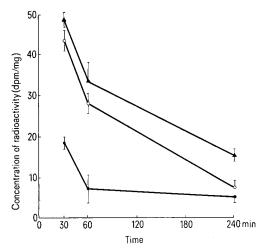
Epididymal fluid was collected as previously described, and separated into epididymal plasma and spermatozoa by centrifugation at  $12,000 \times g$  for 45 min in a microhaematocrit centrifuge (Hawksley Ltd). At autopsy, the following organs were removed and weighed: seminal vesicles, coagulating gland, prostate gland, epididymides, testes and ductus deferentes. The epididymides were divided into caput, corpus and caudae epididymides. A known weight of each organ was taken, solubilized with NCS tissue solubilizer (Amersham/Searle: 0.5 ml) for

Distribution of radioactivity (dpm/g) in the reproductive system of the male rat at various times after i. v. administration of [ $^3H$ ]LSD (24  $\mu\text{Ci/kg}$  b. wt)

Tissue	Time (min) $30 (n = 3)$	60 (n = 4)	180 (n = 3)
	30 (H = 3)	00 (H = 1)	100 (11 = 3)
Blood plasma	$42440 \pm 1370$	$35480 \pm 1850$	$27200 \pm 3370$
Epididymal plasma	$8340 \pm 2440$	$9000 \pm 2450$	$18500 \pm 3580$
Spermatozoa	$10520 \pm 590$	$13750 \pm 1600$	$14870 \pm 2440$
Seminal fluid	$13420 \pm 1300$	$16250 \pm 960$	$13060 \pm 1370$
Seminal vesicle	$31200 \pm 2090$	$31670 \pm 1030$	$23940 \pm 3100$
Coagulating gland	$27930 \pm 1300$	$39750 \pm 2040$	$26120 \pm 4930$
Prostate gland	$51510 \pm 4800$	$49500 \pm 4530$	$21230 \pm 1580$
Caput epididymides	$42640 \pm 5090$	$47000 \pm 5690$	$30470 \pm 3390$
Corpus epididymides	$74360 \pm 27650$	$54670 \pm 2490$	$31190 \pm 4453$
Cauda epididymides	$33360 \pm 7230$	$21330 \pm 1030$	$19560 \pm 1860$
Seminiferous tubules	$23580 \pm 1960$	$27000 \pm 1120$	$14510 \pm 1660$
Vas deferens	$50650 \pm 17940$	$30670 \pm 1030$	$21400 \pm 1080$

Each value represents the mean  $\pm$  SEM; n = number of animals.



Distribution of radioactivity in the placenta ( $\blacktriangle-\blacktriangle$ ) foetus ( $\bullet-\bullet$ ) and maternal blood plasma ( $\bigcirc-\bigcirc$ ) after i.v. administration of [\*\*H]LSD (24  $\mu$ Ci/kg b. wt) to 18 day pregnant rats. Each point represents the mean  $\pm$  SEM of 3 experiments.

12 h and the radioactive content determined by liquid scintillation spectrometry.

Experiment 2. 18 day pregnant Wistar rats were anaesthetized as previously described and catheters inserted into a femoral vein and carotid artery. [³H]LSD (24  $\mu$ Ci/kg) was administered i.v. and blood samples obtained prior to autopsy at 30, 60 or 240 min. At autopsy, both uterine horns were removed, and each foetus weighed and homogenized. A known weight of each placenta and foetal homogenate was solubilized and the radioactive content determined.

Results and discussion. The distribution of radioactivity in the male reproductive system after i.v. administration of [³H]LSD is shown in the table. All tissues contained LSD and/or metabolites. The concentration of radioactivity in epididymal plasma and spermatozoa was greater at 180 min than at 60 min. In contrast, in all other tissues the concentration at 180 min was less than at 60 min. In most tissues the concentration of radioactivity approximated to that in blood plasma.

