placing a thermistor at the edge of the mesentery. Direct readings in degrees centigrade are indicated on a control box meter.‡

Still or cinephotomicrography is accomplished by placing a reflex camera above the field-splitter (Fig. 3). Images of both vascular beds are projected onto a ground glass screen from which observations are made and on which the photograph is composed before exposure. Fig. 4 is an example of the type of micrograph obtained.

For observations which need not be recorded photographically, the field-splitter is removed and a boom-mounted viewing box is centered above the microscope oculars. Two large, circular fields are thus projected side by side upon an opal glass screen. A mirror mounted above the screen at a 45 degree angle reflects the images so the observer may remain seated while viewing.

This method is currently being employed in a comparative study of vascular responses to shock and other stresses.

‡ Thermistor recording unit designed and built by Mr. John Degelman, Boston Univ.

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Quantification of Anaphylaxis in Mice. (23634)

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It has been previously shown that a marked hematocrit rise occurs more frequently than mortality during anaphylaxis in mice(1); hence, in all probability many sub-lethal reactions were recognized. It was the purpose of the present investigation to determine if the degree of hemoconcentration in anaphylaxis is a continuous quantitative function of challenge antigen concentration.

Methods. In this study 2 different sensitizing-challenge procedures were utilized. Typical signs of anaphylaxis as previously described(1,2) were observed in both procedures. In the first procedure female Swiss-Webster mice weighing 22-27 g were sensitized with 2 IP injections (given 4 days apart) of 0.25 ml alum precipitated bovine albumin* with *Hemophilus pertussis* vaccine† (containing 1.0 mg bovine albumin, 10^9 to 10^{10} pertussis vaccine cells, and 2.5% alum). Eight days after the second sensitizing injection, challenge was effected by a rapid IV injection into the caudal vein of a 0.25 ml aliquot containing graded quantities of bovine albumin and pertussis vaccine cells. Controls included mice treated as shown in Table I. In the second procedure, mice of the same strain, sex, and weight as above were sensitized with a 0.2 ml aliquot of bovine albumin—Freund's adjuvant‡ mixture (1 part bovine albumin in saline : 1 part Freund's adjuvant), 0.1 ml of which was injected subcutaneously into each inguinal region. The total sensitizing dose contained 0.1 mg bovine albumin. Twenty-one days later, challenge was effected by injecting 0.2 ml of varying concentrations of bovine albumin in saline into the caudal vein. Controls are indicated in Table II. Hematocrits were determined as previously described(1). Blood samples were drawn from the tails of mice 5 minutes before, and 5, 10, and 15 minutes after challenge. Hematocrit change was defined from the following formula:

$$\frac{\text{Avg post-challenge} - \text{Avg pre-challenge}}{\text{Avg pre-challenge}} \times 100 = \% \text{ hematocrit change.}$$

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

† Pertussis vaccine (Whooping Cough bacterin), Wyeth Labs., Inc. 9 protective units/ml.

‡ Bacto adjuvant, complete (Freund), Difco Labs.

TABLE I. Hematocrit Change in Mice Sensitized and Challenged by Procedure One.

Sensitization	Challenge (mg BSA)		% hematocrit change	% mortality (4 hr)	
APBA + HPV*	BSA + HPV*	4.0	46.2 ± 3.2†	80	8/10
		.38	47.8 ± 3.2	95	19/20
		.125	38.1 ± 3.0	60	17/28
		.050	36.1 ± 4.5	67	12/18
		.030	33.7 ± 3.0	42	8/19
		.025	33.3 ± 3.8	60	12/20
		.020	26.4 ± 3.9	28	5/18
		.015	14.2 ± 2.4	3	1/29
		.008	24.3 ± 3.1	18	5/27
		.004	11.7 ± 3.3	11	2/18
		.002	6.4 ± 2.6	0	0/19
		.001	1.4 ± 1.6	5	1/19
		.0004	−1.0 ± 3.2	0	0/10
Controls:					
APBA + HPV	Saline	0	−1.0 ± 1.0	5	2/43
	HPV	0	−3.0 ± 1.3	0	0/20
APHPV	BSA + HPV	4	−6.1 ± 1.2	0	0/16
	HPV	0	−1.8 ± 1.0	0	0/27
Saline	BSA + HPV	4	−3.9 ± 1.0	3	1/40
	Saline	0	−4.9 ± 1.0	0	0/39

* APBA—Alum precipitated bovine albumin. BSA—Saline solution of bovine albumin. APHPV—Alum precipitated pertussis vaccine. HPV—Pertussis vaccine in saline (10^9-10^{10} cells).
† Stand. error.

