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On the Occurrence of Folic Acid, Folic Acid Conjugates and Folic Acid Conjugases in Pollen

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The contents of folic acid and folic acid conjugates of 10 different pollen samples were determined. The folic acid content ranged between 0.42 and 2.2 μg per g pollen. The amount of folic acid conjugates was in most cases considerably higher — 4 to 12 times as high. In two samples of pollen from grasses, *viz. Zea mays* and *Secale cereale*, the content of folic acid and of folic acid conjugates was of the same magnitude. Compared to other vegetable materials the pollen is relatively rich in folic acid and folic acid conjugates.

The presence of conjugases was established for pollen from *Pinus montana*, *Zea mays*, *Phleum pratense* and *Secale cereale*. For pollen from *Zea mays* the conjugase(s) was found to have its pH-optimum at 4.5 being active between pH 4 and 6. This conjugase must therefore be different from the conjugases in chicken pancreas. The effect obtained is stronger than that obtained with hog kidney and for this reason the conjugase in pollen can hardly be the same as in hog kidney. The content of conjugase in pollen is sufficient to convert all the conjugates in the pollen.

Continuing previous studies on the vitamin content of different pollen samples[1] we have studied the occurrence of folic acid. Very little is known regarding the presence of this vitamin in pollen. As folic acid is believed to be an important component of the so called vitamin T [2] which is reported to be an important growth factor for insects [3], investigations on the occurrence of this vitamin in pollen seemed of *a priori* interest.

In this investigation we wanted to determine both the amount of folic acid * and the amount of folic acid conjugates. As considerable amounts of folic acid conjugates were found (in most pollen samples the content of folic acid conjugates was much greater than the content of folic acid) we also studied the occurrence of folic acid conjugases in the pollen.

* In accordance with Welch and Nichol [4] we understand by folic acid the compounds able to promote the growth of *Streptococcus faecalis* and *Lactobacillus casei, viz.* pteroyl glutamic acid and pteroyl diglutamic acid.

Table 1. The occurrence of folic acid and folic acid conjugates in pollen.

Source of pollen	Folic acid µg/g pollen dry weight	Folic acid + folic acid conjugates	Folic acid in percentage of folic acid + conjugates
Pinus contorta	0.74	3.1	24
Pinus montana	0.42	2.5	17
Picea canadensis	1.4	12.8	11
Alnus glutinosa	0.53	4.3	12
Alnus incana	0.84	10.1	8
Betula alba	1.1	4.3	26
Fagus silvatica	1.9	7.3	26
Zea mays	2.2	4.5	49
Phleum pratense	1.1	5.6	20
Secale cereale	1.1	2.3	48

For the determination of folic acid, 5 g of pollen was suspended in 30 ml of water and heated to 120° for 5 min. Due to this heating the conjugases which might be present in the pollen are inactivated [5]. After heating, the suspension was centrifuged and the pollen residue washed twice with 20 ml of water. The combined extracts were made up to 100 ml for assay.

For the determination of the total content of folic acid plus folic acid conjugates the samples were treated with chicken pancreas conjugase according to the method described by Teply and Elvehjem [6]. The treatment with hog kidney or almond conjugases [7] was also tried, but the values obtained with chicken pancreas conjugase were found to be somewhat higher and this method was therefore preferred. For the assay, *Streptococcus faecalis* ATCC 8043 was used applying the paper-disc method.

Ten pollen samples were analyzed. The samples were collected directly from the plants. The purity of the samples was high, the amount of foreign pollen not exceeding 1 %. The pollen samples were collected in 1955 and stored in a cool and dry place until they were analyzed.

As can be seen from Table 1 the sum of folic acid and folic acid conjugates varied between 2.3 and 12.8 µg per g dry material. These values are relatively high in comparison with those of other vegetable materials [8]. No connection can be seen between the folic acid content and the systematic position of the plant from which the pollen was derived. The pollen samples from the closely related species *Alnus glutinosa* and *A. incana* show a marked deviation in their contents of folic acid and folic acid conjugates. Earlier it was found that the content of six other B-vitamins in pollen from these two species was rather similar [1].

