2 slope lines were hanging at both sides and the histamine hypersensitivity was detectable at doses between 2 μg and 0.002 μg; the latter dose could be acceptable as the minimum sensitizing dose of the LPS preparation, as shown also in another experiment. The lethal rate of the peak on the unimodal curve did not so far reach 100%, showing usually about 60 to 90%. These facts were also applicable to other LPS preparations. PIERONI et al.[11] observed the appearance of lethal rates between zero and less than 100%, but they did not point out the unimodal dose response curve so clearly as we revealed it.

From the unimodal response curve, it is assumed that the mechanism of the histamine hypersensitivity caused by endotoxin would not be simple and that at least two antagonistic activities would be induced in mice by endotoxin administration; the histamine sensitizing activity and the activity of antagonistic factor(s). Roughly speaking, as shown in the Figure, the slope line decreasing from the peak could be dependent on the histamine-sensitizing activity, while the slope line increasing to the peak could represent reflexion of the histamine sensitizing and the predominant anti-histamine sensitizing activities. Therefore, it seems that the peak response may be composed of reflexion of the two independent activities. The entities of the two activities induced by endotoxin are obscure at present. But the anti-histamine sensitizing agent might be glucocorticoids induced by endotoxin, since endotoxin elicited increases of blood cortisol content and of urinary cortisol excretion in dogs[15] and guinea pigs[16], while the histamine hypersensitivity of mice due to endotoxin was inhibited by the administration of hydrocortisone acetate[17].

Other than the *E. coli* LPS preparation, the LPS preparations of *Salmonella typhosa, Salmonella typhimurium, Shigella flexineri* and *Serratia marcescens* were studied in mice. All preparations showed the histamine sensitizing effect beyond the LPS extraction methods and bacterial origins, but not necessarily the same intensity.

The age of mice was found as one of the most important factors, as already described[12]. The older are mice, the more strongly they respond, within the experimental limitations. Therefore, the 11- to 12-week-old mice were used in practice throughout, except in the earlier period of the study. As shown in Table I, the histamine hypersensitivity induced by endotoxin was confirmed at first by using about 17-week-old mice by chance. Formerly we tried to confirm the histamine hypersensitivity several times but the results had not been reproducible. When we think of it now, it is proper that the reproducibility of the results should be poor in consequence of using 4- to 5-week-old mice.

As already reported[9], lentinan induced the histamine hypersensitivity in mice under controlled conditions after the fractionated administration. But the hypersensitivity was not represented in such earlier periods after a single administration of minute dose of lentinan as in the case of endotoxin administration. The fact suggests that the mechanism of histamine hypersensitivity induced by endotoxins may be different from that by lentinan.

Riassunto. È dimostrato che l'apparizione rapida entro 60 min dell'ipersensibilità a istamina è indotta nei topi con dosi minime, come nanogrammi per topo, di tutte le sorti di endotossine esaminate. La risposta dipende dall'età del topo. La relazione fra il logaritmo della dose di endotossina e la mortalità, la dose d'istamine essendo costante, non è lineare; ma risulta una curva con un massimo, «unimodal dose-response curve».

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[15] J. C. MELBY, R. H. EGDAHL and W. W. SPINK, J. Lab. clin. Med. *56*, 50 (1960).
[16] E. M. NADEL, B. YOUNG, A. HILGAR and A. MANDEL, Am. J. Physiol. *201*, 551 (1961).
[17] K. KURATSUKA, R. HOMMA, Y. SHIMAZAKI and I. FUNASAKA, Japan. J. Bact. *29*, 270 (1974) (in Japanese).
[18] Acknowledgment: Thanks are due to Dr. M. KUROKAWA, Director of the Department, for his kind suggestions.

2-Allyl-2-Isopropylacetylurea and its Influence on the Haematopoetic System

After the introduction of drugs containing allyl groups, the occurence of porphyria and thrombocytopenia was observed in patients receiving this type of drugs. Examples of such cases have been described (DUESBERG[1]; DENNIG[2]). Animal experiments led to the discovery, by SCHMID and SCHWARTZ[3], of an acute experimental porphyria in rabbits after oral administration of 2-allyl-2-isopropylacetylurea (AIAU) indicating that this drug must have, at least for porphyria, a disturbing effect on haem-metabolism. Studies with a similar compound, 2-allyl-2-isopropylacetamide (AIA), demonstrated a breakdown of the haem containing cytochrome P-450 in rat liver (DE MATTEIS[4]). Subsequently much work has been carried out on that tissue (LEVIN et al.[5]). Investigations attempting to elucidate the mechanism of the thrombocytopenia have dealt with changes of the thrombocytes stem cell in the bone marrow. The results have tended to be contradictory (ACKROYD[6]). Experiments dealing with whole blood have been very limited (JÜRGENS[7]). Thus in connection with our long-term pigmentation experiments with AIAU, the haematopoetic system has been examined, and the results are reported here.

