parasite and a systemic one, such permeabilyty change brought about by *Sclerospora sorghi* is quite interesting. Though there is considerable leakage of electrolytes on the 45th day from diseased leaves, this may not be adversely affecting the fungus, which by this time enters the oospore stage in the leaves.

Even though there was increased leakage of electrolytes from diseased roots and leaves, there was a suppression of escape of electrolytes from the stem. This could very well be due to accumulation of electrolytes, which may provide nutrition to the fungus which is still in the mycelial stage in the stem. This may also compensate for the loss through the roots.

Loss in permeability is encountered in hosts attacked by facultative parasites where a toxin or enzyme is involved in the pathogenesis[1]. The ionic imbalance and permeability change seen in downy mildew affected sorghum suggests that the elaboration of a principle for the pathogenesis by an obligate parasite like *Sclerospora sorghi* cannot be ruled out.

Résumé. On étudie la perte d'électrolytes dans les plantes de sorghum infectées par le mildiou lanugineux. C'est dans les racines et les feuilles malades qu'elle fut le plus marquée mais elle ne se produisit pas dans la tige malade. Elle eut son maximum dans les feuilles malades et augmenta au fur et à mesure des progrès de la maladie.

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Intrarenally Induced Infection in Rats: Kidney Infecting Dose and Sex Dependence

Experimental research on pyelonephritis requires a suitable animal. However, handy animals are not known to suffer naturally from this disease. It is necessary, therefore, to overwhelm the high local or general resistance of healthy animals against renal infection. Various methods have been developed which in the past 10 to 15 years proved the 2 main pathogenetical mechanisms of this disease in humans. These 2 infection routes are 1. the ascending or retrograde, 2. the haematogenous way. Experimentally there are a few disadvantages in these methods: 1. necessary pre-lesion within the kidney in order to make it sensitive to the infection, 2. the unknown quantity of bacteria reaching the kidney and being responsible for success in infection, 3. an unfixed rate of lethality in the animals caused by the high infection dose necessary.

For special purposes we have found the intrarenal infection model suitable because of its fastness, simplicity, and the possibility of inoculating exactly measured doses of bacteria. This method is an unphysiological one, but the result is apparently the same as in both the other modes of infection, although the special pathomechanisms might be quite different. The method was introduced in rabbits[1], modified for rats[2], and performed by only a few research groups recently[3–5]. Prat has taken it for chemotherapeutical investigations (personal communication).

We have used rats at the age of 5 to 7 months from a Wistar inbred strain held in our institute for about 15 years. For injection procedure the rats were etherized and infected through the ethanol-disinfected skin into the left kidney fixed by 2 of the operator's fingers. Inoculation was done with suspensions of a haemolyzing *Escherichia coli* 06 strain derived from urine of a female patient with urinary tract infection. Overnight at 37 °C bacteria grown on nutrient agar slants in tubes of 16×160 mm size were washed off with 10 ml saline which resulted constantly in a content of 1.5×10^9 bacteria per ml of suspension. This original suspension was diluted stepwise to suitable concentrations which were injected by a fine needle on microlitre syringes. At the same time bacterial counts were performed by plating serial dilutions on blood- and ENDO-agar, resulting next day in the exact amount of bacteria injected. Lastly we have injected planned bacterial concentrations because deviations from the desired amounts controlled by reading the plates on the next day were very small, if any.

In order to find the 'critical' infection dose to give reproducible results for possible biological standardization of this model, an equal volume (0.02 ml) of arithmetically diluted suspensions was injected intrarenally.

Table I. Incidence of infection dependent on the amount of injected bacteria (strain *E. coli* 06 2791/71)

No. of injected bact. per kidney (in 0.02 ml)	No. bacteriol. pos. / No. infected kidneys
$\leqq 1 \times 10^4$	0/30
2×10^4	4/8
4×10^4	8/15
8×10^4	5/10
1.6×10^5	13/15
$\geqq 3.2 \times 10^5$	16/16

Table II. Bacteriological findings from the infected (2×10^7 *E. coli*) left kidneys

Autopsy at days after infection	No. positive / No. infected	Sex of animals	Statistical significance
31–32	24/24	♀	—
	16/20	♂	
83	3/5	♀	—
	0/5	♂	
157–165	8/18	♀	$+(p<0.05)$
	1/13	♂	

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Animals (all female) were sacrificed 2 weeks after inoculation. Homogenized left kidneys were examined bacteriologically. The infection rate in several experiments is demonstrated in Table I.

