close to each other is not a sufficient reason to hypothesize a direct developmental relation between them.

Woronin bodies are commonly believed to act merely as plugs in the septal pore, but it is possible that they play a more complex role. In fact, because of their morphology and location, they are very similar to organelles which, based upon their content of hydrolytic enzymes, were identified as lysosomes[30]. The role of plant microbodies has instead been cleared up to a great degree[25,31]. In a heterotrophic organism such as *T. mentagrophytes*, they are very probably involved in lipid metabolism; therefore the term glyoxysomes might be more appropriate for these organelles.

Even if the biochemical function of the two organelles described above may only be verified on the basis of their respective enzymatic content, it is clear, nevertheless, that their morphology will meet the standards generally accepted for Woronin bodies and microbodies only if the cells of *T. mentagrophytes* are prefixed with TAPO. Furthermore, since the techniques used improve the fixation of the entire cell, possibly increasing the penetration speed of the osmic fixation, they can be suggested for a better vision of other internal structures of the dermatophyte which normal fixative processes do not adequately preserve.

Summary. Microbodies and Woronin bodies, organelles surrounded by a single unit membrane, were identified in the hyphal cells of *Trichophyton mentagrophytes* by employing a fixative containing TAPO. The fine structure of the organelles is described and their possible significance discussed.

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21 March 1975.

[30] C. L. Wilson, D. L. Stiers and G. G. Smith, Phytopathology *60*, 216 (1970).
[31] N. E. Tolbert, A. Rev. Plant Physiol. *22*, 45 (1971).

Acidic Nonsteroid Anti-Inflammatory Drugs Accumulating in Inflamed Tissue

A variety of acidic and non-acidic compounds are potent inhibitors of prostaglandin (PG) synthesis in vitro[1-3]. Some of them, namely the nonsteroid anti-inflammatory drugs (NSAID), exert anti-inflammatory action in vivo[1]. However, only the acidic NSAID have gained acceptance in practice[4,5]. Since inhibition of PG-synthesis is now widely believed to be the main target of NSAID in inflammation[6], the observed clinical usefulness of the acidic in contrast to the non-acidic NSAID remains unexplained. Two explanations are possible. Firstly, inhibition of PG-synthesis is not of decisive importance for the anti-inflammatory effect of NSAID, or secondly, only acidic NSAID inhibit PG-synthesis sufficiently in inflamed tissue.

To test the second hypothesis we measured the inhibition of PG-synthesis in vivo at the site of inflammation by acidic and non-acidic pyrazolone and indole derivatives. The results are given in the Table. Although the 2 pyrazolone and the 2 indole derivatives are almost equally effective in inhibiting PG-synthesis in vitro[6], approximately 10 times higher doses are required for the non-acidic compound to achieve the same effect in vivo as the acidic.

These results indicate that besides PG-synthesis inhibition, there must be an additional characteristic of the acidic NSAID which renders them especially active in inflamed tissue. This kind of selectivity might be the result of specific pharmacokinetics leading to selective accumulation and/or biological activity in certain body compartments. There have been speculations that, e.g.

[1] J. R. Flower, Pharmac. Rev. *26*, 33 (1974).
[2] R. E. Lee, Prostaglandins *5*, 63 (1974).
[3] H. Kunze, E. Bohn and W. Vogt, Biochim. biophys. Acta *360*, 260 (1974).
[4] S. J. Piliero and C. Colombo, J. clin. Pharmac. *7*, 198 (1967).
[5] H. E. Paulus and M. W. Whitehouse, A. Rev. Pharmac. *13*, 107 (1973).
[6] E. A. Ham, K. J. Cirillo, M. Zanetti, T. Y. Shen and F. A. Kuehl, in *Prostaglandins in Cellular Biology* (Eds. P. W. Ramwell and B. B. Pharriss; Plenum Press, New York 1972), p. 345.

Acidic and non-acidic pyrazolone and indole derivatives: Inhibition of PG-synthesis and relative drug content in joint fluid

	Acidity	Dose i.v. (mg/kg)	PG $F_2\alpha$ in inflamed joints (% of untreated controls)	Drug content in inflamed joints (% of control joints)	
				after 3 h	after 5 h
Pyrazolone derivatives					
Antipyrine	Alkaline p.Ka 1.4	200	51 ± 26 [a]	82 ± 18	84 ± 33
Phenylbutazone	Acidic p.Ka 4.4	20	39 ± 14 [a]	429 ± 280 [a]	802 ± 198 [a]
Indole derivatives					
Indoxole	Alkaline p.Ka < 2	50	23 ± 13 [a]	102 ± 31	91 ± 28
Indomethacin	Acidic p.Ka 4.2	5	25 ± 22 [a]	350 ± 224 [a]	631 ± 225 [a]

