Acta vet. scand. 1975, *16*, 24—30.

From the Research Station of the Veterinary Institute, Skara, Sweden.

PYRIMIDINYL NICOTINIC ACID AND CEREBROCORTICAL NECROSIS

By

Claes-Göran Lilja

LILJA, CLAES-GÖRAN: *Pyrimidinyl nicotinic acid and cerebrocortical necrosis.* Acta vet. scand. 1975, *16*, 24—30. — Pyrimidinyl nicotinic acid (PNA) exhibits great structural similarities to amprolium, which is a known thiamine antagonist. Experimental cerebrocortical necrosis (CCN) has been induced by oral administration of amprolium. PNA has been demonstrated in ruminal fluid from animals suffering from CCN.

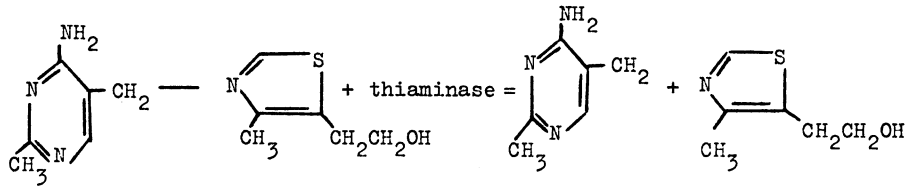
The aim of this investigation was to study whether PNA can act as thiamine antagonist and whether it can give rise to CCN. For this purpose PNA was synthesized and was daily given intravenously to a calf. The dose corresponded to roughly 10 times the content of thiamine in the blood. After three weeks the dose was doubled. During the entire experimental period comprising nine weeks no clinical sign of thiamine deficiency or CCN was noticeable. The values for all recorded blood chemical parameters, with the exception of occasional GOT and PK values, were within the normal limits of variation.

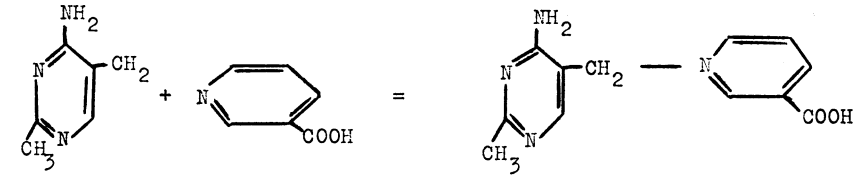
Rats were used in a similar experiment with the same aim. PNA was homogeneously added to their feed in quantities equivalent to five and 10 times the thiamine content. The rats were clinically healthy throughout the experimental period comprising eight weeks. No significant difference in TK activity and TPP effect was observed between the experimental groups and the control group.

cerebrocortical necrosis; polioencephalomalacia; pyrimidinyl nicotinic acid; thiamine; thiamine antagonism.

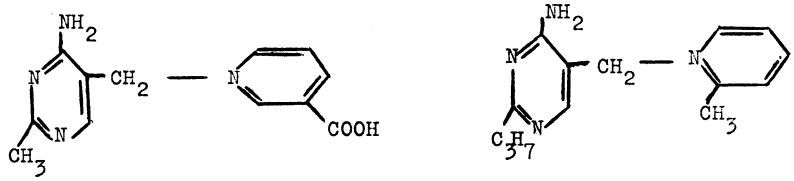
In the search for the aetiology of cerebrocortical necrosis (CCN) in ruminants the main interest recently has been in the study of thiaminases and thiamine antagonists. Under the influence of thiaminase I thiamine is decomposed at the methylene group, so that the thiazole group could be replaced by other nitrogen containing rings. *Edwin & Jackman* (1970) showed that on incubation of thiamine and ^{14}C-labelled nicotinic acid with ruminal fluid from animals suffering from CCN a sub-

stance was demonstrable, both by means of autoradiography and the ultraviolet absorption spectrum, which was identical to synthetic pyrimidinyl nicotinic acid (PNA). The reaction may be illustrated as follows:





The reaction product exhibits great structural similarities to amprolium.



