each and incubated at 20 °C overnight. The mortality of the nematodes was observed using an inverted Leitz microscope at 60 × magnification. The MEYER/SCHNEIDERMAN JH[6] was more active than the RÖLLER JH[7]. A synthetic analogue was active while a synthetic compound lacking the important epoxide group was relatively inactive. These JH type compounds had the same relative activities when tested using inhibition of *C. briggsae* as the assay system.

We conclude that the JH type compounds affect the growth and development of nematodes in axenic culture, and can be compared by toxicity to *N. carpocapsae* or by inhibition of development of *C. briggsae*. No effect on specific developmental stages, moulting, or sex development was detected[9,10].

Table II. Toxicity of juvenile hormone (JH) type compounds to first stage larvae of *Neoaplectana carpocapsae* DD136

JH-type compound (μg/ml)	Survivors at 24 h (%)					
	400	200	100	50	20	0
RÖLLER	67	72	75	72	88	100
MEYER/SCHNEIDERMAN	0	0	0	11	64	100
Synthetic[a]	0	0	0	6	93	100
Compound lacking epoxide[b]	89	–	84	100	100	100

[a] 3,4-methylenedioxyphenyl 6,7-epoxygeranyl ether[8]. [b] Ethyl 6,7, 10,11-tetrahydrofarnesoate. For each hormone level, 5 larvae were inoculated into duplicate tubes containing 0.20 ml of CbMM with hemin and supplemented with hormone treated γ-globulin. Control medium (0 μg/ml JH) was supplemented with ether treated γ-globulin. After 24 h incubation at 20 °C nematodes were examined for viability.

Zusammenfassung. Insektenjuvenilhormone und -analoge wurden an verschiedenen freilebenden und parasitischen Nematoden getestet. Bei *Neoaplectana carpocapsae* wurden toxische Effekte und bei *Caenorhabditis briggsiae* Reifungshemmung festgestellt.

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AD STIMULANDUM

Phenylacetic Acid as a Potential Therapeutic Agent for the Treatment of Human Cancer

In the summary of a previous work, ROBERTS and SIMONSEN[1] remarked that apparently 'tumor cells are operating at a marginal level of glutamine availability by comparison with other tissue'. They believed that glutamine antagonists ought to have anti-tumour activity but found that competitive inhibitors such as γ-L-glutamylhydrazide were not particularly successful as tumour inhibitors. They concluded that 'in order to be able to employ glutamine antagonists in a maximally effective manner it will be necessary to direct attention to methods of decreasing formation and liberation of glutamine into the circulation by normal tissues as well as to disturbing its uptake and utilization in the tumor cells themselves'.

It is now suggested that a simple method for depleting the glutamine reserves of cancer patients might be based on the ability of humans to detoxify phenylacetic acid by conjugation with glutamine. This detoxication reaction which results in the excretion of phenylacetylglutamine in the urine was first detected by THIERFELDER and SHERWIN[2] and later studied in more detail by SHERWIN et al.[3]. POWER[4] found that the same conjugation reaction occurred in the chimpanzee but other mammalian species excreted the acid as a glycine conjugate and birds detoxified phenylacetic acid by conjugation with ornithine[5].

Since phenylacetic acid is not markedly toxic for humans it is considered that this material merits investigation as an anti-tumour agent by itself or in conjunction with glutamine antimetabolites or cancer-chemotherapeutic drugs of other types. Because of the unique mode of detoxication of phenylacetic acid in the human it is expected that there will be no parallel between the results of chemotherapeutic experiments with this acid in tumour-bearing rodents and the outcome of clinical trials in the human cancer patient.

Although the mode of detoxication of phenylacetic acid in the rat has apparently not been investigated in vivo, MOLDAVE and MEISTER[6] found that whereas human liver slices can synthesise radioactive phenylacetylglutamine from [^{14}C]-L-glutamine rat liver slices fail to do so. These experiments suggest that the rat will be unable to conjugate phenylacetic acid with glutamine in vivo. Thus if glutamine deprivation is important for tumour inhibition, phenylacetic acid therapy is likely to have little or no effect on the growth of transplantable rat tumours.

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Gross[7] studied the effect of daily s.c. injections of phenylacetic acid on the growth of the Walker transplantable tumour in rats and found that the substance exerted no significant inhibitory activity. However he showed that injections of ethyl phenylacetate resulted in considerable inhibition of tumour growth. In our own experiments, sodium phenylacetate was supplied in the drinking water to rats bearing subcutaneous transplants of the Rd/3 tumour. As shown in the Table, this treatment failed to inhibit tumour growth. Indeed the results suggest that the treatment caused some enhancement of tumour growth.

