Table 2. [^{3}H]Thymidine incorporation (Bq/mg protein) in adult rat parenchymal hepatocytes in medium added with [^{3}H]thymidine and an effecitve fraction of rat small intestine mucosal homogenate. To L15 plus 30 mg proline-supplemented medium was added 5 % NBCS, [^{3}H]thymidine (2.5 μCi/dish), 10^{-6} *M* insulin, and 5 % effective fraction of supernatant of small intestine mucosal homogenate, control was without homogenate. DNA synthesis was estimated by counting for radioactivity of each 2-day cultivation. Average number of counts for radioactivity was calculated from five dishes

	Culture (Day)					
	2	4	6	8	10	14
Addition of effective fraction	2300	7140	7340	6600	5790	6030
No addition (control)	730	610	330	140	–	–

SP-Sephadex. These are different from EGF and TGF, and are not present in the portal vein, kidney, pancreas, or platelets of normal rats.

In a previous paper the authors reported that HGF appeared in the portal vein within 1.5 h after damage to the liver in rats [7]. In the recently reported works, the so-called hepatocyte growth factor, which accelerates DNA synthesis in primarily cultured hepatocytes, one factor of which the molecular weight was between 76 – 92 kDa, was isolated from the sera of partially hepatectomized rats [8], and the other one with 82 kDa from the platelets of rats [9]. They are similary labile by treatment with heat. But it is unlikely that the small intestine mucosal factor and these two factors from the sera and platelets of rats are identical because the characteristic differences such as molecular weight and stability with heat treatments are observed between these three factors. However, there are some characteristic resemblances such as molecular weight and stability with heat treatments between the small intestine mucosal factors and the hepatopoietin. Therefore, it was suggested that the gastrointestinal tract, including the intestine epithelial mucosa and the Pyer's patches, might be one of the possible organs which produces the enhancing factor for liver regeneration and restoration of liver functions.

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Several Aspects of Bacterial Dicyandiamide Degradation

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The application of dicyandiamide (DCD) in agricultural and horticultural practice, in order to reduce nitrate losses from soils, has gained considerable importance in the past years. DCD specifically inhibits the ammonium oxidation by *Nitrosomonas europaea* [1, 2], thus keeping applied nitrogen in a form which is less prone to leaching.

It has been observed that the degradation of DCD depends mainly on soil temperature, moisture, and clay contents [3 – 5]. Metallic oxides, especially amorphous iron oxides, are able to catalyze the reaction of water with the nitrile moiety to guanylurea. This is supposed to react further to guanidine and then to urea which, in soils, is readily cleaved into ammonia, CO_2, and water [4, 6].

There is, however, a biological degradation of DCD in soils, too. This has recently been confirmed with bacteria isolated from soils [7]. But, so far, detailed information concerning the microorganisms involved and the pertinent reactions are lacking.

To further characterize the catabolism of DCD, different bacteria have been isolated from DCD-treated composts and grown in pure cultures. Two lines have been selected which are able to break down DCD rapidly. The first one (line No. 16-1) is likely to belong to the genus *Rhodococcus* (the identification on the species level is still under way), the second one (line No. 11-1) is presumably a *Pseudomonas* sp.

The *degradation of DCD* by the isolate 16-1 = *Rhodococcus* sp. (conditions: mineral nutrient with DCD as the sole N source, 27 °C, 0.2 ml inoculum at the stationary phase, rotary shaker at 60 rpm) was very rapid: 200 μg DCD-

N/ml were metabolized within 3 days (Fig. 1a). There was no change of DCD concentrations in the sterile controls. Considerable growth was observed only by the DCD-supplied bacteria, as measured by optical density and viable counting by plating. *Pseudomonas* sp. (11-1) behaved similarly: concomitantly with a rapid decrease in DCD concentration there was rapid bacterial growth (Fig. 1b).

DCD metabolism was further followed by thin-layer chromatography (TLC) on silica gel. Plates were run in ethyl acetate : ethanol : glacial acetic acid : water (75 : 10 : 7.5 : 7.5, v/v), and spots visualized by spraying with KI/starch after chlorination. It could be shown that there is no decomposition of DCD in the sterile control (Fig. 2, lane *2*). When incubated with *Rhodococcus* sp., three different degradation products could be seen after 3 days of culture (Fig. 2, lane *3*), which never appeared in the controls. *Pseudomonas* sp. also metabolized DCD in 3 days, yielding two metabolites (Fig. 2, lane *6*).

