., corn pollen might increase egg production in hens (SĂLĂJAN, 1972). It is also remarkable that pollen has been utilized — especially in the Sixties — in therapy, against many man diseases. Sometimes benefits were obtained (STANLEY and LINSKENS, 1974), but it is unknown if they are to be ascribed to pollen *in toto*, or to some of the various and numerous substances localized on its external surface.

As far as it concerns chemical analyses on pollen, they are very numerous, and have been repeated many times in these last fifty years, becoming analytical techniques more and more careful and exact. These studies demonstrate the effective presence of many interesting substances in pollen; in particular, pollen is really rich in proteins and free amino-acids (DE SIMONE *et al.*, 1980; MCCAUGHEY *et al.*, 1980; STANDIFER *et al.*, 1980), and contains also vitamins, fat acids, sterols, and many others (STANLEY and LISKENS, 1974; LOPER *et al.*, 1980; SOLBERG and REMEDIOS, 1980; CERRI *et al.*, 1980/81). But with the adopted analysis methods it has always been necessary one of these treatments: or pollen has been burnt to ash (for minerals determination), or it has been homogenized, that is mechanically crushed, or its content has been extracted with drastic methods, e.g. with organic solvents. In every case, it is never possible to identify which of the substances found in pollen are localized on grain surface (sporophytic recognition proteins, pollekitt, tryphine - HESLOP-HARRISON *et al.*, 1973; PACINI *et al.*, 1985), and which are contained inside. Furthermore, none of these extraction techniques may be in any case compared to a digestion, that is a process naturally occurring under very different chemical and physical conditions.

Therefore, the problems we had to front were various. First, we had to verify if digestive enzymes succeeded in attacking pollen grains and digesting fully or partly their content; on the other hand

we had to perform an objective method by which we could test a great number of kinds of pollen grains, with different walls structures, obtaining comparable results. These researches are still in progress, but the first results are quite interesting, and have been produced during the third meeting of the Italian Society of Pharmacognosy — Alghero, October 1985 (FRANCHI *et al.*, 1985).

OUR RESEARCH - EXPERIMENTAL

Obviously we could not work on pollen loads collected by bees, being mixtures of pollens structurally very different from one another, often showing many impurities (fungal spores, even germinated, various kinds of fragments of vegetable tissues, dusts), and therefore we have begun our tests utilizing pollen of hazelnut (*Corylus avellana* L.).

This pollen is not important for bees (RICCIARDELLI D'ALBORE and PERSANO ODDO, 1978), but shows numerous advantages. It may be obtained with a high purity (we extimated > 98% in particles number), it has a high viability (calculated > 95% with both morphological and enzymatic methods — HESLOP-HARRISON and HESLOP-HARRISON, 1970; FRANCHI *et al.*, 1983; 1984 — so as to be sure of the absence of empty grains at test beginning), it has three pores (which could theoretically facilitate enzymes attack and a quick emptying), it has very few substances on its external surface (at least at a microscopical examination) which could interfere with the analysis of what enzymes have digested from inner content.

We have contemporaneously effected both *in vitro* and *in vivo* tests.

In vitro tests have been firstly performed in test-tubes shaken at regular time intervals and mantained in thermostated bath at 38°C ± 1°C, and successively repeated wih the dissolution test apparatus described in Pharmacopoeia (U.S.P. XXI; F.U. IX).

In a first set of tests, pollen has been subjected to a prolonged permanence in a 0.01 N HCl solution or in a 0.1 N $NaHCO_3$ solution, with or without adding Dioctyl Sodium Sulfosuccinate as surface-active agent, and/or cellulase.

In a second set of tests, pollen has been treated with digestive enzymes: pepsin at pH = 1.2; papain and diastase at pH = 1.2; pan-

creatin and pancreatic lipase at pH = 7.5 and pH = 8.5. Also in this second set, tests were effected with or without surfactant.

At time intervals of 4-6-8 and 24 hours, pollen specimens were taken out, and then microscopically examined under a microscope with interference contrast, or after staining with lacto-phenol Cotton blue (DARLINGTON and LA COUR, 1960).

