STUDIES ON VITAL STAINING

II. The Removal of Brilliant Vital Red from the Blood Stream. Distribution of Dye between Blood Stream and Body Tissues

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INTRODUCTION

In recent years there has developed a voluminous literature which deals with the phagocytosis of foreign materials by various body cells. Ink, bacteria and various dyes have been introduced into the living animal until the tissues were heavily laden with these substances. There has been much discussion about the possibility that one may saturate the tissues to the point that certain of their physiological powers are destroyed, or at least impaired. References to the literature may be found in the monographs of Aschoff (1) and of Börner-Patzelt, Gödel and Standenath (2).

These foreign substances with which we are concerned are taken up and stored in granular form within a vast number of cells, some of which are endothelial in nature, while others are wandering or fixed cells of connective tissues. Details concerning the histological aspects of the problem will be given in a subsequent paper (3). The phagocytic cells scattered throughout the body have been referred to collectively as the "reticulo-endothelial system." Many and varied physiological activities have been ascribed to them. Much has been written of their activity in freeing the blood stream and the tissues generally of objectionable particulate matter, such as bacteria, dead cells and cell debris. There is much evidence to indicate an ability on their part, to transform hemoglobin to bile pigment. It has been supposed that they may play a part in fat metabolism and by some they are thought to be concerned in the elaboration of immune bodies. Still other supposed activities are discussed in the monographs cited above. Many of these theories rest on the most insecure basis. Changes in pigment metabolism, in immune body balance, etc. are often observed to follow injection of certain foreign substances such as ink or carmine. Those who think that ink or carmine paralyze or "block" the reticulo-endothelial system will attribute all of these reactions to changes in the latter, and, totally disregarding possible changes in other organs or tissues, they will find no end of "functions" of this system. Other

workers, more interested in settling the question of "blockade" than in establishing new functions of the reticulo-endothelial system are sometimes rather hasty in assuming that the system has this or that function, and from changes observed they may draw erroneous conclusions in regard to the existence of a "blockade."

Thus, each of these groups of workers assumes the point which the other tries to prove by experiment. It is easily seen that such arguments are in a circle and are utterly futile. We would suggest that much desirable information concerning "blockade" may be obtained by careful study of reactions in which the rôle of the reticulo-endothelial system cannot be questioned. In the present state of knowledge we feel that much uncertainty prevails concerning the great majority of the "functions" of these cells. We feel that more is to be gained by a study of their long-known function of phagocytosis, or "storage" of dyes or other materials.

In the case of many dyes this process can be followed with the microscope, but unfortunately the purely morphological studies are almost entirely qualitative. They furnish little information about the degree or the speed of phagocytosis. The rate at which dyes leave the blood stream gives a better quantitative measure of such activity. However, many dyes and other substances in current use disappear quite rapidly from the blood stream and in many cases the particles tend to agglutinate in capillaries even before they are taken up by phagocytes. Other substances pass out rapidly through the bile or urine or both. Obviously in none of these cases is the rate of disappearance from the blood stream a trustworthy criterion of phagocytic activity. It should be stressed that most of the substances used in the past have had these defects to a striking degree. As yet no ideal test dye has been discovered, though a few approach the ideal much more closely than the rest.

A large number of dyes has been studied by Dawson, Evans and Whipple (4) and much valuable information has been obtained concerning the rate at which they leave the circulating blood. One of these, brilliant vital red, has already received further study from this standpoint by the present author (5). The dye is relatively nontoxic. It is not readily precipitated in neutral or nearly neutral solutions by the salts common in the animal body. The elimination through the kidneys is negligible. However, the dye is largely eliminated through the bile over the course of several days, but in the meantime the tissues become deeply stained and microscopic examination shows the phagocytes everywhere to contain beautiful accumulations of the red dye. During the first 2 or 3 days following injection the amount lost through the liver accounts for distinctly less than half of the dye which leaves the blood stream. The rest is taken up by the tissues, largely within phagocytic cells.

