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Anaphylaxis in Chickens: Evidence for *in Vivo* and *in Vitro* Histamine Release[1] (39952)

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Introduction. Anaphylaxis has been experimentally induced in chickens but the mechanisms are still poorly understood. Immediate cutaneous and systemic reactions have been elicited in actively sensitized birds after challenge with homologous antigen (1, 2). Passive cutaneous anaphylaxis (PCA) was demonstrated in young chickens but not in adults; a short (4-hr) sensitization period was required (3, 4). The PCA reaction could be inhibited with antihistamines and was histologically distinct from the Arthus reaction (4, 5). The antibody which mediated this PCA reaction has been characterized as a heat-resistant γ_1-immunoglobulin (6).

In mammals, knowledge of the mechanisms involved in anaphylaxis has been advanced with *in vitro* models (7). In these experimental systems, isolated tissue or cell suspensions are reacted with immunological and pharmacological reagents and the response is measured as a function of the release of one or more physiologically active substances such as histamine, slow-reacting substance of anaphylaxis (SRS-A), etc. We undertook parallel studies in chickens, using chopped lung, based on reports that histamine-containing mast cells are present in chicken lungs (8, 9). A suspension of small fragments of spongy lung tissue permits experimental conditions which approximate those of isolated cell suspensions (10). In the present study, we examined: (i) the effect of compound 48/80, an agent known to induce an anaphylaxis-like syndrome in chickens (2) and histamine release from mammalian lung (11), on the histamine content of chicken lung and, (ii) the ability of chicken antiserum to mediate the release of histamine and SRS-A from chicken lung.

Materials and methods. Ten-day-old (20–60 g) and adult (1.3–3.2 kg) male *Gallus domesticus* (White Leghorn) chickens, purchased from Truslow Poultry Co. (Baltimore, Md.) and Dover Poultry Co. (Baltimore, Md.), were used. The animals were given standard chicken chow (Lingardf–Klein, Baltimore, Md.) and water *ad libitum*. All chemicals and Evans blue dye were obtained from Fisher Scientific Co. (Pittsburgh, Pa.). Bovine serum albumin (BSA) and human serum albumin (HSA) were from Pentex (Kankakee, Ill.). Tyrode's buffer (12) was used in all *in vitro* experiments. Hyperimmune chicken antiserum to BSA (6) was kindly donated by Dr. William Clem of the University of Florida. Compound 48/80, a condensation product of *p*-methoxyphenethyl methyl amine and formaldehyde, was a gift of Burroughs Wellcome Co. (Tuckahoe, N. Y.). In our laboratory, it released 50% of the histamine from isolated rat peritoneal mast cells at a concentration of 0.5 μg/ml.

For sensitization in the PCA test, 25 μl of antiserum was injected intradermally in the unplucked lateral thoracic area. An equal volume of saline was injected into a control site on the contralateral side of the thorax. Four or 24 hr later, a challenge dose of 2.5 mg of antigen in 0.5 ml of saline containing 0.5% Evans blue dye was injected into the jugular vein. Fifteen to 20 min after this injection, the skin sites were examined and the diameter (mean of the longest and the shortest diameters) of blueing was measured. A diameter greater than 4 mm was considered a positive reaction. The PCA titer of the antiserum was taken as the reciprocal of the highest dilution that yielded a positive reaction.

The antigen binding capacity (ABC) of chicken anti-BSA was determined by the method of Farr (13). BSA was iodinated according to the method of Thorrell and

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Johansson (14) with $Na^{125}I$ (carrier-free, 17 Ci/mg, New England Nuclear, Boston, Mass.). Serial fivefold dilutions of the antiserum were prepared in borate-saline buffer, pH 8, containing 10% normal chicken serum. Antiserum (0.5 ml of each) dilutions were incubated with an equal volume of [^{125}I]BSA (125 ng/ml in 0.1% HSA) for 24 hr at 4°. The resulting immune complexes were precipitated with 50% saturated ammonium sulfate at room temperature. After reprecipitation with the same salt, radioactivity in the washed precipitates was counted in a γ spectrometer (Packard 5330, with 50% efficiency). The reciprocal of the antiserum dilution which was calculated to bind 33% of the amount of antigen in the mixture was designated as the titer.