From these results, it is concluded that kidney infecting dose[6] (KID_{100}) in whole the animals of our *E. coli* strain to be 2... 3×10^5 bacteria in the conditions described. The reproducibility of the results at various times over the year was very good. There was no lethality in about 400 rats infected in the same manner.

Further experiments were performed to get information about duration of the infection by the use of greater amounts of bacteria than the KID_{100}. We wanted to be sure that a great part of the animals should get a longer-lasting infectious process in their kidneys. Rats of either sex were under examination. At various intervals different groups of animals were sacrificed and their kidneys checked for infection. Bacterial strains were identified by haemolysis, fermentation pattern, and serologically. In all the bacteriologically positive cases we found pure cultures of our *E. coli* strain. Saline injected, and suspensions of ground sand in saline intrarenally inoculated, male and female rats in groups up to 15 animals served as controls. They were sacrificed within a range from 2 weeks up to 8 months. In these animals we never observed kidney infection.

Table II shows that there are no great differences between male and female rats up to 4 or 5 weeks. 5 months after infection, nearly half the female animals had positive bacteriological cultures from their infected kidneys, whereas the kidney of only 1 male rat out of 13 exhibited bacterial growth.

Because of the relatively small number of animals available for statistical evaluation, we are not able to decide whether the observed sex difference was possibly present at an earlier stage of the infectious process in the kidneys. Nevertheless, it must be stated from our results that planning and interpretation of similar experiments have to consider the sex of the animals used.

Zusammenfassung. Lebende *E. coli*-Bakterien wurden bei Ratten direkt in die Niere injiziert. Für die Infektion war eine bestimmte Mindest-Keimmenge erforderlich. Bei Injektion höherer Dosen (Chronizität) überstanden die männlichen Tiere die Infektion offenbar schneller und leichter als die weiblichen.

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Sensitivity of some Plant Pathogenic Fungi Towards Plant Metabolites: Antifungal Activity of some Chalcones, Dihydrochalcones and Flavanones

As a part of our programme on the chemical constituents occurring in some plants bearing antifungal properties[1,2], we were able to isolate and characterize 2 new compounds, flemichapparin-B (I) and flemichapparin-C (II)[3] from the roots of *Flemingia chappar* (Fam: Leguminosae) which were found to show antifungal activity[4] against 3 plant pathogens, *Helminthosporium oryzae*, *Curvularia lunata* and *Alternaria solani*. Besides these 2 modified isoflavonoids, 2 new naturally occurring chalcones of unusual aromatic substitution pattern, flemichapparin- (III)[5] and flemichapparin-A (VII)[6], along with 2′,4′-dihydroxychalcone-(IV)[7], were isolated from the aerial parts of *F. chappar*. The antifungal activity of these chalcones and their corresponding derivatives (dihydrochalcones and flavanones) have now been assayed by studying their effects on 4 growth stages of fungi, viz., spore germination, germ tube growth, vegetative growth in colony, and sporulation. The compounds (III–X) have also been studied with a number of fungi representing different classes, and their minimum inhibitory concentration has been determined against a selected test organism.

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Table I. Effect of chalcones and their derivatives at 105 µg/ml concentration on spore germination of 3 plant pathogenic fungi

Compounds	pH of the solutions	Germination (%) of spores of		
		H. oryzae	*A. solani*	*C. lunata*
Flemichapparin (III)	8.5	80	20	0
2′,4′-Dihydroxychalcone (IV)	8.5	100	59	60
Dihydroflemichapparin (V)	10.0	0	90	0
2′,4′-Dihydroxy-dihydrochalcone (VI)	10.0	0	0	0
Flemichapparin-A (VII)	9.8	0	0	0
Tetrahydroflemichapparin-A (VIII)	10.0	0	0	0
6-Methoxy-7-hydroxyflavanone (IX)	9.5	100	0	0
7-Hydroxyflavanone (X)	8.5	100	0	0
Control	7.0	100	95	70