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THE EFFECT OF PTERIDINES ON THE DEVELOPMENTAL CYCLE OF THE PROTOZOAN *NOSEMA APIS* Z.

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SUMMARY

Drones, kept in cages in the absence of bees, were used for the study of the action of pollen, secretion of the pharyngeal glands, and pteridines on the development of *Nosema apis*. On the tenth day from the individual infection the drones were killed and the amount of the spores of the protozoan *Nosema apis* Z. was quantitatively determined.

The experiments did not prove any stimulating effect of pollen and the secretion of the pharyngeal glands on the development of *Nosema*. However, the development of the protozoan was found to be markedly stimulated by tetrahydrofolic acid and by aminopterin. Further eight pteridines tested did not exert a clear and statistically significant influence on the development of *Nosema* in all cases. Two pteridines, 4 - mercaptopteridine and 2 amino-4-hydroxy-6, 7-dimethyl-5, 6, 7, 8-tetrahydroxypteridine, were found to slow down the development rate of *Nosema apis* Z., the latter substance being toxic to the diseased drones. The authors discuss the possibilities of the production of pterins by the intestinal bacterial microflora and their use by the protozoan *Nosema apis* Z.

INTRODUCTION

Investigation into the causes underlying the seasonal occurence of the disease is an important part of the study of the bionomy of the protozoan *Nosema apis* Z. The effect of temperature on the development of *Nosema* was explained as early as 30 years ago (MORGENTHALER, 1938; LOTMAR, 1943). Insufficient attention has, so far, been paid to other causes, especially to the action of pollen, although pollen has been known for more than two decades to have a stimulatory effect on the reproduction of the

parasite (BEUTLER, OPFINGER, WAHL, 1949). It has not been proved until now, whether pollen owes its stimulating effect to the proteins and vitamins it contains, or whether it is due to an indirect action of pollen encouraging the development of the pharyngeal glands which produce high-protein and high-vitamin secretion to be ingested by the parasite, or after all, whether both factors are involved in combination.

The secretion of the pharyngeal glands can be eliminated from the food of caged bees either by the removal of these glands or by the stoppage of their function. Both methods are still impractical in the honey-bee. The only possible solution to this problem is the use of honey-bee individuals in which these glands are not developed, i.e. the drones and the queens.

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It is easier to keep queens without worker bees. However, their mutual aggressiveness and difficulty to obtain them in larger nombers made it impossible to use them in further experimental work. Thus, drones were left as the only material to be used for the experiments. The *Nosema* disease in drones has always been studied in the presence of bees. BAILEY (1972) found, for instance, that drones are as susceptible to the disease as bees. HANKO and LEMÁKOVÁ (1971) also described an invasion of drones by the protozoan *Nosema apis* Z. and their ability to transmit the disease to other colonies. CHEREPOV and KUZNETSOVA (1969) assert that drones are even more susceptible to the disease than are bees.

Drone-keeping in the absence of worker bees for the study of the *Nosema* disease was reported by PEROUTKA in 1975, at the XXVth APIMONDIA Bee-Keeping Congress in Grenoble.

BUTENANT and REMBOLD, 1958, were the first to detect biopterin in the secretion of the pharyngeal glands. HANSER and REMBOLD (1968) found that biopterin and neopterin are important co-factors of phenylalanine hydroxylase and tryptophan hydroxylase in the honey-bee.

The need for the presence of pterins, i. e. 2-amino-4-hydroxypteridines, namely biopterin and neopterin, as a growth factor, is known in the flagellate *Crithidia fasciculata.* In this organism it is particularly the pterins that are involved in the synthesis of unsaturated fatty acids and the majority of these acids are able to meet the protozoan's need for pterins. Palmitooleic acid, inhibiting growth, is an exception (BLAKLEY, 1969).

Analogues of folic acid have been used with success as antimalaric substances and substances used for the control of other protozoans. These derivatives also limited the growth of the flagellate *Crithidia fasciculata.* Biopterin has always been applied with success for breaking the growth inhibition in this protozoan (BLAKLEY, 1969).

The results of these works and the results of experiments with drones, studied for the effect of the secretion of the pharyngeal glands, pollen, and glycides on the development of the protozoan *Nosema apis* Z., justified, in our view, the assumption of the action of pteridines on the reproduction of *Nosema.* Biopterin however had already been tested for influencing the development cycle of the pest by GONTARSKI and MEBS with a negative result in 1964. Action of other pteridines on the developmental cycle of the parasite has not yet been described.

