Rapid Appearance of Histamine Hypersensitivity in Mice by Minute Dose of Endotoxins[1]

It is known that mice have a relatively low sensitivity to histamine. However, PARFENTJEV and GOODLINE[2] discovered that the mice administered with pertussis vaccine had histamine sensitivity enhanced and indicated shock-like symptoms followed by death with small amount of histamine. Since then, a large number of studies[3] on this phenomenon and its agent, the histamine sensitizing factor (HSF)[4] of *Bordtella pertussis*, have been carried out for a good many years. We have also studied the purification and the biological and physicochemical properties of HSF and confirmed it as a protein-like substance having the molecular weight of about 13,000[5,6].

On the other hand, in addition to HSF, a protein-like substance, we found[7] that an antitumour polysaccharide, lentinan, and a few polysaccharides structurally related to this substance, also enhanced the histamine sensitivity of mice[8,9]. The findings that certain polysaccharides were able to induce histamine hypersensitivity in mice, led us to investigate the possible histamine hypersensitivity caused by endotoxins which contain lipid and polysaccharide. After many trials, the adequate controls were also taken. The results thus obtained and described in the present paper indicated clearly that histamine hypersensitivity was induced rapidly in 1 h with even a minute dose such as about 0.001 μg per mouse of endotoxin lipopolysaccharide. In contrast to our results, it was with large amounts (sublethal doses) of lipopolysaccharide 4 days after the endotoxin administration that former workers[10,11] observed the histamine hypersensitivity caused by endotoxin in mice.

Materials and methods. The lipopolysaccharide (LPS) mainly used as endotoxin preparation was prepared from *E. coli* 0111 B4 cells by ether treatment, phenol extraction and ultracentrifugation in our laboratory[13], and it was provisionally named *E. coli* LPS-Y. The other LPS preparations were purchased from Difco Co. Female mice (ddY strain) of several age groups were tested. In order to test the histamine hypersensitivity caused by endotoxin, mice were inoculated i.v. with 0.2 ml of the endotoxin solutions containing various amounts of LPS in pyrogen-free saline, and after varied periods (1 h to 8 days) they were i.p. challenged with histamine solutions in the volume of 0.5 ml. As one dose was given the histamine solution contained 6 mg of histamine dihydrochloride (Sigma Co.) for mice not older than 10 weeks old, or 3 mg for mice not younger than 11 weeks old. Deaths were tabulated 3 h later. The control mouse group, not treated with endotoxin, ran with each test as histamine control. All glass ware, syringes and needles for use were heated for more than 30 min at 250 °C to exclude pyrogen contamination.

Results and discussion. In Table I, the 3 relatively large doses, 200, 20 and 2 μg per mouse of *E. coli* LPS-Y were given i.v. to each group of 40 mice, respectively. 10 mice of each group were i.p. challenged with histamine on days 1, 4, 6 and 8 after the endotoxin administration, respectively. In the histamine hypersensitivity of mice given 200 μg dose of LPS-Y, the lethal rate (5/9) on the 4th day was significantly larger than that of the histamine control, but the other 3 rates were not significantly larger. Therefore, the histamine hypersensitivity was evidently demonstrated only in mice administered with LPS 4 days prior to histamine challenge. This fact was a reconfirmation of the former observations of MALKIEL et al.[10] and of PIERONI et al.[11].

On the other hand, the histamine hypersensitivity of mice given 20 μg dose was not evidently demonstrated on any days tested, while the hypersensitivity of mice given 2 μg dose was clearly demonstrated on the 1st day but not

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Table I. The change of histamine hypersensitivity after *E. coli* LPS administration

LPS dose (μg/mouse[a], i.v.)	Histamine lethality after LPS administration in the following periods (days)			
	1	4	6	8
Experiment 1				
200	3/6[b]	5/9[f]	3/10	2/10
20	5/10	0/10	1/10	0/10
2	6/10[d,e]	2/10	1/10	2/10
Totals on each histamine challenge day	14/26[f]	7/29	5/30	4/30
(%)	(53.8)	(24.1)	(16.6)	(13.3)
Histamine[c] control	1/10	0/10	1/10	1/10

Time response in days. [a] Mouse: female, 17 week old. [b] Numerator presents the number of dead mice, denominator the total number of mice used for histamine challenge. The number of denominator was 10, except in some groups of mice given with 200 μg, in which 5 mice were excluded by accident before the histamine challenge. [c] Histamine challenge dose is 6 mg of histamine dihydrochloride per mouse. [d] According to Fisher's exact test, the rate is significantly different from that of histamine controls at [e] 5%, [f] 2.5% significant levels, respectively.