Apparently there are at least two different ways of DCD degradation by bacteria. The main metabolites formed by *Rhodococcus* sp. are supposed to be cyanourea which appears as the first metabolite (unpublished observations), urea (confirmed by enzymatic testing; Boehringer "Harnstoff-Test"), and a third still unidentified product. When incubating DCD with *Pseudomonas* sp. (line 11-1), we found on the chromatogram a substance together with guanidine and a further, unknown product. It is notable that *Rhodococcus* and *Pseudomonas* seem to be unable to metabolize urea (unpublished observations). In contrast to the DCD degradation with metallic oxides, we never observed guanylurea in biological DCD cleavage.

Cell-free phosphate buffer extracts from *Rhodococcus* sp. (16-1) were able to degrade DCD quantitatively into cyanourea at a very high rate (Fig. 2, lane *4*). Urea was never detected as a degradation product. The DCD-degrading principle is heat-labile, as could be shown by boiling for 30 s (Fig. 2, lane *5*). So far, no buffer-extractable DCD-degrading system could be found in *Pseudomonas* sp. (11-1).

The following conclusions can be drawn from the above mentioned experiments:

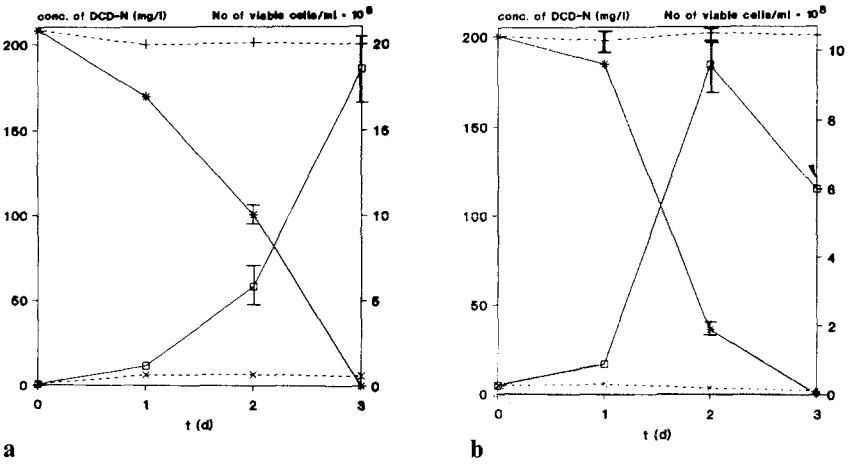

Fig. 1. Degradation of dicyandiamide by a) *Rhodococcus* sp. (line 16-1), b) *Pseudomonas* sp. (line 11-1) and increase of viable count per ml nutrient solution with dicyandiamide as sole N source during incubation time (means ± SD of 4 replicates; *arrowhead* value doubtful; SD ≤ 2 % is not given in the figure). (+) DCD-N (mg/l) sterile, (*) with *Pseudomonas,* (x) growth without N, (□) with 200 mg DCD-N/l

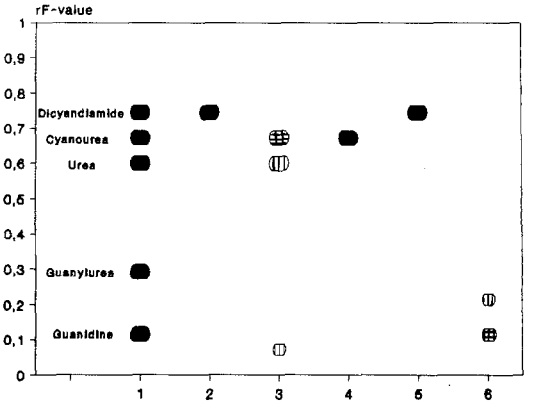

Fig. 2. Thin-layer chromatogram of the metabolism of dicyandiamide. *1* Reference substances, *2* dicyandiamide in nutrient solution without bacteria (sterile control), *3* degradation products of dicyandiamide by *Rhodococcus* sp. (line 16-1) within 3 days of incubation, *4* degradation of dicyandiamide in cell-free phosphate-buffered extracts of *Rhodococcus* sp., *5* dicyandiamide in heated cell-free phosphate-buffered extracts of *Rhodococcus* sp., *6* degradation products of dicyandiamide by *Pseudomonas* sp. within 3 days of incubation

1) DCD can be decomposed by soil bacteria.

2) There are at least two different ways of DCD catabolism. Both seem to be different from the inorganic catalytic DCD breakdown with metallic oxides.

In *Rhodococcus* (16-1), the possible metabolites are cyanourea, urea, an unidentified substance, whereas in *Pseudomonas* sp. (11-1) guanidine and another unknown product appear during the degradation.