To obtain lung fragments, animals were decapitated and the lungs were promptly removed, then immediately placed in chilled buffer. Pleura, large bronchi, and blood vessels were dissected out. The remaining lung tissue was squeezed free of air and residual blood with a gauze sponge. The tissue was blotted dry on filter paper, weighed, and cut into small pieces with scissors. These pieces were then reduced further in size by two cycles of chopping with a McIlwain tissue chopper (Brinkman Instruments Co., Westbury, N.Y.). The resulting lung fragments were washed three times with cold buffer by centrifugation at 600*g* for 10 min. The washed fragments were finally suspended in buffer at a concentration of approximately 600 mg/ml for use in subsequent experiments.

For reaction with compound 48/80 or with antiserum and antigen, 400 μl of a suspension of lung fragments was transferred to a 2-ml microcup (Fisher Scientific No. 2-5442) and then equilibrated to 37° in a water bath before the addition of the appropriate reagents. The final volume was 800 μl. When the reaction was completed, the sample cups were centrifuged at 600*g* for 5 min at 4°. Duplicate 20-μl samples of the resulting supernatant fluid were assayed for histamine as noted below. In experiments in which SRS-A was also determined, all volumes were doubled and 1 ml of the supernatant fluid was frozen and shipped on dry ice to Dr. Robert Orange, Hospital for Sick Children, Toronto, Ontario, Canada, for bioassay (15).

The histamine content of lung fragments was determined after heating 400 μl of chopped lung suspension in a boiling water bath for 15 min, then centrifuging the tissue at 600*g* for 10 min. Duplicate 20-μl samples of the supernatant fluid were removed and assayed for histamine with a radioenzyme method (16). Standard solutions of histamine were assayed in each experiment and the histamine content of test samples was obtained by extrapolation from the resulting standard curve. The percentage histamine released from lung fragments was calculated as follows: percentage release = (histamine in test sample − histamine in control sample)/(histamine content of lung fragments − histamine in control sample) × 100.

Results. Whole lungs were removed from six adult chickens, weighed, and then boiled without chopping. The histamine content of these whole lungs averaged 1.9 μg/g wet weight. Both lungs were then obtained from nine adult chickens and fragments were prepared as described above. The histamine content of the fragments was about 2 μg/g wet weight. The right lungs contained 1.7 ± 0.3 μg/g and the left lungs contained 2.2 ± 0.5 μg of histamine/g wet weight. These values were not significantly different ($P > 0.05$, Students t test). Due to the small size of the lungs of 10-day-old chicks, chopped tissue from these animals was pooled for measurement of histamine. In 11 pools prepared from 12–24 individual lungs, the histamine content (2.5 ± 0.8 μg/g wet weight) was not significantly different from that of adult lungs ($P > 0.05$, t test).

Before studying the effect of compound 48/80 and chicken antiserum to BSA *in vitro*, their effects *in vivo* were examined. Compound 48/80 was injected into the jugular vein of adult chickens in doses ranging from 100 to 1000 μg/kg body weight in 1-ml total volumes. Control animals received an injection of saline only. No abnormal behavior was seen during a 30-min period of observation in the control animals or in birds that received 100 μg/kg body weight (Table I). In all four chickens that received 250 μg/kg body weight, deglutition, salivation, and lacrimation became evident within

TABLE I. EFFECT OF COMPOUND 48/80 IN ADULT CHICKENS.[a]

Compound 48/80 (μg/kg body wt)	No. of animals	Degree of clinical signs[b]	Histamine (μg/g)	Percentage of control
0	6	0	1.8 ± 0.2	–
100	2	0	2.2 ± 0.5	100
250	4	Moderate	1.2 ± 0.3	67
500	4	Severe	0.6 ± 0.1	33
1000	4	Severe/fatal	0.8 ± 0.3	44

[a] Compound 48/80 in saline was injected into the jugular vein. Animals were observed for 30 min and then decapitated. Lungs were prepared and assayed for histamine as described in Materials and Methods.
[b] Refer to text for description.

2 min and subsided within 10 min. More severe systemic reactions, as evidenced by longer duration of the same signs and in several instances by collapse, were noted with doses of 500 and 1000 μg/kg body weight. Although most of the animals appeared to recover completely within 30 min, one bird died 10 min after an injection of 1000 μg/kg body weight. This animal was immediately autopsied and its lungs were removed for determination of histamine content. All the surviving animals were sacrificed 30 min after the injection and their lungs were assayed for histamine. All the chickens which exhibited anaphylactic-like signs also had reduced lung histamine content. The lung histamine levels of animals injected with 250 μg/kg body weight of compound 48/80 were 67% of control (Table I). Those given 500 and 1000 μg/kg body weight of the drug had more than 50% reduction in their lung histamine content.