MATERIAL AND METHODS

Drones of the Carniolan race, produced by the Bee-Keeping Research Institute at Dol, were used for the experiments. In some of the pilot tests we sometimes failed to infect very young drones. For this reason, individuals of different age, coming from a single colony and captured at random on combs in the brood-chamber, were used in all experiments.

The drones were kept in a thermostat at a temperature of 30 ± 1 °C and at a relative humidity of 60 to 80 %, in the same cages as described by PEROUTKA in 1975 (fig. 1). The cages are 120 × 110 × 55 mm in size. The bottom and the two narrow sides are wooden, the upper lid and the two wide side walls of scrim

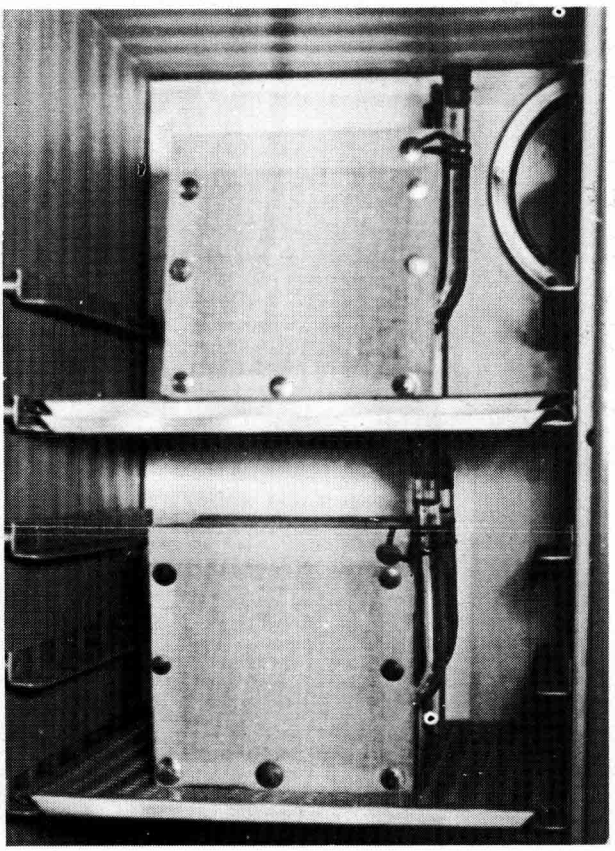


FIG. 1. — *Cages adapted for drones.*

giving the drones better possibility of movement than on glass walls. Each cage was equiped with a test-tube bent to the shape of L. The test tubes contained water and part of intact drone comb, ca 60 × 80 mm in size, bearing food which had been injected into the cells with a syringe. The combs had been exposed to the temperature of 50 °C for 24 hours before use (this temperature was sufficient to devitalize *Nosema* spores (CANTWELL, SHIMANUKI, 1969) in order that the contamination of the comb with *Nosema* spores be eliminated. The cages, the glass feeders and the scrim had also been disinfected with high temperature before use.

The drones were kept in groups of 30 to 50 in cages without bees (fig. 2), with the exception of two groups in which the secretion of the pharyngeal glands was studied for its action on the reproduction of the parasite. In group 1 one worker bee was added per 3 drones, thus the cage contained 30 drones and 10 bees. The drones took their food both from the drone comb and from the bees. In the second group the cage was divided by a queen excluder into two halves. The upper part of the cage contained drones without food and water whereas those in the lower half had access both to food and water. In this group the drones were entirely dependent on the worker bees which had free access to the food and to the drones.

