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# Does Helminth Infection Affect Mental Processing and Educational Achievement?

C. Nokes and D.A.P. Bundy

*In this article, Catherine Nokes and Donald Bundy re-examine the evidence linking intestinal helminth infection to impaired cognitive function and educational outcomes. They consider first the evidence that implies a connection between intellectual dysfunction and the sequelae of infection, then the significance of correlations between infection and poor mental status, and finally the evidence from case-control and double-blind intervention studies. The article is not intended as a comprehensive summary of all the research on helminth infection and mental function – indeed the majority of research undertaken in the early part of this century is not included – but rather as a thought provoking article to highlight the difficulties with interpreting existing data and to stimulate new interest in this field.*

As biomedical researchers, we tend to assess the significance of parasitic infections in terms of their clinical effects and their physical consequences for growth and development. In doing so we may be in danger of overlooking effects which may be of at least equivalent importance for economic and social development.

In 1990, the World Summit for Children set a target of achieving basic primary education for at least 80% of children by the year 2000. In setting this target, it was recognized that providing access to schooling will only result in improved education if these children have the capacity to benefit from the schooling made available to them. There is little point in providing excellent educational facilities if the ability of children to attend school, or to learn while there, is compromised by ill health.

In a UNESCO review[1], intestinal helminth infections were identified as potentially important in this context, not only because they are among the most common infection of school-age children and tend to occur at highest intensity in this age group, but also, and more importantly, because some of the more common consequences of infection (nutritional deficiency and impaired physical development) were likely to have negative consequences for cognitive function and learning ability.

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## Implied evidence

Many of the sequelae of helminth infection are associated with deficits in cognitive functioning. Undernutrition is one of the most common consequences of infection with *Ascaris lumbricoides, Trichuris trichiura, Schistosoma* spp and the hookworms[2], and is also strongly associated with deficits in mental functioning[3]. Iron-deficiency anaemia has a particularly strong link with impaired functioning[4], and is a common component of the clinical picture of hookworm disease, schistosomiasis and intense trichuriasis. Low height-for-age (stunting) has been associated with detriments in cognitive function[5], in mental development[6], in behaviour[7] and in educational achievement[8]; it is a striking feature of intense trichuriasis[9] and a not-uncommon consequence of ascariasis[2]. Low weight-for-age (wasting) and low weight-for-height, two common sequelae of *A. lumbricoides* and schistosome infection[2], have also (though more rarely) been associated with cognitive deficits and impaired concentration in school achievement tests[10], respectively.

These observations imply that many of the commonest nutritional consequences of intestinal helminth infection are likely to impair the ability of children to learn in school. There are also other consequences of infection that may compromise the educational achievement of children. The more minor but commonly observed manifestations of infection, such as diarrhoea and abdominal pain[2], could impair learning through their effects on the general well-being of a child, while heavy infections, though rarely observed, can result in severe complications leading to acute or chronic disability[2].

This implicit evidence indicates that intestinal helminth infection could have a detrimental effect on cognition and educational achievement. It does not, however, provide any indication of how common or how large that effect may be.

## Correlational evidence

Most information on helminth infection and childhood education comes from correlational studies. These studies provide some indication of the possible degree of effect of helminthiasis on educational achievement and are a useful starting point upon which to base subsequent research. For a summary of the studies of nematode infection and mental function, see Table 1.

 

Table 1. Studies of nematode infection on measures of educational achievement and cognitive function[a]

| Location | Infection criteria | Subject No. | Age (years) | Results in index group | Refs |
|---|---|---|---|---|---|
| **Study design – correlational** | | | | | |
| USA | Hookworm (presence/absence) | 78 | 6–17 | Deficit in grade advancement of 0.23 grades per year. | 11 |
| Australia | Hookworm (intensity: uninfected, light and heavy) | 231 | 6.5–15.5 | Negative correlation between the intensity of infection and score on the Binet–Simon test and Porteus Mazes. | 13 |
| Italy | *Trichuris trichiura* (intensity: uninfected, moderate to heavy) | 338 | 6–11 | School performance not affected by moderate and heavy infection, but more by social and hygienic conditions. | 15 |
| Italy | *Trichuris trichiura* | 356 | 6–10 | School performance not affected by moderate and heavy infection, but more by social and hygienic conditions. | 14 |
| South Africa | Poly parasitism (*A. lumbricoides*, *T. trichiura*, hookworm *Schistosoma* spp) | 110 | 10 | Significant association of infection with sustained attention task. No association with memory task or educational attainment. | 28 |
| Central Jamaica | Poly parasitism (*A. lumbricoides*, *T. trichiura*, hookworm) | 593 | 9–12 | Children streamed by teachers according to academic ability. Children with least ability more likely to be infected and heavily infected. | 16 |
| **Study design – intervention** | | | | | |
| Central Jamaica | *T. trichiura* (uninfected, moderate to heavy) | 159 | 9–12 | Nine weeks post-treatment: significant improvement in the treatment group compared to the placebo and uninfected controls in cognitive tests of working memory (fluency, digit-span). No treatment effect in comprehension, arithmetic, MFFT or coding. | 21 |
| Kingston, Jamaica | TDS Uninfected | 38 | 3–6 | One year post-repeated-treatment: significant improvement in the TDS group in the locomotor subscale of the Griffiths tests of mental development. No improvement in three other subscales. | 33 |

