Acta vet. scand. 1963, 4, 156—169.

From the Department of Pharmacology and Toxicology,
The Royal Veterinary and Agricultural College, Copenhagen.

ELIMINATION OF PARATHION IN COWS AFTER ORAL AND DERMAL ADMINISTRATION[1])

By
A.-M. Andersen and *O. Karlog*

Adult ruminants show an unusual tolerance to orally administered parathion. Thus, for instance, daily doses of 32 mg. per kilogram can be given for one week to cows, without any measurable inhibition of cholinesterase (*Pankaskie, Fountaine & Dahm* 1952). Following dermal application of parathion, on the other hand, cows show no greater tolerance than other animal species (*Dalgaard-Mikkelsen* 1956). This discrepancy seems to be referable solely to the fermentative, metabolic processes which parathion undergoes under the influence of the ruminal flora. *Cook* (1957) and *Ahmed, Casida & Nichols* (1958) have shown that parathion in rumen fluid, *in vitro* as well as *in vivo*, by reduction of the nitro-group is converted into aminoparathion. The latter is 100 times less toxic than parathion (*Ahmed et al.* 1958).

The data available concerning a possible excretion of parathion and its metabolites into milk from cows treated with parathion are of particular interest from the standpoint of food-hygiene. *Ahmed, Casida & Nichols* (1958), who worked with radioactively labelled parathion, found about 1 per cent of the given dose to be excreted in the milk. However, *Dahm, Fountaine, Pankaskie, Smith & Atkeson* (1950), *Pankaskie et al.* (1952), and *Gyrisco, Norton, Trimberger, Holland, McEnerney & Muka* (1959) were unable to trace parathion or its metabolites in milk from experimental cows fed with parathion-contaminated lucerne or given large doses in capsules.

---

[1]) Aided by a grant from *Landbrugsministeriets Giftnævn.*

After dermal application, on the other hand, the biotransformation of parathion must be supposed to proceed along other routes than after oral intake. Hence, definite conclusions regarding the mammary excretion cannot be drawn solely from experiments with oral administration. Comparative investigations have therefore been made to throw light on the influence of the two modes of application on the metabolism and excretion of parathion.

ANALYTICAL METHODS

Parathion, paraoxon and p-nitrophenol

Quantitative determination and identification were performed as described by *Karlog* (1957). However, the method for determining total p-nitrophenol was modified in that 1 ml. of 30 % hydrogen peroxide was used at saponification. This appreciably reduced the background in the spectrophotometric analysis (*Buckley & Colthurst* 1954).

Aminoparathion and aminoparaoxon

The total amount of the two compounds was determined quantitatively, using the method of *Averell & Norris* (1948). For analyses of rumen fluid samples it was necessary to modify the procedure as follows:

To a 6.0 ml. sample of rumen fluid, 6.75 ml. of 99 % alcohol was added and the mixture was shaken vigorously for 2 minutes and then centrifugated in 30 min (2000 r.p.m.). An 8.5 ml. sample of the clear supernatant, corresponding to 4.0 ml. of rumen fluid, was removed and to this was added 1.1 ml. of 2 M HCl and 0.1 ml. of 0.25 % sodium nitrite. This was again shaken, allowed to stand for 10 minutes and 0.1 ml. of 2.5 % ammonium sulphamate was added. The solution was again shaken for 15 seconds allowed to stand for ten minutes and 0.2 ml. of 1 % of N-(1-naphtyl) — ethylen diamine dihydrochloride was added. After a final 15 seconds of shaking the solution was allowed to stand for 10 minutes and then read in a spectrophotometer at a wave length of 555 mμ.

Aminoparathion and aminoparaoxon were prepared by reducing parathion and paraoxon with zinc and hydrochloric acid (*Averell & Norris* 1948). These preparations were used for standards and rumen fluid, withdrawn immediately before the experiment, was used as the blank.