In an earlier paper of this series (5) a study was made of the rate at which the dye leaves the blood stream, and curves were plotted to show the relations graphically. The concentration in the blood stream falls off rather rapidly during the first few hours, but much more slowly later on (see also Chart 2C of the present paper). At the end of about 48 hours considerable dye still remains in circulation and from this point on the dye is so slowly eliminated that the curve is almost horizontal. No doubt the curve would approach the horizontal still more closely were it not that a small amount of dye is continuously being lost by excretion into the bile. The tissues are now deep red and it is clear that they are not taking up dye as readily as they would do in their normal unstained condition.

It might have been anticipated that the phagocytes would ingest dye quite rapidly at first when the plasma and lymph about them contain such large amounts of dye, and this is quite in accord with general experience, but there seems to be little recognition of the fact that the phagocytic activity slows up long before all of the dye has been ingested. With larger doses, phagocytosis also slows up but leaving still larger quantities of dye in circulation than before, and along with this one notes that the phagocytes contain more dye. To explain these facts it was suggested that an equilibrium is established between dye in cells and dye in the fluids, and that the diminution in phagocytic activity is in part merely apparent, for renewed activity is observed if we disturb the equilibrium by injecting more dye. Very clearly this concept has important bearings on the question of "blockade" of the body phagocytes and it is of great importance to know whether these dye-laden cells will remove newly injected dye as rapidly as though they themselves were free of dye. Our initial observations about to be presented seemed to indicate that some impairment really exists, for we noted that after a course of vital staining new offerings of dye leave the blood stream less rapidly than normal. We found also that large doses of dye do not leave the plasma as rapidly in proportion as do smaller ones. But certain observations on bile fistula dogs placed these experiments in a new light, and it now seems that the retention of dye in the plasma is associated with inefficient liver elimination which is seen only when large amounts of dye are given. We can offer no evidence to indicate that the liver

tissue is actually injured by the dye. It may be entirely within the realm of normal physiology that 25 per cent of a small dose of dye passes into the bile in 24 hours whereas only about 10 per cent of a large dose will be excreted in such a period. The greater retention of dye within the body may well explain the retention of dye within the plasma, without our having to assume defective phagocytic activity on the part of the tissues. We wish to stress these observations and this interpretation in order to show the danger of assuming a blockade of phagocytes merely because dye leaves the blood stream unduly slowly. This view will be given further emphasis in a subsequent paper (6) where it will be shown that a small injection of India ink will inhibit almost completely for some days the excretion of brilliant vital red by the liver. The great retention of dye in the body during this period seems to be quite sufficient to account for the unduly great dye concentration in the plasma, and there is reason to believe that part of the retained dye has found its way into the tissues and that there is increased coloration there as well as in the plasma. There is little need to assume that the ink had inhibited the activity of the phagocytic system. These observations are in accord with our present contention that large amounts of brilliant vital red within the tissues need not inhibit the entrance into the phagocytic cells of newly injected dye. The disproportion in liver excretion seems to account for all of the findings which might be taken to indicate such impairment in phagocytosis.

Methods

Healthy adult dogs maintained on a mixed diet were used in all experiments. To allow accurate colorimetric measurement of dye in the plasma, feeding hours were so arranged that the plasma would show no lipemia during the morning hours when samples of blood were commonly taken for analysis. The dogs had free access to water at all times. The dye used was brilliant vital red which was obtained from the National Aniline and Chemical Company. Dye from the same bottle was used throughout the course of the experiments. At intervals a filtered two per cent aqueous solution was made up. In each case the color intensity of this stock solution was checked spectrophotometrically just after preparation and also at intervals later on, so that constancy in preparation and absence of fading could be demonstrated.

Blood for analysis was collected in graduated 15 cc. centrifuge tubes containing 2 cc. of a 1.6 per cent sodium oxalate solution to prevent clotting. The tubes

were centrifuged and the amount of plasma read off on the tube, thus making it possible to correct for the dilution occasioned by the 2 cc. of oxalate solution previously added. The amount of dye in the plasma was determined by means of the spectrophotometer. Details of this method of analysis are given in a previous article (7). In all cases the concentration of dye in the plasma is expressed in terms of milligrams of dye per liter of plasma.