[a] Abbreviations: TDS, trichuris dysentery syndrome; MFFT, matching familiar figures test.

Stiles[11], in a study in southern USA, was the first to demonstrate a correlation between helminth infection and the educational advancement of schoolchildren. He found that children infected with hookworm and to a lesser extent with *A. lumbricoides*, advanced through school at a slower rate than did uninfected children, averaging a deficit of 0.23 grades. The deficit was also correlated, however, with poor sanitary conditions. Because there was no recognizable impact of helminths on the memory span of infected children[12], Stiles considered that the higher degree of grade repetition was due to the impact of morbidity on school attendance and not through some direct effect on mental processing. Waite and Neilson[13] examined the relationship between intensity of hookworm infection and the intelligence quotient (IQ) of children in Queensland, Australia. They found that the degree of mental retardation increased in proportion to the intensity of infection and suggested that this was due to 'prolonged anaemia and toxaemia'. However, no potential confounding variables, such as socio-economic status, were measured.

In the 1960s, de Carneri and colleagues[14,15] examined the relationship between geohelminth intensity (particularly that of *T. trichiura*), social and hygienic practices and the mental ability of children in northern Italy. No relationship with infection was observed. This result was attributed to the dominance of social and environmental factors, and the absence of individuals with very heavy worm burdens.

We recently examined the effect of helminth intensity on cognitive function in 9–12 year-old children in three Jamaican schools and found a negative correlation between geohelminth infection and academic achievement[16]. Children with the least academic ability (as assessed by the teachers from examination results) were not only more likely to be infected, but they were also more likely to harbour larger-than-average worm burdens. The level of school absenteeism was also related to infection in these children (Fig. 1) such that the proportion of the year absent from school increased with increasing intensity of infection with *T. trichiura*[17]; the more-heavily infected individuals were absent almost twice as often as were their uninfected counterparts. A similar relationship has been observed for malaria[18] and guinea worm (*Dracunculus medinensis*) infection[19,20], but with both these infections absenteeism was a direct result of incapacity due to disease. This was considered an unlikely cause of absenteeism in the study with *T. trichiura*. However, since infection was associated with poor socio-economic status[21], absence may have been caused by such social factors as an increased need to work with parents. Thus, infection may be a covariate with absenteeism rather than a cause.

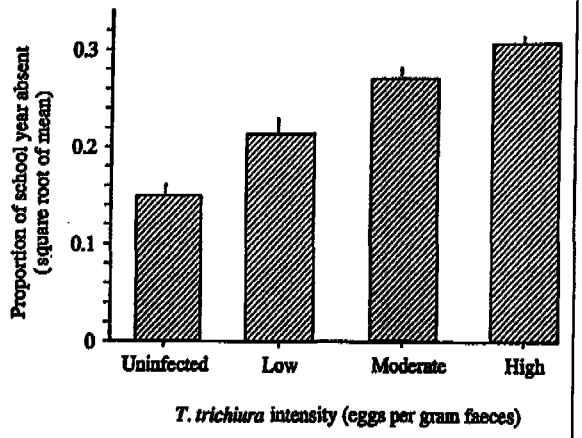


*Fig. 1. Correlation between school absenteeism and infection with* Trichuris trichiura *in three Jamaican schools. Uninfected, 0 epg; low, 1–<2000 epg; moderate, 2000–<7000 epg; high, >7000 epg. (Adapted from Ref. 17.)*

The effects of schistosomiasis on indicators of cognitive function and educational achievement have received relatively more attention than the effects of nematodes, yet the results have often been contradictory and inconclusive. Kieser[22] described mental retardation due to schistosomiasis infection in a few case reports of individuals in South Africa. In addition, a detrimental effect of bilharziasis on psychometric functions of Egyptian children was demonstrated by Abdalla *et al.*[23] Studies using school examination results as a measure of scholastic achievement, however, have found that infection has limited affect[24], no effect[25] or even a beneficial effect[26].