Aminoparathion and aminoparaoxon were identified and separated by paper chromatography. After repeated extraction from an alkaline medium with chloroform and evaporation of the extracts descending chromatography was performed at 27°C, using chloroform saturated with 0.4 M HCl as mobile phase and 0.4 M HCl half-saturated with NaCl and saturated with chloroform as stationary phase. The chromatogram was developed by diazotation with a 2.5 % solution of $NaNO_2$ in 72 % ethanol. Excess $NaNO_2$ was removed with 25 % ammonium sulphamate in 72 % ethanol. After drying the colour was developed by a 10 % solution of N-(1-naphthyl)-ethylenediamine dihydrochloride in 72 % ethanol. The method gave the Rf values: 0.56 for aminoparathion and 0.87 for aminoparaoxon (sensitivity 5 μg).

Intermediates between parathion and aminoparathion

In samples of rumen fluid parathion and possibly existing metabolites at a stage of oxidation intermediate between those of parathion and aminoparathion could be reduced to aminoparathion by zinc and hydrochloric acid. The pool was thereafter determined quantitatively according to *Averell & Norris* (1948). After separate determinations of parathion and aminoparathion, the concentration of these two compounds was subtracted from the total amount. The difference then served as a measure of not fully reduced intermediates of parathion.

Free and conjugated p-aminophenol

Quantitative determination was performed using the method of *Brodie & Axelrod* (1948). The conjugated p-aminophenol was, however, hydrolysed in sealed ampoules by boiling for 6 hours. The amount of p-aminophenol was determined spectrophotometrically after conjugation with o-cresol.

RESULTS

In vitro conversion of parathion in rumen fluid

In preliminary *in vivo* experiments on samples of rumen fluid withdrawn by stomach tube at different intervals after oral administration of parathion, the conversion of parathion was found to proceed too rapidly for homogenous mixing and succeeding quantitative determinations. *In vitro* experiments were therefore conducted.

The animals were fed with the same mixture — hay, beet, straw, rolled oats, and high-protein concentrates, as well as water *ad lib.* — as was given for subsequent investigations of the renal and mammary excretion. Rumen fluid was withdrawn by stomach tube 2 hours after the morning feeding and the pH of the fluid was determined potentiometrically. Parathion, dissolved in 2 ml. of 96 % ethanol, was then added to give a final concentration of 4 mg. per 100 ml. of rumen fluid. The parathion was added within 30 minutes of the time of sample collection. After vigorous shaking the rumen fluid was incubated at 38°C. Samples were drawn for analysis every 10 minutes. Fermentation processes were stopped by adding either 36 % hydrochloric acid, 10 ml. per 100 ml., or 96 % ethanol to a final concentration of 40—50 % ethanol.

The samples were then analysed for parathion, aminoparathion, paraoxon, aminoparaoxon, p-nitrophenol, p-aminophenol and after reduction with zinc and hydrochloric acid, for aminoparathion. Of these compounds, only parathion, aminoparathion, and conjugated p-aminophenol were demonstrated. By calculation of differences intermediates between parathion and aminoparathion were detected. The presence of other hydrolysable p-aminophenol compounds besides aminoparathion was also calculated as the difference between the total amount of conjugated p-aminophenol, determined according to *Brodie & Axelrod*, and aminoparathion. Free p-aminophenol was not demonstrated.

The results of six incubation experiments are shown in Table 1. After 10 minutes of incubation only 12—13 per cent of the added amount of parathion was recovered as the original com-

Table 1.

Biotransformation of parathion in rumen fluid *in vitro* at 38°C.

Incubation period	pH	Total amount recovered % of added	Parathion %	Aminoparathion %	Intermediates between parathion and aminoparathion %	Conjugated p-aminophenol %
10 min.	6.8	94	13	15	57	9
10 min.	6.9	100	12	15	60	9
10 min.	6.9	112	12	14	64	22
20 min.	6.9	103	5	65	28	5
20 min.	6.8	108	5	48	30	25
20 min.	7.1	107	7	57	22	21

pound, and after 20 minutes no more than 5 per cent. Intermediates were found in large amounts after 10 minutes, whereas after 20 minutes the change into aminoparathion was so advanced that the intermediates showed a corresponding fall. Other conjugated p-aminophenol compounds which, unlike aminoparathion, could be regarded as reduced hydrolytic products, constituted maximally one-fourth of the total amount of metabolites.