The drugs were dissolved in DMSO and infused slowly (10 min) i.v. at zero time. 1 h later urate crystals (UC) were injected (4% w/v in saline) into the right intertarsal joint of the chicken (2 kg body wt.) the left joint receiving 0.3 ml saline as a control. 3 h later joint washes were performed, the PG $F_2\alpha$ content measured in the UC injected joints as described previously[16] and the drug dependent inhibition of PG-synthesis expressed in percent of DMSO treated controls. On other animals having received the same treatment, the drug content in the inflamed and control joints was measured 3 or 5 h after drug administration by fluorophotometric methods (antipyrine and indoxole)[14,15] or using ^{14}C-labelled drugs. Means and standard deviations of 5 and more experiments are given. [a] $p < 0.01$.

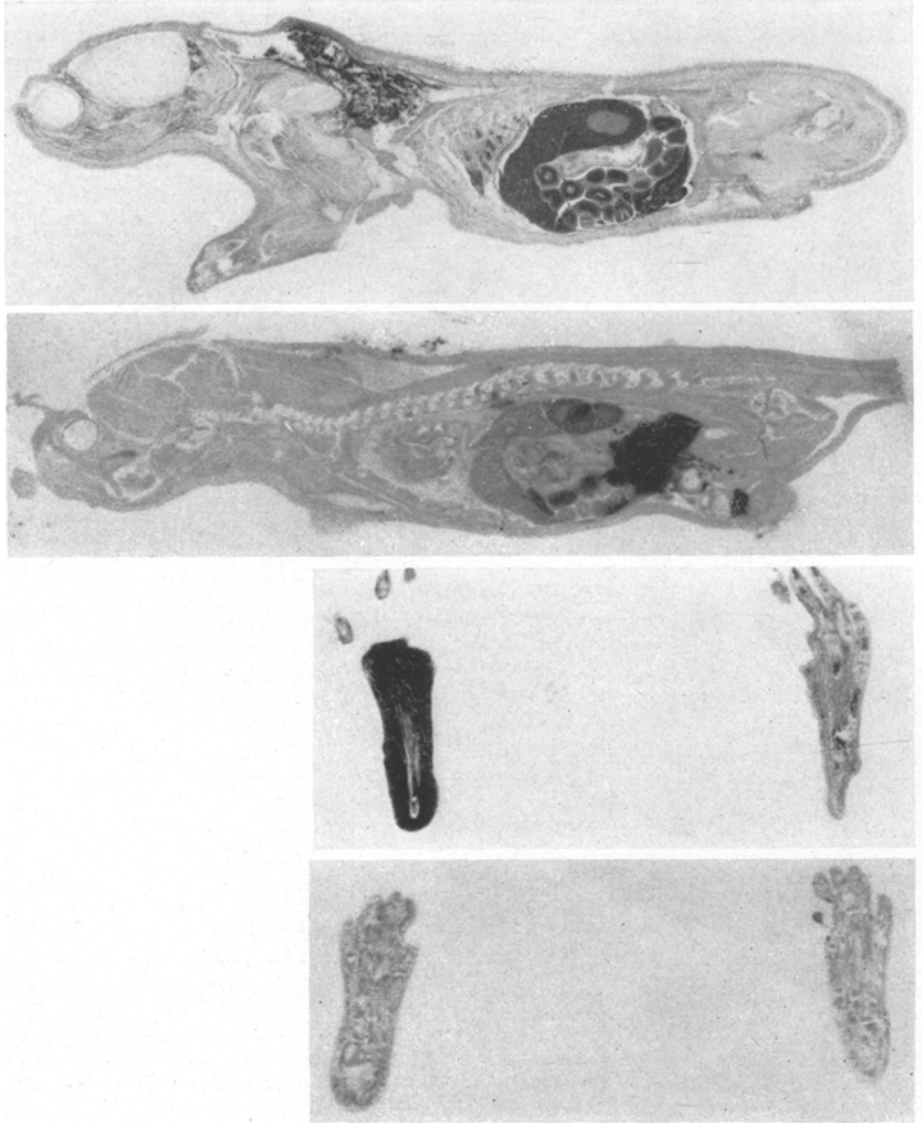
phenylbutazone 'has a specific relationship to inflamed tissue'[8]. To find out whether there is accumulation of acidic NSAID in contrast to the non-acidic congeners, we assayed the concentration of the same drugs in the washes from inflamed and non-inflamed joints of chickens 3 and 5 h after drug administration, at 2 and 4 h after eliciting the inflammation respectively. The results are given in the Table. Clearly, there is accumulation of the two acidic NSAID tested in the fluid of the inflamed joints. The concentration of these drugs in the fluid of the inflamed joints was already about 3 times higher than in the control joints at 3 h and 6 to 8 times higher at 5 h after drug administration. On the other hand, the non-acidic congeners did not show such behaviour. The concentration of these drugs was equal or only slightly higher in the fluid of the inflamed as compared to the control joints.