In contrast to the results observed in the preceding experiments, compound 48/80 induced only minimal histamine release when reacted with chopped lung fragments *in vitro*. Equal volumes of washed lung suspension from either 10-day-old chicks or adult chickens were incubated at 37° for 10 min with 400 μl of varying concentrations of compound 48/80 or buffer alone. The reaction was stopped by centrifugation at 4°. Spontaneous histamine release (i.e., without the drug) averaged 6.4 ± 0.9% in five separate experiments. This "blank" value was subtracted from experimental values in the following sets of data. Lung fragments from adult chickens released negligible amounts of histamine (<2%, Fig. 1). Those from 10-day-old chicks relased a small proportion of their lung histamine (up to 6%) when reacted with 50 μg/ml of compound 48/80 or more.

The antibody activity of chicken anti-BSA was determined by ABC and PCA tests. The ABC of this antiserum was 3125. The PCA titer of the antiserum after a 4-hr sensitization period was 250. There was no detectable PCA reaction after a 24-hr sensitization period.

The capacity of chicken anti-BSA antiserum to sensitize lung fragments of adult chickens to release histamine was then investigated. Chopped lung fragments were suspended in buffer containing antiserum (1:10) or in buffer alone for 20 min at 37°. The samples were then centrifuged and washed three times. The fragments were resuspended with 400 μl of buffer and equal volumes of varying amounts of BSA (from 0.5 to 500 μg/ml) for another 20 min at 37°. In four separate experiments, no detectable histamine release was observed.

Since the PCA reaction of 10-day-old chicks was detected only after a short sensitization period, experiments were done in which the antiserum was not washed from the lung fragments before reacting them with antigen. Chopped lung pieces were incubated with antiserum (1:10) or buffer alone for 20 min at 37°. The samples were centrifuged and the pellets resuspended

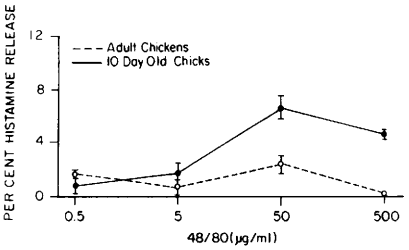

FIG. 1. Compound 48/80-induced histamine release from chopped lung. Duplicate 400-μl volumes of washed chopped lung suspensions were incubated with equal volumes of compound 48/80 or buffer for 20 min at 37°. The reaction was stopped by centrifugation and duplicate 20-μl samples of the supernatant fluid were assayed for histamine. Each point represents the mean ± SEM of four separate experiments.

(without washing) in 400 μl of buffer. An equal volume of compound 48/80 (containing from 0.5 to 500 μg/ml) was added to these suspensions and the reaction mixtures were reincubated for another 20 min at 37°. After centrifugation, the supernatant fluids were assayed for histamine. The release of histamine from these lung fragments was a function of antigen dose and of the age of the lung donor (Fig. 2). Maximum release was 12 ± 4% at 50 μg of BSA/ml with chopped lungs from 10-day-old animals. Adult chicken lungs released 7 ± 1% of their total histamine with 5 μg of BSA/ml. In three experiments, there was no detectable SRS-A in the supernatant fluids.

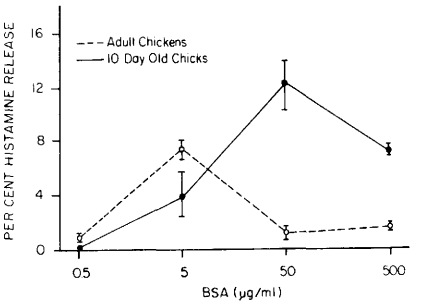

FIG. 2. Antigen-induced histamine release from chopped lung. Duplicate 400-μl volumes of washed chopped lung suspension were incubated with a 1:10 dilution of chicken anti-BSA antiserum for 20 min at 37°. The tubes were centrifuged and resuspended with 400 μl of buffer. BSA (400 μl) was added to the mixtures and reincubated for another 20 min at 37°. The reaction was stopped by centrifugation and 20-μl duplicate samples were assayed for histamine. Each point represents the mean ± SEM of three experiments.