The tendency to attach insufficient weight to the choice of outcome variable may be an important reason why the results from correlational studies have been so variable. School examination results, in particular, are very complex measures of intellectual development and may be affected by many factors other than the health of the individual child, such as the availability of schooling or the quality of teaching[27,28]. Tests should only be used if good repeatability can be achieved, and if they are sensitive and culturally appropriate. The use of relatively crude measures of academic achievement or of subjective indicators such as the relative 'aliveness' of a child[25] are more likely to be subject to errors. Kvalsvig[27] suggests that, in the past, researchers have often selected school performance and intelligence tests not because they were appropriate measures but simply because the tests were readily available. Future studies should place more emphasis on the choice of outcome with a view to defining specific areas of cognition or mental development which are affected, and to identifying underlying mechanisms.

There has also been a tendency to overlook the importance of intensity of infection; many studies investigate the effects of minor subclinical infections without distinguishing these from cases of severe disease[25,29], yet it is well established in the health literature that the severity of disease is largely dependent on the intensity of infection. But perhaps the major difficulty in interpreting correlational studies arises because both infection and intellectual achievement are causally related to socio-economic status. It is not surprising, therefore, that social and hygienic conditions are important predictors of scholastic achievement and intellectual development[14,15,30,31]. The important question is the extent to which helminth infection also contributes to this effect.

### Intervention studies

The possibility of a causal association between helminth infections and education or cognitive function may be addressed through intervention studies. These studies entail measuring the change or improvement in performance following expulsion of the parasite infection by treatment, while not changing other parameters of the relationship. Unfortunately, very few studies have used this type of experimental design.

Castle *et al.*[31] used a variety of mental ability tests measuring accuracy and speed in productivity, which were thought to be sensitive to fatigue resulting from schistosome infection. On finding a significant deficit in infected children compared to those uninfected at baseline, infected children were treated with hycanthone and compared with an uninfected control group that had been pair-matched for age, sex and socio-economic status. Three years later, the treated children had improved significantly more than the uninfected controls in their performance on the spatial relationships test which required strong visual imagery skills. Although this study was a marked improvement on previous work, a case-control study is not the strongest design because the number and choice of confounding variables that can be measured and controlled for is limited. Socio-economic status was undoubtedly an important variable but other co-variates that were not assessed, such as improved nutritional status, may also have contributed to the observed improvement.

A stronger experimental design was employed, in separate studies, by Jordan and Randall[29] and Bell *et al.*[32], who conducted clinical trials involving a placebo group. By recruiting a control group that was infected with *Schistosoma* spp, it was assumed that any confounding variables would be equally distributed between these individuals and the group who were to receive treatment. Jordan and Randall[29] measured scholastic achievement and Bell *et al.*[32] measured IQ, with respective periods of six and 12 months between receiving treatment and repeating the tests. Both studies found marked improvements in the performance of children who had received treatment as opposed to those who had received a placebo and remained infected, although only Bell *et al.*[32] tested this using statistical tools. It is not clear, however, whether or not Jordan and Randall[29] randomly assigned children to treatment, and if either study gave the untreated children an identical placebo. Thus, it is not possible to differentiate the unique effect of *Schistosoma* spp from that of confounding variables that may have differed between the groups at baseline (such as socio-economic status) or have concomitantly changed or improved in response to treatment (such as nutritional status).

The results of two clinical trials and a case-controlled study on the effects of geohelminth infection on cognitive function and mental development have recently been reported. Callender *et al.*[33] investigated the effects of very heavy infections of *T. trichiura* on

the mental development of Jamaican children. Recruiting children with intense and symptomatic trichuris dysentery syndrome (TDS)[34] would be expected to maximize the probability of finding an effect, because of the relationship between the intensity and severity of infection. Such was the severity of infection in these children, that to have selected an infected placebo group as a control would have been unethical. Therefore, to control for confounding variables, a case-controlled study was conducted whereby members of the infected TDS group were pair-matched to an uninfected control group on the bases of age, sex, locality and socio-economic status. Treatment was given at three-month intervals to the infected TDS group only. At baseline, the TDS treatment group differed significantly from the control group in a wide range of developmental measures. After one year, regular treatment had led to significant improvements in measures of locomotor development and nutritional status. These findings raise a number of important issues: (1) they show that *T. trichiura* expulsion leads to an improvement in locomotor development; (2) they highlight the difficulty of differentiating the effects of worms from that of nutritional status; and (3) they demonstrate that controlling for nutritional status may be inappropriate since this may be an important mechanism by which infection could affect mental development.

