12. Trelease, S. F., and Beath, O. A., 1949, Preface to *Selenium*, published by the authors, New York.
13. Pinsent, J., *Biochem. J.*, 1954, v57, 10.
14. Poley, W. E., Wilson, W. O., Moxon, A. L., and Taylor, J. B., *Poultry Sci.*, 1941, v20, 171.
15. Schwarz, K., *Ann. N. Y. Acad. Sci.*, 1954, v57, 878.

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Hematocrit Change as Indication of Anaphylactic Shock in the Mouse. (23309)

JOHN D. FULTON, WILLIAM E. HARRIS, AND CHARLES E. CRAFT
Department of Microbiology, School of Aviation Medicine, USAF, Randolph Air Force Base, Texas

Because of their complexity, methods for quantitation of anaphylaxis in large hypersensitive animals(1) are not readily adapted for the mouse. Consequently, the occurrence of anaphylaxis in mice is customarily determined using death as end point. Attempts have been made to estimate sublethal anaphylaxis in mice by observing the appearance of certain gross signs (*i.e.*, cyanosis and convulsions). Because of their subjectivity, these observations are inadequate. Kind(2) found that the rectal temperatures of mice surviving anaphylactic shock were markedly decreased.

In our laboratory we consistently observed that hemoconcentration occurs in mice undergoing anaphylaxis. We observed signs of anaphylaxis similar to those described by Weiser *et al.*(3). Immediately after challenge most of the mice became quiet and huddled in one position. The fur became ruffled and there was mild lacrimation. There soon followed partial paralysis of the limbs and marked cyanosis of the limbs, tail, and snout. Many animals experienced brief but violent convulsions separated by short periods of complete prostration. Nearly all mice which underwent convulsions died within 30 minutes. Nelson *et al.*(4) reported that the hematocrit of pooled mouse blood was markedly increased following fatal anaphylactic shock. The present study was designed to determine whether or not hematocrit change might be a suitable criterion for determination of anaphylaxis in mice.

Methods. Sensitization and challenge. Following randomization, female Swiss-Webster mice (22-27 g) were sensitized with 2 IP injections of 0.25 ml of antigen administered 4 days apart. Twelve days after receipt of the initial sensitizing injection the animals were challenged with an injection in the caudal vein of 0.25 ml of antigen. Two antigen preparations were employed: 1) alum precipitated bovine serum albumin* with *Hemophilus pertussis* vaccine† (APBA + HPV), each 0.25 ml containing 1 mg of bovine serum albumin suspended in 2.5% alum (pH 6.5 to 7.5) with 10^9 to 10^{10} *H. pertussis* vaccine cells; and, 2) the same as above except that *H. pertussis* vaccine was not included. This antigen preparation is referred to as APBA. A single challenge antigen preparation, bovine serum albumin with *H. pertussis* vaccine (BSA + HPV) was used. Each 0.25 ml of this preparation contained 4 mg of bovine albumin and 10^9 to 10^{10} cells *H. pertussis* vaccine. Controls included groups of mice sensitized with one or the other of the 2 sensitizing antigen preparations and challenged with saline, mice injected with saline instead of sensitizing antigen preparation and challenged with BSA + HPV, and groups given saline instead of both sensitization and challenge. *Hematocrit determination*: A modification of the microhematocrit method described by McGovern *et al.*(5) was employed. Four-tenths mm ID microprecipitin capillary

* Bovine serum albumin, 30%, sterile, for use as diluent in Rh testing. Armour Labs.