In order to secure further information on the effect of glutamine deprivation on the growth of rat tumours, we tested the therapeutic action of the sodium salt of *para*-aminosalicylic acid (PASA) which, according to Kawamata and Hiratani[8] is detoxified at least in part by conjugation with glutamine in the rat. When this compound was fed to Rd/3 tumour rats at the level of 6 mg/ml of drinking water, some evidence of tumour inhibition was obtained. Two of the tumours failed to grow and 1 tumour was markedly retarded (3.6 g at 14 days). One rat died on day 13 with a tumour of 35.4 g and the remaining 2 rats which were killed on day 14 had tumours of 37.5 and 36.6 g. In our experience with the Rd/3 tumour we have occasionally found one spontaneous regression per group of 6 transplant rats. Our results suggest that PASA may be capable of exerting some inhibitory action against Rd/3 sarcoma but it would be premature to relate this action to the supposed ability of PASA to sequester glutamine in the rat.

Effect of sodium phenylacetate and sodium *para*-aminosalicylate on the growth of Rd/3 sarcoma in rats

Group	Mean body weight (g) on Day 0	Day 14	During 14 days mean consumption per rat of Food (g)	Fluid (ml)	Compound in fluid (g)	Mean tumour weight (g) ± S.D.
A	237	263	196	353	–	42.0 ± 8.4
B	216	236	173	384	1.213	49.1 ± 18.3
C	213	214	186	284	1.704	22.6 ± 17.7

Albino male rats (3 groups of 6) each received a s.c. transplant of 0.05 ml of Rd/3 tumour mince along the right flank on Day 0. All groups were fed Diet No. 86 (Oxoid Ltd., London) ad lib. Group A (controls) given 500 ml of fresh tap water daily. Group B given daily a fresh solution of 1.58 g of sodium phenylacetate in 500 ml of tap water starting on Day 1. Group C given daily a fresh solution of 3 g of sodium *para*-aminosalicylate in 500 ml of tap water starting on Day 1. Food and fluid consumption per group measured daily. Experiment terminated on Day 14.

In 1958 Neish[9] reported some studies on the formation of glutamine conjugates by normal subjects and cancer patients who took small oral doses of sodium phenylacetate. No marked differences could be found between the optical rotations of the phenylacetylglutamine conjugates excreted by the 2 groups but it was established that the ability of cancer patients to excrete phenylacetylglutamine was not impaired and that small doses of phenylacetic acid were well tolerated by all subjects. It may be of interest to note that one of the patients (patient M. Table[9]) experienced a feeling of well-being after he ingested sodium phenylacetate.

Apart from the fact that phenylacetic acid acts as a plant growth hormone, the compound has not received extensive pharmacological study. Mirsky et al.[10] found that a number of plant growth hormones including indole acetic acid (which, incidentally, forms a glutamine conjugate in the human[11]) have insulinase-inhibitory and hypoglycemic activity in the rat. Effects of this kind may have to be looked for in future projects relating to the metabolism of phenylacetic acid in humans.

In conclusion, it may be noted that, in extensions of the studies of Gross cited above, anti-tumour activity[12] has been claimed for esters of other plant growth regulators such as indole acetic acid and 2,5-dichlorophenoxyacetic acid in animal experiments and in humans. It would appear that there are reasonably good grounds for attempting to inhibit the growth of human cancers by the application of the relatively non-toxic compound phenylacetic acid and its esters.

Résumé. On a montré que l'acide phenylacétique peut être utile pour le traitement du cancer humain.

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PRO EXPERIMENTIS

Eine Methode zur Trockenfixation grossflächiger Kryostatschnitte

Dünne Grossflächenschnitte von Organen und Ganztieren sind – bei entsprechender Vorbehandlung des Gewebes – durch Gefrierschneiden flüssig fixierten Materials[1,2] und tiefgefrorenen Nativmaterials in der Regel einfach anzufertigen. So können flüssig fixierte Grossschnitte nach einem speziellen Verfahren beispielsweise feucht auf Papier aufgezogen und konserviert werden[2].

Die Aufbewahrung von nativen Kryostatschnitten ist bei Raumtemperatur nur nach Trocknung möglich, wobei die Haltbarkeit jedoch limitiert ist. Es wurde daher ein Verfahren entwickelt, Kryostatschnitte von Ganztieren durch Gefriertrocknen und Bedampfen mit Formaldehyd in eine bei Raumtemperatur haltbare Form zu über-

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