Having demonstrated an effect of very intense infections of *T. trichiura* on the mental development of children, the question arises whether or not a similar improvement would follow expulsion of less-severe infections. We examined this issue[21] with respect to moderate to heavy infections of *T. trichiura*, also in Jamaican children. The study differed from the one described above in that cognitive function was measured as opposed to mental development, improvement was examined after a single, rather than repeated, treatment, and the time period over which an improvement was measured was nine weeks as opposed to one year. In addition, a double-blind clinical trial was conducted with an uninfected control group included for comparative purposes. To ensure that the random allocation of treatment or placebo to infected children had distributed any confounding variables, which may also affect cognitive function, equally between groups, some confounding variables were measured around the time of testing. These variables included socio-economic status, nutritional status (height and weight), iron status (haemoglobin and free erythrocyte protoporphyrin), educational opportunity, age, sex and IQ (Ravens Coloured Progressive Matrices). Nine weeks after intervention, children who received anthelmintic treatment scored significantly better than children in the placebo group, who had remained infected throughout the study, in tests of auditory short-term memory (digit-span forwards and backwards) and in the scanning and retrieval of long-term memory (fluency). Furthermore, on completion of the study, the treatment group and the uninfected controls no longer differed significantly in their performance of these tests. The effects were most marked for the fluency test and are represented in Fig. 2, and suggest that moderate to heavy infections with the human whipworm have a detrimental and reversible impact on a child's working memory. The impact of light infections and the impact of other parasitic helminth species on mental function still requires investigation.

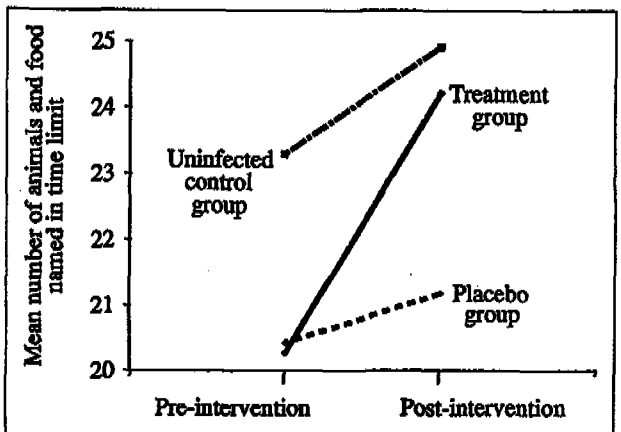


*Fig. 2. Effect of moderate to heavy infections of* Trichuris trichiura *on verbal fluency in Jamaican schoolchildren. Fluency is scored by adding the number of different animals the subject names in one minute to the number of different foods the subject names in one minute. (Adapted from Ref. 21.)*

Whether or not similar effects on cognitive function are observed from multiple species infections of parasitic helminths was examined by Kvalsvig *et al.*[28] A double-blind clinical trial was conducted, but ineffective cure rates and problems with confounding effects of age and nutrition resulted in the authors rejecting the post-intervention results as invalid. They therefore repeated the study in order to remove the design problems encountered, but this second attempt had to be abandoned due to serious flooding of the study community. Analysis of the baseline data in the second study revealed that poor performance on attentional tasks was associated with parasite status but there was no association with educational attainment and, in contrast to the study by Nokes *et al.*[21], there was no association with memory function.

## Conclusion

The implied evidence for an effect of helminth infection on cognitive function is persuasive, but the evidence from the correlational and intervention studies leaves many uncertainties concerning the extent and nature of the effect. We can only concur with the view of Baddeley[35] that 'any attempt to come up with a neat theoretical interpretation of the findings ... is premature'. More, and more-carefully designed, studies are clearly required.