3) From *Rhodococcus,* a heat-labile substance could be extracted with buffer which is able to decompose DCD readily, suggesting enzymatic control.

Experiments are under way to further characterize the different metabolic pathways of DCD degradation and the identification of the bacteria on a species level. The results might be of importance for the application of DCD in clean water areas and predicting the behavior of DCD in groundwaters.

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Identification of a Brood Pheromone in Honeybees

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The ability of social insects to identify the brood, and more precisely its sex, caste, and developmental stage is essential for the workers in charge of the brood care. This identification implies the combination of a few signals, particularly mechanical and chemical signals which play a fundamental role for ants [1], wasps [2], and bees [3].

The existence of brood pheromones has been postulated often for the social Hymenopterae, but only two have been identified with certainty [4, 5].

The honeybee brood care is secured through a few behavioral sequences including the feeding of the larvae, the capping of the cells, and the thermoregulation of the brood area in the colony. A triglyceride, glyceryl-1,2-dioleate-3-palmitate, induces the attraction and the clustering of the worker bees on artificial queen cells impregnated with this component [5].

The capping of the cells is a collective behavior which is displayed by numerous workers closing the top of the cells with wax [6].

Chemical signals produced by the brood are detected by an acarian, *Varroa jacobsoni* Oudemans, which induces significant damage in the honeybee colonies all over the world. In order to reproduce, the mites penetrate the cells a few hours before the capping, when the larvae are 4 – 5 days old. We have recently identified ten fatty esters in the honeybee drone larvae analyzed just before capping [7]. These compounds are also present in smaller amounts in the worker larvae (unpublished results). Three of these substances, methyl palmitate, ethyl palmitate, and methyl linolenate are involved in the attraction of the mites by the larvae [7]. Because these compounds represent a kairomone for the parasite, we assumed that they could have a pheromonal role for the honeybees, in relation to the brood care, particularly the capping of the cells, and have confirmed this hypothesis.

The influence of these esters in triggering the capping of the cells by the worker bees was studied through topical applications on worker larvae of the same age. About 24 h before capping, 0.25 µl of the mixture of the ten esters in the natural proportions [7], which corresponds to 150 larvae equivalent (lar-eq), was applied, without solvent, on each larva. Worker bees capped the cells containing the treated larvae significantly faster than the control ones (Fig. 1).

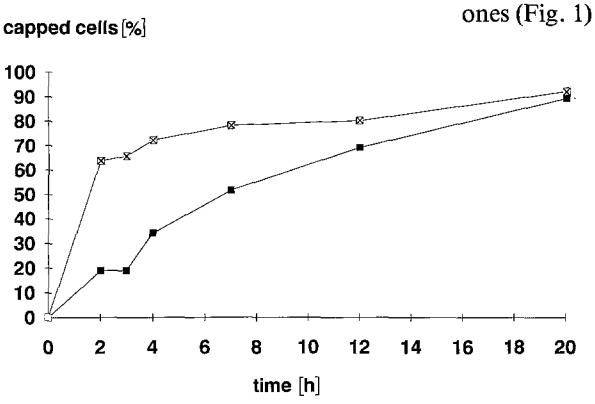

Fig. 1. Capping of the cells after topical applications of a mixture of the 10 esters on worker bee larvae of the same age. A queen was isolated on a comb for 24 h in order to obtain eggs of the same age. When larvae were 5 days old, 1 day before capping (last larval stage), they were individually located with a transparent film, and separated at random in two sets, each with 80 to 200 larvae. Topical applications were realized with a microsyringe, and 0.25 µl of the mixture was spread on each larva of one of the set chosen at random (⊠); the other set was used as control (■). Then the comb was replaced in the center of the colony and the capping of the cells by worker bees was observed as a function of time. The experiment was repeated 5 times with 5 different colonies, and a total of 687 treated and 1013 untreated (control) cells was observed. Percentages of capping were significantly different between the treated and the control cells (Mann-Whitney test, $p < 0.001$), at any time, except at 20 h. Amount of aliphatic esters in one drone larva [7]: methyl oleate (MO), 0.07 µg; ethyl oleate (EO), 0.03 µg; methyl linoleate (ML), 0.05 µg; ethyl linoleate (EL), 0.01 µg; methyl linolenate (MN), 0.59 µg; ethyl linolenate (EN), 0.18 µg; methyl palmitate (MP), 0.26 µg; ethyl palmitate (EP), 0.09 µg; methyl stearate (MS), 0.26 µg; ethyl stearate (ES), 0.08 µg

