REFERENCES

1. R. H. Michell, *Biochim. biophys. Acta* **415**, 81 (1975).
2. P. Greengard, *Science* **199**, 146 (1978).
3. S. S. Jafferji and R. H. Michell, *Biochem. J.* **160**, 163 (1976).
4. A. A. Abdel-Latif, in *Function and Metabolism of Phospholipids in CNS and PNS* (Eds. G. Porcellati, L. Amaducci and C. Galli), p. 227. Plenum Press, New York (1976).
5. A. A. Abdel-Latif, R. A. Akhtar and J. P. Smith, in *International Conference on the Cyclitols and the Phosphoinositides* (Eds. F. Eisenberg and W. W. Wells), p. 121. Academic Press, New York (1978).
6. R. A. Akhtar and A. A. Abdel-Latif, *J. Pharmac. exp. Ther.* **204**, 655 (1978).
7. H. D. Griffen, J. N. Hawthorne and M. Sykes, *Biochem. Pharmac.* **28**, 1143 (1979).
8. S. F. Fisher and B. W. Agranoff, *J. Neurochem.* **34**, 1231 (1980).
9. C. Van Breeman, P. Aaronson and R. Loutzenhiser, *Pharmac. Rev.* **30**, 167 (1979).
10. A. A. Abdel-Latif, R. A. Akhtar and J. N. Hawthorne, *Biochem. J.* **162**, 61 (1977).
11. K. Ahmad and J. D. Judah, *Biochim. biophys. Acta* **104**, 112 (1965).
12. G. Keryer, G. Herman and B. Rossignol, *Lett. Fedn Eur. Biochem. Soc.* **102**, 4 (1979).
13. A. A. Abdel-Latif and B. Luke *Biochim. biophys. Acta*, **673**, 64 (1981).
14. C. Van Breeman, P. Aaronson and R. Loutzenhiser, in *Vascular Neuroeffector Mechanisms* (Eds. J. A. Bevan, T. Godfraind, R. A. Maxwell and P. M. Vanhoutte), p. 227 Raven Press, New York (1980).
15. D. F. Cole, *Br. J. Ophthal.* **45**, 202 (1961).
16. M. V. Riley and V. Baroncelli, *Nature, Lond.* **201**, 621 (1964).
17. M. V. Riley, *Expl Eye Res.* **3**, 76 (1964).
18. C. L. Winder and M. W. Weiner, *J. Pharmac. exp. Ther.* **213**, 375 (1980).
19. M. V. Riley, *Biochem. J.* **98**, 898 (1966).
20. J. W. Putney, Jr., *Pharmac. Rev.* **30**, 209 (1979).
21. G. Biamino and B. Johansson, *Pflügers Arch. ges. Physiol.* **321**, 143 (1970).
22. L. B. Rosenberger and D. J. Triggle, *Can. J. Physiol. Pharmac.* **57**, 348 (1979).
23. R. A. Akhtar and A. A. Abdel-Latif, *Biochim. biophys. Acta* **527**, 159 (1978).
24. A. A. Abdel-Latif, B. Luke and J. P. Smith, *Biochim. biophys. Acta* **614**, 425 (1980).
25. R. A. Akhtar and A. A. Abdel-Latif, *Biochem. J.* **192**, 785 (1980).

Biochemical Pharmacology, Vol. 30, No. 11, pp. 1374–1378, 1981.
Printed in Great Britain.

0006-2952/81/111374-05 $02.00/0

Acute and chronic effects of barbiturates and ethanol on phospholipid and sulfatide content of rat brain regions

(*Received* 14 *July* 1980; *accepted* 11 *December* 1980)

The cellular mechanisms underlying the sedative effects of barbiturates and ethanol remain obscure. It is generally accepted, however, that the major sites of anesthetic action reside within the synapse [1].

Recent studies have shown that both acute and/or chronic administration of barbiturates and ethanol produce changes in a number of membrane lipid parameters. Acute pentobarbital administration, for example, significantly increases the ratio of triphosphatidylinositol (TPI) to diphosphatidylinositol (DPI) in rat brain microsomes [2]. Barbiturates, added *in vitro*, decrease the incorporation of ^{32}P into synaptosomal phosphatidylinositol and phosphatidic acid [3]. Both short-term (2 hr) and long-term (10 days) ethanol administration to mice have been shown to reduce the relative proportion of polyunsaturated fats in synaptosomal phospholipids [4]. *In vitro* ethanol increases the fluidity of spin-labeled synaptosomes, whereas the result of chronic ethanol administration is an adaptational resistance to the *in vitro* fluidizing action of ethanol [5]. Chronic administration of ethanol also causes an increase in the cholesterol content of erythrocyte and brain membranes [6]. This adaption by membranes may expalin the development of resistance [5] to the fludizing actions of *in vitro* ethanol exposure, although more recent work suggests that the presence of cholesterol in membranes may be required for the expression of tolerance but not for the attenuation of ethanol-induced membrane fluidization [7].