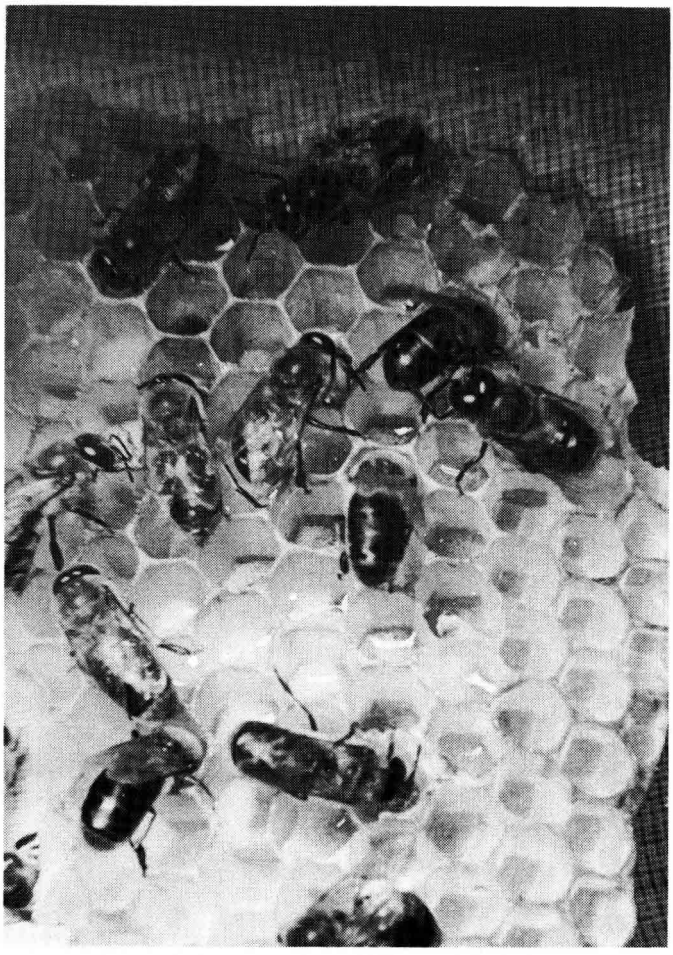


FIG. 2. — *Drones on the small comb in the cage.*

When the drones had been removed from the colony, they were left to fast for 30 minutes; after this period, they were individually infected by 3×10^4 spores of the protozoan *Nosema apis* Z. (fig. 3). The dose was individually administered by means of a micro-pipette, 10 ul. of 50 % sugar solution being used as carrier. The tenth day after infection the drones were killed and the quantity of the spores of the parasite was determined. The preparation of the alimentary tract was not always successful, owing to considerable damage; hence we prepared whole abdomens of the drones. The abdomen, placed in a calibrated test-tube, was macerated for ca. 12 hours in distilled water. Then it was crushed by means of a glass stick, the remains of chitin were removed from the test-tube, and water was added to obtain the final content of 2 ml. The spores were counted in Bürker's cell.

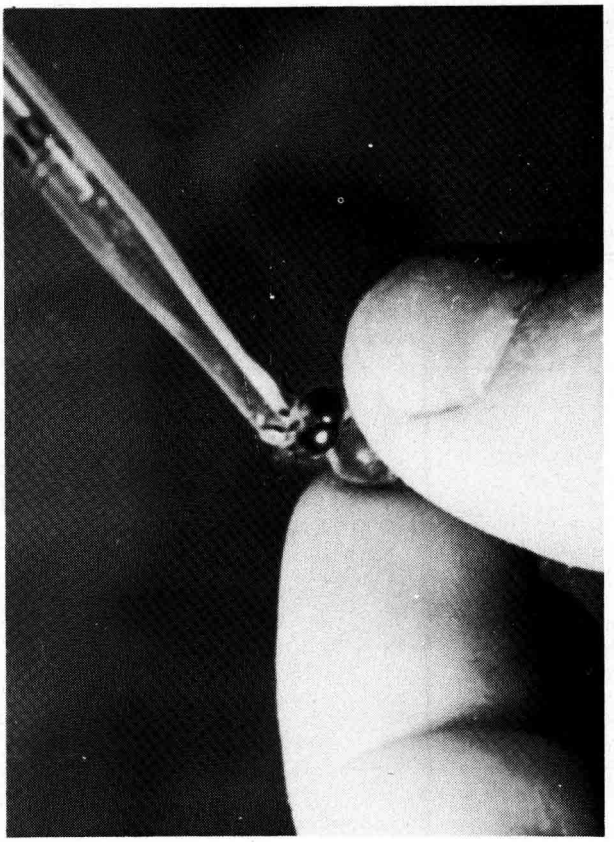


FIG. 3. – *Individual invasion of drones.*

The drones were fed honey to which 3 % goat-willow pollen, which had been stored at minus 30 °C, was added. *Nosema* consumes the nitrogenous compounds from the host's food, as found by LOTMAR (1939). Pollen was added to honey in all experiments in order that the amount of these substances be sufficient. The use of glycides alone for the nutrition of the drones implied the hazard of nitrogenous compounds becoming a limiting factor, with the intensive schizogony of the protozoan *Nosema apis*. Goat-willow pollen was used for its major stimulatory effect on the developmental cycle of the protozoan in the bees (PEROUTKA, 1975 a).

