Increased Lung Serotonin and Pulmonary Edema in Mice Injected with Antiserum to Ehrlich's Ascites Tumor.* (27050)

L. S. KIND, A. BURKHALTER AND R. SHERINS

Departments of Microbiology and Pharmacology, University of California Medical Center, San Francisco

Redfern(1) described reactions of guinea pigs injected with rabbit sera containing Forssman antibodies. The guinea pigs showed symptoms resembling those of anaphylaxis but the condition of their lungs was markedly different from the lungs of anaphylactic animals. The lungs of the guinea pigs killed by the heterophile antisera were hemorrhagic and edematous and fluid often extended into the trachea and exuded from the nose. We have recently observed a similar condition in mice injected with serum from a rabbit immunized with Ehrlich's ascites tumor cells (EATC) grown in tissue culture(2). An additional finding in our experiments, however, is the presence of a markedly elevated serotonin content in the lungs of the test animals.

Materials and methods. A rabbit received an intravenous injection of 1 x 10^4 EATC administered every fourth day for 12 days. Thereafter, 1 x 10^7 EATC mixed with Freund's adjuvant were injected subcutaneously twice a week. Eight weeks after initial injection, the rabbit was bled and its serum found to be toxic for mice (see below). Additional doses of EATC were administered weekly or bi-weekly, and the serum used in the experiments to be described was obtained 5 months after initial injection of EATC. This serum had a cytotoxic titer of 1:50 when tested against EATC *in vitro*.

Swiss-Webster, 20-25 g female mice were injected intraperitoneally with 5 billion *B. pertussis* organisms since this procedure increases the susceptibility of these animals to the lethal effects of serotonin, histamine, and anaphylaxis(3). Five to 8 days after inoculation with *B. pertussis*, mice were injected intravenously with 0.5 ml of the rabbit antiserum to EATC. Other pertussis inoculated mice were challenged with 0.5 ml of normal rabbit serum or 0.5 ml of rabbit antiserum to mouse globulin. The latter antiserum had an LD_{50} similar to that of the antiserum to EATC, and killed mice (reversed passive anaphylaxis) in a similar time period (5-15 minutes). Lungs were removed upon death of the animals, weighed, and analyzed for histamine by the method of Shore *et al.*(4), and for serotonin by the method of Bogdanski *et al.*(5).

Results. A few minutes after administration of rabbit antiserum to EATC the mice became still, then prostrate, went into convulsions, and died gasping for air. A large percentage of the animals had bloody, frothy fluid exuding from the nose, and had lungs that were larger than normal with patchy hemorrhagic areas on their surface interspersed with areas of emphysema. Histologic studies confirmed these gross observations.

Hemorrhagic edematous lungs are not seen in mouse anaphylaxis. Indeed, Treadwell *et al.* (6), in describing the widespread visceral congestion in anaphylactic mice, made a point of mentioning the paucity of lung changes in this species. The data in Table I indicate that the mean lung weight of mice injected with antiserum to EATC was significantly greater (350 ± 15 mg) than the mean lung weight of anaphylactic mice (212 ± 17 mg) or mice injected with normal rabbit serum (182 ± 7 mg).

The data in Table I also demonstrate a 10-fold increase in serotonin in the lungs of mice injected with the antiserum to EATC as compared with mice injected with normal rabbit serum. Total lung serotonin of the mice injected with rabbit antiserum to EATC was also significantly greater (4.09 ± 0.51 μg) than the lung serotonin of mice injected with rabbit antiserum to mouse globulin (1.00 ± 0.09 μg). In contrast, there were no significant differences in the total lung histamine content of the various groups of mice.

Discussion. On the basis of the data presented there seems little doubt that hemorrhagic edematous lungs with a high serotonin

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TABLE I. Serotonin and Histamine Content of Lungs of Pertussis Inoculated Mice Injected with Various Rabbit Antisera.

Serum Injected	No. of animals	Lung wt. (mg)	Serotonin Content			No. of animals	Lung wt. (mg)	Histamine Content		
			μg/lungs	μg/g lung	μg/100g body wt.			μg/lungs	μg/g lung	μg/100g body wt.
None (Control)	5	186 ± 9.0	.48 ± .08	2.59 ± .37	2.40 ± .40	5	203 ± 7.5	.13 ± .01	.64 ± .04	.54 ± .06
Normal rabbit serum	4	182 ± 7.5	.54 ± .09	2.91 ± .53	2.57 ± .46	5	196 ± 7.5	.15 ± .02	.76 ± .08	.65 ± .06
Antiserum to mouse globulin	5	212 ± 17.2	1.00 ± .09*	4.80 ± .51*	5.50 ± .39*	5	209 ± 13.4	.17 ± .02	.81 ± .09	.72 ± .08
Antiserum to EATC	5	350 ± 14.7*‡	4.09 ± .51*‡	11.78 ± 1.65*‡	20.44 ± 2.89*‡	5	335 ± 31.5*‡	.13 ± .01	.42 ± .07‡	.57 ± .07

