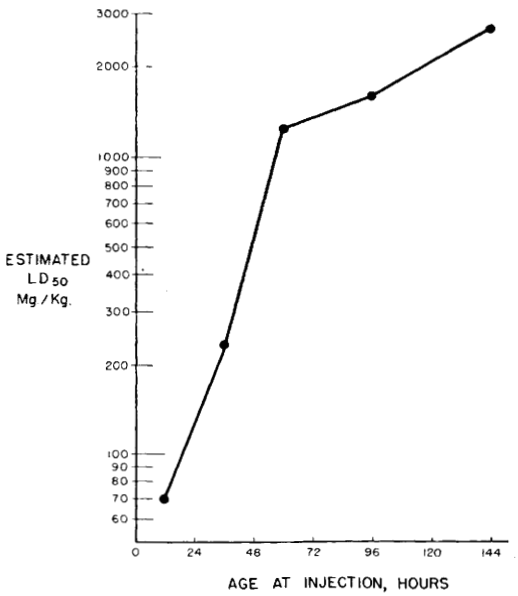


FIG. 1. Rise in tolerance of the baby mouse to progesterone with increasing age.

method of progesterone detoxification and catabolism is poorly understood(4). The data obtained on the development of resistance to progesterone within 2 to 3 days after birth may lead to an understanding of the mechanism whereby progesterone is inactivated or excreted.

*Summary and conclusions.* The new-born mouse is highly susceptible to the toxic and lethal action of progesterone but, within 2 to 3 days, resistance develops rapidly, and by the 7th day the mouse can tolerate about 100 times as much progesterone as when one day old. Closely related steroids, at equivalent and even higher doses, do not cause the acute lethal action of progesterone in the new-born mouse. The report that progesterone given during the last third of gestation will cause fetal death has been confirmed, and it is suggested that this is due to a direct toxic effect of progesterone on the fetus. The rapid development of resistance to progesterone within a few days after birth suggests that a system or mechanism has appeared which is capable of detoxifying, catabolizing, counterbalancing or accelerating the excretion of progesterone.

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## An Evaluation of the Role of Ferritin (VDM) in Traumatic Shock.* (19472)

JOHN K. HAMPTON, JR.,† J. J. FRIEDMAN, AND H. S. MAYERSON.
(With the technical assistance of Elizabeth Holbrook.)
*From the Physiology Department, Tulane University School of Medicine, New Orleans, La.*

*Introduction.* In studies of shock, a correlation has been noted between irreversibility of the syndrome and a phase of hyporeactivity of the finer vessels of the exteriorized omentum and mesentery(1,2). The latter has been attributed to a blood-borne vasodepressor material (VDM) from ischemic liver, etc., which has been identified as a form of ferritin(3-5). The inference has been made that a release of ferritin occurring during shock contributes to the syndrome by rendering vessels unreactive (3). Few other vascular sites, however, have been demonstrated to react to VDM the way the mesoappendix does. The present experiments were designed to test whether 1) increasing the concentration of ferritin in tissues or 2) removing it from the circulation affects the ability of rats to survive after shock-producing trauma.

*Materials and methods.* Ferritin was crys-

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† Markle Scholar in Medical Science.

TABLE I. Effect of Increasing Ferritin in Tissues (Exp. I) and Removing it from Circulation (Exp. II) on Ability of Rats to Withstand Trauma.

| Exp. | Group | No. of rats | Injection | % mortality | Mean death time (hr) ± stand. error |
|---|---|---|---|---|---|
| I | A | 80 | — | 70 | 2.03 ± .216 |
| | B | 68 | 3 mg Fe as Hb | 77.94 | 1.87 ± .187 |
| II | C | 46 | .5 ml normal rabbit serum | 76.09 | 1.39 ± .173 |
| | D | 43 | .5 ml rabbit anti-dog ferritin serum | 79.07 | 1.44 ± .148 |

talized by the method of Granick(6). It was recrystalized 4 times and finally reprecipitated 4 times by 50% saturation with ammonium sulfate. Thorough dialysis was carried out following each precipitation. Antiserum was prepared by injection of alum-precipitated ferritin into rabbits, according to the method described by Mazur and Shorr(4). The amount of ferritin was determined by the specific precipitin reaction(7) and by the slide technic modified after Granick(6). Estimation of ferritin was done by analysis of iron in the ammonium sulfate precipitate of the supernatant formed by heating tissue homogenate to 80°C. The Wong(8) method for iron was used. The hemoglobin solutions used were prepared from human cells and were supplied us by Sharp and Dohme. The Noble-Collip drum(9,10) was used to produce traumatic shock. Our drum was constructed of ¼ inch lucite. The inside diameter was 16 inches and the depth 6.5 inches. Two 2 inch baffles placed opposite each other were inside the drum, projecting inward at about a 45° angle. The drum was rotated at 45 r.p.m.