Results. Hematocrit changes, mortalities, and other pertinent data obtained from groups of mice treated according to the first procedure are shown in Table I. In the various control groups hematocrit change was negative in all cases and mortality at 4 hours postchallenge was insignificant. None of the 3 mice which died in the control group experienced a significant hematocrit rise. It is apparent from these results that pertussis vaccine and/or the manipulations incidental to treatment did not cause either significant hematocrit rise or mortality. In the test groups it is apparent that there is a positive relationship between the degree of hematocrit change and the dose of bovine albumin. Mor-

TABLE II. Hematocrit Change in Mice Sensitized and Challenged by Procedure Two.

Sensitization	Challenge (mg BSA)		% hematocrit change	% mortality (4 hr)	
BSA + F*	BSA*	8.0	24.2 ± 3.8†	15	3/20
		2.0	39.2 ± 3.4	22	4/18
		1.0	39.8 ± 3.1	44	4/ 9
		.5	38.1 ± 2.9	32	6/19
		.25	30.1 ± 3.4	15	3/20
		.125	24.8 ± 3.2	11	2/18
		.063	18.8 ± 2.6	10	3/29
		.032	15.5 ± 4.3	0	0/19
		.016	5.0 ± 2.4	0	0/10
		.008	3.0 ± 1.0	0	0/10
		.002	−2.1 ± 1.0	0	0/16
		.0005	−3.9 ± 1.0	0	0/10
Controls:					
Saline + F	BSA	2	−1.8 ± 1.0	0	0/ 9
BSA + F	Saline	0	−4.0 ± 1.2	0	0/20
Saline + F	"	0	−2.9 ± 1.3	0	0/16

* BSA + F—Saline solution of bovine albumin emulsified in equal vol of Freund's adjuvant. Saline + F—Emulsion of equal volumes of saline and Freund's adjuvant. BSA—Saline solution of bovine albumin.
† Stand. error.

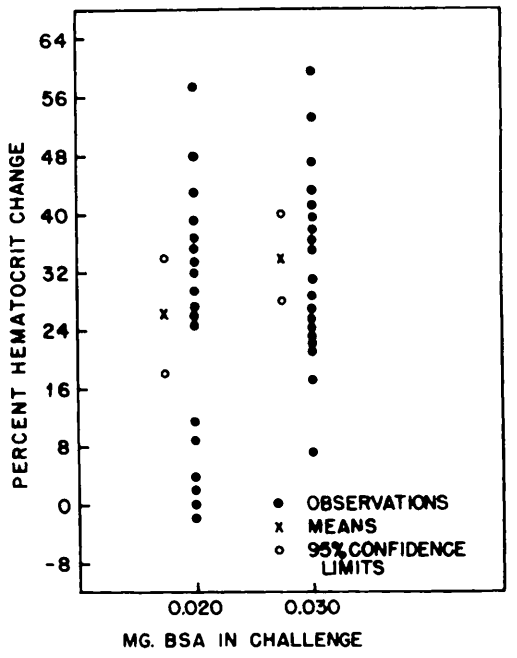

FIG. 1. Individual observations, means, and 95% confidence limits for two representative groups from Table II.

tality varies with antigen concentration in a like manner. Individual observations, means, and the 95% confidence limits, as determined by Student's T test, for 2 representative groups from Table I are plotted in Fig. 1. These observations seem to be continuous and normally distributed about the mean. Individual observations from the other test groups of Table I presented essentially the same pattern when plotted in a like manner. Hematocrit change is plotted against $\log_{10}$ dose bovine albumin in Fig. 2. The relationship between the two appears to be sigmoidal. A linear regression line was fitted to the points from 0.001 mg to 0.38 mg. Above and below these points, the curve seems to level off. An analysis of variance, performed on the data between 0.001 and 0.38 mg bovine albumin, showed that the means differ significantly ($P<0.01$) and that there is a highly significant linear trend to the relationship between log dose bovine albumin and hematocrit change ($P<0.001$). Significance of the deviation of individual points from the calculated regression line was determined by Student's T test. Only the mean observation for 0.015 mg bovine albumin deviated significantly ($P<0.05$).

