undiluted state, chyle lost none of its susceptibility to clearing. Saline-washed chylomicra from chyle, on the other hand, were significantly less susceptible to clearing after storage for one month at −18°C, although they suffered less in this respect than did saline-washed chylomicra from plasma. It was found necessary to warm the frozen chyle 2-3 hours at 37°C before testing, otherwise it continued to equilibrate during the testing and erroneously gave the appearance that its clearing susceptibility improved after heating at 50°C or 65°C. After the 3 hours prewarming, however, it was found that the previously frozen chyle showed the same degree of very slight "damage" after 15 minutes heating at 50°C or 65°C as the fresh chyle.

Discussion. Our results showed that the susceptibility of the chylomicra to clearing by post-heparin plasma may be reduced by storage at 4°C or −18°C or by heating. Whether this is due to changes in the fat of the chylomicron or in its protein envelop, or to changes in a lipoprotein complex cannot be concluded from our studies. If the changes are due to denaturation of a protein film on the chylomicron the question would still arise as to whether this film is essential in the sense that it is an essential factor for maximal clearing, or whether the denatured protein coating interferes with the clearing reaction by making the fat unavailable. It is of interest that loss of susceptibility to clearing brought about by heating the chylomicra was not completely reversed by the addition of normal plasma, but the presence of certain amounts of plasma during heating lessened this change.

The possibility must also be considered that a specific protein or lipoprotein complex is involved. This is suggested by the fact that the heating of chyle (diluted 1:100 in saline) caused much less change in the susceptibility to clearing of the chylomicra than the same heat applied to washed chylomicra from lipemic plasma. This might suggest that the protein moiety of the chylomicron is fundamentally important to the clearing reaction, or that chyle itself contains a specific substance necessary to maximal clearing that is either absent or present in relatively low concentration in plasma.

Conclusion. Heat or storage at −18°C is attended by a significant reduction of the susceptibility to clearing of saline washed chylomicra from plasma. Thoracic duct chyle is affected much less by the same treatments.

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Plasma Volume Changes in Egg-White Injected Rats. Effect of Cortisone.* (21014)

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Selye described an "anaphylactoid" condition produced by intraperitoneal injection of egg-white to rats(1) characterized by the development of edema. Further studies(2,3) have shown that adrenalectomized rats reacted to egg-white with a more severe condition, usually fatal. It was also observed that adrenal cortical extract(4) and cortisone (5) had a protective effect on the systemic actions of egg-white. The present report deals with plasma volume changes in egg-white-treated rats and attempts to explain the mechanism of the protective action of corti-

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sone in terms of these changes and their effect on capillary function.

Methods. Plasma volume was evaluated by means of the Evans blue method. Albino rats weighing between 135 and 150 g were anesthetized with Nembutal (30 mg/kg intraperitoneally). Evans blue was injected by way of the left jugular vein at the dose of 10 mg/kg. Thirty minutes after injection of the dye, the animals were bled by sectioning the blood vessels of the neck. Blood was collected into small beakers containing 0.6 ml of a 1.1% solution of sodium oxalate. The sample was immediately transferred to a Kolmer type sedimentation tube and centrifuged for 10 minutes at 2500 r.p.m. After taking a hematocrit reading (taking into account the volume of anticoagulant solution), the plasma-oxalate mixture was pipetted off and distilled water was added to reach a 1/20th dilution of plasma. The plasma dilution was read against a distilled water blank on a Coleman Model 14 spectrophotometer at 600 mμ. The readings were converted into μg of dye per ml of plasma by means of a standardization curve of Evans blue dissolved in distilled water. Plasma volume was calculated from the equation: $V_{ml/kg} = \frac{\text{dye injected } (\mu g/kg)}{\text{plasma dye } (\mu g/ml)}$ Plasma volume was also calculated from the hematocrit by equating the plasma ratio (P.R. = 100 — Hematocrit) with the plasma volume obtained by the dye method. In any given experimental group the plasma volume was calculated from the following equation: $V_{ml/kg} = \frac{(P.R.)_{exp}}{(P.R.)_C} \times V_C$; where $(P.R.)_{exp}$ is the plasma ratio of the given experimental group, $(P.R.)_C$ is the plasma ratio of the corresponding control group and V_C is the plasma volume of this control group as calculated from the dye concentration. No corrections were made for plasma trapping which can be considered negligible compared to the experimental changes. Microscopic observations were made on the rat meso-appendix using the method described by Chambers and Zweifach(6). The Krebs-Ringer formula(7) was used with addition of 3% gelatin for drip solution. Observations were made with the Spencer phase microscope at 100× and 200× for gross observations and 400× for a more detailed view of circulation in individual blood vessels. Adrenalectomies were performed under ether anesthesia and the adrenalectomized rats were maintained on 1% NaCl in their drinking water. All the animals were treated with egg-white prepared from pooled eggs, dialyzed overnight in the cold and lyophilized. For each experiment fresh solutions of the lyophilized material were made up in saline. Cortisone (Cortone acetate Merck) was given subcutaneously according to the following schedule: First injection 2 days prior to experiment, second injection the day before and third injection 2 hours before administration of egg-white. The dose indicated is the amount given in each injection.