One of the main difficulties with interpreting the results from studies to date, is that a whole myriad of cognitive and educational outcomes have been used. Some of the outcomes may potentially be very sensitive indicators for psychological assessment, but unless there is some theoretical reasoning behind the choice of outcomes, it will be impossible to define effects in any specific area(s) of mental functioning. One way forward could be to start by defining the simpler cognitive functions which are affected, and then go on to investigate whether these effects are carried through to more-complex tasks or abilities such as educational achievement.

Another difficulty to be faced is in defining a mechanism for an effect. At least three different, though not mutually exclusive, pathways by which undernutrition may affect mental functioning have been proposed by Simeon and Grantham-McGregor[3]. First, there may be a direct anatomical or biochemical change to the central nervous system (CNS). For example, iron deficiency is postulated to have a biochemical link with behavioural alteration due to impaired catabolism of catecholamines, electron transport and porphyrin synthesis[36]. There is some evidence that immune responses to influenza may have a similarly direct effect on the CNS[37] and it is tempting to speculate that the chronic elevation of immune responses in helminthiasis might have similar consequences. Second, illness may reduce the activity of a child and thus reduce exposure to stimulation and opportunities for learning. Third, illness may alter the behaviour of the child such that there is a reciprocal lack of responsiveness in the caretaker (or teacher?) which further aggravates the problem. If helminth infection has an equally complex interaction with learning ability, then the search for a unique effect of infection is unlikely to be rewarded. Indeed, if nutrition *per se* plays a central causative role, studies which attempt to control for nutritional changes may actually remove the very mechanism by which worms affect cognition or educational achievement.

The major practical question is whether the effect of helminth infection on cognition is likely to be large enough to have developmental significance. If the correlational data are to be accepted uncritically, then they suggest a highly significant relationship between infection and practically important outcomes such as examination results and school grades. However, such interpretation is not valid since the relationships are undoubtedly confounded by socio-economic factors. One important finding in the Jamaican intervention study, however, was that infected children showed such an improvement in their performance in some tests that they became indistinguishable from children who were socio-economically much better placed. These results clearly need replicating, and the study also warrants further investigation to determine whether the improvement observed is reflected in any educational improvement.

We should expand our examination of the impact of parasitic helminths beyond the narrow definitions of clinical and physical health to include assessment of the effects of infection on the ability of children to benefit from primary schooling. It is difficult to overstate the importance of this. For many children in the developing world, those few years of basic education may be the only education they ever receive.

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# A comparison of some methods for the determination of methoxyl groups in commercial pectin preparations

M. Kujawski and T. Tuszyński

The results of methoxyl groups determination in pectin preparations were compared. The methods of direct (I, II) and indirect (III, IV) methanol determination connected with enzymatic (II, IV) or alkaline (I, III) pectin demethylation were applied. Higher values were obtained using indirect methanol methods than in the case of a direct methanol determination. The difference between these groups of methods was statistically significant. The precision of all the methods was high. The degree of methylation (DM) was calculated based on methanol content; moreover in the methods III and IV it was also determined from the ratio of methylated carboxyl groups to a total of acidic groups. From the difference (%) between these two ways of calculating DM the purity of pectin can be determined. The method based on enzymatic demethylation and direct methanol analysis is recommended as a precise and selective one.

## Introduction

The presence of pectin in plant raw material and the activity of pectin esterases lead to the liberation of methanol and cause its occurrence in food products. Of the three possible factors — thermal, chemical and enzymatic — which induce the deesterification of pectins, the latter have the largest share in the release of methoxyl groups [3, 7, 11, 12]. The heterogeneity of pectin esterases [4, 8, 9] as well as the diversity of pectic substances as regards their chemical structure and degree of methylation [1, 7, 11, 16] lead finally to some difficulties in the determination of the methanol released from pectins. Wucherpfennig et al. [15] differentiate in fruit juices the methyl alcohol already existing, potential, and total alcohol.

The determination of the content of methoxyl groups in pectins is of considerable importance in both theory and practice because it enables us to know better the structure of pectic substances, to determine the degree of pectin esterification, and to forecast the amount of methyl alcohol, which theoretically may be released under some determined conditions of the technological process [3, 6, 10, 11]. The precision of determining methoxyl groups in pectins depends not only on the purity of preparations and pectic substances but also on the analytical procedure. It may be assumed that all methods which serve to determine the activity of pectin esterases are — under suitable conditions — useful with the determination of methoxyl groups in the material investigated. We may distinguish here the methods based on direct and indirect determination of methanol. To the former group may be assigned the micromethod described by Wood et al. [14], and an automatic manner based on that principle to determine the activity of pectin esterases in the commercial preparations of that enzyme [13]. The methyl alcohol originating as a result of pectin deesterification may also be determined after its distillation [10, 12]. However, these methods are neither suitable for small samples nor convenient in the case of serial analyses.

