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THE EFFECTS OF HEMORRHAGE ON BODY COMPOSITION*

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THE aqueous phase of body composition — the total body water and its solutes — includes as its prime component the great working mass of body cells, participating in energy exchange and substrate oxidation for work performance. The body-cell mass is surrounded, infiltrated and bathed by a second watery component, slightly smaller in volume, and concerned with the bulk movement of oxygen, carbon dioxide, metabolites and regulators, via the circulation: the extracellular fluid. Whatever other differences exist between these two reservoirs of body water, none is more clear cut than the fact that within cell water the predominant cation is potassium (scarcely contaminated by sodium) at an approximate concentration of 150 milliequiv. per liter, while in the water of the extracellular phase, potassium is present in very small quantities and the predominant cation is sodium, at an approximate concentration of 150 milliequiv. per liter. Despite this distinction in the distribution of these two similar alkaline cations on the two sides of the cell membrane, total solute concentration is identical in both phases; there is no resting gradient for water distribution despite the large amount of energy expended to maintain this cationic distinction.[1] In the steady state, partition within body water, as between cells and extracellular fluid, depends upon genetic determinants of body build, energy requirements and sex-linked regulators.

As a third component, around and within body water is stored a large nonaqueous or anhydrous component consisting of neutral triglycerides in a sparse matrix of fat-cell protoplasm: the body fat. The protoplasmic matrix of fat is counted as a part of the body-cell mass, and shows no fundamental difference from other cells, as regards chemical composition. The weight of neutral triglycerides in the body is likewise determined by genetic inheritance of degrees of obesity, intake-expenditure balance of calories and sex-linked factors.

The sex-linked determinants of body composition become evident in the remarkable change in body-water partition that occurs at puberty, the female gaining weight in lipid adiposity after that time, while the male continues for several years to gain weight in terms of potassium-rich muscular tissues. These are largely skeletal muscles whose relatively greater development is a secondary sex characteristic related to androgen dominance. This leaves the male as an adult, with a body potassium content about 15 per cent higher (approximately 450 milliequiv.) than his female counterpart.[2]

Standing upright within this structure of cells, fluid and fat is a fourth distinct component of body composition, a special tissue that is heavy, dry, rigid and predominantly extracellular: the skeleton. Its size is also determined by genetic and sex-linked components. As would be expected with any extracellular tissue, energy balance is of far less significance in determining its magnitude.

Of particular interest and importance within the aqueous phase is a small but special component: the blood volume. This carries within it two representatives, each being a circulating manifestation of one phase of body water. The red-cell volume is a special division of the body-cell mass, rich in potassium, requiring energy locally for maintenance of membrane integrity and cation distribution, and bearing a fixed volume-relation to the body-cell mass to which it brings oxygen and from which it removes carbon dioxide. The plasma volume is a special division of the extracellular fluid, rich in sodium, its absolute size regulated by renal-endocrine activity and fluid availability, and bearing a fixed relation to the size of the total extracellular fluid.

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A hemorrhage distorts this steady state of body composition: a circulating segment of body composition is suddenly removed. Autoregulation of body composition is challenged so that its activities become more clearly evident. A multifaceted response ensues, whose understanding is essential to treatment.

The studies reviewed here have been carried on over a period of twelve years, and have involved observations of the response to venous blood loss induced in 65 normal human volunteer subjects. A few studies in patients with stabilized anemia have also been included. The magnitudes of the venesections have varied from that of an ordinary blood-bank donation (about 500 ml., or 10 per cent of the blood volume) on up to about 1500 ml., or about 30 per cent of the blood volume. In this work I am deeply indebted to the help of a group of young men and women who have studied in our laboratory during this period, and I should particularly like to mention the assistance of Dr. John Lyons, Dr. Fuad Dagher, Dr. John Skillman, Dr. John Olson, Dr. Julius Lister, Dr. Ian McNeill, Dr. William Walker, Dr. Louis Plzak, Mrs. Caryl Magnus Boyden and Miss Margaret Ball.

Most of these data are based on observations in man, but in some cases (such as those involving catheterization of the adrenal vein), we have of necessity moved to the laboratory animal, maintaining an awareness of important species differences. We have drawn freely on the scientific work of other laboratories interested in the same problem, to whom we acknowledge our indebtedness.

The methods used here will not be reviewed in detail since the study of body composition has recently been described in monograph form.[2] The blood chemical values were measured by standard methods. Basic to all these studies of volume regulation has been the remarkable accuracy and reproducibility of the radiochromate method for the measurement of the red-cell volume, as originally described by Sterling and Gray[3] and later modified by Read.[4] The constancy and holistic integrity of the red-cell volume itself, and of this method for its measurement (even after hemorrhage, transfusion, infusion or dehydration), provide the basis for sequential calculation of plasma-volume changes on a continuous basis as the reciprocal of the large-vessel hematocrit; these derived expressions have held up under the scrutiny of repeated direct plasma-volume measurement; sequential correction for the starting whole-body hematocrit ratio is a necessary refinement.[5] Measurements of the plasma volume itself have been based on sequential determinations in multiplicate of several points on the tracer disappearance slope and on repeated new injections of either blue dye or iodinated albumin. Measurements of the extracellular volume have been done with radiobromide, employing the single-exponential decay slope between sixty and a hundred and twenty minutes, reverse-extrapolated to zero time. This yields a measurement of extracellular water volume that is constant and reproducible, undergoes easily measured fluctuations in disease, returns readily to steady state normalcy and is reproducible on multiple sequential measurements in the same individual.

