were aerated, changed regularly and maintained at room temperature. After the roots had made at least 4 cm of new growth they were fixed in Helly's fluid, embedded in wax and sectioned transversely.

Normally, pea roots have a symmetrical triarch (rarely tetrarch) vascular pattern. Of 13 roots grown after colchicine treatment, 8 showed a deviation from this normal pattern in the regenerating portion of the root. The abnormal patterns resulted from an increase in the number of protoxylem poles from the usual 3 poles to 4, 5 or 6 poles (figure 1). One root showed a reduction of the number of poles from 3 to 2.

Examination of serial sections of the regenerating roots reveals how the abnormal patterns arise and their subsequent histories. The new patterns may originate immediately distal to the c-tumour (i.e. the swelling induced by the colchicine immediately behind the root apex) or they may occur after the root has made up to 2 cm of additional growth. The increases in the number of protoxylem poles occur in 2 ways. One is by the splitting of an existing pole: that is, where there was previously 1 cell, or 1 group of cells, differentiating as a protoxylem pole, 2 arise (figure 2). The sites of inception of the 2 poles then diverge and become independent of one another. The other way is by the inception of a new pole that is unrelated to any pre-existing pole. Here, a single lignified cell is at first seen equidistant from the existing poles (figure 3) and, as root regeneration continues, new metaxylem cells differentiate to complete the additional xylem arm (figure 4).

All the abnormal vascular patterns found in the regenerating roots showed a tendency to revert eventually to the original triarch condition by loss of the supernumerary protoxylem poles. Changes in patterning also involved changes in the symmetry of the placement of the xylem arms. For instance, an asymmetrical pentarch pattern which arose in 1 regenerating root (figure 5) subsequently converted to a symmetrical pattern (figure 6) by changes in both the orientation of metaxylem differentiation and the positioning of the protoxylem poles relative to one another.

Although the period of exposure to colchicine is brief, the drug probably persists in cells of the apex for some time afterwards[10]. This persistence may account for some of the long-term changes in mitotic activity found in root meristems following a 3 h treatment[11,12], and, in part, for the changes in vascular pattern. Alterations to the meristem include a reduction of its size[13] and the possible stimulation into division of cells located in the quiescent centre (QC)[12,14]. The cells from the QC eventually repopulate the meristem.[14,15]. The size of the QC has been postulated to control the complexity of the vascular pattern[3,6]; therefore, the change in the number of xylem arms in the regenerating root may reflect changes in the number, or activity, of cells in the reforming QC after its stimulation by colchicine. It is also conceivable that cells in the procambial cylinder that would never normally become lignified are stimulated to do so by the colchicine treatment[16]. The patterning of the vascular system is likely to involve some type of interaction between presumptive vascular cells in the region of their inception. Whether the repatterning that is observed relates to changes in the ploidy of the presumptive xylem initials or their neighbours, or to disturbances to gradients of morphogenetic determinants within the apex, remains to be elucidated.

10 J. S. R. Nelson, Can. J. Genet. Cytol. *14*, 279 (1972).
11 R. D. MacLeod, Öst. bot. Z. *120*, 15 (1972).
12 R. D. MacLeod, Chromosoma *35*, 217 (1971).
13 R. D. MacLeod, Planta *71*, 257 (1966).
14 D. Davidson, Chromosoma *12*, 484 (1961).
15 T. Shimamura, Cytologia *9*, 486 (1939).
16 E. R. Witkus and C. A. Berger, Bull. Torrey bot. Club *77*, 301 (1950).

A new drug against Paragonimus infection

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Summary. An oral dose of 50–100 mg/kg b.wt of a new anthelmintic, albendozole, for 2–3 weeks killed adult flukes and stopped shedding of ova of Paragonimus kellicotti in experimentally infected cats. No clinical signs related to treatment were recognized. This low toxicity of albendozole may be useful in treating human paragonimiasis.

Paragonimiasis is a serious parasitic disease of human beings in Asia, Africa and South America[1]. 3 or more hosts are involved in the life cycle: the definitive host (man) becomes infected by ingesting intermediate hosts (crabs, crayfish) or paratenic hosts[2] (wild pigs) that have eaten crabs. Several carnivorous hosts (cats, dogs) also act as the definitive host for species of Paragonimus that infect man. Young flukes migrate to the lungs of the definitive host via intestinal wall, peritoneum and diaphragm. The most common symptoms are a cough, profuse expectoration, hemoptysis, and chest pains[3,4]. Infection may not be confined to the lungs as Paragonimus frequently invades eyes and brain causing vision impairment, seizures and paralysis[3,4]. Bithionol is considered the drug of choice at the present time[3,4]. However, this drug causes unpleasant side effects such as diarrhea, nausea, vomiting and urticarial eruptions[3,4]. We report promising paragonimocidal properties of a recently discovered anthelmintic, albendazole[5], in cats experimentally infected with Paragonimus kellicotti.

Each of 6 specific-pathogen free cats were inoculated orally with 25 metacercariae dissected from the hearts of naturally infected crayfish. The cats developed radiographically demonstrable cysts in their lungs 28 days after

1 I. Miyazaki, Jap. J. Med. *10*, 8 (1969).
2 I. Miyazaki and S. Habe, J. Parasit. *62*, 646 (1976).
3 M. Yokogawa, Adv. Parasit. *3*, 99 (1965).
4 M. Yokogawa, Adv. Parasit. *7*, 374 (1969).
5 V. J. Theodorides, R. J. Gyurik, W. D. Kingsbury and R. C. Parish, Experientia *32*, 702 (1976).