EXPERIMENTAL

If brilliant vital red be injected into the blood stream, the blood plasma taken several minutes later will be found to be bright red. If the amount of dye in such plasma be measured by colorimetric means we find that the concentration is almost exactly proportional to the amount of dye injected. With given dosage, proportional to body weight, this original dye concentration varies somewhat in different dogs depending on variations in blood volume. If such a standard dose be adopted as routine, it is found that this original high dye concentration is not maintained, but the concentration falls off, so that only 15–25 per cent of the dye remains 24 hours later. From this point on the fall is much more gradual, and we believe that this slowing up is related to the fact that the tissues are becoming stained with dye and on this account do not take up dye from the fluids as readily as normal. To be sure, the amount of dye now in circulation is not large, but we know that normal unstained tissues will attract such small amounts of dye quite readily, as one can demonstrate by injecting small doses into normal unstained dogs. This delay on the part of the stained tissues has been considered briefly in a previous publication (5). It was suggested that dye passes from plasma to tissues until an equilibrium partition is approached. We may believe that the amount of dye in the blood stream would finally become quite stationary were it not that a small amount of dye is constantly spilling over into the bile and is lost from the body.

Dog 24–74. Shepherd, 30 kgm.

May 23, 1927. Thirty cubic centimeters of a 2 per cent solution of brilliant vital red injected intravenously. Five minutes after the injection the concentration of dye in the plasma was 440 mg. per liter plasma; after 1 hr., 370; 6 hrs., 250; 24 hrs., 130; 48 hrs., 42; 72 hrs., 15.

May 26 to June 2 inclusive, daily injections of 30 cc. of a 2 per cent solution of the same dye. Five minutes after the last injection the plasma contained 670

mg. dye per liter plasma; 6 hrs. later, 525; 24 hrs., 335; 48 hrs., 190. Immediately following the collection of this last sample 30 cc. more of the dye were injected. Five minutes after this injection the plasma contained 540 mg. dye per liter plasma. After 6 hrs., 370; 24 hrs., 270; 48 hrs., 160; 72 hrs., 100.

Several months later almost all of the dye had been eliminated from the body and the tissues were again in a normal condition, suitable for further experiments.

August 3, 1927. Thirty cubic centimeters of a 2 per cent solution of the dye were injected intravenously. Five minutes later the plasma contained 415 mg. dye per liter plasma. One hour later, 335 mg.; 6 hrs., 245; 24 hrs., 84; 48 hrs., 30; 72 hrs., 19.

August 6 to 13 inclusive, daily injections of 30 cc. of the 2 per cent dye. Five minutes after this injection the plasma contained 740 mg. dye per liter plasma; 6 hrs. later, 550; 24 hrs., 360; 48 hrs., 185; 72 hrs., 102; 96 hrs., 67; 120 hrs., 45.

Dog 24–96. Shepherd, 18 kg.

May 24, 1927. Twenty cubic centimeters of a 2 per cent solution of brilliant vital red injected intravenously. Five minutes after the injection the concentration of dye in the plasma was 470 mg. dye per liter plasma; after 1 hour, 410; 6 hrs., 310; 24 hrs., 160; 48 hrs., 103; 72 hrs., 62.

May 27 to June 3 inclusive, daily injections of 20 cc. of the 2 per cent dye solution. Five minutes after the last injection the plasma contained 990 mg. dye per liter plasma; after 6 hrs., 735; 24 hrs., 520; 48 hrs., 420; 72 hrs., 300; 96 hrs., 205; 120 hrs., 180.

After several months had elapsed and the tissues had rid themselves of almost all of the dye the animal was used for the following experiments.

August 6, 1927. Twenty cubic centimeters of the 2 per cent dye solution were injected intravenously. Five minutes later the plasma contained 480 mg. dye per liter; after 1 hr., 430 mg. dye; 7 hrs., 295; 24 hrs., 138; 48 hrs., 76; 72 hrs., 48.

