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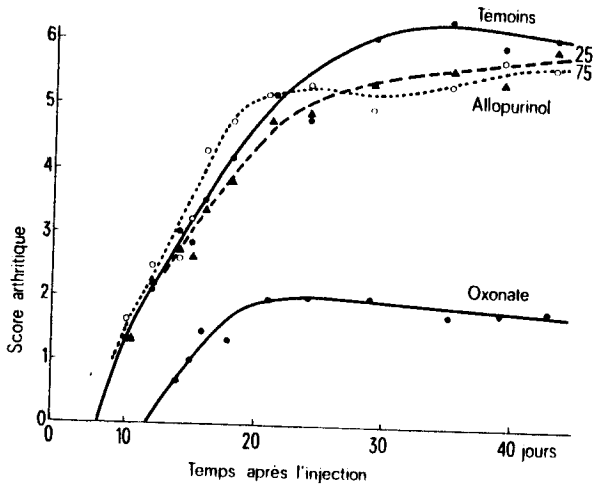
Specialia

Influence de l'oxonate de potassium et de l'allopurinol sur l'arthrite à adjuvant*

Régime	Œdème de la patte injectée Jours 0–9	Jours 9–45	Œdème de la patte non-injectée Jours 9–45	Score arthritique Jours 0–45
Normal	100	100	100	100
Allopurinol 25 mg/kg jour	92	97	97	90
Allopurinol 75 mg/kg jour	94	103	115	94
Oxonate	73	36	19	32

* Surfaces relatives des courbes montrant l'évolution de l'œdème des pattes arrière et du score arthritique.



Variation du score arthritique chez les rats témoins (● — ●, courbe supérieure), les rats à l'oxonate (● — ●, courbe inférieure) et les rats à l'allopurinol (▲ — ▲, 25 mg/kg jour et ○ — ○, 75 mg/kg jour).

(inflammation de la patte injectée) et de la réaction secondaire (polyarthrite induite) a été suivie pendant 6 semaines par la mesure du poids des animaux, du volume des pattes arrière et d'un score arthritique. Les rats non-injectés ont servi de référence pour le volume des pattes. *Résultats.* L'injection d'adjuvant produit un accroissement rapide du volume de la patte injectée chez tous les rats injectés. La polyarthrite périphérique se développe après une période de latence d'environ 9 jours chez les rats témoins et chez les rats à l'allopurinol et de 13 jours chez les rats à l'oxonate (figure). Pour faciliter les comparaisons, nous avons mesuré (tableau) les surfaces relatives des différentes courbes (score arthritique et œdème des pattes arrière injectées et non-injectées) en prenant pour référence le groupe témoin injecté. L'oxonate a une légère action anti-inflammatoire sur la réaction primaire non spécifique (réduction de 27% de l'œdème de la patte injectée) et présente un effet immunosuppresseur important sur la polyarthrite (réduction de 64 et de 85% de l'œdème des pattes arrière injectées et non-injectées, réduction de 68% du score arthritique).

Les pertes de poids au cours des réactions primaire et secondaire sont plus faibles pour le groupe de rats à l'oxonate (10 et 27 g par rat) que pour les 3 autres groupes (32 et 65 g). Chez ces 3 derniers groupes uniquement, une ankylose de la colonne vertébrale caudale apparaît au cours de la 3e semaine qui suit l'injection. Cette spondylite atteint environ 80% des animaux entre le 30e et le 40e jour.

Dans une expérience de courte durée (4 jours), Riesterer et Jaques[9] ont montré que l'allopurinol n'a pas d'effet anti-inflammatoire sur l'arthrite à adjuvant déjà établie. Nos expériences montrent qu'il n'a pas non plus d'influence sur le développement de cette polyarthrite, même lorsqu'il est administré quotidiennement à des doses subléthales pendant plusieurs semaines. Il semble donc que le métabolisme des pyrimidines ne soit pas impliqué dans le mécanisme de l'inhibition de l'arthrite à adjuvant par l'oxonate.

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LSD: The distribution of [^{3}H]LSD in the reproductive system of the male rat and placental transfer in the female rat

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Summary. After administration of [^{3}H]LSD to male rats, radioactivity was present in all tissues of the reproductive system, notably associated with spermatozoa in the epididymis. In addition, in pregnant rats LSD and/or metabolites readily crossed the placental barrier.

The general distribution of LSD in mice and rats has been studied by several workers[1–6]. The purpose of the present investigation was to study a) the distribution of [^{3}H]LSD in the tissues of the reproductive system of the male rat and b) the placental transfer of [^{3}H]LSD in late pregnancy. *Materials and methods.* Experiment 1. Mature male rats of the Wistar strain (250–350 g) were anaesthetized with urethane (14% w/v in 0.9% saline; 10.0 ml/kg i.p.). Polyethylene catheters were inserted into a femoral vein and a carotid artery. [2(n)-^{3}H]lysergic acid diethylamide ([^{3}H]LSD; specific radioactivity 15.8 Ci/mmole; obtained from the Radiochemical Centre, Amersham) was dissolved in saline : methanol (19 : 1 v/v) after evaporation of the organic vehicle. Radiochemical purity was shown to be 97% by thin layer chromatography in solvent system chloroform : methanol : acetic acid (40 : 30 : 30). [^{3}H]LSD (24μCi/kg; 0.5μg/kg) was injected i.v. and blood samples obtained prior to autopsy at 30, 60 or 180 min.