These experiments clearly show that LSD and/or metabolites is distributed in the male accessory sex organs. Moreover, the presence of radioactivity has been shown both in the seminiferous tubules, where spermatozoa are being formed, and associated with spermatozoa undergoing maturation in the epididymis. These findings raise the interesting question as to whether or not LSD and/or metabolites is actually present within the sperm nucleus, and if so whether the presence of the drug could adversely influence spermatozoa development and maturation.

Radioactivity present in maternal blood plasma, placental tissue and the foetus at different time intervals is shown in the figure. The concentration, highest in the placenta, declined between 30 and 240 min. The percentage of administered radioactivity recovered per foetus was as follows: 0.11  $\pm$  0.01% (mean  $\pm$  SEM) at 30 min;  $0.04 \pm 0.02\%$  at 60 min;  $0.03 \pm 0.01\%$  at 240 min. Autoradiographic studies on the placental transfer of LSD in mice 5 have indicated that in late pregnancy 0.5% of the radioactive dose passed the placental barrier into the foetus in 5 min. The teratogenic effects observed in rats when LSD was injected in early pregnancy 9, 10 indicate placental transfer in this species. The present experiments have confirmed this and demonstrated the appearance of a significant fraction of the dose in the near-term foetus.

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## Anti-arthritic activity of bredinin, an immunosuppressive agent

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Summary. Bredinin has been found to have an inhibitory effect upon the secondary lesions occurring from adjuvant injection in rats.

Bredinin, a nucleoside antibiotic isolated from Eupenicillium brefeldiaum, was found to have potent immunosuppressive effects, causing suppression of both primary and secondary immune responses in mice<sup>3</sup>. With respect to the mechanism of the immunosuppressive action of bredinin, Sakaguchi et al.<sup>4,5</sup> reported a growth inhibitory action of bredinin on mammalian cells due to blockade of the pathway from inosine 5'-monophosphate to xanthosine 5'-monophosphate or from xanthosine 5'-monophosphate. Recently,

Effects of bredinin and 6-mercaptopurine (6-MP) on swelling of hind paw in adjuvant arthritic rats

Dose (mg/kg)	Percent increase of foot volume				
	3rd day	20th day	30th day	40th day	
Adjuvant injected foot					
Control	$96.4 \pm 5.5$	$140.8 \pm 6.7$	$164.3 \pm 7.8$	$128.6 \pm 8.2$	
Bredinin 2.5	$103.1 \pm 7.3$	$99.0 \pm 16.4*$	$107.1 \pm 16.9**$	$94.9 \pm 13.0*$	
5.0	89.8 + 5.1	104.1 + 19.6	116.3 + 26.7	118.4 + 23.1	
10.0	102.5 + 4.7	102.1 + 18.5	99.0 + 18.7**	$121.4 \pm 29.8$	
6-MP 10.0	$102.0 \pm 5.1$	$100.0 \pm 14.5*$	$105.1  \frac{-}{\pm}  14.7  ext{**}$	$102.0 \pm 26.9$	
Adjuvant non-injected for	oot				
Control		$39.8 \pm 12.1$	$58.2 \pm 16.1$	$49.0 \pm 11.9$	
Bredinin 2.5		$17.3 \pm 9.6$	25.5 + 7.1	17.3 ± 3.4*	
5.0		$13.3 \pm 7.9$	$21.4 \pm 6.6$	$20.4 \pm 7.9$	
10.0		$9.2 \pm 5.1*$	$7.1 \pm 5.2*$	$11.2 \pm 3.4*$	
6-MP 10.0		5.1 + 4.0*	17.4 + 5.4*	8.1 + 2.9**	

Drugs, suspended in 0.5% carboxymethylcellulose solution, were administered i.p. once a day for 30 days starting from the day of adjuvant injection (0 day). Each value represents the mean  $\pm$  SE of 7 rats. \*p < 0.05, \*\*p < 0.01.