The pollen was pelleted by the bees. Before use, the pellets were crushed, the pollen was thoroughly mixed in honey, and on the first and third day of the experiment 8 ml of this food was put into each cage so that the drones could take in the food *ad libitum*. The food should fill not more than 3/4 of the volume (height) of the cells. When the cells contained more food, or when the food was spilled over the comb, the drones quickly got sticky and died in 48 hours.

The only exceptions were the third and fourth experimental groups. Honey with 3 % secretion of the pharyngeal glands was administered to the drones in the third group and honey alone to the fourth group. The secretion of the pharyngeal glands had been kept in dark glass bottle at minus 30 °C.

In groups which were studied for the effect of pteridines (tabl. 1) 5 mg of the substance was mixed with each amount of 8 ml of food, always on the first and third day of experiment. Thus the drones consumed food, containing the studied pteridine, throughout the duration of the experiments.

TABLE 1. — *The number of the spores of* Nosema apis Z. *ascertained 10 days after the infection*

The first year of experiments
F — table = 1,69

Order	Food	No. of observations	Mean*	Standard deviation	Mortality %	Significant differences between means for groups - S-method
1.	honey + 3 % pollen + bees .	72	14,68	12,12	18,1	5, 6
2.	drones fed bv bees	40	18,97	6,24	0	
3.	honey + 3 % royal gelly ...	16	13,31	7,53	82,6	5
4.	honey + 3 % pollen	64	13,73	14,06	59,7	5, 6
5.	neopterin	23	35,06	17,19	63,4	1, 3, 4, 7, 8, 10
6.	aminopterin	34	32,81	21,15	46,8	1, 4, 7, 8
7.	2, 4-diamino-6-tetrahydroxy-butylpteridine	19	13,39	9,38	69,8	5, 6
8.	folic acid	29	15,14	10,88	53,2	5, 6
9.	2-amino-4-hydroxy-6-tetrahy-droxybutylpteridine	22	21,01	11,27	51,1	
10.	6, 7 dimethylpterin	34	16,85	12,62	65,3	5
11.	honey alone	83	19,90	17,26	59,1	
12.	honey + 3 % pollen	57	0		64,7	non-contaminated control drones

* The number of spores per drone, in millions

The effort to find whether or not pteridines are substances exerting a significant influence on the developmental cycle of *Nosema* was based on the knowledge of the action of four substances. Most important among them was tetra-hydrofolic acid in which the action on protozoans is relatively well known (BLAKLEY, 1969).

Further substances, involved in these studies, were neopterin, contained in the secretion of the pharyngeal glands and affecting the protozoan *Crithidia fasciculata* in a manner similar to the action of biopterin (BLAKLEY, 1969), 6-polyhydroxyalkylpteridines, and 6, 7-dimethylpterine.

Another substance which could markedly affect the developmental cycle of the protozoan *Nosema apis* Z. was aminopterin which is, in numerous species, an inhibitor of folic acid (BLAKLEY, 1969). However, there may be cases in which aminopterin can replace folic acid.

The fourth group of substances tested were mercaptopteridines which were assumed to show an inhibitory effect in biochemical reactions influenced by pteridines (SLAVÍK, personnal communication).

Tabl. 2 shows the results of experiments in which pteridines were added to food at a rate of 5 mg : 2 ml of 0.1 molar solution of ascorbic acid, containing 5 mg pteridine, was added to 8 ml of honey with 3 % goat-willow pollen. Owing to the lower solubility of some pteridines in acid medium, the *p*H was adapted with $NaHCO_3$ to the value of 6,8. Again, the food was administered on the 1st and 3rd day of experiment so that the drones might eat *ad libitum*.

TABLE 2. *The number of the spores of the protozoan* Nosema apis, *as determined after 10 days from infection*