In control analyses of boiled rumen fluid 99 per cent of the added parathion was recovered as the original compound after 30 minutes' incubation at 38°C.

Renal and mammary excretion after oral administration of parathion

Two Jersey cows, weighing 350 and 435 kg., were used as experimental animals. At the start of the experiment a Rüsch balloon catheter was introduced into the bladder for the continuous collection of urine during the first 50 hours. Milk samples were drawn at the ordinary evening and morning milkings. Blood samples were withdrawn from the jugular vein for cholinesterase determination on whole blood, using the ordinary Warburg technique (*Augustinsson* 1948).

Parathion was administered by stomach tube as a 1 % aqueous parathion emulsion in doses of 1 and 10 mg. per kilogram body weight respectively.

The cows displayed no signs of poisoning during the experimental period and no inhibition of blood cholinesterase activity was demonstrated in any of the blood samples.

Analyses of urine specimens for parathion, aminoparathion, paraoxon, aminoparaoxon, p-nitrophenol, and p-aminophenol revealed the presence of p-nitrophenol and conjugated p-aminophenol, whereas none of the above mentioned oxidation or reduction products were demonstrable. The results are illustrated in Figs. 1 and 2, which show that conjugated p-aminophenol was the dominant metabolite (97 per cent), and that the excretion was complete in about 40 hours. The metabolites recovered in the urine corresponded to 38 and 25 per cent respectively of the amounts of parathion given to the animals.

No parathion, paraoxon, aminoparathion, aminoparaoxon, p-nitrophenol, and p-aminophenol could be demonstrated in the milk samples.

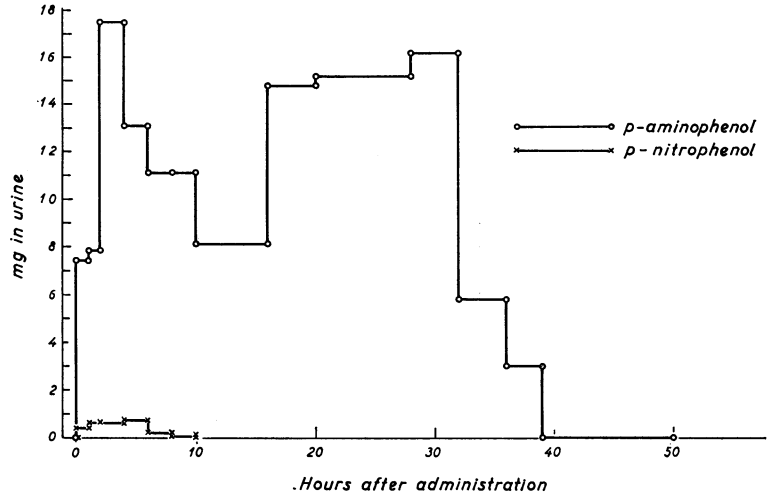


Fig. 1. Urinary excretion of p-nitrophenol and conjugated p-aminophenol in a cow after oral administration of parathion (1 mg./kg. b.w.).
Abscissa: Time after administration.
Ordinate: Total amounts of p-nitrophenol and conjugated p-aminophenol.