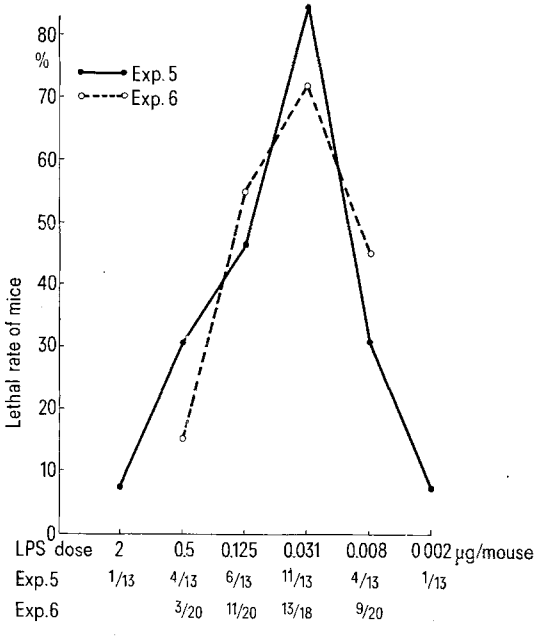
Table II. The change of histamine hypersensitivity after *E. coli* LPS administration

Experiment	LPS dose (μg/mouse[a])	Histamine lethality after LPS administration in the following periods (h)								Histamine[c] control
		1	4	5	6	7	8	10	24	
2	20	0/12[b]	1/12		1/12				3/12	
	2	0/12	2/12		7/12[d,g]				3/12	0/12
3	2	0/12	0/12		6/12[f]		5/12[g]	7/12[g]	1/12	
	0.2				7/12[g]		4/12[e]			0/12
4	0.5	0/20	3/20	4/20		7/20[g]				
	0.125	2/20	12/20[h]	10/20[h]		12/20[h]				0/20
	0.031	5/20[e]	13/20[h]	12/20[h]		9/20[h]				

Time response in hours. [a] Mouse: female, the age in Exp. 2 is 14 weeks, the ones in Exp. 3 and 4 are 11 weeks. [b] Numerator presents the number of dead mice, denominator the total number of mice used for histamine challenge. [c] Histamine challenge dose is 3 mg of histamine hydrochloride per mouse. [d] Statistical significance level against histamine control. [e] $\alpha = 0.05$; [f] $\alpha = 0.025$; [g] $\alpha = 0.01$; [h] $\alpha = 0.001$.

on any other days tested. Among total lethal rates including the rates of 3 LPS doses, the total lethal rate on 1 day was significantly different from those on the other days. Furthermore, in the rates on 1 day after the LPS administration, it could be noticed that the sensitivity was significantly demonstrated only in the mice given the smallest LPS dose (2 μg).

The above-mentioned facts caused us to investigate the histamine hypersensitivity of mice due to much smaller doses of endotoxin, especially within 24 h after the administration. The data of 3 experiments are presented in Table II. Experiment 2 shows that, in the mice given 2 μg of LPS-Y, the hypersensitivity was significantly demonstrated only 6 h after the administration, but not at other times, while in the mice given larger dose (20 μg) the change of the sensitivity was not demonstrated at any times tested after the administration. In Experiment 3, the histamine hypersensitivity of the mice given 2 μg of endotoxin was significantly demonstrated at least for 6 to 10 h after the administration, and it could not be demonstrated again 24 h thereafter. Such a time response of the histamine hypersensitivity to endotoxin is similar to that of SCHAYER's[14] induced histamine in tissues caused by endotoxin. The relation between our findings and SCHAYER's induced histamine remains to be solved in further studies. Then our attention focussed on the hypersensitivity responses with much more minute doses of endotoxin. In Experiment 4, the histamine hypersensitivity due to the smallest dose tested (0.031 μg) was significantly demonstrated in 1 h and later after the administration; that due to 0.125 μg in 2 h and later, and that due to 0.5 μg only in 7 h, respectively. These results suggested that the more minute the endotoxin dose was, the earlier the histamine hypersensitivity was detectable within the ranges of doses tested. In other words, the histamine hypersensitivity due to relatively large dose cannot be detected in early hours after the endotoxin administration. The real reason for these phenomena is quite obscure, but these findings would be explainable by the characteristic dose response curve of the endotoxin-induced histamine hypersensitivity to be mentioned later.



Dose response curve of histamine hypersensitization induced by *E. coli* LPS administration.

In order to measure the histamine-sensitizing activity of endotoxin, that is, in order to determine the dose-response relation, the mice pretreated with endotoxin must be challenged with a constant histamine dose in a certain hour period after endotoxin administration. We arbitrarily adopted the 5-hour period after the administration as a rule in the following experiments.

In Experiment 5, mice were administered i.v. with *E. coli* LPS-Y doses ranging from 2 to 0.002 μg per mouse in 4-fold serial dilutions and 5 h later they were i.p. challenged with histamine. Experiment 6 was carried out about 2 months after the Experiment 5 in order to check the reproducibility of the results. The lethal rates of mice in both experiments were tabulated at the bottom of the Figure. In the Figure, the 2 dose- response curves were very similar and were shown as a unimodal curve the peaks of which corresponded to 0.031 μg dose per mouse in both Experiments 5 and 6. From the peak of the curve,

[14] R. W. SCHAYER, in *Bacterial Endotoxins* (Eds. M. LANDY and W. BRAUN; Rutgers Univ. Press, New Brunswick, New Jersey (1964), p. 182.

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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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

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