August 9 to 16 inclusive, daily injections of 20 cc. of 2 per cent dye solution. Five minutes after the last injection the plasma contained 830 mg. dye per liter plasma; after 6 hrs., 740 mg.; 24 hrs., 575; 48 hrs., 320. Immediately following the collection of this last sample 20 cc. more of the dye solution were injected. Five minutes after this injection the plasma contained 695 mg. dye per liter plasma; after 6 hrs., 625 mg.; 24 hrs., 390; 48 hrs., 250; 72 hrs., 210.

Dog 25–16. Airedale, 23 kgm.

May 22, 1927. Twenty cubic centimeters of a 2 per cent solution of brilliant vital red injected intravenously. Five minutes after the injection the concentration of dye in the plasma was 400 mg. per liter plasma; after 1 hr., 325 mg.; 6 hrs., 240; 24 hrs., 105; 48 hrs., 54; 72 hrs., 31.

May 25 to June 1 inclusive, daily injections of 20 cc. of a 2 per cent solution of the same dye. Five minutes after the last injection the plasma contained 790 mg. dye per liter plasma; 6 hrs. later 670; 24 hrs., 450; 48 hrs., 325. Immediately following the collection of this last sample 20 cc. more of the dye were injected. Five minutes after this injection the plasma contained 660 mg. dye per liter plasma. After 6 hrs., 530; 24 hrs., 375; 48 hrs., 280; 72 hrs., 200.

Several months were now allowed to elapse so that the dye might be eliminated from the body and the tissues become pale once more in preparation for the experiments to follow.

August 4, 1927. Twenty cubic centimeters of a 2 per cent solution of the dye were injected intravenously. Five minutes later the plasma contained 340 mg. dye per liter; 1 hr. later, 320; 6 hrs., 205; 24 hrs., 95; 48 hrs., 71; 72 hrs., 28.

August 7 to 14 inclusive, daily injections of 20 cc. of the 2 per cent dye. Five minutes after this injection the plasma contained 700 mg. dye per liter; 6 hrs. later, 510; 24 hrs., 355; 48 hrs., 225; 72 hrs., 155; 96 hrs., 123; 120 hrs., 100.

Dog 25–29. Collie, 28 kg.

May 21, 1927. Twenty-four cubic centimeters of a 2 per cent solution of brilliant vital red injected intravenously. Five minutes after the injection the con-

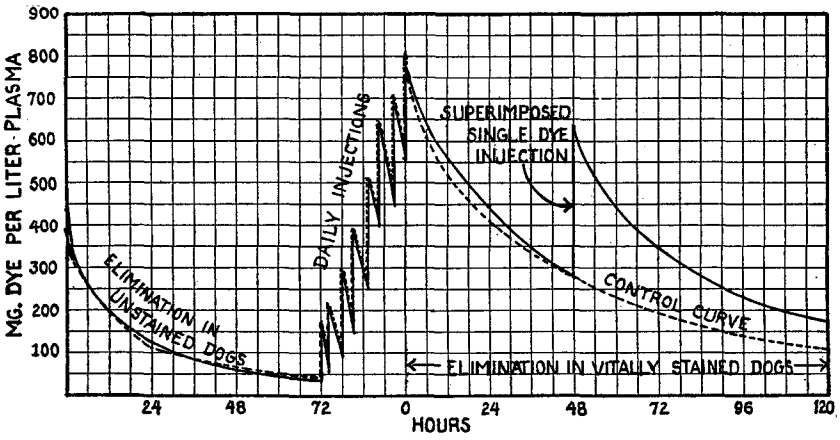

CHART 2A. Concentration of brilliant vital red in plasma (average of four experiments).

centration of dye in the plasma was 360 mg. per liter; after 1 hr., 340; 6 hrs., 280; 24 hrs., 114; 48 hrs., 59; 72 hrs., 43.