The other group of methods includes those based on alkaline titration of carboxyl groups liberated during pectin deesterification [2, 5, 7, 11]. Demethylation can be performed by using acids or alkalies as well as enzymes. Considering the slow course of the former process, the alkaline or enzymatic demethylation is recommended [3].

The aim of the present study was to define the precision, accuracy and time intensity of the four methods of determining methanol and the degree of pectin esterification.

## Material and methods

Six commercial pectin preparations were examined: (A) "A" — Københavns Pektinfabrik (Denmark); (B) "LMC", sugarfree — POMOSIN (FRG); (C) "LM-350", degree of amidation 20—25% — CESALPINIA spa (Italy); (D) "PEKTOWIN" — (Poland); (E) "M4/FA" — UNIPECTINA spa (Italy); (F) "Genu Pectin, citrus", sugarfree — Københavns Pektinfabrik (Denmark). In order to remove sugar and other accompanying additives the pectin samples were washed with 5% HCl in 60% ethanol, next in neutral 60% ethanol, and at last in neutral 96% ethanol; finally they were dried at room temperature.

Demethylation of pectin samples and determination of methanol content were performed by applying four different methods. The first method (I) described by Wood et al. [14] consists in the saponification of esters with NaOH and determination of the methanol released in consequence of the reaction with acetylacetone (pentane-2,4-dione). The second method (II) of the deesterification of pectins made use of the pectin esterase preparation from orange peels, with an activity of 14.4 U/mg, produced by Sigma Chemical Company. The amount of enzyme and the duration of reaction were matched to make deesterification complete. The procedure was based on the paper by Wood et al. [14]. The third method (III) consisted in the alkaline demethylation of pectin by means of NaOH [2]. At first the free acidic groups were determined by titrating the sample with 0.1 N NaOH towards phenolphtaleine ("a" $cm^3$). Next the sample was strongly alkalified in order to obtain complete demethylation, and 2 h later there was determined the amount of alkalies ("b" $cm^3$) used to neutralize the demethylated carboxyl groups. The amount of the released methanol was calculated stoichiometrically, assuming that 1 $cm^3$ 0.1 N NaOH corresponds to 3.204 mg $CH_3OH$. At the application of the fourth method (IV) enzymatic pectin demethylation was carried out according to Kertesz [7]. The pectin esterase preparation described in method II was used. The pectin solution was brought up to pH 7.5 by means of 0.05 N NaOH ("a" $cm^3$) and next the enzyme was added. The reaction was carried out at room temperature with continuous mixing and titrating the reacting mixture with 0.05 N NaOH at such a rate to keep pH 7.5. The pectin deesterification was regarded to be finished when pH underwent no changes in the course of 15 min. The amount of the released methanol was calculated stoichiometrically, based on the "b" $cm^3$ value, like in method II.

The degree of pectin methylation — in % of the theoretical one — was calculated from the amount of the liberated methanol, assuming that the completely esterificated polygalacturonic acid contains 16.32% of methoxyl groups [3, 6]. Moreover, in the methods III and IV, the degree of carboxyl groups' methylation was calculated basing upon the ratio of the methylated carboxyl groups ("b") to a total of carboxyl groups ("a" + + "b") which were present in the pectin [2, 5].

## Results and discussion

The investigated commercial pectin preparations differed in their origin, the way they were obtained, purity and degree of methylation. It was known, that pectin D had been obtained from apples, and the others from citrus fruits. One pectin (E) was isolated as salt, another one (C) contained 20—25% of the carboxyl groups in the form of carbamyl. Two pectins (B and F) contained no sugar addition. Preliminary washing of these pectins in slightly acidified 60% ethanol resulted in obtaining preparations free of additives but containing ester-bound methanol, as well as pectin components, which were not polyuronide. The purity[1] of pectins determined by the authors varied, being in the range from 63,6% for pectin D to 100% for pectin B.

[1] In their considerations, the authors mean the "purity" of pectin as the percentage of polyuronide in pectin.

