Changes in Protein-Bound Dye Disappearance Rate as a Result of Evisceration and Nephrectomy

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This study presents data which indicate that evisceration results in a marked decrease in the rate of disappearance of Evans Blue (T-1824) from the circulation of rabbits. Nephrectomy causes a significant increase in the rate of removal of dye from the bloodstream. The possible explanations for these changes are considered, and the objections to the use of dye disappearance as a measure of capillary permeability are discussed.

Several recent papers report changes in "capillary permeability" on the evidence of measurements of the rate of escape of protein-bound dyes from the circulation. Retention in the circulation of these dyes, like the retention of proteins may be determined by a large number of independent factors. It is, therefore, hazardous to draw conclusions concerning capillary permeability from these measurements alone.

Although protein-bound dye leaves the circulation, at least in part, by transfer across the capillary wall, the degree of leakage is probably not uniform across all capillaries of the body; thus, the disappearance rate may be largely determined by escape across certain excessively leaky capillary walls in specific tissues. In addition, active mechanisms like phagocytosis may account for the removal of a significant amount of the dye. Since the activity of the reticuloendothelial system may be subject to physiologic regulation, this factor may play a significant and grossly variable role in modifying the rate of dye removal. Finally, the loss of dye along excretory pathways, that is, into the urine or into the gastrointestinal tract, must also be considered.

The liver may contribute significantly to the removal of dye by each of the mechanisms suggested above. The excessive leakiness of the liver capillaries would make this organ a likely place for the escape of protein-bound dyes. Direct measurements show significantly higher dye concentrations in thoracic duct lymph than in the cervical lymph. The Kupffer cells of the liver by their phagocytic activity may remove much of the dye; however, results of experimental attempts to assess the role of the reticuloendothelial system are inconclusive. The more recent studies suggest that this mechanism is probably of only minor importance in determining the rate of dye removal.

Qualitative and quantitative evidence exists to support the theory that some small fraction of the dye is eliminated in the bile. However, this route accounts for only 0.4 per cent of all the Evans Blue (T-1824) lost from the circulation.

Although traces of dye have been found in urine of dogs and humans after rather large injections, no visible staining of urine has ever been detected in cats or rabbits.

The present investigation was undertaken to determine the importance of extrahepatic pathways and to clarify the role of the kidney in the removal of T-1824 from the circulation of the rabbit. The experiments involved com-
Comparison of dye disappearance rates in normal, eviscerated, and nephrectomized rabbits.

**Methods**

Disappearance rates over a period of 120 minutes were determined from arterial plasma concentrations of T-1824 on pentobarbital sodium anesthetized rabbits. Immediately after the control rate was established, one group of animals, to be eviscerated later, was surgically prepared by coarctation of the vena cava. Such animals were designated as "V Rabbits." When an adequate collateral circulation had been established a second determination of dye disappearance was made on these animals. A minimum of two weeks was permitted to elapse between the initial and final stage of evisceration. Disappearance rates were again measured beginning one hour after the evisceration was completed. In another group of animals bilateral nephrectomies were performed by a simple ventral approach about one week after determination of the control disappearance rate. Data were also obtained on a group of rabbits which was treated in all respects like the eviscerated animals except that the kidneys also were removed at the second stage of evisceration. Thus data were obtained on five different groups of animals, namely, controls, "V Rabbits," eviscerated, nephrectomized, and eviscerated-nephrectomized rabbits. In some cases arterial pressures were taken by direct cannulation of the carotid artery.

For each determination, the animal was prepared by exposure of the femoral artery from which a single control sample was withdrawn immediately after completing the preparation. Soon thereafter about 2.5 mg. of T-1824 per kilogram of body weight was administered via the marginal ear vein. At precisely measured intervals during the 120 minutes after the dye injection, further arterial blood samples were taken.

For each sample about 1.0 ml. of blood was withdrawn into a syringe containing about 0.2 ml. of 1.6 per cent sodium oxalate; the exact amounts were determined gravimetrically. The thoroughly mixed, oxalated blood was delivered into a Wintrobe tube and centrifuged at 3000 r.p.m. for 30 minutes. The hematocrit levels were read, corrected for oxalate dilution, and recorded. Exactly 0.2 ml. of the supernatant plasma was diluted with 2.0 ml. of 0.9 per cent sodium chloride and the dye concentration of plasma determined colorimetrically (590 m\u00b5).

The dye concentrations as measured were adjusted by correcting each reading for oxalate dilution and transcapillary fluid shifts according to the following expression:

\[
C = C_m \times \frac{(100 - \%rbc)(\%wb - \%rbc)(\%wb)}{\%wb - \%rbc)^2(\%wb)}
\]

Where \(C\) = corrected dye concentration
\(C_m\) = the apparent dye concentration
\(wb\) = whole blood
\(rbc\) = red blood cells
\(t\) refers to a sample taken at time \(t\) and 
\(o\) refers to the control sample.

This expression was derived by combining an expression for effects of oxalate dilution, based on measured weights and volumes of blood and oxalate, and an expression for the effects of fluid shifts based on hematocrit measurements.