May 24 to 31 inclusive, daily injections of 24 cc. of a 2 per cent solution of the same dye. Five minutes after the last injection the plasma contained 806 mg. dye per liter plasma; 6 hrs. later, 625 mg.; 24 hrs., 405; 48 hrs., 315; 72 hrs., 230; 96 hrs., 170; 120 hrs., 110.

After several months had elapsed and the tissues had rid themselves of almost all of the dye the animal was used for the following set of observations.

August 5, 1927. Twenty-four cubic centimeters of a 2 per cent solution of the dye were injected intravenously. Five minutes later the plasma contained 390 mg. dye per liter; after 1 hr., 370 mg.; 6 hrs., 240; 24 hrs., 114; 48 hrs., 62; 72 hrs., 49.

August 8 to 15 inclusive, daily injections of 24 cc. of the 2 per cent dye solution. Five minutes after the last injection the plasma contained 810 mg. dye per liter plasma; after 6 hrs., 605 mg.; 24 hrs., 425; 48 hrs., 280. Immediately following the collection of this last sample 24 cc. more of the dye were injected. Five minutes after this injection the plasma contained 620 mg. dye per liter plasma; after 6 hrs., 540 mg.; 24 hrs., 350; 48 hrs., 220; 72 hrs., 180.

In order to learn more about the behavior of dye-stained tissues we have made daily dye injections into a number of dogs until their tissues were quite heavily stained. Under these circumstances the

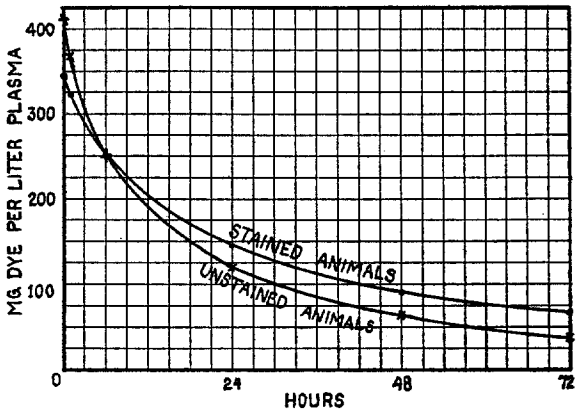

CHART 2B. Concentration of brilliant vital red in plasma (average of four experiments).

amount of dye circulating in the plasma may be very large. Even for 48 hours after the last injection rather large amounts of dye are still present in the plasma, but the rate at which the dye is leaving the circulating blood is very much reduced. The tissues are now deep red in color—a result of the dye granules present in the phagocytes. We might expect that these cells, partially filled with dye, would refuse further offerings, or at least would show a sluggish response. That they do not refuse altogether is most readily demonstrated by the injection of a test dose of dye. We are presenting 4 experiments which show this quite convincingly. The protocols are presented individually and Chart 2 A shows the average of all four dogs (Dogs 24–74, 24–96, 25–16, and 25–29).

The jagged peaks in the mid-portion of the chart illustrate schematically the rise in dye in the plasma accompanying the 8 daily intravenous injections of dye. At the end of this period the plasma was found to contain approximately 800 mg. dye per liter. The dotted control curve proceeding from this point shows that the dye leaves the plasma rather rapidly at first, but it is noted that 48 hours later the plasma still contains nearly 300 mg. dye per liter, and there are 100 mg. per liter at the end of 120 hours. It is seen that the curve is rapidly approaching a plateau. We believe that the elimination of dye by the liver is largely responsible for the fall noted in the last few hours of this curve. The tissues are still deep red. This is well seen in the skin and mucous membranes.

If several months are allowed to elapse the tissues will become almost entirely free of dye and the experiment may be repeated with almost identical results. Such a duplication is shown by the continuous line in this same chart. As in the control experiments it will be noted that following 8 daily injections the plasma again contained about 800 mg. of dye per liter, and that from this point on the two curves are very similar, and as before described the curve begins to flatten out decidedly after about 48 hours, and as before we also note that the tissues are deep red.