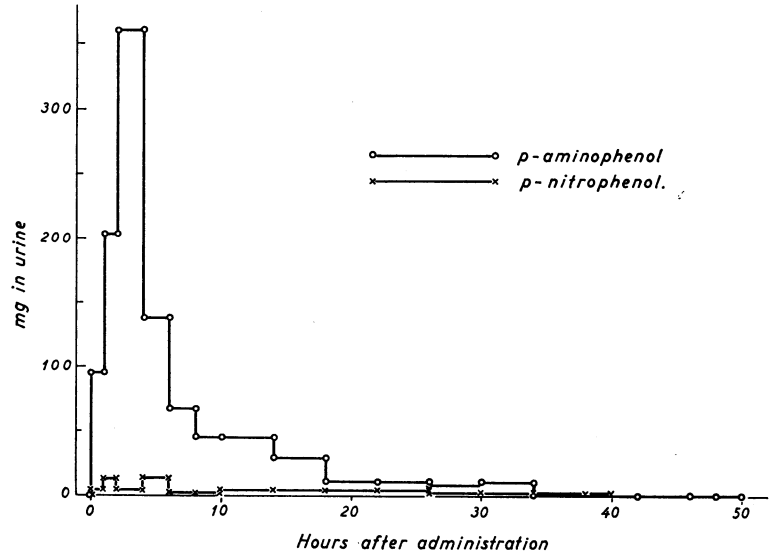


Fig. 2. Urinary excretion of p-nitrophenol and conjugated p-aminophenol in a cow after oral administration of parathion (10 mg./kg. b.w.).
Abscissa: Time after administration.
Ordinate: Total amounts of p-nitrophenol and conjugated p-aminophenol.

Renal and mammary excretion after dermal application of parathion

The cows used were the same as in the experiments with oral administration. During the experimental period the animals were tied in box stalls in a manner which prevented them from licking the body. A Rüsch balloon catheter was introduced into the bladder for quantitative and continuous collection of urine. Milk samples were drawn morning and evening, and blood samples were taken at regular intervals.

Parathion was applied as a 0.05 per cent aqueous emulsion in a dose corresponding to 1 mg. per kilogram body weight, distri-

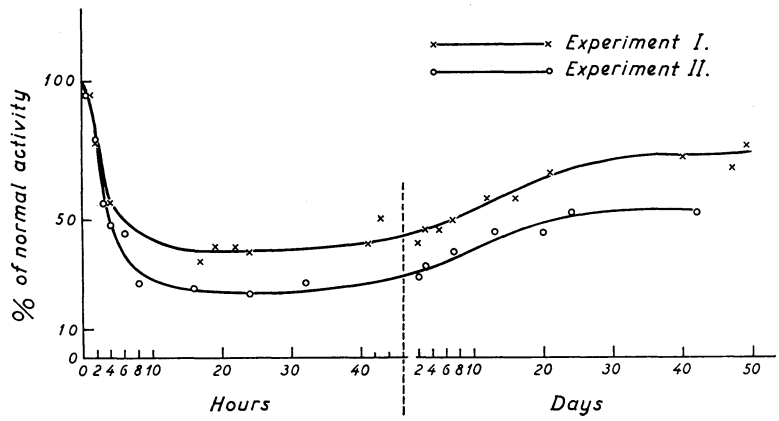


Fig. 3. Cholinesterase inhibition in whole blood from two cows after dermal application of parathion (1 mg./kg. b.w.).
Abscissa: Time after application.
Ordinate: Relative activity.

buted over back and flanks. Samples of hair were cut daily for analysis.

The animals had diarrhoea and a poor appetite 14 to 24 hours after the application. The development of these symptoms coincided, as shown in Fig. 3, with an appreciable inhibition of the cholinesterase activity in whole blood. The graph also shows that the activity had not returned to normal 50 days after the application.

Examination of the hair samples showed an uneven distribution of parathion in the coat. Therefore elimination from the body in this way could not be followed quantitatively. The analysis revealed, however, that parathion is not converted in the

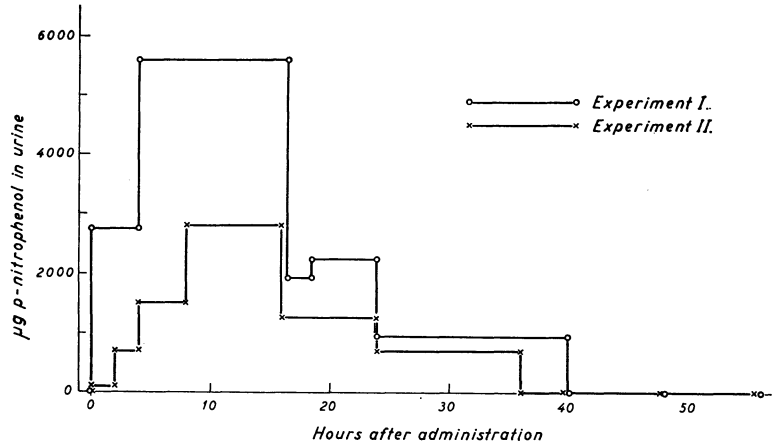


Fig. 4. Urinary excretion of p-nitrophenol after dermal application of parathion (1 mg./kg. b.w.). Experiments on two cows.
Abscissa: Time after administration.
Ordinate: Microgram p-nitrophenol.