The second year of experiments
F – table = 1,75

Order	Food	No. of observations	Mean*	Standard deviation	Mortality %	Barlet test (differences between groups at alpha = 0,01	alpha = 0,05)
1.	folic acid	57	10,69	8,70	24		
2.	6, 7-dimethylpterin	46	9,60	8,93	33,3		
3.	tetrahydrofolic acid	32	17,08	14,66	53,6	9, 4, 2	9, 4, 2, 1, 10 11
4.	2-amino-4-hydroxy-6, 7-dimethyltetrahydropteridine	13	6,56	7,50	83,3		
5.	tetrahydroneopterin	34	12,16	13,43	53,4		
6.	neopterin	59	15,33	8,24	25,3	9, 4	9, 4, 2
7.	aminopterin	37	17,11	10,76	7,5	9, 4, 2	9, 4, 2, 1, 10, 11
8.	4-mercaptopteridino-6, 7 : 1′, 2′-cyclohexan	66	15,71	11,85	13,8	9, 4	9, 4, 2
9.	4-mercaptopteridine	31	6,11	7,79	20,5		
10.	4-mercapto-6, 7-dimethylpteridine	42	11,18	10,17	34,3		
11.	honey + 3 % pollen	46	11,18	11,38	10,2		
12.	2-amino-4-hydroxy-6-tetrahydroxybutylpteridine	54	17,89	13,10	37,2	9, 4, 2, 1	9, 4, 2, 1, 10, 11, 5
13.	2, 4-diamino-6-tetrahydroxybutylpteridine	41	14,09	12,21	48,1	9, 4	9, 4
14.	honey + 3 % pollen	47	0		17	non-contaminated control drones	

* The number of spores per drone, in millions

Some reduced pteridines : tetrahydrofolic acid, 2-amino-4-hydroxy-6, 7-dimethyl-5, 6, 7, 8-tetrahydropteridine, and tetrahydroneopterin, were used in experiments the results of which are presented in Tabl. 2. Due to their very low stability, these substances had been dissolved in 0.2 molar solution of ascorbic acid, neutralized with $NaHCO_3$ to the *p*H value of 6, 8. Sugar solution was added after the dilution of the pteridine so that the resultant food could contain an 0,1 molar concentration of ascorbic acid. After 30 minutes' starvation, upon the dilution of pteridines, drones were individually subject to the administration of these substances, in each case 24 and 48 hours after infection. On the remaining days the drones consumed just the basic food, the honey with pollen.

Tetrahydropteridines had to be consumed within one hour after dilution. For the mentioned period, they were protected against oxidation by the 0,1 molar solution of ascorbic acid. The dose was determined so that the given amount of pteridines per drone could be maintained — 10 mg per cage.

Owing to the limited amount of pteridines and great demands for work, it was impossible to keep control along with each group for studying the action of pteridines on the mortality of healthy drones. For this reason we present, for each group, the mortality percentage of diseased individuals for the 10-day post-invasion period.

Single classification analysis of dispersion was used for the evaluation of the results obtained, and the S-method at alpha = 0,05 was used for the comparison of the effect of individual substances on the reproduction of *Nosema*. In tabl. 2 the results were compared by the Barlet test.

RESULTS

The experiments on drones did not reveal any stimulatory effect of pollen in food on the reproduction of the protozoan *Nosema apis* Z., in comparison with glycides used alone. Even the drones fed pure honey, had more spores of the protozoan (19.19 million per drone) than those fed honey with 3 % goat-willow pollen (13.73 million spores per drone). However, this difference is statistically insignificant.

The tests also proved no effect of the secretion of the pharyngeal glands on the developmental cycle of *Nosema*. The drones which could consume honey with 3 % pollen and, at the same time, food from bees, had 14.68 million spores each. Similar values were obtained in the drones which got honey with royal jelly (13.31 million spores) and in those fed honey with 3 % goat-willow pollen (13.73 million spores). The greatest amount of spores per individual (18.97 million) was found in the drones which were fed just by the bees. However, the difference is not statistically significant even in this case.

Neopterin and aminopterin showed a marked stimulatory effect on the development of the protozoan *Nosema apis* Z. The drones fed a food with addition of neopterin had the largest amount of spores of *Nosema* (35.06 million per drone).

The influence of this pterin on the reproduction of the protozoan is statistically significant, if compared with the drones kept together with bees, and with the groups of drones given honey with 3 % royal jelly, honey with a 3 % pollen, and drones given 2,4 diamino-6-(1′, 2′, 3′, 4′-tetrahydroxybutyl) pteridine, folic acid and 6,7 dimethylpterin. Aminopterin also stimulated the developmental cycle of *Nosema apis* Z., but the degree of this stimulation was lower. The other pteridines did not exert any statistically significant influence on *Nosema,* although it may be said that 2-amino-4-hydroxy-6 (1′, 2′, 3′, 4′-tetrahydroxybutyl)-pteridine slightly encouraged the development of the protozoon, producing 21.01 million spores per drone.

In the first year, the lowest mortality was ascertained in the groups of drones kept together with bees (18.1 %), and no mortality at all was obtained in the drones fed by the bees through the queen excluder. In that period, the method of keeping the drones alone had not yet been refined in all its detail. These drones, still not invaded by the

spores of the parasite, died within 10 days and their mortality rate was 64.7 %. Also the drones which had been invaded by the spores and which had been given honey alone, honey with pollen, and honey with pteridines, died within 10 days at a rate of 60 %. A higher mortality rate, 82.6 %, was ascertained in the group of drones which were fed honey with 3 % royal jelly. Similar data are shown in Tabl. 1.