During *in vivo* tests we have utilized mice (NMRI albino mice), to which a water suspension of hazelnut pollen was administered by means of gastric intubation. This, in order to prevent that directly pollen-feeded mice could dirty their muzzles and forefeet, and afterwards the cage-bottom, altering our results. In fact, after a 24-hours fast (15 before and 9 after the experience), mice were allowed to feed freely, and pollen was sought in faeces, where it was present especially 10-15 hours after consumption. For this test faeces were disgregated in water, and examined under microscope after staining pollen with lacto-phenol Cotton blue. Obviously the test was preceeded by a blank, to be sure of the absence of pollen or similar figures (in form or stainability) in usual mice-feed.

RESULTS

In the first test set, without enzymes, pollen remained unaffected and, even after a 24-hours treatment, is completely similar to a control mantained in water.

In gastric enzymes tests (pH = 1.2), pollen poral area has been emptied (it may be more easily reached), whilst cytoplasm has remained apparently unaffected.

The treatment with intestinal enzymes (pancreatin and pancreatic lipase at pH = 7.5 or pH = 8.5) led instead to a partial emptying of grains.

The addition of surfactant speeded up the beginning of process, without modifying the apparent amount of emptying in 24 hours. A greater emptying (never a complete one) has been obtained prolonging the dissolution apparatus test up to 48 hours: anyway, so long a test has no biological significance.

As concerns *in vivo* tests, the pollen present in mice faeces was partly emptied, with a feature strictly similar to pollen after the *in vitro* treatment with pancreatin and pancreatic lipase.

Conclusions

First of all, it is remarkable that with both *in vitro* and *in vivo* tests we have obtained the same results (both kinds of tests were repeated many times). This fact ensures on the validity and repeatibility of the chosen methods, and will let us carry on the one or the other of the tests even alone, in the prosecution of our researches, according to each single research and to the disposable kinds of pollen (in regard to purity or to availability).

On the other hand, it is evident that, while substances located on the external surface of grains or even in the poral area, in exine cavities or intine tubules (Knox and Heslop-Harrison, 1970), may be easily reached by enzymes and are digested with all the tested methods, the most grain content, that is cytoplasm, is not disposable, remaining inside grains after the *in vivo* tests, or after a 24-hours treatment *in vitro*.

It is also remarkable — but it is difficult to give it a well-defined significance — that grain emptying seems to go on from the pore zone to grain interior, and no vacuole-like structures are formed, similar to those described during pollen digestion by bees (Stanley and Liskens, 1974).

In prosecution to these researches, we have two aims.

First, we intend to test with the same methods other kinds of grains, with different-structured exine and intine in respect to hazelnut, to verify if there are grains particularly easy or difficult to be digested. In fact, while bee-collected pollen pellets consist of mixed grains, if we could prove that specific kinds of grains are digested much more than others, a hand-collection or a mechanized collection could be proposed, to be made directly from plants, improving quality and perhaps reducing costs.

On the other hand, we are carrying on chemical studies on pollen extracts obtained by means of digestive enzymes. Until now, we have separated a certain number of spots by thin layer chromatography, and we are trying to identify the constituent substances.

When they are identified, not only shall we see whether they are or not of dietetic or pharmaceutical interest, but we shall also pass to the analysis of what remains inside grains, to quantify in some way emptying amount.

At last, we shall research those same substances (or even others,

if more suitable) operating on other pollens, as to develop a sort of classification of pollen digestibility and, if possible, of their actual utility as food.

REFERENCES

Celletti M., Civetta Beccaria C., De Gennis L., Formigoni L., Lombardi A., Marchetti R., Parisi V., Pugno A., Raggi A.M., Virgili E., Sforza B., Ghiglieri A., Bianchini M., Sturma M., Formigoni A. (1960) - Nel Mondo della Natura. Zoologia. Vol. 1, 188-192. Federico Motta Editore, Milano.

Cerri R., Cordella G., De Simone F., Senatore F. (1980-81) - Acidi grassi e steroli nel pollini di *Castanea sativa, Cedrus silani* e *Cucurbita pepo. Rend. Acad. Sci. Fis. Mat.* (Napoli) Ser. IV, **48**, 445-451.

Darlington C.D., La Cour L.F. (1960) - The handling of chromosomes, 3rd Ed. George Allen & Unwin Ltd., London.