Results. Plasma volume changes. In 8 normal rats the mean dye concentration was found to be 126.0 (±6.7) μg/ml of plasma, giving a mean plasma volume of 79.5 ml/kg of body weight. Hematocrit determinations in the same group of animals gave a mean plasma ratio of 51.7 (±1.1)%. It is legitimate to assume that this plasma ratio corresponds to a plasma volume of 79.5 ml and that any change in plasma volume, other factors being equal, will be reflected by a corresponding change in plasma/cell ratio. This assumption, although probably valid for the purposes of this study, calls for certain reservations which will be discussed below.

When groups of rats were injected intraperitoneally with 2 g/kg of egg-white, simultaneously with the development of edema, plasma volume changes were observed with both the dye and the hematocrit methods. Blood samples were taken from animals killed at varying intervals after the egg-white injection. The interval between dye injection and blood collection was 30 minutes all through the experiments. The results shown in Fig. 1 indicate that, on the whole, when dye concentration increases the plasma ratio falls; both indicating loss of plasma from the circulating blood. A discrepancy is noted, however, for the first half-hour when the dye concentration drops simultaneously with the plasma ratio. At the peak of the reaction (between 1½ and 2 hours) the plasma loss

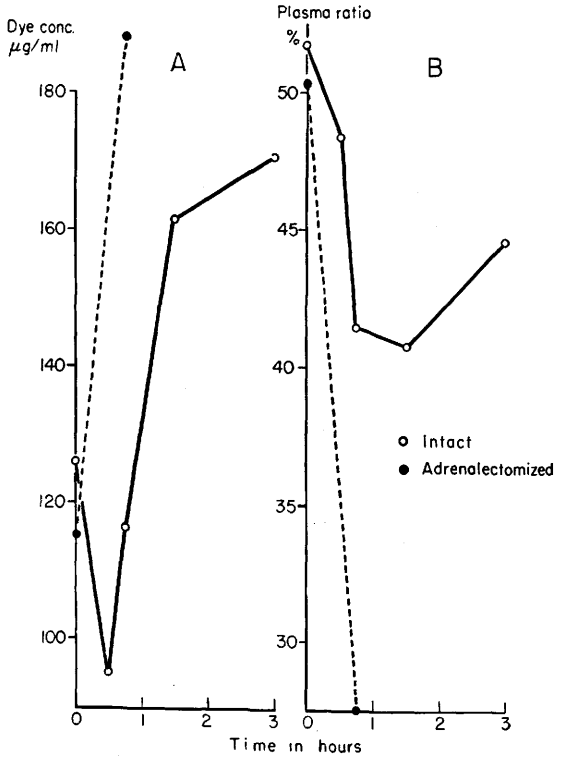

FIG. 1. Action of egg-white on plasma concentration of Evans blue and on plasma ratio in intact and adrenalectomized rats. Abscissa: time after intraperitoneal injection of 2 g/kg of egg-white (hr); ordinates, dye concentration in μg/ml of plasma (A), plasma ratio (= 100 − hematocrit) % (B). The rats were injected with 10 mg/kg of Evans blue 30 min. before the blood samples were collected. Each point represents the mean of 6 to 10 observations.

calculated from either the dye concentration or the hematocrit was close to 20%.