Although it is clear that barbiturates and ethanol alter a variety of important membrane variables, few studies have directly compared the acute or chronic effects of these drugs on brain membrane lipids. Johnson *et al.* [8] have shown recently that artificial membranes, formed from lipid extracts of synaptosomal membranes from ethanol-tolerant mice, exhibit tolerance to the fluidizing effect of ethanol and cross-tolerance to pentobarbital, but not to morphine. In the present paper, we report the results of a comparative study on the effects of acute and chronic administration of ethanol and barbiturates on the content of brain regional phospholipids and sulfatides.

Male Spargue–Dawley rats (250–350 g) were kept on a 12/12 hr light–dark cycle with food and water *ad lib.* for at least 5 days. The rats were then randomly divided into three experimental groups: control, acute, and tolerant. For the barbiturate studies, acute animals received a single dose of pentobarbital (50 mg/kg, i.p.) and were killed 30 min later. Animals in the tolerant group received a food cup containing a milled diet (Purina Lab Chow) thoroughly mixed with 2.5 to 3.5 mg phenobarbital/g diet as their sole food source. Animals in the tolerant group were maintained on the phenobarbital diet for 13 days and were killed on the morning of day 14.

A treadmill apparatus ("moving belt") was used to determine the degree of intoxication and tolerance development for animals in the tolerant group. Briefly, this

Table 1. Chronic phenobarbital performance scoring using the moving belt apparatus*

Days on phenobarbital diet	Performance scores (% time on moving belt) (N = 5)	Phenobarbital consumed/day (mg/kg body weight)
1	57.7 ± 17.4	143.1 ± 7.3
2	75.0 ± 14.3	130.0 ± 4.5
3	73.5 ± 11.6	147.8 ± 4.5
4	78.1 ± 15.2	183.0 ± 18.2
5	83.3 ± 10.0	188.7 ± 19.1
6	81.9 ± 11.7	193.2 ± 9.5
7	83.3 ± 15.9	193.4 ± 11.3
8	92.3 ± 4.9†	205.1 ± 9.4
9	93.7 ± 3.4†	212.7 ± 11.1
10	88.4 ± 7.1	224.5 ± 12.6
11	96.2 ± 1.6†	232.2 ± 10.7
12	97.7 ± 1.0†	208.6 ± 17.8
13	98.4 ± 1.3†	246.2 ± 21.5

* Each value is the mean ± S.E.

† Performance scores were significantly increased ($P < 0.05$) compared to day 1 (Student's *t*-test).

procedure, somewhat modified from Gibbins *et al.* [9], involved a determination of the percentage of time (during a continuous 6-min measurement period) each rat remained on a moving belt located in a closed chamber with an electrical grid floor. Each rat was trained once daily for at least 4 days and until 100 per cent of the measurement time was spent on the moving belt. After the initial training period, the phenobarbital diet was started and measurements were taken once daily for 13 days.

For the ethanol studies, acute animals received a 4.0 g/kg, i.p., dose of ethanol (20%, w/v) 30 min prior to decapitation. Animals in the tolerant group were kept on a Sustacal™ and 10% (w/v) ethanol diet for 20 days. The control group was pair-fed a diet containing Sustacal™ with sucrose substituted isocalorically for ethanol. Rats fed the liquid diet consumed an average of 11.9 $g \cdot kg^{-1} \cdot day^{-1}$ of ethanol, and the mean blood ethanol concentration (measured by the method of Friedman *et al.* [10] between 8:00 and 9:00 a.m.) during the diet period was 118.4 ± 38.1 mg/dl ($\bar{x}$ ± S.E.). Blood alcohol levels remained relatively constant throughout the 20-day regimen. The average weights of rats fed a Sustacal™ ethanol diet for 20 days did not differ significantly from those of the control animals. Ethanol "sleep-time" (loss of righting reflex, unconsciousness) was used to assess tolerance development. Animals in the tolerant groups were given a 3.0 g/kg, i.p., dose of ethanol (20%, w/v) on days 3, 12 and 19 of the diet regimen. Sleep-time was defined as the interval between loss of righting reflex and its recovery, as indicated by the ability of the animal to turn over from the supine position twice within 1 min.