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Epididymal fluid was collected as previously described[7], and separated into epididymal plasma and spermatozoa by centrifugation at 12,000 × g for 45 min in a micro-haematocrit 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 12 h and the radioactive content determined by liquid scintillation spectrometry[8].

Experiment 2. 18 day pregnant Wistar rats were anaesthetized as previously described and catheters inserted into a femoral vein and carotid artery. [^{3}H]LSD (24 μCi/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 [^{3}H]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.

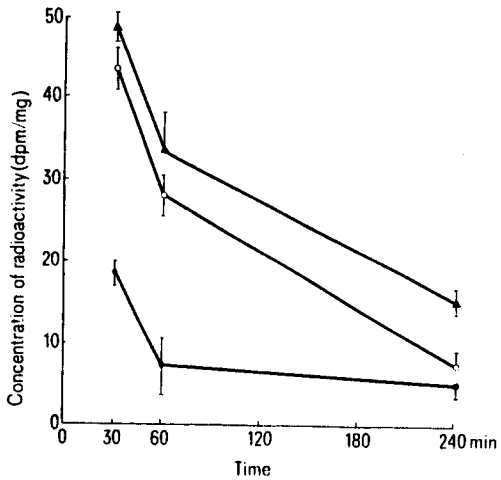
These experiments clearly show that LSD and/or metabolites is distributed in the male accessory sex organs. Moreover, the presence of radioactivity has been shown both in the seminiferous tubules, where spermatozoa are being formed, and associated with spermatozoa undergoing maturation in the epididymis. 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 ± 0.01% (mean ± SEM) at 30 min; 0.04 ± 0.02% at 60 min; 0.03 ± 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.

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

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

Each value represents the mean ± SEM; n = number of animals.



Distribution of radioactivity in the placenta (▲–▲) foetus (●–●) and maternal blood plasma (○–○) after i.v. administration of [^{3}H]LSD (24 μCi/kg b. wt) to 18 day pregnant rats. Each point represents the mean ± SEM of 3 experiments.

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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[3]. With respect to the mechanism of the immunosuppressive action of bredinin, Sakaguchi et al.[4,5] 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 to guanosine 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 ± 5.5	140.8 ± 6.7	164.3 ± 7.8	128.6 ± 8.2
Bredinin 2.5	103.1 ± 7.3	99.0 ± 16.4*	107.1 ± 16.9**	94.9 ± 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 ± 29.8
6-MP 10.0	102.0 ± 5.1	100.0 ± 14.5*	105.1 ± 14.7**	102.0 ± 26.9
Adjuvant non-injected foot				
Control		39.8 ± 12.1	58.2 ± 16.1	49.0 ± 11.9
Bredinin 2.5		17.3 ± 9.6	25.5 ± 7.1	17.3 ± 3.4*
5.0		13.3 ± 7.9	21.4 ± 6.6	20.4 ± 7.9
10.0		9.2 ± 5.1*	7.1 ± 5.2*	11.2 ± 3.4*
6-MP 10.0		5.1 ± 4.0*	17.4 ± 5.4*	8.1 ± 2.9**

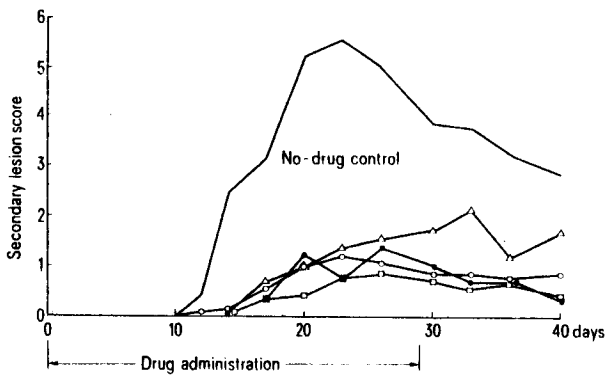
Drugs, suspended in 0.5% carboxymethylcellulose solution, were administered i.p. once a day for 30 days starting from the day of adjuvant injection (0 day). Each value represents the mean ± SE of 7 rats. *$p < 0.05$, **$p < 0.01$.

Steinberg[6] 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:

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

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



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: ○—○, 5.0 mg/kg: △—△, 10.0 mg/kg: □—□, 6-MP, 10.0 mg/kg: ●—●. 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.

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 azathioprine. 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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