The logarithms of the final corrected concentrations were plotted against time. Although in most cases the points could be fitted to a straight line by inspection, a regression line was calculated for each plot. The calculated slopes of these were taken as the percentage of dye disappearing from the blood stream per minute (reciprocal minutes).

**Results**

A total of 41 disappearance rates were determined on 35 rabbits. A preliminary series (I) includes experiments in which measurements of blood pressure were not taken. Since the possibility existed that this factor might be responsible for the changes noted, direct measurements of carotid arterial pressure were taken in a second series (II).

Table 1 represents the disappearance rates in per cent per minute of each individual experiment and the means of the different groups. The rates of dye disappearance obtained from the control animals and the "V Rabbits" showed no significant differences. The mean rate of dye loss from the blood stream of the eviscerated animals was significantly lower than in the control group. Nephrectomized animals had a mean dye disappearance rate which was significantly higher than the control group, and those animals which were both eviscerated and nephrectomized gave a mean value that was again indistinguishable from the controls.

No systematic change in the hematocrit was noted in any of the groups investigated, indicating that only minor shifts of fluid had taken place.

**Discussion**

The data obtained in all cases confirm the essential exponential character of the disappearance curve. In the present investigation
the problem of delimiting the mixing time was avoided by fitting the linear portion of the disappearance rates was considered. However, data obtained from the second series of animals summarized in figure 1 show that there is no relationship between disappearance rates and the average blood pressure taken during the time of the disappearance determination.

Further, evidence exists that there are very few physiologic conditions which affect the removal of T-1824 from the blood stream with the possible exception of shock due to severe burn. It is apparent that the dye disappearance is not altered by shifts of water in or out of the vascular system. It was assumed that no major transcapillary fluid shifts could occur without some changes in the hematocrit values. Figure 2 represents the changes in hematocrit values noted in four different groups of animals. The shifts seem to occur in a rather random fashion. Increases or decreases in the water content of the plasma diluted or concentrated the dye in the circulation; nevertheless, the curves representing the dye disappearance rates are quite regular when properly corrected for fluid shifts.

The close correspondence between the disappearance rates of the control animals and curve to points well beyond the longest estimates for mixing. The possibility that the changes in blood pressure might explain the differences noted in
the "V Rabbits" suggests that there is no profound change in the perfusion of the liver of the operated animals.

It has been established that following evisceration the rate of loss of a protein-bound dye from the blood stream is significantly decreased. It is possible that a deficiency of some component which favors the removal of dye, or conversely, the accumulation of a substance which assists in retention of the dye, results from removal of the liver and abdominal viscera. Evidence exists that histamine increases the dye disappearance rate. It is possible that evisceration results in depletion of this substance. As has been pointed out above, much of the activity of the reticuloendothelial system is removed with the abdominal viscera.

Evisceration may deprive the animal of a major "leak" for the escape of dye from the circulation. The several ways in which the liver might serve as such a leak have been discussed. In our opinion the most likely explanation for the results obtained is that half of the dye normally lost from the circulation either escapes across the liver sinuses and the visceral capillaries into the thoracic lymph or is phagocytosed by tissue in the abdominal viscera.

The augmentation of the rate of dye removal in the nephrectomized group was entirely unpredicted. The possibility that postnephrectomy-hypotension might disturb the dynamics of circulation and, by altering the effective mixing time, lead to the apparent increase in the disappearance rate was not supported by data obtained in the second series of experiments. It is quite possible that the accumulation of some materials in the short period between the nephrectomy and the time of the rate determination may be responsible for the increase noted. However, neither the substance involved nor the exact mechanism whereby the increased dye disappearance is effected is yet clear. The possibility that histamine and histamine-like substances may be involved is under study.

The fact that the augmented leakage of T-1824 in the nephrectomized animals is not limited to a local change in the liver or viscera is suggested by the data of the fifth group of animals in which the combined procedures were employed. The disappearance rate after evisceration-nephrectomy is considerably greater than in eviscerated animals; that is, loss of dye into the "carcass" is more rapid after nephrectomy.

Since the rate of disappearance of a protein-bound dye can be markedly altered by evisceration, and since the mechanisms which are probably responsible for the decrease may not be related to changes in vascular permeability, it seems highly presumptive to us to use the disappearance rate of a protein-bound dye from the circulation of an intact animal as a measure for capillary permeability. We believe that a change in the disappearance rate may be considered as an indication of altered blood flow through specific organs or perhaps altered reticuloendothelial activity so that the disappearance data obtained from the intact animals can have only indirect value as a measure of capillary permeability.*

**SUMMARY**

The rate at which T-1824 disappears from the circulation of anesthetized rabbits is decreased to about half the control value as a result of complete evisceration and is markedly increased after nephrectomy. On the basis of

*These experiments were performed in laboratories generously provided by the Allan Hancock Foundation.
these results and a survey of the literature, we concluded that the use of dye disappearance rate is inadmissible as a measure of capillary permeability.

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