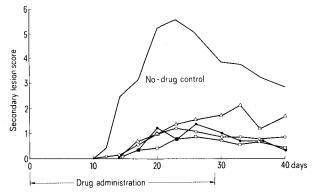
Steinberg<sup>6</sup> has shown that some immunosuppressive agents have beneficial effects on rheumatoid arthritis and systemic lupus erythematosus. Here we report on our study which investigates whether bredinin has an inhibitory effect on adjuvant-induced polyarthritis in rats.

Materials and methods. Female rats of the Sprague-Dawley strain (SPF), aged 7 weeks, were used for the experiments. The arthritic syndrome was induced by an intradermal injection of 0.1 ml of a fine suspension of heat-killed Mycobacterium butyricum (Difco) in liquid paraffin (concentration: 6 mg/ml) into the right foot pad of the animal. Foot volumes (ml) were measured to the level of the lateral malleolus by water displacement. The percent increase of foot volume was calculated as follows:

 $\label{eq:continuous} \mbox{Volume of swollen foot} - \mbox{Volume of foot before adjuvant injection}$ 

Percent increase =  $\frac{\text{adjuvant injection}}{\text{Volume of foot before adjuvant injection}}$ 

Results. The table shows the effect of bredinin on the intensity of swelling in the paw, due to the development of adjuvant polyarthritis. In rats which received bredinin, the primary phase of inflammation observed 3 days after the adjuvant injection was not suppressed at all, but the secondary phase of inflammation at 20 and 30 days,



Effects of bredinin and 6-MP on the development of the secondary lesions in rats injected adjuvant. Drugs, suspended in 0.5% carboxymethylcellulose solution, were administered intraperitoneally once a day for 30 days starting from the day of adjuvant injection (0 day). Bredinin, 2.5 mg/kg:  $\bigcirc-\bigcirc$ , 5.0 mg/kg:  $\triangle-\triangle$ , 10.0 mg/kg:  $\bigcirc-\bigcirc$ , 6-MP, 10.0 mg/kg:  $\bullet-\bigcirc$ . The degree of secondary phase of inflammation found at sites in the non-adjuvant-injected hind-leg, both fore-legs, and both ears were scored on a 0 to 2.0 scale as follows: 0: nil, 0.5: moderate, 1.0: moderately severe, 2.0: severe. The scores from each check point were added, giving each rat a maximum total score of  $2.0 \times 5 = 10$ . Each point represents the mean of the total scores from 7 rats.

especially in adjuvant non-injected foot, was remarkably suppressed. 6-Mercaptopurine (6-MP) was found to exhibit an inhibition similarly.

The development of secondary lesions in adjuvant-induced polyarthritic rats is represented in the figure. Bredinin was found significantly to inhibit lesioning at 2–3 weeks and 30 days after adjuvant injection. Furthermore, at 40 days, i.e. 11 days after the last drug administration, significant suppression of the arthritis was still observed in the groups injected with bredinin as well as 6-MP. The body weight curve in the bredinin group (2.5 and 5.0 mg/kg) was found to be improved as compared to the no-drug control group. But this was not seen in the groups treated with high dose of bredinin (10 mg/kg) as well as 6-MP (10 mg/kg).

Discussion. Many studies suggest that the immune mechanism participates in the development of adjuvant polyarthritis 7-9. Additionally, Mizuno et al.3 have demonstrated that bredinin suppresses the multiplication of several mammalian cells through inhibition of nucleic acid synthesis, and that it possesses a greater suppressive effect upon antibody formation against sheep red blood cells than does azathiopurine. This suggests that the suppression of the developing adjuvant polyarthritis by bredinin is due to the inhibition of antibody formation and/or multiplication of sensitized lymphocytes by a possible antigen in adjuvant polyarthritic rats. The specific character of bredinin seems to be immunosuppressant, as we previously noted that the drug did not possess any anti-inflammatory activity against carrageenan edema nor activity against the capillary permeability increase induced by a few chemical mediators 10. In conclusion, our present findings suggest the possibility of a clinical application for bredinin in rheumatoid arthritis.

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