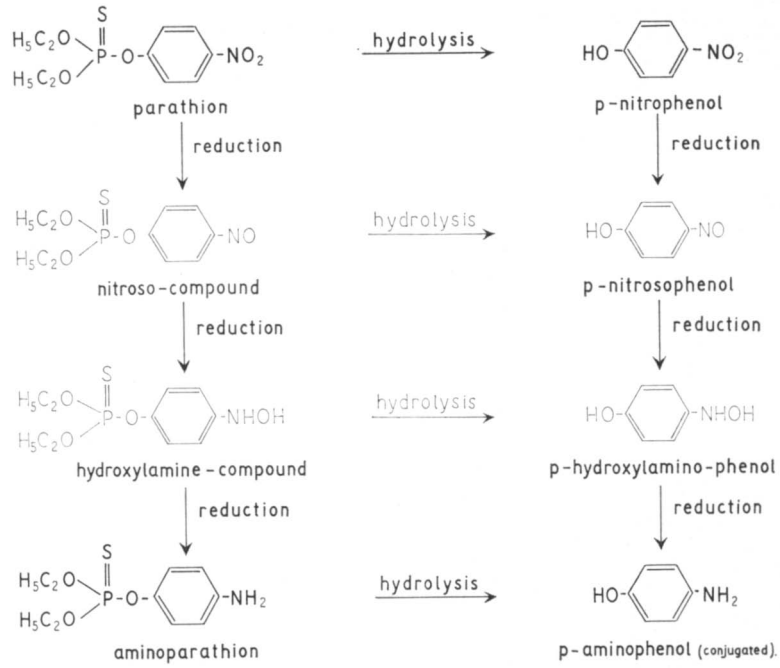
coat, as only parathion was demonstrable, but no paraoxon, aminoparathion, aminoparaoxon, nor p-nitrophenol or p-aminophenol. The amount in the hair decreased from an initial concentration of 300—900 µg. per gram hair to zero within 12 days.

In the urine samples p-nitrophenol was the only metabolite detectable. The excretion is illustrated in Fig. 4, which shows that p-nitrophenol was already present in the urine after the first hour, and that by far the largest amount was excreted within 16 to 17 hours. Forty hours after the start of the experiment the p-nitrophenol concentration was too low to be measured by this method, *i. e.* under 0.1 µg./ml.

As considerable amounts of parathion were removed by cutting hair samples for analysis, no attempt was made to calculate the proportion of applied parathion which was excreted as p-nitrophenol. Neither parathion nor any of the metabolites previously mentioned could be detected in the milk samples.

DISCUSSION

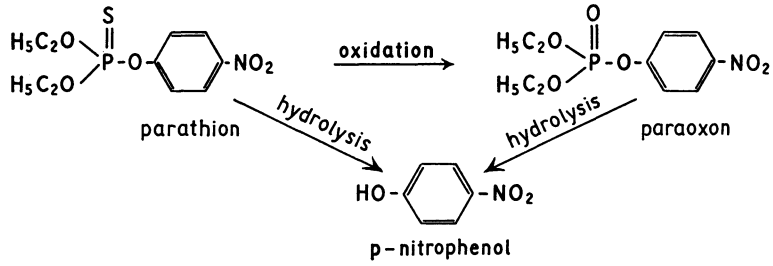
The results of the present investigation bear out the view that the metabolism of parathion depends on the mode of administration. After *oral* administration parathion, like various other nitro compounds (see *Phillipson* 1955), was reduced to the cor-



F i g. 5. The possible transformation of parathion after oral administration in cows.