*Results. Effect of increased concentration of tissue ferritin upon the outcome of traumatic shock.* The ferritin content of tissues may be raised by injection of hemoglobin(11). In an early experiment, 1 mg doses (10 ml of the solution) of elemental iron as hemoglobin were injected intraperitoneally into 4 male Wistar rats every other day for 3 doses. One week after the final injection, the rats were sacrificed and their livers perfused with saline. The livers were then homogenized with twice their weight of distilled water and the homogenate heated to 80°C. The precipitate which formed was washed twice with water, which was pooled with the original supernatant. This supernatant was treated with 35 g of ammonium sulfate per 100 ml. The precipitate which formed was washed with 35% ammonium sulfate and finally analyzed for iron. Four controls were treated in the same manner. The control livers showed concentrations of iron in the crude ferritin fraction of 0.047-0.059 mg/g of liver. The iron concentration in these fractions from injected rats ranged from 0.072-0.108 mg/g. Additional analyses, done by the slide technic of estimation of ferritin crystals produced by cadmium sulfate, also indicated a significant increase in apoferritin in the rats receiving 3 mg of hemoglobin iron. The iron analyses showed the mean value of the injected group to be 69% higher than control. Results of the slide technic were 0 for controls and 3+ (2+ to 5+) for injected rats. Male Wistar rats of Carworth Farms' stock, weighing 150-200 g, were subjected to trauma in a Noble-Collip Drum one week after the injections of hemoglobin, employing the same dosage as above. Normal control rats were traumatized in the same manner. Each animal remained in the drum for 9 minutes, giving a total of 405 revolutions. Table I, Exp. I, gives the results of this experiment.

The significance of the difference between the mortalities of the two groups has been tested. The probability of 0.49 indicates that the difference between the two groups could have easily occurred by chance alone. To further analyze the possibility of a difference between the groups, the difference in the mean death times was tested. A probability of 0.28 again indicates the difference to be insignificant. Indeed, it is surprising that the large volume of hemoglobin injected (30 ml total) was not manifest as an undesirable insult.

*Effect of decreased circulating ferritin upon the outcome of traumatic shock.* An excess of antiserum of good titer to dog ferritin gives a recovery of about 99% of added rat ferritin (12). An intravenous injection of 0.5 ml of pooled anti-dog ferritin serum was given to 150 g rats immediately after drumming for 450 revolutions. The sera of these rats, sacrificed 30 minutes to 5 hours after drumming, retained readily detectable antibodies which precipitated rat ferritin. Those which survived the drumming showed antibodies in their sera which precipitated rat ferritin up to 24 hours.

To determine the effect of ferritin neutralization by antibody on the outcome of shock one group of rats was drummed for 450 revolutions and received 0.5 ml of the pooled anti-dog ferritin serum intravenously immediately upon removal from the drum. A similar group received 0.5 ml of normal rabbit serum after drumming and served as a control. Table I, Exp. II, shows the results of this experiment. The probabilities (0.83 and 0.73) calculated from these data indicate no significance in the differences in mortality or mean death time, respectively.

Since it has been our observation as well as Zweifach's(10) that animals dying within a few minutes after drumming or in the drum usually do so because of hemorrhage in the viscera or intracranially, only animals that survived at least 15 minutes are included in the tables. Mortality figures are based on a 24-hour survival time. However, the analyses of the data are unchanged when all animals are included.

*Discussion.* Chambers(1) pointed out that the omental circulation was selected for microscopic observation in the mesoappendix test because, in contrast to cutaneous tissue, its circulation is maintained throughout the greatest part of the hemorrhagic syndrome. Conclusions drawn from the reactivity of these vessels may in no way represent the reactivity of vessels generally in the organism. We feel that before a causal role in any phase of the shock syndrome can be assigned to ferritin (VDM), evidence other than its vasodepressor activity to certain vessel areas and its appearance correlated with the hyporeactive phase of shock must be obtained. Frank *et al.*(13) were unable to obtain correlation of VDM with irreversibility when morphine was used as the anesthetic. When unanesthetized animals were subjected to irreversible hemorrhagic shock, VDM failed to appear in the circulation. A correlation of blood borne VDM with the hyporeactive and irreversible phases of shock is suggested by them as an artefact produced by anesthesia. They offer evidence that the intravenous injection of VDM in hepatectomized, nephrectomized dogs had no effect on arterial pressure or survival. Similar experiments in this laboratory support their findings.