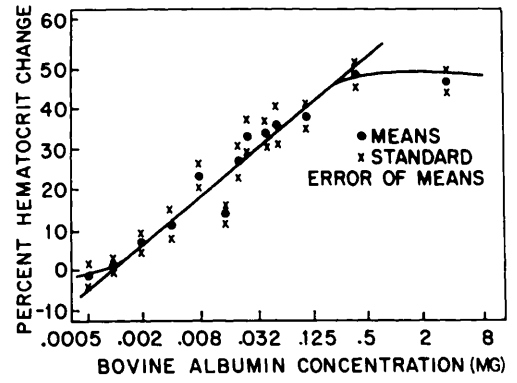

FIG. 2. Relationship of hematocrit change to log dose bovine albumin in challenge antigen preparation. (Data from Table I.)

Hematocrit changes, mortalities, and other information in groups of mice treated according to the second procedure are shown in Table II, and hematocrit change is plotted against $\log_{10}$ dose bovine albumin in Fig. 3.

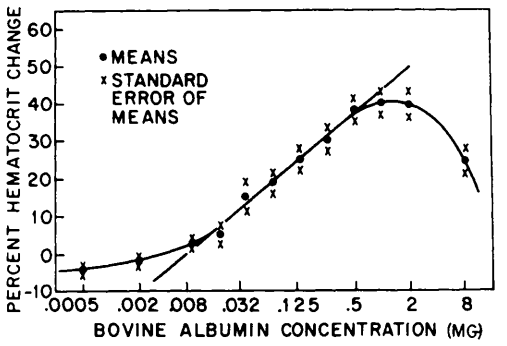

FIG. 3. Relationship of hematocrit change to log dose bovine albumin in challenge antigen preparation. (Data from Table II.)

When individual observations were plotted (as in Fig. 1) they appeared to be normally distributed about the mean. An examination of Table II and Fig. 3 reveals that the results obtained by the second procedure are very similar to those obtained by the first procedure. Hematocrit change and mortality are positively related to challenge antigen concentration (Table II), a segment of the dose response curve could be fitted to a straight line (Fig. 3), and the range of hematocrit change and standard errors were of the same order (Tables I, II).

Discussion. Anaphylaxis in mice is cus-

tomarily measured by mortality. Data collected by this method are often inadequate because only the existence or non-existence of anaphylaxis is recognized. A method of observation which measures the extent of the reaction, rather than its mere presence or absence, is more satisfactory because variation within groups of mice can be more precisely estimated. An efficient method for determining the extent of anaphylaxis should have these characteristics: 1) There should be regularity in the dose-response relationship; 2) experimental error should be small enough so that moderate differences in the response can be measured in rather small groups of animals; and 3) individual observations should be distributed continuously so as to rule out qualitative observations. The data presented herein indicate that these criteria are approached when anaphylaxis in mice is determined by change in hematocrit. Furthermore, this method was shown to be reliable for two widely divergent anaphylactogenic procedures. This method has other advantages in that the collection of blood samples does not seem to harm the animal; each mouse serves as its own prechallenge control, and the apparatus required is simple, inexpensive, and seems to have little intrinsic variation(1). Therefore, the use of hematocrit determinations for drawing quantitative comparisons in mouse anaphylaxis studies is recommended.

Summary. Results indicate that hematocrit rise in anaphylactic mice is a sigmoidal function of the log dose bovine albumin in the challenge antigen preparation. Over a given range, the relationship tends to be linear. Indications are that hematocrit change is a continuous function, and not a ratio of "all or none" responses. This method is recommended for drawing quantitative comparisons in studies of mouse anaphylaxis.

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Effect of Artificial Changes in Egg Composition on Hatchability and Chick Growth.* (23635)

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Egg size. as well as the weight and physical characteristics of yolk and albumen, is known to affect the hatchability of hen's eggs. The earlier work on this subject has been reviewed by Landauer(1). Lerner(2) has shown that optimal hatchability is found in eggs slightly below the mean egg size for any stock in which egg size has been favored by artificial selection.

The weight and quality of albumen in the hen's egg can be reduced artificially by local X-irradiation of the oviduct(3). The influence of these changes in the egg on hatchability and chick growth is considered in the present report.

Materials and methods. Mature White Leghorn hens were used in these experiments. Oviducts were exposed by laparotomies and X-irradiated; other parts of the hen's body were protected from irradiation by lead shielding. The details of the irradiation procedure have been described elsewhere(3). Surgical controls were prepared for each irradiated group. In the initial experiments the entire oviducts of experimental birds were irradiated. However, this procedure resulted in such numerous and severe shell defects in eggs laid following treatment that only a few were suitable for hatchability studies. This report deals only with later experimental groups in

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