De Simone F., Senatore F., Sica D., Zollo F. (1980) - Free amino acids from pollens. *Biochem. Syst. Ecol.*, **8**, 77-79.

Ducker S.C., Knox R.B. (1985) - Pollen and pollination: a historical review. *Taxon*, **34**, 401-419.

Franchi G.G., Franchi G., Giorni G. (1983) - Influenza sul polline dell'inquinamento atmosferico da piombo. *Boll. Chim. Farm*, **122**, 589-597.

Franchi G.G., Pacini E., Rottoli P. (1984) - Pollen grain viability in *Parietaria judaica* L. during the long blooming period and correlation with meteorological conditions and allergic diseases. *Giorn. Bot. Ital.*, **118**, 163-178.

Franchi G.G., Franchi G., Corti P. (1985) - Indagini preliminari sulla cessione del contenuto del polline nell'apparato digerente. Summaries of papers presented at «3° Congresso Nazionale della Società Italiana di Farmacognosia», p. 54. Università degli Studi, Sassari. The extensive form of the paper is in press in the proceedings volume of the same meeting.

F.U. IX - Farmacopea Ufficiale della Repubblica Italiana, IX Ed. (1985). Vol. 1, pp. 416-423. Istituto Poligrafico e Zecca dello Stato, Roma.

Gisin H. (1960) - Collembolenfauna Europas. Ed. Museum d'Histoire Naturelle, Genève.

Heslop-Harrison J., Heslop-Harrison Y. (1970) - Evaluation of pollen viability by enzymatically induced fluorescence; intracellular hydrolysis of fluorescein diacetate. *Stain. Technol.*, **45**, 115-120.

Heslop-Harrison J., Heslop-Harrison Y., Knox R.B., Howlett B. (1973) - Pollen-wall proteins: «gametophytic» and «sporophytic» fractions in the pollen walls of the Malvaceae. *Ann. Bot.* (London), **37**, 403-412.

Knox R.B., Heslop-Harrison J. (1970) - Pollen-wall proteins: localization and enzymic activity. *J. Cell Sci.*, **6**, 1-27.

Loper G.M., Standifer L.N., Thompson M.J., Gilliam M. (1980) - Biochemistry and microbiology of bee-collected almond (*Prunus dulcis*) pollen and bee bread. I. Fatty acids, sterols, vitamins and minerals. *Apidologie*, **11**, 63-73.

McCaughey W.F., Gilliam M., Standifer L.N. (1980) - Amino acids and protein adeguacy for honey bees of pollens from desert plants and other floral sources. *Apidologie*, **11**, 75-86.

Pacini E., Franchi G.G., Hesse M. (1985) - The tapetum: its form, function and possible phylogeny in Embryophyta. *Pl. Syst. Evol.*, **149**, 155-185.

Ricciardelli d'Albore G., Persano Oddo L. (1978) - Flora apistica italiana. Istituto Sperimentale per la Zoologia Agraria, Roma.

Sălăjan Gh. (1972) - Cercetari cu privire la eficienta utilizarii polenului de porumb ca supliment proteic si biostimulator in hrana tineretului porcin si aviar. Ph. D. Thesis, Inst. Agron. Ian Ionescu de la Brad, Iasi, Romania.

Solberg Y., Remedios G. (1980) - Chemical composition of pure and bee-collected pollen. *Meld. Norg. LandbrHøgsk.*, **59**, (18).

Standifer L.N., McCaughey W.F., Dixon S.E., Gilliam M., Loper G.M. (1980) - Biochemistry and microbiology of pollen collected by honey bees (*Apis mellifera* L.) from almond, *Prunus dulcis*. II. Protein, amino acids and enzymes. *Apidologie*, **11**, 163-171.

Stanley R.G., Linskens H.F. (1974) - Pollen. Biology, biochemistry, management. Springer Verlag, Berlin.

U.S.P. XXI - The United States Pharmacopeia, XXI Ed. (1985); pp. 1243-1244. United States Pharmacopeial Convention Inc., Rockville, MD.

(ms. pres. il 15 dicembre 1986; ult. bozze il 23 aprile 1987)