The presence of VDM in the circulation in shock and many other states(3), such as congestive heart failure, cirrhosis of the liver, etc., does not eliminate the possibility that it is the loss of some liver function which is responsible for the abnormal condition. In fact, our studies of ferritin storage in the liver suggest to us that after anaerobiasis and other liver injury ferritin would be found in the blood stream, especially in such small quantities as noted by bioassay. Our results indicate that the amount of ferritin in the tissues and its presence or absence in the blood are, however, not determinants of fatality due to trauma, nor is the time required for the animals to succumb to the insult significantly affected.

*Summary.* Increased tissue ferritin produced by intraperitoneal hemoglobin or precipitation of circulating ferritin by antiserum failed to affect mortality rate and mean death time in rats subjected to trauma in the Noble-Collip drum.

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## An Instrument Suitable for Measurement of Osmotic Pressure of Biological Fluids. (19473)

ALEXANDER J. SCHAEFFER.
*From Los Angeles, Calif.*

In previous papers(1) I have reported on measurements of osmotic concentration of the extraocular and intraocular fluids. The instrument constructed for this special need has not been described and might be a valuable tool for similar investigations.

Niederl and Levy(2) published a simplified method for the determination of molar concentrations, a modification of the well known original method of Barger. The method is based upon the fact that in a closed system, harboring two solutions of different molarity,

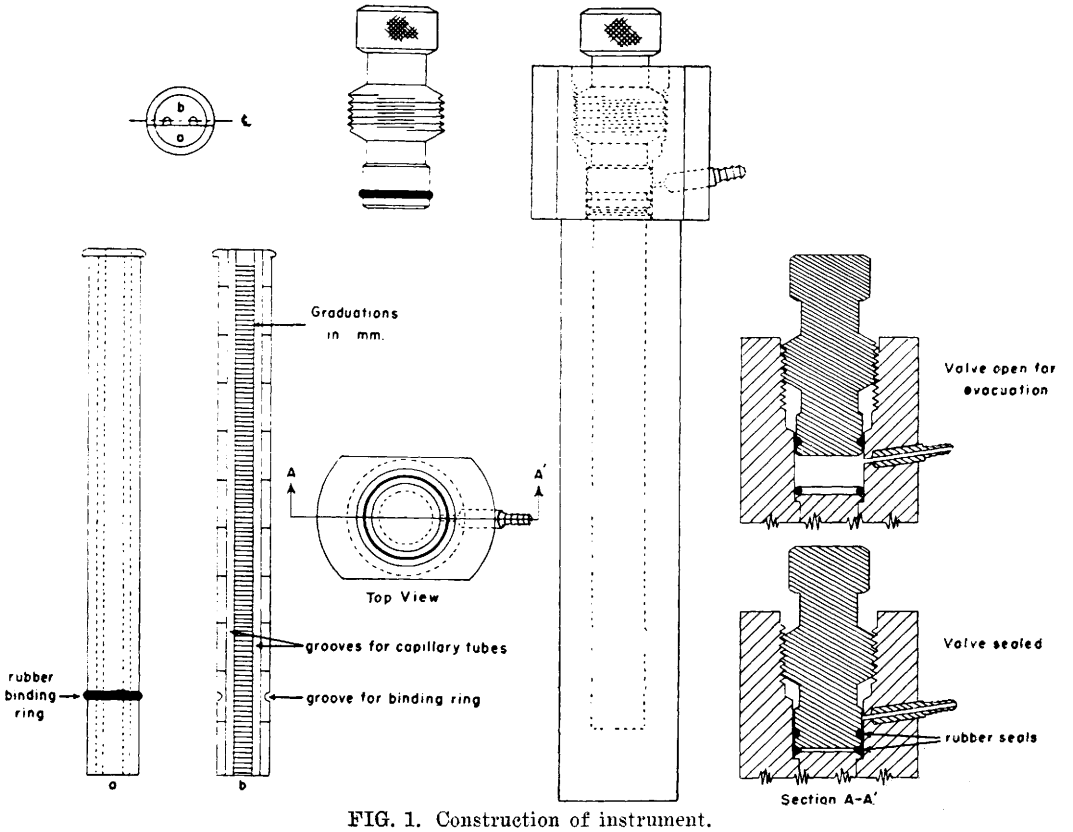


FIG. 1. Construction of instrument.