Each number is mean ± standard deviation of mean. * $P < .01$—compared to control. ‡ $P < .01$—compared to antiserum to mouse globulin.

content are not characteristic of reversed passive anaphylaxis in the mouse. As already mentioned, Redfern(1) differentiated guinea pig anaphylaxis from the reaction of these animals to Forssman antibody (hemorrhage and edema of the lungs). Recently, Humphrey(7) observed that Forssman antibodies would not cause mast cell degranulation in the guinea pig although this occurs in anaphylaxis. Also, blood taken just before death of animals injected with Forssman antibodies contained no detectable histamine. Humphrey(7) quotes unpublished experiments of Mongar which indicate that no histamine is released when Forssman antibodies are perfused through the isolated guinea pig lungs. Forssman antigen is present in many tissues of the mouse including the lung(8) as well as in certain mouse tumors(9), but it is doubtful that Forssman antibodies are responsible for the mouse lung pathology which we have observed. Our doubt is based on the fact that to this date, we have been unable to absorb out the lethal effects of rabbit antiserum to EATC with sheep red blood cells. This serum has likewise proved to be nonlethal for guinea pigs. Absorption of rabbit antisera to EATC with various mouse tissues is now in progress. Thus far, the only other rabbit antiserum which has produced hemorrhagic edematous lungs in mice is that of a rabbit injected with mouse mast cell tumor P 815†. A rabbit antiserum to normal mouse spleen cells killed mice but did not produce hemorrhagic edematous lungs. Possible differences in the ability of malignant mouse cells and normal mouse cells to produce rabbit antisera with the property of damaging mouse lungs are now under investigation. The association of pulmonary edema with increased lung serotonin has also been noted by Skillen *et al.*(10) in rats exposed to ozone.

Although the role of serotonin in the etiology of pulmonary edema is still in doubt, preliminary experiments in our laboratory have shown that cyproheptadine, a compound with antiserotonin activity(11), can block the pulmonary edema and hemorrhage produced by rabbit antiserum to EATC.

Summary. Mice injected with a rabbit anti-

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serum to Ehrlich's ascites tumor developed hemorrhagic edematous lungs with a markedly increased serotonin content. Such lung findings were not seen in mouse anaphylaxis.

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Excretion into Bile of Intrinsic Factor and Its Vit. B_{12} Complex Following Intravenous Injection. (27051)

KUNIO OKUDA

Department of Medicine, Yamaguchi Medical College, Ube, Japan

It has been shown *in vitro* (1,2,3) that uptake of cyanocobalamin (B_{12}) by liver tissue is enhanced by intrinsic factor concentrate (IF), but whether this phenomenon has any bearing on the biological function of naturally occurring IF is not known. We demonstrated that $Co^{60}B_{12}$, when premixed with IF and injected to rats intravenously, was taken up by the liver in almost absolute preference to other organs (4). It was also shown that degree of the preferential uptake might be related to purity of IF but not to the abundance of reticuloendothelial cells, and that formation of an IF-B_{12} complex was prerequisite to this phenomenon. Our observation has been confirmed by others (5,6). Since IF used in these studies was of hog origin and therefore a heterologous protein to the animals, interpretation of the results seems to be limited because of the possibility of nonspecific uptake of such proteins by the liver. Our further attempt to clarify the mechanism by which the liver captures IF-B_{12} complex has disclosed that part of the complex is immediately excreted into bile following the trapping by the liver, and that the same is true of IF without B_{12}. These findings will not only throw light on the mode of action of IF in hepatic uptake of B_{12}, but will provide a clue as to the mechanism of disposal of foreign substances by the liver. Such data are reported here.

Materials and methods. Young adult rats of Wistar strain which had been raised on a stock diet and rabbits of about 2 kg that had been fed a soy-bean curd diet were used. The IF used was a hog stomach preparation (WES #727) *, and the activity was such that 1 to 2 mg was active in pernicious anemia patients when administred together with 15 μg of B_{12}. The B_{12} binding capacity of this preparation was 500 mμg/mg as measured by dialysis method. The $Co^{60}B_{12}$ used had a specific activity of approximately 0.8 μcurie per μg. Rats were anesthetized with ether and intraperitoneal injection of urethan, 1 ml per 100 g body weight; no anesthesia was given to rabbits. Laparotomy was performed and a glass cannula was inserted into the common bile duct and was tied with the duct. Bile was collected directly from the cannula in the case of rats, or through a polyethylene tubing in the case of rabbits. After securing the basal bile

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