The content of folic acid calculated as per cent of the sum of the contents of folic acid and folic acid conjugates varies considerably. For most of the pollen samples it varied between 10 and 25 %, *i.e.* the folic acid conjugates were present in considerably higher amounts than was folic acid. For two of the samples, however, the relative amounts of folic acid were very high. In the pollen from the two grasses *Zea mays* and *Secale cereale* the folic acid content was as high as that of the folic acid conjugates. In the third sample of grass-pollen investigated, *i.e.* pollen from *Phleum pratense*, the amount of folic acid

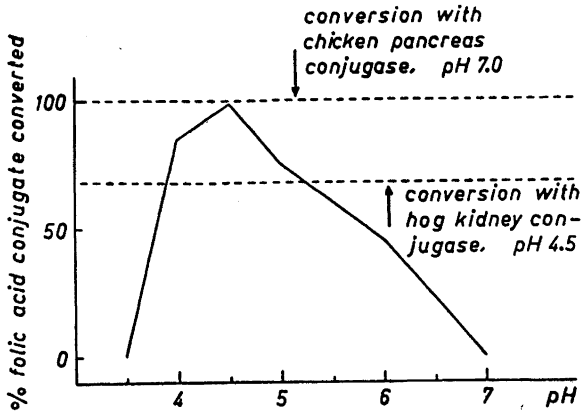

Fig. 1. Conversion of folic acid conjugates.

was low compared to the value for the folic acid conjugates. The number of pollen samples investigated was to small to justify any conclusion as to whether pollen from grasses have a tendency to be relatively rich in folic acid compared to folic acid conjugates.

Weygand and Hofmann [9] studied the content of folic acid in some pollen samples and found the amount to vary between 3.4 and 6.4 μg per g pollen. It is uncertain whether the folic acid conjugates are included.

As the content of folic acid conjugates appeared to be high in pollen it was of interest to investigate the presence of conjugates in the pollen. Since conjugases are widely distributed in nature [7] their occurrence in pollen was to be expected.

The investigation of pollen conjugases was performed mainly with pollen from *Zea mays*. A number of samples were prepared, each containing 5 g pollen in 25 ml of water. One of the samples was heated to 100° immediately in order to inactivate the conjugases, and was then used to determine the amount of folic acid present. One other sample was similarly heated and chicken pancreas conjugase added to determine the total content of folic acid and folic acid conjugates. Other samples were kept for 18 h at 25° at different pH values.

As can be seen from Fig. 1, the amount of folic acid was unaltered in the samples kept at pH 3.5 and 7.0 whereas there was an increase in the folic acid activity of the other samples. This indicates that the pollen contains conjugase (or conjugases) active between approximately pH 4.0 and pH 6.0, the optimum being about pH 4.5.

The conjugase present in the pollen from *Zea mays* differs from the conjugase in chicken pancreas in having the optimum at pH 4.5. The chicken pancreas conjugase has the optimum at pH 7-8 [5]. Although the conjugase extracted from hog kidney has its optimum at pH 4.5 [7,10] it can, however, hardly be the same conjugase as that in pollen. Our experiments showed that the hog kidney conjugase cannot split the conjugates in pollen to the same extent as

can the pollen conjugase. Thus the conjugase found in pollen must differ from both the conjugase in chicken pancreas and that in hog kidney.

It is seeen from Fig. 1 that at pH 4.5 the same amount of folic acid was obtained with pollen conjugases as with chicken pancreas conjugase. If it be assumed that chicken pancreas conjugase can split all the conjugates, then the amount of conjugase present in the pollen would suffice to convert all the conjugates of the pollen into folic acid.

It is of interest that pollen conjugase has its optimum at a pH not naturally occurring in pollen.

Experiments with other pollen samples, *viz.* pollen from *Pinus montana*, *Phleum pratense* and *Secale cereale*, demonstrated that the content of folic acid increased and that the content of folic acid conjugates decreased when the pollen was incubated in water at 25° and at pH 5. This indicates that conjugases are also present in these pollen samples.

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