It may be argued that our results are only pertinent for a very specific model of acute inflammation and cannot be generalized. Therefore we extended our experiments to a more widely used model of inflammation, the carrageenin edema in the rat. ^{14}C-labelled phenylbutazone or antipyrine was administrated p.o. to young rats and at the same time an inflammation elicited by the injection of carrageenin into the left hind-paw and the subcutaneous tissue of the neck. 5 h later the animal was anaesthetized, exsanguinated, deep frozen and cut into thin (100 μm thick) slices. The slices were freeze dried and then mounted on X-ray-film. The radioautographs so obtained for phenylbutazone (Figure a) show high radioactivity in the inflamed tissue of the neck and the left hind-paw, but also in liver, small intestine and kidney. Other tissues contain only traces of activity. In contrast, experiments using ^{14}C-labelled antipyrine (Figure b) do not show accumulation of activity in the inflamed tissue, again indicating that only acidic NSAID accumulate in inflamed tissue.

An explanation for this observation may be that the two common physioco-chemical characteristics of acidic NSAID, namely their unusually high degree of binding to plasma proteins in vivo[1,5] and their pKa values around

[7] R. J. FLOWER, H. S. CHEUNG and D. W. CUSHMAN, Prostaglandins *4*, 325 (1973).

[8] G. WILHELMI and R. PULVER, Drug Res. *5*, 221 (1955).



Radioautographs of phenylbutazone (a) and antipyrine (b) treated rats. Young rats (30 g body wt.) were given 100 μCi (10 mg/kg) ^{14}C-labelled phenylbutazone or antipyrine by stomach tube. At the same time inflammation was elicited by the injection of 0.05 ml of a 2% (w/v) suspension of carrageenin into the left hind-paw or 0.2 ml suspension plus 0.2 ml air into the subcutaneous tissue of the neck. 5 h later the animals were exsanguinated, deep frozen and cut into thin (100 μm) slices which were mounted on X-ray film. After 8 days of exposure, the pictures given were examined. The phenylbutazone treated animal (a) shows high activity in the inflamed tissue of the neck and the left hind-paw, whilst the antipyrine treated does not display high activity in the inflamed tissue.

4[9,10], cause two effects. Firstly, capillary damage with extravasation of plasma proteins, as seen in inflammation[11], will lead to concommittant extravasation of drug bound to these proteins and thus cause elevated concentrations of drug in inflamed tissue. Secondly, weak acids show increased lipophilicity in acidic compartments, which may result in increased membrane-concentration[9], and biological activity. Acidic compartments are known to exist in the stomach[9], the kidney[9] and inflamed tissue[11]. Whether these explanations together are sufficient to explain the accumulation and pharmacological activity of acidic NSAID, however, remains a matter of speculation at present[12].

Nevertheless it can be concluded that our observations add a new aspect to the understanding of the mode of action of acidic NSAID. It appears that only acidic drugs which are highly bound to plasma proteins accumulate specifically in inflamed tissue and thus inhibit PG-synthesis in vivo at the site of inflammation. On the other hand, the same physico-chemical characteristics may lead to high membrane concentrations and/or biological activity of these drugs in small intestine, kidney and liver causing the known unwanted side effects in these organs[5]. As a consequence of these observations, it appears almost impossible to dissociate the desired anti-inflammatory action of acidic NSAID from their harmful side effects. Also, since the degree of accumulation in the inflamed tissue may vary from species to species (depending e.g. on the degree of protein binding of therapeutic doses), search for optimal pharmacokinetic behaviour in humans may serve as a useful additional guide line for the development of better anti-inflammatory drugs[13–16].

Zusammenfassung. Es wird gezeigt, dass saure Antiphlogistika im entzündeten Gewebe angereichert werden. Alkalische Strukturverwandte zeigen diesen Effekt nicht. Die Bedeutung dieses Befundes für das Verständnis der Wirkung bekannter und die Entwicklung neuer nicht steroidaler Antiphlogistika wird diskutiert.

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Department of Pharmacology, Biozentrum der Universität, Klingelbergstrasse 70, CH-4056 Basel (Switzerland), 19 March 1975.