Materials and methods. 42 male Sandoz OFA-SPF rats, 120–140 g, were dosed twice daily with a s.c. injection of AIAU[8] in propandiol (50 mg/ml). The overall daily dose

[1] R. DUESBERG, Münch. med. Wschr. *79*, 1821 (1932).
[2] H. DENNIG, Münch. med. Wschr. *80*, 562 (1933).
[3] R. SCHMID and S. SCHWARTZ, Proc. Soc. exp. Biol. Med. *81*, 685 (1952).
[4] F. DE MATTEIS, FEBS Lett. *6*, 343 (1970).
[5] W. LEVIN, M. JACOBSON and R. KUNTZMAN, Arch. Biochem. Biophys. *148*, 262 (1972).
[6] J. F. ACKROYD, Clin. Sci. *7*, 249 (1948).
[7] R. JÜRGENS, Arch. exp. Path. Pharmak. *212*, 440 (1951).
[8] We thank Hoffmann-La Roche AG Basel for their generous gift of the AIAU.

was 400 mg/kg; 42 control rats were injected with similar quantities of propandiol MERCK only. At days 1, 3, 7, 10, 14, 17 and 21, after the first dose 3 animals of each group were removed and a sample of blood taken from the retroorbital plexus. Besides haematocrit erythrocytes, reticulocytes and Heinz-bodies were counted by means of Coulter counter, methylene blue staining and nile blue staining respectively. Haemoglobin was estimated by the haemoglobin-cyanide method. The remaining animals were allowed to recover for 21 days during which time blood was taken in a similar manner as above, and the same parameters measured at similar times after stopping dosing. Another group of 5 animals were treated in the same way with AIAU in a daily overall dose of 500 mg/kg for 42 days, and on the 43rd day the thrombocytes were counted by the cocaine method and compared to 5 control animals.

Results and discussion. If rats are treated for a relatively long period with AIAU, they show marked disturbances in liver haem metabolism, which is paralleled by a EM and histologically visible pigmentation of this tissue[9], and increased urinary excretion of porphyrins and their precursors. Variations were also noted in serum enzymes and other serum parameters (RENTSCH and JOHNSTON[10]).

In our experiments we were unable to find major changes in the haematopoetic system. Erythrocytes, haematocrit and haemoglobin variations were within the margin of error of our techniques. During the experiment the reticulocytes were in the range of 21–145 cells/1000, which is within the physiological norm for the rats used. Heinz-bodies, markers of intra-erythrocyte haemoglobin destruction (RENTSCH and WITTEKIND[11]), were not found. Hence it can be seen that AIAU produces no significant alterations in the parameters measured, except for a slight elevation of the thrombocytes in the high-dose animals, a result contrary to that found in humans hypersensitive to AIAU (see Table).

Thrombocytes of rats dosed with AIAU 500 mg/kg (T) compared with those of controls (K)

Animal number	Thrombocytes per µl
K1	836×10^3
K2	772×10^3
K3	974×10^3
K4	782×10^3
K5	1102×10^3
K average	893×10^3 [a]
T1	1268×10^3
T2	1366×10^3
T3	1252×10^3
T4	1068×10^3
T5	1204×10^3
T average	1232×10^3 [b]

[a] SD: $\pm 142 \times 10^3$; [b] SD: $\pm 109 \times 10^3$; $P<0{,}01$
See text for full experimental details.

LEVIN et al.[12] suggest that AIAU exerts its pathophysiological effects for 2 reasons: 1. there is an allyl group in the molecule, and 2. the compound is converted into an active metabolite. It is also suggested that the active form is an epoxide on the allyl group. This epoxide is then thought to react with haem causing its destruction in liver.

Why then does AIAU not cause haemoglobin destruction in blood? One answer may be that red cells are unable to metabolize it and the active metabolite formed in other tissues never reaches the circulatory system. In this context it should be mentioned that other tissues besides liver can produce the active metabolite. We have found a green pigmentation in isolated microsomal preparations of lung and kidney from rats treated with AIAU[10]. This indicates a similar pattern to that in the liver where AIAU causes haem breakdown products from cytochrome P-450, colouring the microsomes.

Tissues capable of producing the active molecule probably bind it tightly and rapidly to structures near the site of its formation. This means that the effective half-life of the new compound formed must be extremely short. In the kidney, there is the possibility of an allyl group epoxide forming and being rapidly excreted due to the special nature of the tubular system. Epoxides found in rat urine after administration of AIA support this[13,14].

Zusammenfassung. Wenn Ratten längere Zeit mit 2-Allyl-2-isopropylacetylharnstoff behandelt werden, finden sich in verschiedenen Organen grün-braune Pigmente. Diese vorwiegend in Microsomen lokalisierten Produkte stammen vom Häm des Cytochroms P-450 sowie anderen Hämoproteinen. Am hämatopoetischen System konnten demgegenüber keine Veränderungen gefunden werden. Damit ist der Schluss gestattet, dass der für die Hämdenaturierung verantwortliche aktive Metabolit eine sehr kurze Halbwertzeit haben muss.

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[9] G. RENTSCH, A. JOHNSTON, CH. HODEL and A. PATAKI, in preparation.
[10] G. RENTSCH and A. JOHNSTON, in preparation.
[11] G. RENTSCH and D. WITTEKIND, Toxic appl. Pharmac. *11*, 81 (1967).
[12] W. LEVIN, M. JACOBSON, E. SERNATINGER and R. KUNTZMAN, Drug Metab. Dispos. *1*, 275 (1973).
[13] D. J. DOEDENS, Diss. Abstr. *32*, 2951 B (1971).
[14] The authors wish to thank Dr. G. RÜTTIMANN and Miss H. MAURER for their technical help.

Inhibition of the Local Hemorrhagic Shwartzman Reaction by an Acid Proteinase Inhibitor, Pepstatin

When bacterial endotoxin is injected into rabbits intracutaneously, followed in 24 h by an i.v. injection of another endotoxin preparation, an inflammatory and necrotic reaction is manifested at the site of intracutaneous implantation of the material[1,2]. The mechanism of this local hemorrhagic SHWARTZMAN phenomenon is unclear. However, evidence indicates that a granulocyte mediated inflammatory process[3–5] is involved along with the effect of endotoxin upon the blood vessels and lymphoreticular cells[6,7]. Complement[8] as well as the blood coagulation