Discussion. The present investigation confirms the earlier report of Lecompte and Beaumarriage (2) that compound 48/80 is capable of inducing an anaphylaxis-like syndrome in chickens. The response is dependent on the drug dose. The chicken seems to be relatively sensitive to the action of compound 48/80, perhaps more like the rat than the guinea pig and mouse in this regard (17). Since the response of chickens to the drug was associated with reduction in lung histamine content, our data lend support to previous suggestions that histamine may be a mediator of anaphylaxis in these animals (2, 4, 6). However, this association does not prove the definitive involvement of histamine in the reaction.

It was possible to prepare small fragments of chicken lung which contained as much histamine per unit weight as whole lung, and such fragments released very little histamine spontaneously. When reacted with compound 48/80, only minimal additional amounts of histamine were released from the lung fragments, and this required an amount of drug which was probably cytotoxic to histamine-containing cells. In contrast, the intravenous administration (500–1000 μg/kg body wt), which presumably resulted in drug levels well below the level achieved *in vitro*, was associated with the loss of more than half of the histamine content of the lungs. We cannot explain the resistance of lung tissue *in vitro* to the action of compound 48/80.

Evidence was obtained that chopped chicken lung was capable of releasing histamine in response to an immunologic stimulus. The fractional histamine release was within the range reported to occur with *in vitro* antigen-induced release from chopped mammalian lung (15, 18). The difference between the responsiveness of the lungs of 10-day-old chicks and adult chickens is consistent with a similar difference observed in the PCA tests of antiserum activity. One possible explanation for these differences is that adult animals have higher levels of the immunoglobulins that mediate anaphylactic reactions and that these nonspecific immunoglobulins may competitively inhibit the binding of specific anaphylactic immunoglobulin to the requisite tissue sites (19). Alternatively, it is possible that metabolic differences between the tissues of young and adult animals may affect one or more steps in the reaction. For example, adult chickens are less responsive than young chicks to intradermal or intravenous administration of histamine (2, 6). An identical phenomenon was observed with isolated tissues (20, 21). Sections of gut from 1-week-old chicks have been shown to be about 100–1000 times more sensitive to histamine than tissue from adult birds (21). This suggests that tissue histamine receptors are altered as a function of age.

The release of SRS-A from chopped lung was not detected in this study. In contrast, chopped guinea pig lung and chopped human lung have been shown to release SRS-A *in vitro* (15, 18). It is possible that chicken lung does not generate or release SRS-A during an anaphylactic reaction *in vitro*, or that the amount released is not measurable. Furthermore, SRS-A might be released from chicken lung but, because of structural differences from mammalian SRS-A, it might not be detectable in the bioassay; or it might be highly labile and therefore lost before the assay. The present experiments therefore do not eliminate the existence of an avian SRS-A.

Since the antiserum used in this study mediated a PCA reaction after a 4-hr but not after a 24-hr sensitization period, and since chopped lung that had been washed after incubation in this antiserum did not respond to antigen, it is unlikely that the antibody mediating the *in vivo* and *in vitro* reactions was a reaginic (IgE)-type antibody. Rather, it appeared to behave more like IgG-1 (22) and IgGa (19) antibodies of rodents, in that mediator release was observed only when specific antigen was added to tissue that still contained the sensitizing antiserum. In previous reports, the antibody which mediates the short-latency PCA reaction in chickens was shown to be a γ_1-immunoglobulin, and to be resistant to mercaptoethanol and to heating at 56° for 4 hr (6). The antibody used in the present study is most likely of the same type. The nature of its interaction with tissue remains unknown. The chopped lung system described in this report provides a suitable means for studying the function of this antibody and for investigating the mechanism of anaphylaxis in chickens.

Summary. An anaphylaxis-like syndrome was induced in adult White Leghorn chickens by intravenous administration of compound 48/80. This reaction was associated with a reduction in histamine content of the lungs, suggesting that histamine is a mediator of this syndrome. Compound 48/80 induced minimal histamine release from chopped lung *in vitro*, probably at concentrations which were cytotoxic. When chopped lung fragments were incubated with chicken anti-BSA antiserum and then with BSA, histamine but not SRS-A was detected in the supernatant fluid. The percentage of histamine released was a function of antigen concentration, and was greater with lung fragments from 10-day-old chicks as compared to adult chickens. No histamine was released when the lung fragments were washed after sensitization, suggesting that the antibody was not firmly bound in the tissue sites. The antibody probably is IgG-like rather than IgE-like. The precise nature of this *in vitro* reaction is still not known.

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