[9] A. Goldstein, L. Aronow and S. M. Kalman, *Principles of Drug Action* (Harper and Row, New York 1969).
[10] H. Levitan and J. L. Barker, Science *176*, 1423 (1972).
[11] T. J. Williams and J. Morley, Br. J. exp. Path. *55*, 1 (1974).
[12] N. A. Cummings and G. L. Nordby, Arthrit. Rheumat. *9*, 47 (1966).
[13] K. Brune, Agents Actions *4*, 230 (1974).
[14] D. G. Kaiser, B. J. Bowman and A. A. Forist, Analyt. Chem. *38*, 977 (1966).
[15] B. B. Brodie, J. Axelrod, R. Soberman and B. B. Levy, J. biol. Chem. *179*, 25 (1949).
[16] M. Glatt, B. Peskar and K. Brune, Experientia *30*, 1257 (1974).
[17] We thank the Ciba-Geigy Corp. for providing labelled phenylbutazone, the Merck, Sharp and Dohme for labelled indomethacin and the Upjohn Comp. for indoxole.
[18] Supported by Grant 3.8450.72 from the Swiss National Faundation for Scientific Research.

Microsomal Metabolism as a Determinant of Aflatoxin Toxicity

Aflatoxins are a group of toxins elaborated by the fungus *Aspergillus flavus* which are natural contaminants in foods and feeds. They are known to produce acute necrosis, cirrhosis and carcinoma of the liver in a variety of animal species including non-human primates[1–3]. However, there is a marked variation in the response to the toxin between various species of animals[4]. It has not been possible to correlate these differences with differences in the status of the drug metabolizing system[5]. Qualitative changes in the metabolism of the toxin rather than quantitative differences in a single enzyme system may be one of the explanations for the species variations. Examples of such species-specific metabolism have, in fact, been documented[6]. Such a possibility also explains the variation with respect to the zones affected in the livers of different animal species. Aflatoxin liver damage is periportal in the rat, duckling, and monkey, midzonal in the rabbit and centrilobular in the pig, guinea-pig and dog[4]. These observations, therefore, indicate that aflatoxin is metabolically handled differently by different species of animals.

In spite of intensive and extensive studies, there is, as yet, no complete understanding regarding the site and nature of aflatoxin metabolism. It has been suggested that aflatoxin requires to be activated for its acute toxicity[5]. It has also been suggested that its toxicity is dependent upon its being inactivated[7]. Similarly, divergent speculations have been made with respect to its carcinogenic potential. On the basis of reported data from controlled studies in rats subjected to various experimental procedures, an attempt has been made here to explain the metabolic basis for aflatoxin toxicity.

Microsomal drug metabolism is relatively ineffective in young animals[8] and in protein depleted animals[9,10]. In both these situations, susceptibility to acute aflatoxin toxicity is increased[11,12]. Administration of DDT is associated with increased rate of drug metabolism[13] and such treatment results in protection from aflatoxin-induced liver injury[14]. The behaviour of carbon-tetrachloride in these conditions is exactly opposite to that of aflatoxin[7,15]. Such a reciprocal behaviour of these two toxins is also observed from the zones affected in the rat liver during their acute toxicity. In acute aflatoxin toxicity, the lesion is periportal, while in carbon tetrachloride, it is centrilobular. It is usual for the toxins requiring

[1] P. G. Tulpule, T. V. Madhavan and C. Gopalan, Lancet *1*, 962 (1964).
[2] C. Gopalan, P. G. Tulpule and D. Krishnamurthi, Food Cosmet. Toxic. *10*, 519 (1972).
[3] R. H. Adamson, P. Correa and D. W. Dalgard, J. natn. Cancer Inst. *50*, 549 (1973).
[4] W. H. Butler, in *Progress in Liver Diseases* (Popper and Schaffner, New York 1970), vol. 3, p. 408.
[5] D. S. P. Patterson and R. Allcroft, Fd. Cosmet. Toxic. *8*, 43 (1970).
[6] D. V. Parke and R. T. Williams, Br. med. Bull. *25*, 256 (1969).
[7] A. E. M. McLean and E. K. McLean, Br. med. Bull. *25*, 278 (1969).
[8] R. Kato, P. Vasanelle, G. Frontino and E. Chiesara, Biochem. Pharmac. *13*, 1037 (1964).
[9] A. E. M. McLean and E. K. McLean, Biochem. J. *100*, 564 (1966).
[10] W. J. Marshall and A. E. M. McLean, Biochem. J. *122*, 569 (1971).
[11] G. N. Wogan, Bact. Rev. *30*, 460 (1965).
[12] T. V. Madhavan and C. Gopalan, Arch. Path. *80*, 123 (1965).
[13] L. G. Hart and J. R. Foots, Proc. Soc. exp. Biol. Med. *114*, 388 (1963).
[14] A. E. M. McLean and E. K. McLean, Proc. Nutr. Soc. *26*, 12 (1967).
[15] J. R. Meyer and S. B. Pessoa, Am. J. trop. Med. *3*, 177 (1923), cited by Rouiller in *The Liver* (Academic Press New York 1964), vol. 2, p. 429.