For brain lipid extraction, rats from each of the three groups were decapitated, and whole brains and brain regions (cerebral cortex, cerebellum, brain stem, hypothalamus, striatum, and midbrain) were dissected quickly, weighed, and lipids were extracted by the method of Trifaro [11]. Tissue samples from whole brain and brain regions were homogenized in 10 vol of 5% trichloroacetic acid, and the acid-soluble and acid-insoluble materials were separated by centrifugation at 8000 *g* for 10 min at 4°.

The acid-insoluble material was extracted with 15 vol. of chloroform–methanol (2:1, v/v) containing 0.25% HCl. The extract was vortexed with 0.2 vol. of cold KCl (0.8%), and the upper and lower phases were separated by centrifugation at 500 *g* for 20 min at room temperature. The upper, aqueous phase was removed and discarded. The interphase was then washed twice with "theoretical upper phase" solution [12]. Finally, the lipid extract (lower phase) was diluted to a constant volume with chloroform–methanol (2:1, v/v). Fifty to seventy-five microliters of each extract containing approximately 4–5 μg of lipid-phosphorus was spotted on a silica-gel G thin-layer chromatographic plate. Each plate was developed in a rectangular chamber (28 × 23 × 7.5 cm) that contained about 100 ml of solvent mixtures. The solvent systems consisted of chloroform–methanol–conc. ammonium hydroxide (65:30:4, by vol.) in the first dimension and chloroform–methanol–acetic acid–water (170:25:25:6, by vol.) in the second. After development in the first direction, the plate was dried under warm air for 3 min and then was developed in the second solvent system at a right angle to the first direction. Co-chromatography of tissue extract with standard phospholipids was used to identify the lipids. The phospholipids were detected by means of iodine vapor. Phospholipid spots on the silica gel plates were scraped off, and phospholipid phosphorus was assayed by the method of Bartlett [13]. Sulfatide content of the lipid extract was determined by the method of Kean [14].

To demonstrate that rats receiving 13 days of phenobarbital diet developed behavioral tolerance, the amount of phenobarbital consumed was measured and compared with the ability of the animal to walk on a treadmill (in the moving belt apparatus). The results of phenobarbital consumption and the moving belt scores are shown in Table 1.

On day 1 of the diet the rats remained on the moving belt 57.7 per cent of the time and consumed 143.1 mg phenobarbital/kg body weight. By day 8 the rats exhibited

Table 2. Sleep-time of chronic Sustacal™–ethanol-treated rats*

Days on ethanol	Sleep-time (min)
3	23.6 ± 2.2
12	9.5 ± 2.0†
19	0.0 ± 0.0†

* Rats maintained on the Sustacal™–ethanol liquid diet were given ethanol (3.0 g/kg) (20%, w/v) on days 3, 12, and 19 of the diet. Values are means ± S.E.; N = 11.

† Significantly different ($P < 0.01$) from value on day 3 (Student's *t*-test).

Table 3. Effect of chronic phenobarbital administration on phospholipid and sulfatide contents of whole brain and brain regions*