Plasma volume changes showed a development closely parallel to edema formation. Cutaneous edema started about 40 minutes after injection, reached its peak in 90 minutes and all but disappeared between 3 to 5 hours.

Removal of the adrenals, by itself, does not alter plasma volume significantly. In 9 adrenalectomized rats the mean dye concentration was 115.7 (±2.4) μg/ml with a plasma ratio of 50.4%. The calculated plasma volume was 87.0 ml/kg, not significantly different from the intact group. Under the influence of egg-white, the adrenalectomized rats developed within 30 to 60 minutes edema, cyanosis and irreversible shock. Since most of these animals were dead one hour after the injection of egg-white, study of their plasma volume was done only once, at 45 minutes. The results shown in Fig. 1 indicate a rapid and marked rise of dye concentration and a steep fall of the plasma ratio. The loss of plasma appears to be higher according to the hematocrit readings than according to dye concentration.

Table I shows that plasma loss is related to the dosage of egg-white but, even at the lower dose level the hematocrit gives a higher plasma loss than the dye method. This can probably be explained by the fact that the dye also escapes from the blood together with the proteins to which it becomes bound.

When cortisone was given either to intact or to adrenalectomized rats under the conditions specified above, the plasma volume changes were considerably decreased or even completely prevented. As seen in Table I, 5 mg/kg of cortisone given to intact rats (group 3) prevented the increase in dye concentration, although the hematocrit still indicated a slight plasma loss. Similar results were obtained in adrenalectomized rats pretreated with 10 mg/kg of cortisone (groups 6 and 8). It should be noted that adrenalectomized rats required higher amounts of cortisone to protect them and also that there is a rough proportionality between the dose of egg-white and the concentration of cortisone required for protection.

Microscopic observations. Results shown by direct observations of small blood vessels paralleled the plasma volume changes. These observations extend over a period of 5 hours following the egg-white injection. The intact rats, treated with egg-white, did not show any marked circulatory changes until about 90 minutes after injection. After this interval the flow was slowed down in the dilated and hyperemic metarterioles and arteriovenous capillaries and some clumping of blood cells was seen in the venules. Normal circulation was restored 3 to 5 hours after injection.

In the adrenalectomized animals these changes were both earlier and more severe. Thirty minutes after egg-white injection capillary function was deeply depressed with sludging of blood and stasis. These changes persisted until death of the animals.

TABLE I. Effect of Egg-White on Plasma Volume in Intact, Adrenalectomized and Cortisone-Treated Rats.

No.		From dye conc. Plasma vol.	From dye conc. Plasma loss, %	From hematocrit Plasma vol.	From hematocrit Plasma loss, %	N
1	*Intact rats*, controls	79.5 ± 4.2*	—	79.5 ± 1.7*	—	8
2	Egg-white 2 g/kg	62.3 ± 3.5	22.0	64.0 ± 4.1	19.5	10
3	" " , C† 5 mg/kg	82.8 ± .8	+2.4‡	72.1 ±12.5	9.3	10
4	*Adrenalectomized rats*, controls	87.0 ± 1.8	—	87.0 ± 4.4	—	9
5	Egg-white 1.5 g/kg	68.6 ± 4.8	21.2	54.7 ± 3.3	37.0	7
6	" " , C 10 mg/kg	85.8 ± 4.5	1.0	70.4 ± 9.6	19.2	7
7	" 2 mg/kg	55.8 ± 4.6	35.8	42.6 ± 5.3	51.2	6
8	" " , C 10 mg/kg	73.9 ± 3.2	15.2	58.0 ± 3.0	33.3	6

* Stand. error. † C = Cortisone. ‡ Increase in plasma vol.

Blood samples collected close to peak of reaction: 90 to 120 min. from intact rats, 40 to 50 min. from adrenalectomized animals. Plasma volume values are given in ml/kg body wt. Significance of differences between means was tested with the *t* test. Comparison between groups 2 and 1, 3 and 2, 5 and 4, 7 and 4, 6 and 5, and 8 and 7 gave P values of .01–.001, except for the hematocrit between groups 2 and 1 where the P value was .02–.01.