At this time a large test dose of dye was injected in order to ascertain how readily these dye-laden tissues would dispose of additional amounts of dye. As a result of this superimposed injection the dye concentration in the plasma promptly rose from about 300 mg. per liter to somewhat over 600. The curve from this point on shows rather rapid elimination at first, proving beyond doubt that the phagocytic activity was far from being abolished by the presence of the dye which these cells contained. We note, however, that the initial rapid loss of dye from the plasma is not maintained and the curve ultimately approaches a plateau which is nearly twice as high as that of the control curve shown by the dotted line.

We may look upon the space between these two curves as representing the reaction of the dye-stained tissues to this final superimposed dye injection. This difference may be measured at a number of points on the curves and the differences plotted separately. It is of great interest to compare the curve so obtained with a curve show-

ing the results in unstained dogs, and for purposes of comparison we have determined the normal elimination rate for these same dogs. The results are shown in the first portion of the chart, before the tissues had been stained by the daily injections. This normal curve is plotted separately in Chart 2 B and alongside this curve is the other which shows the arithmetical differences between the two curves just discussed. It will be observed that the slopes of the curves are decidedly different, and it is seen that when the dogs are vitally stained the dye leaves the plasma more slowly than normal, evidence

TABLE 21

	Dye per 1000 cc. plasma						
	5 min.	1 hr.	6 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Large dose (40 mg. per kg.)							
Dog 28–161	1020	885	700	410	225	147	71
Dog 28–158	850	710	570	325	152	94	42
Dog 28–146	865	745	530	305	165	84	52
Dog 28–107	1150	880	785	440	220	89	45
Av. all dogs	971	805	646	370	190	101	53
Small dose (8 mg. per kg.)							
Dog 28–161	157	150	118	56	26	8	
Dog 28–158	178	157	110	38	18	8	
Dog 28–146	154	133	95	37	21	7	
Dog 28–107	205	181	128	75	44	6	
Av. all dogs	174	155	113	47	27	7	

which might be accepted as proof that the tissues were becoming saturated with dye were it not that we know that liver excretion may greatly alter the amount of dye remaining in the body and hence in the plasma also. Even this factor of liver excretion could be disregarded if it were known that the amount of dye excreted into the bile were always directly proportional to the amount present in the plasma. Recent work in this laboratory shows quite conclusively that this is not the case. Part of this data will be presented in a later paper of this series (6) and part is left for future publication. We can show (6) that a small injection of India ink may have little or no effect on the volume of bile excreted, nor on the daily output

of bile salts and bile pigments, and despite this the liver may be almost completely unable to excrete brilliant vital red for 48 hours or longer. We know little of other conditions which may affect one function of the liver so specifically to the apparent exclusion of certain others. It is hoped that experiments now in progress will shed light on a field so little understood. Our recent studies also show that increasing