Effect of albendazole therapy in cats infected with Paragonimus kellicotti

Cat No.	Albendazole (mg/kg b.wt)	Eggs per g of feces on post-treatment days 0	3	6	9	12	14	Autopsy examination DAIP	No. of patent cysts	No. of live flukes*
1	100	860	200	80	0	0	0	107	None	None
2	100	2120	340	40	0	0	0	107	None	None
3	20	720	700	200	20	60	20	108	5	9
4	20	2660	960	780	220	80	20	108	3	7
5	None	1300	2360	960	920	1460	1240	106	9	17**
6	None	1420	480	980	1440	1060	1240		Not done	

* Each cat given 25 metacercariae. DAIP = Days after inoculation with Paragonimus metacercariae. ** Percent recovery of adult flukes from 7 cats not included in this paper was: 87, 84, 80, 68, 60, 58 and 50.

inoculation with Paragonimus (DAIP). Ova of Paragonimus were seen beginning 56–59 DAIP. Starting 80 DAIP, 4 Paragonimus-infected and 2 uninfected control cats were administered an oral aqueous suspension of albendazole in 2 divided doses of 20 or 100 mg/kg b.wt daily for 14 days. Faeces were examined daily for trematode ova[6] and radiographs and hemograms were taken weekly. 4 weeks after the start of chemotherapy 5 cats were killed and necropsied. The results are shown in the table.

It is apparent that the administration of 100 mg/kg b.wt of albendazole for 14 days (total dose, 1400 mg) killed the adult flukes and stopped shedding of ova. A dramatic resolution of the patent cysts in the lungs was apparent both radiographically and at necropsy. The administration of 20 mg/kg b.wt of albendazole (total dose, 280 mg) killed half of the flukes and partially suppressed the shedding of ova.

The control cat number 6 that was not necropsied (table) was then treated with albendazole 50 mg/kg b.wt, daily for 21 days beginning 101 DAIP. Paragonimus ova were not detected in the feces of this cat 9 days after administering the drug and there was a dramatic resolution of lesions in the lungs as detected by radiographic examination and necropsy 2 weeks after the cessation of chemotherapy.

No clinical signs related to treatment were recognized. Hematological examination of all 8 cats were within normal limits except at one sampling interval (92 DAIP) when cat numbers 1 and 2 were leukopenic and neutropenic. The cause of the transient neutropenia was not determined. Histopathological examination of sections from all major organs of all treated cats failed to reveal any changes related to albendazole treatment.

6 F. A. Happich and J. C. Boray, Aust. Vet. J. *45*, 326 (1969).

Dichloropyrimidines: Specific inhibitors of virus growth[1]

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Summary. Dichloropyrimidines can be considered as a new group of antiviral substances having a common spectrum of inhibitory action.

The antiviral activity of 2-amino-4,6-dichloropyrimidine has been reported previously[2–4]. Certain features of this action deserve consideration: 2-amino-4,6-dichloropyrimidine acts on virus growth at concentrations which have little or no effect on macromolecular metabolism of unifected cells; rather unrelated viruses, such as Polio, Vaccinia and Herpes simplex viruses, are inhibited; inhibitory action is not due to fraudulent replacement of nucleic acid precursors. Research now in progress indicates that the antiviral action of 2-amino-4,6-dichloropyrimidine is shared by other bichlorinated pyrimidines. Preliminary data from this research are referred to below.

Material and methods. Compounds studied are listed in tables, for brevity. Virus strains (NIH, Bethesda) were: Polio 1 Brunenders, Coxsackie B_1, Encephalomyocarditis (EMC), Newcastle Disease (NDV), Vesicular stomatitis (VSV), Vaccinia and Herpes simplex 1 (HSV). Experiments were carried out on human aneuploid HEp 2 cells (American type culture collection, Rockville) and on primary mouse embryo cells, both grown in Eagle's MEM (Hank's base, pH 7.3) supplemented with 7% calf serum. Eagle's MEM (Earle's base) and aminoacid free Eagle's MEM (AFE) both brought up to pH 7.3 and supplemented with 2% calf serum were used in the tests. Maximum non-cytotoxic doses (MNCTD) of the drugs were determined by incubating 16-h-old HEp 2 cell monolayers (10^7 cells/sample) at 37 °C in Eagle's MEM 2% serum in the presence of scalar drug dilutions. After 48 h, gross cell damages were checked under light microscope and cell vitality was determined by measuring intracellular incorporation of neutral red[4]. Drug ability to interfere with cell growth was established by adding colchicine (Simes, 0.1 µg/ml) to the cultures 3 h after

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2 M. A. Marcialis, M. L. Schivo, P. Uccheddu, A. Garzia and B. Loddo, Experientia *29*, 1442 (1973).

3 M. A. Marcialis, M. L. Schivo, A. Atzeni, A. Garzia and B. Loddo, Experientia *29*, 1559 (1973).

4 M. A. Marcialis, O. Flore, A. Firinu, P. La Colla, A. Garzia and B. Loddo, Experientia *30*, 1272 (1974).