Reduced pteridines and mercaptopteridines were used for influencing the developmental cycle of *Nosema apis* Z. in further tests. Due to the fact that an 0.1 molar solution of ascorbic acid had been used for the protection of pteridines from oxidation, the effect of other pteridines used in the previous tests was also tested.

It was revealed during the evaluation of the results by the Barlet test at alpha = 0.05 that statistically significant differences existed in the stimulation of the development of *Nosema apis* by tetrahydrofolic acid (17.08 mil, spores per drones), aminopterin (17.11 mil. spores per drone), and 2-amino-4-hydroxy-6- (1′, 2′, 3′, 4′-tetrahydroxybutyl) pteridine (17.89 mil. spores per drone), in comparison with the control drones fed just honey with 3 % goat-willow pollen (11.18 mil. spores per drone). The other substances did not exert a significant influence on the development of the parasite, although neopterin and 4-mercaptopteridino-6, 7 : 1′, 2′-cyclohexan slightly encouraged its development (15.33 and 15.71 mil. spores per drone, respectively).

4-mercaptopteridine was found to reduce statistically significantly the number of the spores of *Nosema,* as compared with the food containing tetra-hydrofolic acid, neopterin, aminopterin, 4-mercaptopteridino-6.7 : 1′, 2′-cyclohexan, 2-amino-4-hydroxy-6-tetrahydroxybutylpteridine, and 2,4-diamino-6-tetrahydroxybutylpteridine, even at significance level of alpha = 0,01. 4-mercaptopteridine also reduced the number of spores of the protozoan *Nosema apis* Z. in comparison with the control group of drones fed just honey and pollen. However, the reduction of the number of spores was statistically insignificant. Similarly, the quantity of the spores of *Nosema apis* was reduced by 2-amino-4-hydroxy-6, 7-dimethyl-5, 6, 7, 8-tetrahydroxypteridine.

In the second year of experiments, the method of keeping drones alone was already refined so that the 10-day mortality rate was 17,0 % in the non-infected drones and just 10,2 % in the infected drones. Considering that the same food was used in all further groups it can be assumed that the higher mortality of the experimental individuals was due either to the intensive development of the protozoan *Nosema apis* Z. or to the toxicity of the pteridine given to the drones, or, after all, to the combination of both.

Detail data are shown in Tabl. 2.

DISCUSSION

Tests on drones did not prove that the developmental cycle of *Nosema apis* Z. is stimulated either by the pollen in the food or by the secretion of the pharyngeal glands,

in comparison with glycides administered alone. The drones which were fed honey with royal jelly and were invaded by the spores of the parasite showed an 82.6 % mortality rate of the experimental individuals which might indicate a vigorous development of the protozoan in the cells of stomach epithelium. However, the examination of the remaining living drones and those which were fed only by bees and did not suffer from a significant increase of the number of the spores justified the assumption that the drones given the honey enriched with royal jelly died from the changes in the royal jelly which is known to be a quickly decomposing substance. These were the reasons why we eliminated royal jelly from further experiments, and pollen, which was assumed not to have any adverse influence on the development of *Nosema apis* Z., was used as the source of nitrogenous compounds.

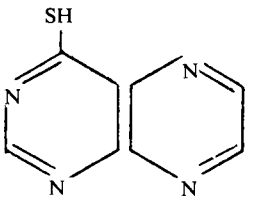
The results of the tests with pteridines proved the hypothesis, that these substances have an effect on the developmental cycle of the parasite. The development was found to be markedly stimulated by tetrahydrofolic acid. Neopterin was another substance found to exert a stimulative effect, especially in the first year of experiments.

As assumed, the development of the causal agent of the *Nosema* disease was to be stimulated also by tetrahydroneopterin. However, we failed to obtained a pure preparation. Small amounts of platine got into the substance in the course of preparation and, apparently, this admixture was responsible for the higher mortality rate of the experimental individuals.

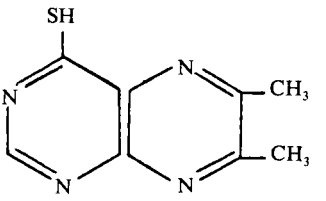
The experiments did not prove an inhibitory action of aminopterin. On the contrary, this substance always stimulated the development of the protozoan *Nosema apis* Z. It is interesting, in this connection, that in the second year of experiments when the drones contained more than 17 million spores each, their mortality rate was at the lowest level (just 7.5 %).