† Pertussis vaccine (whooping cough bacterin), Wyeth Labs.

tubes‡ were heparinized by immersing them in a 1% solution of heparin sodium salt.§ then drying at 100°C for 60 minutes. The dried, heparinized tubes were stored in a desiccator until use. To take the blood sample, the tail of an immobilized mouse was severed the minimum distance from the tip required for moderate bleeding. Following collection of blood, one end of the partially filled capillary tube was sealed by flaming, and the open end broken off. The tubes were then centrifuged for 4 minutes at 10,000 rpm in a standard micro-hematocrit centrifuge.‖ Hematocrit values were determined by using the standard reading template. Two sequential hematocrits were taken approximately 5 minutes prior to administering the challenge injection. The mean of these 2 hematocrit values is considered to be the prechallenge, normal hematocrit of the animal. For approximately 550 duplicate samples, a standard deviation of ± 1.57 units (approximately 3%) was observed. It can be inferred from this that a hematocrit change of 5% or more is not likely to be due to chance; therefore, a change in hematocrit of 5% or more has been arbitrarily selected as being significant. Approximately 5 minutes after the duplicate prechallenge hematocrits, the challenge dose of antigen or saline was injected. Blood samples were taken at 5, 10, and 15 minute intervals after challenge. In earlier studies blood samples were taken at 2 minutes and at each 5 minute post-challenge interval through the 30 minute period. This was discontinued as it was found that the maximum hematocrit change occurred, if there was a change, within 15 minutes post-challenge. Hematocrit changes were calculated according to the following formula:

$$\% \text{ change} = \frac{\text{Mean post-hematocrit} - \text{Mean pre-hematocrit}}{\text{Mean pre-hematocrit}} \times 100$$

‡ Capillary tubes, 0.4 mm ID, 90 mm long. Schiefelin & Co., N. Y.

§ Liquaemen, Roche-Organon, Nutley, N. J.

‖ International hematocrit centrifuge, Interntl. Equipment Co., Boston, Mass.

Results. Sensitivity and specificity of hematocrit rise during anaphylaxis. Test animals were treated as follows:

Sensitization	Challenge	Approx. No. mice
APBA + HPV	BSA + HPV	150
APBA	" "	150

Control animals were treated as follows:

Sensitization	Challenge	Approx. No. mice
APBA + HPV	Saline	100
APBA	"	100
Saline	BSA + HPV	150
"	Saline	100

Seventy-five % (221/295) of test animals showed a hematocrit increase of 5% or more, and 41% (121/295) showed a hematocrit increase of 30% or more (Fig. 1). In the test group, there were 47% (139/295) deaths within 24 hours after challenge (Fig. 2). Of the 139 test animals that died, 133 showed a hematocrit rise of 5% or more. Thirty % (88/295) of test animals had a hematocrit increase of 5% or more but did not die. In the control group, 3% (12/446) had a hematocrit rise of 5% or more (Fig. 1). In this group 3% (13/446) died (Fig. 2), but only one of the 13 was among the 12 control mice showing a 5% hematocrit rise. The 12 remaining control animals that died showed a decreased hematocrit (Fig. 2).

Comparison of hematocrit change and mortality rate as methods for measuring anaphylaxis. In this experiment, 2 groups of approximately 45 animals each were employed for each of 2 sensitizing procedures,

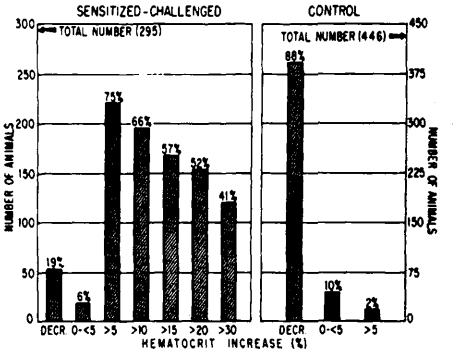

FIG. 1. Hematocrit response of sensitized-challenged animals *vs* control animals.