	Phospholipid and sulfatide contents						
	Whole brain	Cerebellum	Brain stem	Cerebral cortex	Striatum	Hypothalamus	Midbrain
			(% content)				
Pair-fed control							
Phosphatidic acid	2.5 ± 0.4	1.9 ± 0.5	3.3 ± 0.7	2.1 ± 0.5	3.4 ± 1.3	2.8 ± 1.7	2.9 ± 0.9
Phosphatidylcholine	30.8 ± 1.5	31.2 ± 2.7	30.0 ± 2.1	35.0 ± 2.1	32.7 ± 2.9	33.7 ± 3.8	30.9 ± 1.3
Phosphatidylethanolamine	16.0 ± 1.8	14.3 ± 1.3	14.0 ± 1.4	18.9 ± 1.2	17.8 ± 3.0	17.0 ± 1.0	16.2 ± 0.8
Phosphatidylinositol	2.3 ± 0.5	1.8 ± 0.5	1.8 ± 0.5	1.5 ± 0.5	3.2 ± 0.7	2.2 ± 0.8	2.2 ± 0.4
Phosphatidylserine	10.9 ± 0.9	11.3 ± 1.3	12.7 ± 1.3	12.8 ± 0.6	13.0 ± 2.1	11.4 ± 1.4	11.4 ± 1.2
			(mg/g wet tissue)				
P_i	1.97 ± 0.07	1.77 ± 0.18	2.41 ± 0.06	1.64 ± 0.09	1.63 ± 0.12	1.63 ± 0.12	1.85 ± 0.14
Sulfatide	4.30 ± 0.03	5.14 ± 0.31	9.94 ± 0.54	4.85 ± 0.35	5.84 ± 0.24	5.28 ± 0.69	6.12 ± 1.03
			(% content)				
Chronic phenobarbital treatment							
Phosphatidic acid	2.7 ± 0.7	2.0 ± 0.3	3.4 ± 0.8	2.9 ± 0.8	3.4 ± 0.6	3.0 ± 0.6	3.3 ± 0.8
Phosphatidylcholine	31.2 ± 0.9	32.7 ± 2.2	27.0 ± 2.5	34.3 ± 1.3	35.3 ± 1.7	32.4 ± 1.2	30.1 ± 1.9
Phosphatidylethanolamine	16.2 ± 1.5	15.4 ± 1.8	13.4 ± 1.2	19.5 ± 3.2	18.8 ± 1.0	16.6 ± 0.8	15.8 ± 1.4
Phosphatidylinositol	2.5 ± 0.9	2.0 ± 0.4	1.8 ± 0.5	2.2 ± 0.5	3.5 ± 1.5	2.0 ± 0.7	2.0 ± 0.4
Phosphatidylserine	11.3 ± 0.2	11.4 ± 1.2	12.1 ± 1.2	11.9 ± 0.9	13.6 ± 1.7	11.5 ± 1.4	11.6 ± 1.2
			(mg/g wet tissue)				
P_i	2.02 ± 0.05	1.76 ± 0.16	2.64 ± 0.11†	1.72 ± 0.03	1.78 ± 0.12	1.72 ± 0.21	2.05 ± 0.17
Sulfatide	4.31 ± 0.15	6.03 ± 0.44†	11.29 ± 0.69†	4.93 ± 0.24	6.44 ± 0.48‡	5.70 ± 0.65	7.07 ± 0.92

* Each value is the mean ± S.D. of five separate experiments.
† $P < 0.01$ (Student's t-test).
‡ $P < 0.05$ (Student's t-test).

Table 4. Effect of acute and chronic ethanol administration on sulfatide content of whole brain and brain regions*

	(mg sulfatide/g wet tissue)						
	Whole brain	Cerebellum	Brain stem	Cerebral cortex	Striatum	Hypothalamus	Midbrain
(A) Control	5.6 ± 0.25	5.23 ± 0.64	10.09 ± 0.80	4.02 ± 0.20	4.96 ± 0.89	5.11 ± 0.52	8.01 ± 0.77
Acute ethanol treated (4 g/kg, i.p.)	4.76 ± 0.26	5.12 ± 0.34	9.65 ± 0.57	3.93 ± 0.39	5.05 ± 0.41	4.51 ± 0.33†	7.28 ± 0.55
(B) Pair-fed control	5.04 ± 0.27	5.57 ± 0.33	10.59 ± 0.60	4.43 ± 0.33 (N = 4)	6.50 ± 0.54 (N = 4)	5.04 ± 0.63	7.11 ± 0.59
Chronic ethanol treated	5.53 ± 0.44‡	5.33 ± 0.30	10.42 ± 0.82	4.38 ± 0.30 (N = 4)	6.40 ± 0.30 (N = 4)	4.88 ± 0.46	7.27 ± 0.54

* Each value is the mean ± S.D. of five separate experiments.
† $P < 0.02$ (Student's *t*-test).
‡ $P < 0.05$ (Student's *t*-test).

a significantly greater ability to remain on the moving belt (92.3 per cent, $P < 0.05$ compared to day 1) and they consumed 205.1 mg phenobarbital/kg body weight. On day 13 the animals stayed on the moving belt with 98.4 per cent accuracy ($P < 0.05$ compared to day 1) and the phenobarbital consumption increased to 246.2 mg/kg body weight. A significant increase in the ability to walk on the moving belt over the 13-day period was evidence of behavioral tolerance development. The phenobarbital consumption increased by 72 per cent over the diet period. During the entire period there was no apparent increase or decrease in body weight. These data agree closely with our recent work in which moving belt scores were correlated with brain levels of phenobarbital to characterize functional tolerance development [15].