responding amino compound *via* the microbial fermentative processes in the rumen. *Cook* (1957) has shown that this reduction is almost complete after one hour of incubation *in vitro*. In our experiments 75 to 90 per cent of the parathion had already been reduced to aminoparathion or intermediates after 10 minutes. The chemical configuration of these intermediates has not been established, but, like the conversion products between p-nitrophenol and p-aminophenol, they may very likely, as shown in fig. 5, be nitroso or hydroxylamine derivatives (*Gardocki & Hazleton* 1951). Like *Ahmed, Casida & Nichols* (1958), we found hydrolytic products in rumen fluid, and conjugated p-aminophenol as the dominant metabolite in the urine. While *Ahmed et al.* (1958) regard aminoparathion as the principal compound in the urine, *Pankaskie et al.* (1952) take p-aminophenylglucoronide to be the main product after oral administration of parathion. *Ahmed et al.* (1958), using their isotope technique, also demonstrated parathion and paraoxon in blood samples after administration of 6.7 mg. parathion per kilogram body weight to a cow. For paraoxon they stated values of 0.29 p.p.m. 30 minutes after the admini-

stration and 0.14 p.p.m. 2 hours after. This finding seems, however, to be inconsistent with the fact that they found an uninhibited cholinesterase activity in the withdrawn blood samples, as was also the case in the present investigation. *Fallsheer & Cook* (1956), on the other hand, found 50 per cent cholinesterase inhibition in *in vitro* experiments at paraoxon concentrations of 0.006 p.p.m. The reduction processes in the rumen are therefore likely to be so complete that very large doses are required for intact parathion to be absorbed in such an amount that sufficient paraoxon can be produced for cholinesterase inhibition. The feeding experiments of *Pankaskie, Fountaine & Dahm* (1952) in which daily doses of parathion as high as 32 mg./kg. did not



F i g. 6. Transformation of parathion after dermal application in cows.

inhibit blood cholinesterase activity, seem to justify this hypothesis.

After *dermal* application of parathion the only metabolite demonstrable in the urine was the hydrolytic product p-nitrophenol, *vide* Fig. 6. This compound is also the principal metabolite *e. g.* in the dog, following intravenous injection of parathion (*Gardocki & Hazleton* 1951), in monkeys after subcutaneous and dermal application (*Lieben, Waldman & Krause* 1952), and in rabbit and man after oral administration (*von Eicken* 1954). In the above mentioned species the bioconversion of parathion proceeds by oxidation and hydrolysis, chiefly in the liver (*Gage* 1953; *Karlog* 1957; and others), resulting in production of paraoxon and p-nitrophenol. The marked inhibition of the cholinesterase activity observed in the blood samples from cows showed that a considerable amount of paraoxon is produced following absorption of parathion through the skin. The detoxicating reduction processes after oral administration to cows are thus referable solely to microbial, fermentative processes in the rumen.

The absorption proceeds at a slow rate, as pointed out by *Frederiksson* (1961) on the basis of skin absorption experiments, using P^{32}-labelled parathion applied topically to cats. No metabolites were detectable in the coats of our experimental cows. This is in agreement with observations made regarding the stability of parathion in impregnated gauze tapes suspended in cow sheds (*Karlog* 1958). The deposit in the coat was demonstrable up to 12 days after the application. This means in practice that a poisoning developed by dermal application of parathion can be maintained for several days, unless the deposit in the coat is removed (*Dalgaard-Mikkelsen, Karlog & Poulsen* 1958).

Neither after oral nor after dermal application was parathion or its metabolites detected in the milk by the analytical method employed, which has a sensitivity of 0.1 μg./ml. or 0.1 p.p.m. This result is in agreement with most previous observations following oral administration of parathion to dairy cattle (*Dahm et al.* 1950; *Pankaskie et al.* 1952; *Gyrisco et al.* 1959). Only *Ahmed et al.* (1958), using P^{32}-labelled parathion, detected up to 0.9 p.p.m. of the hydrolytic products and 0.06 p.p.m. of the intact parathion. Thus, there should be no risk in feeding young animals with milk from mildly parathion-poisoned cows.

REFERENCES

Ahmed, M. K., J. E. Casida & R. E. Nichols: Bovine metabolism of organophosphorus insecticides: Significance of rumen fluid with particular reference to parathion. J. Agric. Food Chem. 1958, *6*, 740—746.