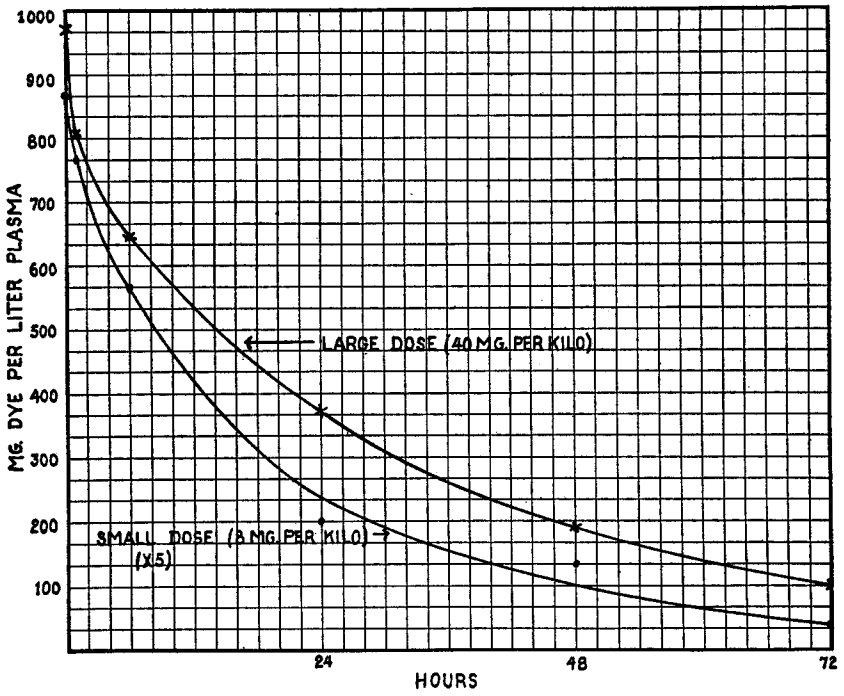

CHART 2C. Varying dose of brilliant vital red (average of four dogs—see Table 21).

the dye concentration in the plasma 5 fold does not increase liver excretion by a like amount. In fact the excretion is little more than doubled under these conditions. From this it must be clear that with large dye doses the dye is retained in the body relatively much more effectively. Such conditions must prevail in vitally stained animals where there is much dye in the plasma as well as in the tissues. The inefficient liver excretion under these conditions must go far to account

for the longer retention in the plasma of test doses injected to study the disappearance rate. In fact we believe that much of the retained dye does pass into the tissues, giving them as well as the plasma a much more intensive coloration.

Experiments like these to compare large and small injections have also been carried out on normal dogs without the bile fistula operation, and in these experiments we have made it a special point to determine the rate at which the dye leaves the blood stream. The results with 4 such dogs are given in Table 21. All gave essentially similar results, the averages of which are shown graphically in Chart 2C. With a large intravenous injection (40 mg. per kilo body weight) we observe that the plasma taken 5 minutes later is deep red in color. It contains nearly 1000 mg. dye per liter. Twenty-four hours later there are still nearly 400 mg. per liter and at the end of 72 hours about 100 mg. still remain in circulation. This curve is to be contrasted with one with a small dye injection made a number of weeks previously into these same dogs. At that time only 8 mg. per kilo had been injected. This dosage is only one-fifth that given in the later experiment, and in order to simplify comparison of the curves we have multiplied the values observed by 5, and in Chart 2C we have plotted the results alongside the curve resulting from the large injection. The curves thus brought to the same scale are quite similar for the first hour or so, but one observes that after that they tend to separate rather rapidly and at the end of the experiment one curve is almost twice as high as the other. It is noted that the marked lag is shown in the curve resulting from the large injection. It is quite obvious that dye does not leave the circulation five times as rapidly as with a dose one-fifth as large. We feel that the phagocytic activity may not be quite proportional to the dosage of dye and that with large dosage the undue delay in elimination may be due in part to this, but we are convinced that much if not all of the discrepancy between the two curves should be attributed to the fact that the liver does not excrete the large dose five times as rapidly as the smaller one.

DISCUSSION

The experiments just presented have shown that following multiple daily injections or, indeed, after a single large injection, much of the

dye remains in circulation for many days. The rapid elimination during the first hours stands quite in contrast to the slower elimination later on. After several days the amount in the plasma remains almost constant. A state has been reached such that the tissues take up little dye unless the concentration in the plasma is artificially raised by further dye injection. We feel that this relative inactivity of the tissues must be taken to mean that an approximate equilibrium has been established between dye in plasma and dye in the tissues. We know that this inactivity is not due to a lowering of the plasma dye concentration below a hypothetical functional threshold value, for we have shown that with small doses the normal unstained tissues will attack such amounts of dye with great vigor. The concept of an equilibrium between dye in plasma and dye in tissues is also borne out by work still in progress which goes to show that by rapid bleeding and transfusion of vitally stained dogs one can reduce the dye concentration in the circulating plasma to a very low level, and in such cases we have observed that dye passes back from tissues to blood stream, and for a number of hours the dye concentration in the blood stream increases. We can thus demonstrate the essential features of an equilibrium reaction, namely that the progress is reversible and the tendency is to resist or compensate for a displacement produced in either direction.