The ethanol sleep-time data (Table 2) were obtained from animals that were given 3.0 g/kg, i.p., of ethanol (20%, w/v) on days 3, 12 and 19 of the ethanol liquid diet. Their sleep-times had decreased significantly by days 12 and 19 (9.5 ± 2.0 and 0.0 ± 0.0 min respectively) compared to day 3 (23.6 ± 2.2 min).

Acute administration of pentobarbital (50 mg/kg, i.p.), in contrast to chronic phenobarbital administration, did not alter total phospholipid-phosphorus content, the proportions of individual phospholipids, or the sulfatide content of whole brain or brain regions. Similarly, neither acute nor chronic administration of ethanol affected the total phospholipid-phosphorus content or the proportions of individual phospholipids of whole brain and brain regions.

Table 3 shows the effects of chronic phenobarbital administration on brain phospholipid and sulfatide content. The total phospholipid-phosphorus content and the proportions of individual phospholipids of whole brain and brain regions from the tolerant groups were identical to controls, except in brain stem. In brain stem, the total phospholipid-phosphorus concentration was increased by 9.5 per cent without altering the proportions of individual phospholipids. In tolerant animals, a significant increase of sulfatide concentration was found in cerebellum (17.3 per cent, $P < 0.01$), brain stem (13.6 per cent, $P < 0.01$), and striatum (10.3 per cent, $P < 0.05$). No significant change in sulfatide content of whole brain, cerebral cortex, hypothalamus or midbrain was observed in comparison to pair-fed controls.

The acute administration of ethanol reduced sulfatide content in hypothalamus (Table 4A). No significant change in sulfatide content, however, was observed in whole brain, cerebral cortex, striatum, brain stem, cerebellum or midbrain in comparison to controls. Chronic ethanol administration increased the sulfatide content of whole brain but no such increase was detectable when brain regions were analyzed (Table 4B).

The results of the present study show that, whereas acute barbiturate administration had no effect on brain phospholipid or sulfatide concentrations, chronic administration of barbiturates increased the phospholipid content of brain stem and increased the sulfatide content of cerebellum, brain stem and striatum but not midbrain, hypothalamus, or cerebral cortex. This observation may be of importance for several reasons. First, the fact that changes in phospholipid and sulfatide concentrations occurred after chronic barbiturate treatment but not after acute administration suggests that, if changes in concentration of these lipids are involved in the central actions of barbiturates, they are probably involved in an adaptive process. Second, the increase in the lipid concentrations after chronic barbiturate treatment exhibited brain regional selectivity. The adaptive increase in sulfatide concentration occurred in the same brain regions (brain stem and cerebellum) that, in our previous work [15], showed the most marked adaptation to inhibition of synaptosomal calcium influx after chronic phenobarbital administration. Furthermore, recent work

has shown that, although all acidic membrane lipids bind calcium, sulfatide is the most potent [16]. Sulfatide, as a result, has been suggested as a possible membrane calcium ionophore [16].

Acute administration of ethanol reduced the sulfatide content of hypothalamus (although no other brain region), but it was without effect on brain regional phospholipid content. Chronic ethanol administration increased the sulfatide content of whole brain, but no such action was observed when brain regions were analyzed for sulfatide. One would expect that an increased sulfatide content in whole brain would be reflected in a similar change in at least some of the brain regions. Since this was not the case, sulfatide involvement in the actions of ethanol must be considered questionable.

Acknowledgements—This work was supported by NIAAA Grant AA05132-01.

Department of Pharmacology and Neurosciences Program
University of Alabama in Birmingham, The Medical Center
Birmingham, AL 35294, U.S.A.

ANDREW Y. CHWEH
STEVEN W. LESLIE*

* Author to whom all correspondence should be addressed.