Amprolium is a known thiamine antagonist, and experimental CCN has been induced by oral administration of the substance (*Markson et al.* 1972, *Lilja* 1973). If PNA as well can act as thiamine antagonist, the above reaction will have the character of so-called lethal synthesis. This means that the presence of thiaminase I in the rumen gives rise to a rapid thiamin deficiency. In the first place thiamine administered or formed is decomposed, and secondly an active antagonist is formed.

The aim of this investigation was to study whether PNA can act as thiamine antagonist and whether it can give rise to CCN.

MATERIAL AND METHODS

PNA was synthesized ad modum *Matsukawa & Yurugi* (1954). The substance was identified as follows. The decomposition temperature was determined and found to accord closely with that given in the literature. Paper electrophoresis showed a distinct band which clearly differed both from thiamine and nicotinic acid. The synthesized substance showed a positive reaction to Dragendorff's reagent. After spraying with potassium ferrocyanide a bluish-white fluorescence was obtained in long-wave u.v. light both for the reaction product and thiamine.

Through intravenous injections a calf was daily given 6 mg PNA, which corresponds to roughly 10 times the content of thiamine in the blood. The blood volume of the calf was estimated at 1/13 of its body weight, 75 kg, to 5.8 l. The normal figure for total thiamine in the blood of calves aged two to nine months is 8.9 ± 3.5 γ/100 ml ($\bar{x} \pm 2$ s, own investigations). After three weeks the PNA-dose was doubled. Weekly blood samples were taken for determination of the following enzyme reactions: creatine phosphokinase (CPK), glutamic oxaloacetic transaminase (GOT), pyruvate kinase (PK), transketolase (TK), and thiamine pyrophosphate (TPP) effect. The blood glucose was also determined.

Rats were used in a similar experiment with the same aim. They were divided into three groups of nine. All were given pelleted feed for laboratory animals (Astra-Ewos, Södertälje), containing a specific quantity of thiamine (5.1 mg/kg). In the feed for the experimental groups I and II PNA was added homogeneously in concentrations five and 10 times the quantity of the thiamine constituent. Once a week one animal from each of the two experimental groups and one from the control group were sacrificed by tapping of blood direct from the heart for determination of TK activity and TPP effect.

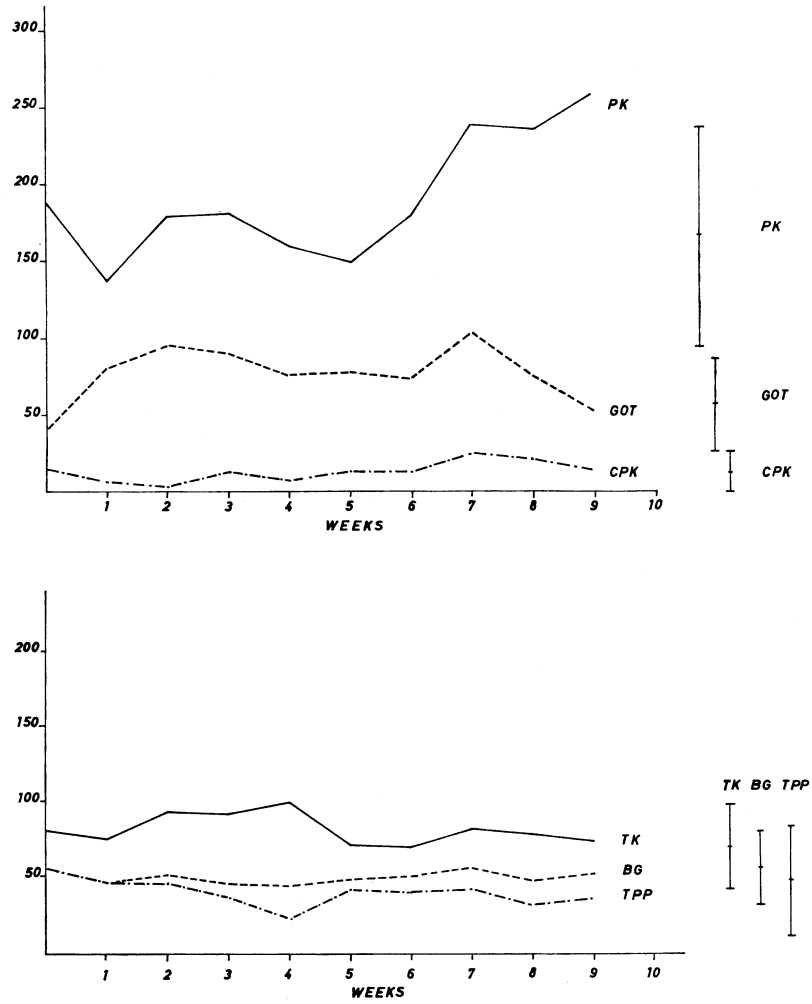
The following analytical methods were used:

Blood glucose and serum GOT:	AB KABI's standard method
CPK and PK:	Boehringer's standard method
TK and TPP:	ad modum *Schouten et al.* (1964)

RESULTS AND DISCUSSION

During the entire experimental period comprising nine weeks the calf was healthy by clinical assessment and no sign of vita-

min B_1 deficiency was noticeable, nor any symptoms similar to those seen in CCN. During the first seven weeks of the experiment the calf increased in weight 1.13 kg per day. Throughout the experimental period the values for all recorded blood parameters, with the exception of occasional GOT and PK values, were within the normal limits of variation (Figs. 1 and 2).



Figures 1—2. PK (mu/ml), CPK (mu/ml) and GOT (Karmen u/ml) in serum, glucose (mg/100 ml), TK activity and TPP effect (i.u. as given by *Schouten et al.* 1964) in blood from a calf given pyrimidinyl nicotinic acid intravenously for some weeks. The vertical lines indicate normal values (mean ± 2 s).

The rats were clinically healthy throughout the experimental period. No significant difference was observed between the experimental groups and the control group. A tendency to increasing transketolase activity with rising age of the animals was noticeable for all groups (Fig. 3). The mean TK values showed close conformity between the groups but had large standard deviations (Table 1).

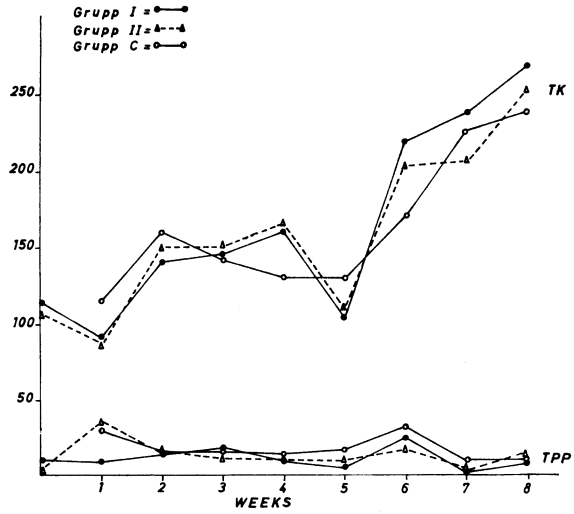


Figure 3. TK activity and TPP effect in blood (i.u. as given by *Schouten et al.* 1964) of rats fed pyrimidinyl nicotinic acid for eight weeks.

Table 1. TK activity and TPP effect in blood of rats fed various concentrations of PNA.