Interesting results were also obtained with the use of mercaptopteridines. 4-mercaptopteridine reduced the development of the protozoan by 45.5 %, as compared with the control drones given just honey with pollen. The group of drones fed 4-mercapto-6, 7-dimethylpteridine in their food had the same number of spores as the control group and the third groupe given 4-mercaptopteridino-6, 7 : 1′, 2′-cyclohexan in food had even a greater number of spores per drone than the control group. It is inferred from the experiments that in 4-mercaptopteridines the inhibitory effect on the development of the protozoan decreases with the increase of non-polar substituents at levels 6 and 7. It is interesting to compare the formulae of these mercaptopteridines with biopterin.

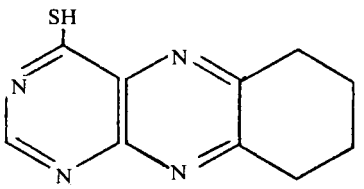
As to the remaining pteridines, 6, 7-dimethylpterin and folic acid apparently do not influence the development of the parasite, although 6, 7-dimethylpterin is responsible for a higher mortality rate of the drones. It can be assumed, that this pteridine caused the greatest mortality among the attacked individuals which is suggested, in this group, also by a generally lower number of the spores of *Nosema apis* Z.



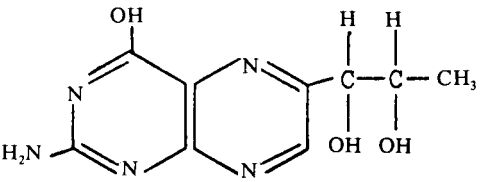
4-mercaptopteridine



4-mercapto-6, 7-dimethylpteridine



4-mercaptopteridino- 6, 7 : 1′, 2′-cyclohexan



biopterin

A small number of the spores of the protozoan *Nosema apis* Z. was found in the groups of drones given 2-amino-4-hydroxy-6, 7-dimethyl-5, 6, 7, 8-tetrahydropteridine in their food. Since the mortality rate of drones during the experiments was 83.3 %, it can be assumed that the preparation either caused a vigorous development of the parasite, resulting in a high mortality of the drones, or had a toxic effect on the drones.

Interesting results were obtained with the use of 2-amino-4-hydroxy-6-tetrahydroxybutylpteridine and, in part, with the use of 2, 4-diamino-6-tetrahydroxybutylpteridine. It may be that the protozoan *Nosema apis* Z. is able to utilize these pteridines directly or to metabolize them after some conditioning. In this connection one problem remains unsolved : the possibility of the absorption of pteridines from the alimentary tract and perhaps the conversion of pteridines by the intestinal bacterial microflora of drones.

No such experiments with pteridines have, so far, been published. GONTARSKI and MEBS (1964) are the only authors who have tested the effect of biopterin on the development of the protozoan *Nosema apis* Z. in the honey-bee. These authors did not describe their experiments in detail. They just state that 6 mg biopterin was added to 100 ml of food and that this mixture did not influence the developmental cycle of the parasite.

It is inferred from the results of the experiments that it will be necessary to study mecaptopteridines as possible inhibitors of the developmental cycle of the protozoan *Nosema apis* Z. 4-mercaptopteridine deserves greatest attention, since it not only reduced the development of the parasite but also had no toxic effect on the host. However, further scrutiny will be necessary in this connection in the study of queens' oviposition, since mercaptopteridines may also act as chemosterilants.

Another problem still unsolved is the effect of pteridines, particularly aminopterin, upon the intestinal bacterial flora. Their inhibition might manifest itself in a reduced mortality of the drones from septicaemia in the initial stages of the disease and, as a secondary manifestation, in an increased number of spores per living drone as a result of survival inspite of a high infection rate.

The most important problem now being studied is the explanation of the role of the intestinal microflora as a possible source of pterins for the protozoan *Nosema apis* Z. The results of the work by HARTWIG (1976) on the cultivation of the parasite on tissue cultures suggest that bacterial flora must be involved in the developmental cycle of the protozoan *Nosema apis* Z.

CONCLUSION

Test on drones revealed that 3 % additions of goat-willow pollen to food and 3 % additions of the secretion of the pharyngeal glands do not influence the developmental cycle of the protozoan *Nosema apis* Z., in comparison with food containing glycides alone.