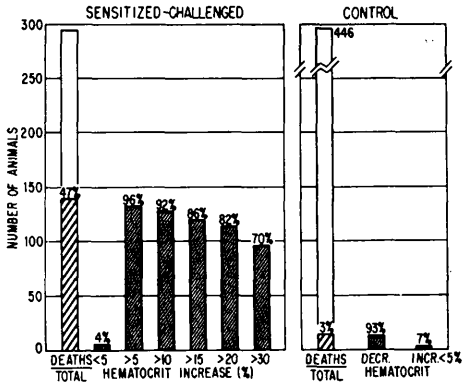

FIG. 2. Relationship between anaphylactic death and hematocrit rise.

viz., sensitized with APBA + HPV, and sensitized with APBA (Fig. 3). In both procedures, the same challenge antigen (BSA + HPV) was employed. Control groups to complete the factorial design (as in the preceding section) were included. One group of mice was sensitized and challenged, and hematocrits taken as described previously. The second group of mice was sensitized and challenged, was observed, and deaths within the 24-hour post-challenge period recorded. The animals in this group were immobilized for the same period of time as was the "hematocrit group."

Comparison of results obtained in this experiment indicates that the incidence of hematocrit rises of more than 5% was higher than the incidence of deaths ($P<.001$) (Fig. 3). It had previously been observed (unpublished results) that the 2 hypersensitizing technics described above produced significantly different death rates following challenge; this was confirmed in the present study ($P<.05$) (Fig. 3). The 2 different sensitizing procedures also resulted in differences in the number of animals showing hematocrit increases of 5% or more ($P<.01$) (Fig. 3).

Discussion. It has been shown that, with the technics employed, an increase in hematocrit regularly accompanies anaphylaxis in mice. In a group of animals of which 47% died in anaphylaxis, 75% showed a hematocrit increase of 5% or more. This would indicate that increased hematocrit occurs both in mice that subsequently die in anaphylaxis and in those which survive. Control animals

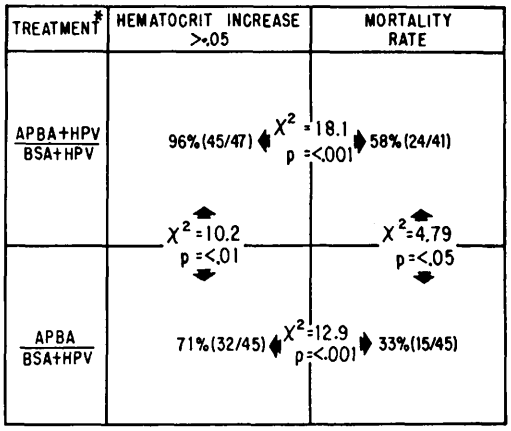

FIG. 3. Comparison of hematocrit change and mortality counts as methods for determining degree of anaphylactic shock.

consistently showed a decrease in hematocrit. When the incidence of mortality and hematocrit change in different groups of mice is compared, hematocrit increases of 5% or more were found to occur more frequently than death in groups of anaphylactic animals. Differences were observed in the incidence of anaphylactic responders produced after challenge of animals made hypersensitive by 2 different sensitizing procedures when either death or hematocrit increase was used as an end point. It appears, therefore, that hematocrit increase may be a more sensitive index of anaphylaxis than death.

Summary. An increase in hematocrit regularly accompanies anaphylaxis in mice. The incidence of hematocrit increase is higher than the incidence of death in groups of anaphylactic mice. It is suggested that hematocrit change is a useful index of anaphylaxis in mice.

1. Noelpp-Eschenhagen, I., and Noelpp, C., *Progress in Allergy*, v4, 361-456; S. Karger, Basel-N.Y., 1954.

2. Kind, L. S., *J. Immunol.*, 1955, v74, 387.

3. Weiser, R. S., Golub, O. J., and Hamre, D. M., *J. Inf. Dis.*, 1941, v68, 97.

4. Nelson, C. T., Fox, C. L., Jr., and Freeman, E. B., *J. Invest. Dermatol.*, 1952, v18, 113.

5. McGovern, J. J., Jones, A. R., and Steinberg, A. G., *New England J. Med.*, 1955, v253, 308.

Received January 21, 1957. P.S.E.B.M., 1957, v95.