Augustinsson, K. B.: Cholinesterases. A study in comparative enzymology. Acta physiol. scand. 1948, *15*, suppl. 52.

Averell, P. R. & M. V. Norris: Estimation of small amounts of 0,0-diethyl-0-p-nitrophenyl thiophosphate. Anal. Chem. 1948, *20*, 753—756.

Brodie, B. B. & J. Axelrod: The estimation of acetanilide and its metabolic products, aniline, N-acetyl-p-aminophenol and p-aminophenol (free and conjugated) in biological fluids and tissues. J. Pharmacol. exp. Therap. 1948, *94*, 22—28.

Buckley, B. & J. P. Colthurst: The determination of 0,0-diethyl-0-p-nitrophenyl thiophosphate residue in tomatoes. Analyt. 1954, *79*, 285—289.

Cook, J. W.: In vitro destruction of some organophosphate pesticides by bovine rumen fluid. J. Agric. Food Chem. 1957, *5*, 859—863.

Dahm, P. A., F. C. Fountaine, J. E. Pankaskie, R. C. Smith & F. W. Atkeson: The effects of feeding parathion to dairy cows. J. Dairy Sci. 1950, *33*, 747—757.

Dalgaard-Mikkelsen, S.: Maladies et accidents chez le bétail causés par les insecticides et herbicides utilisés dans la pratique agricole. Office International des Epizootiés, 1956, R. No. 423.

Dalgaard-Mikkelsen, S., O. Karlog & E. Poulsen: Behandling af alkylfosfatforgiftninger med acetylkolinesteraseaktivatoren P-2-AM. VIII Nordisk veterinärm., Helsingfors, 1958, B. rapp. 15.

von Eicken, S.: Zur Ausscheidung von p-Nitrophenol im Urine nach Einwirkung von Pflanzenschutzmittel „E 605". Angew. Chem. 1954, *66*, 551—553.

Fallscheer, H. O. & J. W. Cook: Report on enzymatic methods for insecticides. J. Ass. offic. Agric. Chem. 1956, *39*, 691—697.

Fredriksson, T.: Studies on the percutaneous absorption of parathion and paraoxon. Acta Derm.-Venereol. 1961, *41*, 353—362.

Gage, J. C.: A cholinesterase inhibitor derived from 0,0-diethyl-0-p-nitrophenyl thiophosphate in vivo. Biochem. J. 1953, *54*, 426—430.

Gardocki, J. F. & L. W. Hazleton: Urinary excretion of the metabolic products of parathion following its intravenous injection. J. Amer. Pharm. Ass. (Sci. Ed.) 1951, *40*, 491—494.

Gyrisco, G. G., L. B. Norton, G. W. Trimberger, R. F. Holland, P. J. Mc Enerney & A. A. Muka: Effects of feeding low levels of insecticides residues on hay to dairy cattle on flavor and residues in milk. J. Agric. Food. Chem. 1959, 7, 707—711.

Karlog, O.: Determination of parathion, paraoxon and p-nitrophenol in organic tissue material. Acta pharmacol. toxicol. 1957, *14*, 92—100.

Karlog, O.: Elimination of parathion in inpregnated gauze tape against flies. Acta pharmacol. toxicol. 1958, *14*, 236—239.

Lieben, J., R. K. Waldman & L. Krause: Urinary excretion of paranitrophenol following parathion exposure. Archiv. Indust. Hyg. & Occup. Med. 1952, *6*, 491—495.

Pankaskie, J. E., F. C. Fountaine & P. A. Dahm: The degradation and detoxication of parathion in dairy cows. J. Econ. Entomol. 1952, *45*, 51—60.

Phillipson, A. T.: Rumen dysfunction. Advanc. vet. Sci. 1955, *2*, 212—261.

SUMMARY

Incubation *in vitro* of rumen fluid, to which had been added 40 µg. parathion per milliliter resulted in a rapid reduction of the nitro-group of the parathion to an amino group. After 10 minutes of incubation 12—13 per cent was recovered as unconverted parathion, and after 20 minutes 5 per cent. The administered parathion was in part converted to aminoparathion and intermediates, while up to 25 per cent was recovered as conjugated p-aminophenol.