Quantitative concepts of dye partition between cells and the fluids which surround them received scant attention in the older literature. So much of the work concerns the permeability of cell membranes and here the problem of equilibrium is neglected. Other workers have been more concerned with the power of cytoplasm to dissolve dyes or to unite with them in one manner or another. Certain of these workers have had the equilibrium concept clearly in mind; others have been less specific, though in certain cases the concept seems to be implied. Attempts to compile quantitative data are almost completely lacking until very recently. Within the past few years Irwin (8) has made notable contributions to the study of the distribution of certain basic dyes between plant cells and the fluids in which they are immersed. Many factors were found which influence this distribution, but she is convinced that with constant conditions the amount of dye within the cell is proportional to the concentration of dye in the sur-

rounding fluid. Collander (9) has also made studies on plants, but with acid dyes. He gives much thought to the question of permeability, and the equilibrium concept is much less fully treated than in the work of Irwin. These quantitative studies of Collander and of Irwin involve plant cells only. The diffuse coloration of the sap of plant cells may well be comparable to the diffuse staining sometimes seen in animal cells, but it is difficult to relate this process to the granular storage in macrophages of certain acid dyes such as brilliant vital red. The brilliant and deeply colored granules which form within the macrophages are almost certainly not formed from simple staining of preexisting protoplasmic granules within the cell, though we must admit that the dye may exist in union with substances not previously segregated into discrete foci. Schulemann (10) and Evans and Scott (11) have stressed the view that the behavior of this group of dyestuffs in the body may be closely akin to the process of phagocytosis of small bits of particulate matter so familiar to all. There is the added feature that during the process of granule formation the cells must take the dye from solution and build it up into small concentrated microscopic aggregates. We wish to stress our observation that even in this type of staining, so closely related to phagocytosis, we can demonstrate the existence of an equilibrium between the dye in cells and the dye in the surrounding fluids. It is perhaps less surprising that such a principle holds where the dye seems more clearly to enter into union with preexisting materials within the cells. We should be more surprised to find it in the case of acid dyes where the granules are built up *de novo* within the cells by a process of concentration and storage far more elaborate in character.

We feel that studies concerning equilibria should be extended to other types of cells and to other dyes. Such studies should illuminate the questions regarding whether in various cases the dye within the cell is merely dissolved in fluids and lipoids of the cell or whether it exists in the form of chemical or physico-chemical combination with elements present in the cell. Very possibly no single rule holds for all dyestuffs and for all cells.

SUMMARY

Brilliant vital red injected into the blood stream of dogs is slowly taken up by phagocytes in various parts of the body, but eventually

an equilibrium is established, after which the concentration as measured in the plasma remains almost constant for long intervals of time.

This equilibrium can be disturbed by injecting more dye, and in this case the phagocytes resume ingestive activity, apparently with normal or nearly normal vigor. This activity continued until a rather large part of the newly injected dye has been removed, and as the reaction again slows up we note that both plasma and tissues contain more dye than before. It is difficult to be certain that the distribution ratio of dye between plasma and tissues remains unaltered with dosage, but evidence indicates that for non toxic doses, at least, this is approximately true.

This study of this partition ratio is complicated by the fact that the liver slowly excretes dye into the bile, and this helps to reduce the amount of dye in the body. Partial correction for this factor can be made by ascertaining the dye output in bile fistula dogs. These latter studies show that dye elimination into bile is *relatively less efficient* when large doses of dye are given to the animal than with smaller dosage. This undue retention of dye in the body with large dosage helps to maintain the dye concentration in the plasma at unduly high levels. These peculiarities in liver excretion have an important bearing on liver physiology in general, and in addition they also have an important application in connection with the theory of "blockade of the reticulo-endothelial system." It is now obvious that prolonged retention of dye in the blood stream does not of itself prove that this group of phagocytic cells is "blocked" against the entrance of foreign material. Altered excretion by liver, kidney, etc. must be ruled out before we can accept such data as evidence of "blockade."

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