	Group I (≈ 25 p.p.m.)	Group II (≈ 50 p.p.m.)	Control group (0 p.p.m.)
TK	n = 9 $\bar{x}$ = 164.4 s = 62.7	n = 9 $\bar{x}$ = 160.1 s = 55.0	n = 9 $\bar{x}$ = 163.8 s = 45.1
TPP	n = 9 $\bar{x}$ = 5.7 s = 3.5	n = 9 $\bar{x}$ = 6.7 s = 4.9	n = 8 $\bar{x}$ = 9.1 s = 4.2

Smith & Healy (1968) recorded elevated CPK concurrently with normal GOT values in spontaneous cases of CCN. Elevated PK values have also been reported (*Edwin* 1970). The same pat-

tern has been observed in amprolium-induced CCN (*Lilja* 1973). The transketolase activity appears to vary directly with the level of available co-enzyme (cocarboxylase), but may possibly be affected by metabolic disorders other than a thiamine deficiency. The TPP effect, however, is very specific and a significant rise must therefore reflect a thiamine deficiency (*Dreyfus* 1962).

CONCLUSIONS

Under the experimental conditions described PNA showed no thiamine-antagonistic effect nor the ability to induce cerebrocortical necrosis.

REFERENCES

Dreyfus, P. M.: Clinical application of blood transketolase determinations. New Engl. J. Med. 1962, *267,* 596—598.

Edwin, E. E.: Plasma enzyme and metabolite concentrations in cerebrocortical necrosis. Vet. Rec. 1970, *87,* 396—398.

Edwin, E. E. & R. Jackman: Thiaminase I in the development of cerebrocortical necrosis in sheep and cattle. Nature (Lond.) 1970, *228,* 772—774.

Lilja, C. G.: Cerebro-cortical necrosis (C.C.N.) in calf. An experimental reproduction of the disease. Acta vet. scand. 1973, *14,* 464—473.

Markson, L. M., G. Lewis, S. Terlecki, E. E. Edwin & J. E. Ford: The aetiology of cerebrocortical necrosis: The effects of administering antimetabolites of thiamine to preruminant calves. Brit. vet. J. 1972, *128,* 488—498.

Matsukawa, T. & S. J. Yurugi: On thiamine destructive factor. J. Vitaminol. 1954, *1,* 27—38.

Schouten, H., L. W. Statius van Eps & A. M. Struyker Boudier: Transketolase in blood. Clin. chim. Acta 1964, *10,* 474—476.

Smith, J. B. & P. J. Healy: Elevated serum creatine phosphokinase activity in diseases of the central nervous system in sheep. Clin. chim. Acta 1968, *21,* 295—296.

SAMMANFATTNING

Pyrimidinyl nikotinsyra och cerebrocortical nekros.

Pyrimidinyl nikotinsyra (PNS) uppvisar stora strukturella likheter med amprolium, som är en känd tiaminantagonist. Experimentellt har cerebrocortical nekros (CCN) hos idisslare framkallats genom per oral tillförsel av amprolium. PNS har påvisats i våminnehåll från djur med CCN.

Syftet med föreliggande undersökning var att studera om PNS kan verka som tiaminantagonist och om den kan ge upphov till CCN. För detta ändamål syntetiserades PNS och gavs dagligen intravenöst till en kalv. Dosen motsvarade c:a tio gånger blodets innehåll av

tiamin. Efter tre veckor dubblerades dosen. Under hela försöksperioden som varade nio veckor kunde inga kliniska tecken på tiaminbrist eller CCN påvisas. Värdena för alla registrerade kliniskt-kemiska parametrar, med undantag av enstaka GOT- och PK-värden, låg inom de normala variationsgränserna.

I ett annat försök inblandades PNS i fodret åt råttor i mängder motsvarande fem och tio gånger fodrets innehåll av tiamin. Råttorna var kliniskt friska under hela den åtta veckor långa försöksperioden. Ingen signifikant skillnad i TK-aktivitet och TPP-effekt i blodet förelåg mellan försöksgrupperna och kontrollgruppen.

(Received May 24, 1974).

Reprints may be requested from: Claes-Göran Lilja, Research Station of the Veterinary Institute, S-532 00 Skara, Sweden.