Cortisone pretreatment was seen to prevent disturbances in capillary circulation. In the intact rats (group 3 of Table I) only slight and transitory red cell clumping was observed. In the adrenalectomized groups treated with cortisone (groups 6 and 8) a slight slowing down of blood flow was also observed.

It should be noted that some clumping of white cells was seen in all groups even in the controls. This was, however, noticeably increased in all egg-white treated animals which have not received cortisone.

Discussion. The purpose of the present studies is to explain the mechanism by which egg-white causes edema and shock. The results obtained also suggest an explanation for the protective action of cortisone. The Evans blue method of plasma volume determination has been examined in critical reviews(8,9) and is considered satisfactory for static evaluations of plasma volume. In the present work the method is used under conditions which introduce at least one more degree of complexity. In acute shifts of fluid, as are produced in the egg-white syndrome, permeability of the vascular wall is increased to protein molecules. Evans blue is known to combine with proteins and will therefore share the fate of these molecules. This may result in a decrease in dye concentration, interpreted as an increased plasma volume.

The same criticism applies to the hematocrit method. If blood cells escape from circulation into the tissues or are stored in reservoir areas the change in plasma ratio may be interpreted as an increased plasma volume. It is possible therefore that the actual plasma loss is greater than indicated by either the dye or the hematocrit methods. With these reservations, it can be stated that the results obtained in this study give a fair indication of gross plasma volume changes. If the changes recorded were smaller their interpretation would be open to doubt.

Plasma loss caused by egg-white was significantly decreased by cortisone. This suggests that cortisone may exert its protective action primarily on the capillary wall and thus maintaining the plasma volume within limits compatible with survival. It is known that when a critical plasma volume is reached a vicious circle is established resulting in irreversible circulatory collapse.

It has been stated(4,5,10) that, although cortisone decreases mortality in adrenalectomized egg-white treated rats, it has no effect on the edema. In all these studies, however, edema was evaluated only by gross inspection by which quantitative changes could not have been detected.

Summary. Egg-white administration to rats results in loss of plasma from circulating blood. In adrenalectomized rats egg-white injection causes a greater loss of plasma than in intact animals. In animals pretreated with cortisone the loss of plasma is significantly smaller than in unprotected animals. The observations suggest that cortisone may act by

preserving capillary function and maintaining plasma volume above the critical level.

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Metachromasia in the Living Cell.* (21015)

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It has been generally agreed that metachromatic granules are not observed in the cytoplasm of fixed fibroblasts(1). They, as well as other cells, however, may show diffuse cytoplasmic metachromasia, which has been shown to be due to RNA(2-4). This might indicate that the metachromatic material of the ground substance(5,6) (not due to RNA), which is presumed to be produced by the fibroblast, acquires the quality of a chromotrope only when outside the cell(1). On the other hand, fixed mast cells show intracellular granular metachromasia. They have been presumed to produce both heparin(7,8) and hyaluronic acid(9-11). Mast cells in tissue culture have been identified by the presence of supravital metachromatic granules(12-17). The purpose of this communication is to describe the presence of metachromatic granules in living cells other than mast cells. It is further shown that their presence does not constitute definitive evidence for the identification of cells as mast cells on this basis alone.

Methods. Toxicity of toluidine blue (ToB), and thionin in low concentrations to culture cells has been found to be small. When ToB was added in a final concentration of 1/200000 to a medium of plasma and 20% embryo extract in chick amniotic fluid at the time of explantation, growth observed for 4 days was not inferior to growth in control cultures. Easily observable intracellular metachromasia, however, was not produced until dye concentrations of 1/50000 to 1/100000 were reached, when some toxicity to cells resulted. These concentrations, however, offer the best opportunity for observation of metachromasia and were employed in these studies. Fibroblasts from human skin and from chick skin and heart were cultured in a liquid medium of 20% embryo extract in chick amniotic fluid (18), using the coverslip method. At the second to fourth day of cultivation, the medium was changed to one of 1/50000 ToB in Ringer's solution. A dye concentration of 1/40000 ToB was used when cultures were grown in a plasma medium. Under these conditions, on direct microscopic observation with high power magnification, vital staining started at one side of the fibroblast near the nucleus. First a faintly rose-stained spot appeared in which tiny pink granules were embedded. The granules soon became more distinct and gradually, close to them, violet- or purple-colored granules appeared; the latter

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