Table 1
Dispersion of results of methanol determination by four methods (I—IV) in six different pectins

| Pectin | MeOH content [%] | |
|---|---|---|
| | Dispersion of means | $\bar{x}$ (methods I—IV) |
| A | 8.91—9.82 | 9.36 |
| B | 4.18—4.60 | 4.46 |
| C | 3.59—4.45 | 3.94 |
| D | 6.10—6.57 | 6.36 |
| E | 2.13—4.34 | 2.99 |
| F | 8.96—9.82 | 9.36 |

A = Pectin "A" (Denmark)
B = Pectin "LMC" (FRG),
C = Pectin "LM-350" (Italy),
D = Pectin "PEKTOWIN" (Poland),
E = Pectin "M4/FA" (Italy),
F = Pectin "Genu Pectin, citrus" (Denmark)

For each of the investigated pectin preparations (A—F) the results differed, depending on the analytical method applied (I—IV). The dispersion of results (Table 1) was influenced by the kind of pectin. Neither the purity of pectin nor the degree of methylation had any influence on those differences. Instead, it may be assumed, that the fact of binding the carboxyl groups by other than methyl groups brings about certain difficulties in the analytical procedure.

In order to assess the precision of the methods applied the standard deviation $\sigma$ and variation coefficient V [%] were calculated for each of the series determinations (Table 2, A—F). All the four methods proved to be precise enough, but the results obtained by enzymatic methods (II and IV) showed a lesser variation. It is characteristic that the methods III and IV, in which methanol is calculated on the basis of titrimetric determination of released carboxyl groups, result in higher values than the methods I and II, in which methanol is determined directly. The differences between the methods were statistically calculated using the STUDENT's t-test. At the level of significance 0.05 the differences between the methods I/II and III/IV are not significant in four out of six pectins investigated. Only in one out of six pectins there are no statistically significant differences between the methods II/III and II/IV. The differences observed between the methods I/III and I/IV are statistically significant for every pectin. It seems that of the two pronouncedly different groups of methods the former, (I, II) in which methanol is determined directly, is more accurate, because the concentration of that alcohol is compared with standard solutions. The alkalimetric determination of acidic compounds (methods III and IV) which are present in such indefinite substances as pectins, is — according to the authors — less specific.

The degree of methylation of pectins (DM) is a result of the determined content of methoxyl groups. For that reason the values obtained by the methods III and IV are higher than those by the methods I and II. It is interesting to compare the two manners of calculating the DM which were applied to obtain the results by the methods III and IV. Great differences

Table 2
Methanol liberated as a result of pectin deesterification and determined by four different methods

| Method | Number of samples | Methanol content $\bar{x}$ [%] | Standard deviation $\sigma$ | Variation coefficient V [%] |
|---|---|---|---|---|
| Pectin A | | | | |
| I | 15 | 8.91 | 0.39 | 4.38 |
| II | 6 | 8.91 | 0.15 | 1.68 |
| III | 6 | 9.82 | 0.21 | 2.14 |
| IV | 6 | 9.80 | 0.06 | 0.56 |
| Pectin B | | | | |
| I | 16 | 4.18 | 0.17 | 4.07 |
| II | 6 | 4.47 | 0.21 | 4.70 |
| III | 6 | 4.60 | 0.40 | 8.70 |
| IV | 9 | 4.57 | 0.08 | 1.75 |
| Pectin C | | | | |
| I | 15 | 4.00 | 0.22 | 5.50 |
| II | 6 | 3.59 | 0.09 | 2.51 |
| III | 6 | 4.45 | 0.24 | 5.39 |
| IV | 6 | 3.70 | 0.05 | 1.30 |
| Pectin D | | | | |
| I | 16 | 6.27 | 0.26 | 4.15 |
| II | 6 | 6.10 | 0.16 | 2.62 |
| III | 6 | 6.49 | 0.19 | 2.93 |
| IV | 6 | 6.57 | 0.05 | 0.76 |
| Pectin E | | | | |
| I | 13 | 2.13 | 0.45 | 21.10 |
| II | 6 | 2.40 | 0.06 | 2.50 |
| III | 6 | 4.34 | 0.39 | 8.99 |
| IV | 6 | 3.09 | 0.10 | 3.24 |
| Pectin F | | | | |
| I | 15 | 8.96 | 0.36 | 4.02 |
| II | 6 | 8.98 | 0.21 | 2.34 |
| III | 6 | 9.66 | 0.20 | 2.07 |
| IV | 6 | 9.81 | 0.18 | 1.84 |

are observed here, depending on the kind of pectin (Table 3). Attention should be drawn to the fact, that the ratio b:(a + b) is independent of the amount of non-polyuronide substances and of the presence of additives contained in the commercial pectin preparations (except acid substances). The DM calculated in that way by DRZAZGA [2] is not reliable with respect to the amount of methoxyl groups really present in the pectin preparation. The purity of pectin — defined by the authors above — may, according to them, be calculated by comparing DM obtained from the amount of methanol "b" with DM calculated from the ratio "b:(a + b)" in the methods III or IV. The greater the differences [%] the less pure is the pectin. The degree of pectin methylation and the determined purity of the studied preparations are shown in Table 3.