The drones fed exclusively by bees as well as those which could take their food from bees and eat honey with 3 % goat-willow pollen from combs, also had not an increased number of the spores of the parasite in their alimentary tract, in comparison with the drones eating honey.

Tetrahydroxyfolic acid and aminopterin exerted a statistically significant stimulative effect on the development of *Nosema apis* Z. The development of the protozoan *Nosema apis* Z. was also stimulated by neopterin, 2-amino-4-hydroxy-6-(1′, 2′, 3′, 4′-tetrahydroxybutyl) pteridine, 2, 4-diamino-6(1′, 2′, 3′, 4′-tetrahydroxybutyl) pteridine, and 4-mercaptopteridino-6, 7 : 1′, 2′-cyclohexan, although this stimulation was not always statistically significant.

Folic acid, 6, 7-dimethylpterin, 4-mercapto-6, 7-dimethylpteridine, and tetrahydroneopterin did not influence the development of *Nosema,* although the latter three were responsible for a higher mortality of the attacked individuals.

2-amino-4-hydroxy-6, 7-dimethyl-5, 6, 7, 8-tetrahydropteridine limited the developmental cycle of the protozoan *Nosema apis* Z., but was responsible, at the same time, for a high mortality rate in the host. The development of the protozoan *Nosema apis* Z. was also reduced by 4-mercaptopteridine, without any greater increase of drone mortality.

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ZUSAMMENFASSUNG

DER EINFLUSS VON PTERIDINEN AUF DEN ENTWICKLUNGSZYKLUS DES PROTOZOON *NOSEMA APIS* Z.

Drohnen, ohne Arbeitsbienen in Käfigen gehalten, wurden zum Studium der Wirkung von Pollen, des Sekrets der Pharynxdrüsen und von Pteridinen auf die Entwicklung von *Nosema apis* benutzt. Am 10. Tag nach der individuellen Infektion wurden die Drohnen getötet und die Anzahl der Nosemasporen quantitativ bestimmt.

Die Versuche ergaben keinerlei stimulierenden Effekt von Pollen oder von den Sekreten der Pharynxdrüsen auf die Entwicklung der *Nosema.* Hingegen wurde die Entwicklung des Protozoon deutlich durch Tetrafolsäure und Aminopterin stimuliert. Es wurden acht weitere Pteridine geprüft, aber in allen Fällen konnte kein deutlicher und statistisch signifikanter Einfluss auf die Entwicklung von *Nosema* gefunden werden. Für zwei Pteridine, 4-Mercaptopteridin und 2-Amino-4-hydroxy-6, 7-dimethyl-5, 6, 7, 8-tetrahydroxypteridin, wurde im Gegenteil eine Verlangsamung der Entwicklungsrate von *Nosema apis* Z. nachgewiesen, wobei sich die letztere für die erkrankten Drohnen als toxisch erwies. Die Autoren diskutieren die Möglichkeit der Produktion von Pteridinen durch die bakterielle Mikroflora des Darms und deren Aufnahme durch das Protozoon *Nosema apis* Z.

RÉSUMÉ

INFLUENCE DES PTÉRIDINES SUR LE CYCLE DE DÉVELOPPEMENT DU PROTOZOAIRE *NOSEMA APIS* Z.

Des mâles d'abeilles, conservés en cages sans ouvrières ont été utilisés pour étudier l'action du pollen, de la secrétion des glandes pharyngiennes et des ptéridines sur le développement de *Nosema apis.* Dix jours après les avoir infecté individuellement, on a tué les mâles et dénombré la quantité de spores du protozoaire *Nosema apis.*

Les expériences n'ont montré aucun effet stimulant du pollen ni de la secrétion des glandes pharyngiennes sur le développement de *Nosema.* On a pourtant trouvé que le développement du protozoaire était fortement stimulé par l'acide tétrahydrofolique et par l'aminoptérine. Les huit autres ptérines testées n'ont pas exercé d'influence nette et statistiquement significative sur le développement de *Nosema* dans aucun des cas. On a mis en évidence que deux ptéridines, la 4-mercaptoptéridine et la 2-amino-4-hydroxy-6, 7-diméthyl-5, 6, 7, 8-tetrahydroxyptéridine, ralentissaient le taux de développement de *Nosema apis* Z., la dernière substance étant toxique pour les mâles contaminés. Les auteurs discutent des possibilités de la production des ptérines par la microflore bactérienne intestinale et leur utilisation par le protozoaire *Nosema apis* Z.

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