REFERENCES

1. C. D. Richards, *Trends Neurosci.* **3**, 9 (1980).
2. R. Natsuki, R. J. Hitzemann, B. A. Hitzemann and H. H. Loh, *Biochem. Pharmac.* **26**, 2095 (1977).
3. J. C. Miller and I. Leung, *Biochem. J.* **178**, 9 (1979).
4. J. M. Littleton and G. John, *J. Pharm. Pharmac.* **29**, 579 (1977).
5. J. H. Chin and D. B. Goldstein, *Science* **196**, 684 (1977).
6. J. H. Chin, L. M. Parsons and D. B. Goldstein, *Biochim. biophys. Acta* **513**, 358 (1978).
7. D. A. Johnson, N. M. Lee, R. Cooke and H. H. Loh, *Molec. Pharmac.* **15**, 739 (1979).
8. D. A. Johnson, N. M. Lee, R. Cooke and H. Loh, *Molec. Pharmac.* **17**, 52 (1980).
9. R. J. Gibbins, H. Kalant and A. E. LeBlanc, *J. Pharmac. exp. Ther.* **159**, 236 (1968).
10. M. B. Friedman, C. K. Erickson and S. W. Leslie, *Biochem. Pharmac.* **29**, 1903 (1980).
11. J. M. Trifaro, *Molec. Pharmac.* **5**, 382 (1969).
12. J. Folch, M. Less and G. H. Sloane Stanley, *J. biol. Chem.* **226**, 497 (1957).
13. G. R. Bartlett, *J. biol. Chem.* **234**, 466 (1959).
14. E. L. Kean, *J. Lipid Res.* **9**, 319 (1968).
15. S. V. Elrod and S. W. Leslie, *J. Pharmac. exp. Ther.* **212**, 131 (1980).
16. D. E. Green, M. Fry and G. A. Blondin, *Proc. natn. Acad. Sci. U.S.A.* **77**, 257 (1980).

Biochemical Pharmacology, Vol. 30, No. 11, pp. 1378–1382, 1981.
Printed in Great Britain.

0006-2952/81/111378-05 $02.00/0
Pergamon Press Ltd.

Inhibition of antigen-induced histamine release and thromboxane synthase by FPL 55712, a specific SRA-A antagonist?

(*Received* 10 *July* 1980; *accepted* 22 *December* 1980)

FPL 55712, sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylic acid, has been described to be a selective antagonist for SRS-A, *in vitro* [1]. For this reason it has been commonly used to ascertain the involvement of SRS-A in anaphylactic reactions in various animal model systems [2–6]. In this communication we describe two other properties of FPL 55712 that should be considered when interpreting such studies: (1) FPL 55712 is a potent inhibitor of antigen-induced histamine release *in vitro*, and (2) FPL 55712 inhibits thromboxane synthase.

For assessment of the ability of this drug to inhibit antigen-induced histamine release, two model systems were employed. One was an IgE-dependent system utilizing passively sensitized rat peritoneal cells. The second was an IgG-dependent system employing chopped guinea pig lung fragments from actively sensitized animals. The IgE-dependent passively sentized rat peritoneal system was similar to that described by Herzig and Kusner [7]. Reaginic antiserum was prepared by injecting (i.p.) male OLA-1, Hooded Lister rats (Oxfordshire Laboratories, Oxfordshire, England), weighing 150–200 g, with 10 μg egg albumin (Nutritional Biochemical Corp., Cleveland, OH) in 0.5 ml of Pertussis Vaccine (10 ou of heat-killed bacteria, Cannaught Laboratories, Willowdale, Toronto, Canada). On day 30 the animals were boosted with a second injection of 1 μg egg albumin in 0.5 ml saline (i.p.), and on day 34 they were anesthetized with CO_2 and exsanguinated by cardiac puncture. The sera from 150 rats were inidividually tested by passive cutaneous anaphylaxis (PCA) in rats, using a 48-hr sensitization period [8]. Sera with a titer of at least 1:50 were combined. The titer of the resulting combination was 1:256. The pooled serum was stored at −70°. The reaginic nature of the antiserum was demonstrated by heating for 4 hr at 56°. This reduced the titer to less than 1:4 in the PCA test. For passive sensitization, a mixed population of peritoneal cells (containing 5–10% mast cells) was harvested by lavage from eight to twelve male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA), weighing between 180 and 220 g, which were fasted overnight. The buffer used was Hanks' balanced salt solution containing 50 μg/ml sodium heparin (Sigma Chemical Co, St. Louis, MO) buffered to pH 6.9 with 5% (v/v) of 0.1 M sodium phosphate. The cells were isolated by centrifugation at 350 *g* for 5 min (0–4°) and resuspended in 2 ml of the reaginic serum. After sensitization for 2 hr at 37°, 1 ml of buffer was added and the mixture was recentrifuged as described above. The sensitized cells were then resuspended in buffer to a concentration of approximately 1×10^6 cells/ml, and triplicate