Summing up, it should be stated that all the methods presented and applied for the determination of methoxyl groups in pectin preparations are sufficiently precise. However, the micromethods I and II seem to be more accurate because they are based on direct determination of methanol, which makes them more specific. The analytical procedure is not

Table 3
Degree of pectin esterification (DM) and their purity determined by different methods

| Pectin | DM [%] | | | | | | Purity [%] | |
|---|---|---|---|---|---|---|---|---|
| | Method I | Method II | Method III | | Method IV | | Method III | Method IV |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| A | 54.59 | 54.59 | 60.17 | 64.94 | 60.05 | 64.46 | 92.6 | 93.2 |
| B | 25.61 | 27.39 | 28.19 | 27.78 | 28.00 | 27.45 | 100 | 100 |
| C | 24.51 | 22.00 | 27.27 | 36.17 | 22.67 | 32.53 | 75.4 | 69.7 |
| D | 38.42 | 37.38 | 39.77 | 62.54 | 40.26 | 62.78 | 63.6 | 64.1 |
| E | 13.05 | 14.71 | 26.59 | 26.01 | 18.93 | 21.45 | 100 | 88.2 |
| F | 54.90 | 55.02 | 59.19 | 66.36 | 60.17 | 67.81 | 89.2 | 88.7 |

1 and 2 — based on direct determination of MeOH
3 and 5 — based on indirect determination of MeOH
4 and 6 — based on the ratio $b:(a + b)$
7 = 3:4
8 = 5:6

complicated in these cases, and the methods are suitable for serial analyses. The enzymatic demethylation of pectin samples using pectin esterase destined for analytical purposes should be preferred.

**Zusammenfassung**

M. KUJAWSKI und T. TUSZYŃSKI: Vergleich einiger Methoden zur Bestimmung der Methoxylgruppen in kommerziellen Pektinpräparaten

Es werden die Ergebnisse der Bestimmung von Methoxylgruppen in Pektinen mit direkten (I, II) und indirekten (III, IV) Methanolbestimmungen in Verbindung mit enzymatischen (II, IV) und alkalischen (I, III) Pektindemethylierungen verglichen. Bei der indirekten Methanolbestimmung (III, IV) werden höhere Werte als bei der direkten Methode (I, II) erhalten. Die Unterschiede zwischen diesen Methodengruppen sind statistisch signifikant. Die Präzision der Methoden ist in allen Fällen sehr hoch. Der Grad der Pektinmethylierung wird auf Grund des Methanolgehalts berechnet. Bei den Methoden III und IV wird dieser zusätzlich aus dem Verhältnis der methylierten zu den Gesamtcarboxylgruppen ermittelt.

Aus dem Unterschied (in %) zwischen beiden Berechnungsweisen kann der Reinheitsgrad der Pektinproben bestimmt werden. Als präzise und genaue Verfahrensweise wird die der enzymatischen Demethylierung und der direkten Methanolbestimmung empfohlen.

**Резюме**

М. Куявски и Т. Тушынски. Сравнение некоторых методов для определения метоксиловых групп в коммерческих препаратах пектина

В настоящей работе сравниваются результаты определения метоксиловых групп пектина, полученных четырьмя разными методами. Исследованы методы прямого (I, II) и непрямого (III, IV) определений метанола в соединении с ферментативным (II, IV) и щелочным (I, III) деметилированием

пектина. Из данных вытекает, что значения полученные при помощи прямого метода определения метанола превышает те, которые были получены непрямым методом. Различия между этими группами методов являются статистически достоверными. Определена точность метода, которая была во всех случаев очень высока. Подсчитана степень метилирования пектина на основе содержания метанола, а в методах III и IV дополнительно из соотношения метилированных групп к общему числу карбоксиловых групп. Из разницы (в %) между этими двумя способами подсчетов можно определить степень чистоты образцов пектина (в %). Для достижения точных результатов рекомендуется применение метода ферментативного деметилирования и метода прямого определения метанола.

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