After oral administration to two Jersey cows of 1 mg. and 10 mg. parathion per kg. respectively, no signs of poisoning were observed and no inhibition of the blood cholinesterase activity was detected. In the urine conjugated p-aminophenol constituted 97 per cent of the excreted metabolites and p-nitrophenol the remaining 3 per cent.

Neither parathion, nor the metabolites paraoxon, aminoparathion, aminoparaoxon, p-nitrophenol or p-aminophenol could be detected in the milk samples.

Dermal application of 1 mg. parathion per kilogram body weight caused inhibition of the cholinesterase activity in the blood and development of mild poisoning. The deposit in the coat consisted exclusively of parathion and was measurable for up to 12 days. In the urine p-nitrophenol was found as the only metabolite. Neither parathion nor metabolites were detected in milk samples.

ZUSAMMENFASSUNG

Elimination von Parathion bei Kühen nach peroraler und dermaler Applikation.

Inkubierung von Pansensaft, dem 40 µg Parathion pro ml *in vitro* zugesetzt waren, bewirkte schnelle Reduktion der Nitrogruppe des Parathions zu einer Aminogruppe. Nach 10 Min. langer Inkubierung wurden 12—13 %, nach 20 Min. nur 5 % als unumgebildetes Parathion wiedergefunden. Ausser Aminoparathion und intermediären Reduktionsprodukten wurden bis zu 25 % der zugesetzten Menge an Parathion als gekoppeltes p-Aminophenol wiedergefunden.

Nach peroraler Verabfolgung von 1 mg/kg bzw. 10 mg/kg Parathion an zwei Jerseykühe wurden weder Vergiftungssymptome noch Herabsetzung der Cholinesteraseaktivität im Blutt wahrgenommen. Im Harn entfielen 97 % auf die ausgeschiedenen Metaboliten gekoppelten p-Aminophenols, der Rest auf p-Nitrophenol.

In Milchproben wurden weder Parathion, Paraoxon, Aminoparathion, Aminoparaoxon noch p-Nitrophenol oder p-Aminophenol nachgewiesen.

Dermale Applikation von 1 mg Parathion/kg verursachte Hemmung der Cholinesteraseaktivität im Blut und Entwicklung leichterer Vergiftungssymptome. Das Depot in der Haarschicht bestand ausschliesslich aus Parathion und liess sich bis zum 12. Tage nachweisen. Im Harn wurde p-Nitrophenol als alleiniger Metabolit festgestellt. In Milchproben fanden sich weder Parathion noch Metabolite.

RESUMÉ

Elimination af parathion hos køer efter peroral og dermal applikation.

Inkubering af vomsaft tilsat 40 µg parathion pr. ml *in vitro* medførte hurtig reduktion af parathionets nitrogruppe til en aminogruppe. Efter 10 minutters inkubering genfandtes 12—13 %, efter 20 min. 5 %, som uomdannet parathion. Ud over aminoparathion samt intermediære reduktionsprodukter blev op til 25 % af den tilsatte mængde parathion genfundet som koblet p-aminofenol.

Efter peroral indgift af henholdsvis 1 mg./kg. og 10 mg./kg. parathion til to jerseykøer blev der ikke iagttaget forgiftningssymptomer eller nedsættelse af kolinesteraseaktiviteten i blodet. I urinen udgjordes 97 % af de udskilte metabolitter af koblet p-aminofenol, resten af p-nitrofenol.

I mælkeprøver påvistes hverken parathion, paraoxon, aminoparathion, aminoparaoxon, p-nitrofenol eller p-aminofenol.

Dermal applikation af 1 mg. parathion/kg. forårsagede hæmning af kolinesteraseaktiviteten i blodet samt udvikling af lettere forgiftningssymptomer. Depotet i hårlaget bestod udelukkende af parathion og kunne efterspores i indtil 12 dage. I urinen påvistes p-nitrofenol som eneste metabolit. I mælkeprøver påvistes ikke parathion eller metabolitter.

(Received October 29. 1962).