Resting Phase-Volume Relations

Based on the gross reference of body weight or size, blood volume shows the same variability seen in body water as a whole. The two vary together as reciprocals of the body fat, as do most other measurements of aqueous-phase parameters. Several clinical methods are available to estimate normal values despite this inherent variability. For bedside use the simplest corrector is an estimate of the degree of obesity, permitting one to arrive at useful predictions for blood volume. A recent review compares a number of methods for these predictions, as well as the result of several methods of blood-volume measurement.[6]

Of greater significance for body compositional study is the relation of the blood volume or its two major components to the aqueous phase as a whole, the variable of obesity having been removed thereby; total body water is now the reference standard. With body composition thus stripped down to its nonlipid components, the range of normal blood volumes is greatly reduced. For example, the plasma volume varies as a function of body weight in males according to the regression $PV = 927 + 31.5$ B.Wt.,* and for females according to the regression $PV = 849 + 25.9$ B.Wt.; the correlation coefficients for these two regressions are 0.61 and 0.65 respectively, and the sex difference (both in slopes and in means) is statistically significant at a p value less than 0.001. Expressed as a function of body water, the plasma volume shows a regression relation indicated by $PV = 640 + 63.4$ TBW; the correlation coefficient has now risen to 0.77, the correlation itself is statistically significant at a probability value less than 0.01, and there is no statistically significant sex difference whatsoever. As corollary data, the regression of red-cell volume on total body water is given by $RV = 53.0$ TBW $- 158$. Here the correlation coefficient is 0.88, again with no statistically significant sex difference. For blood volume the analogous equation is $BV = 647 + 111.8$ TBW, with a correlation coefficient at 0.84, a highly statistically significant correlation and no sex difference.

Having removed the variable of obesity, we find that three basic relations are of outstanding importance in expressing the blood volume as a component of the body composition it represents and through which it circulates.

*In these equations, volumes are in milliliters, body weight (B.Wt.) in kilograms, total body water (TBW) and extracellular water (ECW) in liters and total exchangeable potassium (K_e) in milliequivalents.

First is the fact that the red-cell mass bears a recognizable relation to the mass of body cells to which it supplies oxygen. Several workers have sought to express this relation in a variety of ways. For example, there is a highly significant regression relation between the red-cell mass and total body water, indicated in the foregoing paragraph. Yet, because total body water has a fixed partition as between cells and extracellular fluid in the normal person, the overall size relation between the red-cell volume and the total body water may mask a more significant underlying relation between the red-cell mass and the body-cell mass itself. Muldowney and his co-workers[7] sought to express this as a regression of the red-cell volume on intracellular water, the latter derived merely as total body water minus an extracellular-phase measurement. They termed the latter entity the "lean body mass," a semantic confusion remaining from the densitometric methods of body composition developed by Behnke and his group[8] at the Navy diving tank in Bethesda. Unfortunately, the term "lean body mass" includes everything in the body that is not fat, and therefore includes the heavy nonaqueous skeleton, as well as tendon, fascia, collagen and elastin, none of which are energy requiring. As a reference for body cells, total exchangeable potassium is far preferable. The wet weight of normal body cells is related to the total amount of potassium in the intracellular phase because the concentration of potassium in body cells is held remarkably constant in a wide variety of tissues and through a wide variety of diseases. Using the total exchangeable potassium as a basis of expression to signify the body-cell mass, there is a very close relation for both the red-cell volume and the blood volume, expressed by the following two regressions: $RV = 189 + 0.51\ K_e$; and $BV = 1451 + 1.1\ K_e$. The correlation coefficient for these two regressions is 0.86 and 0.80 respectively.

Second is the relation of the plasma volume to extracellular fluid. This can be expressed as regression for plasma volume on total extracellular water, as $PV = 78 + 173.2$ ECW. This likewise shows a high correlation coefficient at 0.83. A further expression for the relation of plasma volume to the extracellular fluid is expressed as the PV:IF ratio. This is the ratio of the volume in the plasma to the volume of fluid in the interstitial phase,* the latter being the total extracellular fluid minus the plasma volume. With any of the standard plasma-volume methods, together with the radiobromide technic for extracellular-water volume, the PV:IF ratio in the normal adult male is 0.230.

Third is the sex-linked component, reflecting in the blood volume the fact that adult males have a larger body-cell mass and a smaller extracellular-fluid volume than females. Males, therefore, have a larger total blood volume and a higher cell:plasma ratio, with a distinctly elevated red-cell volume, as compared with the female of the same weight. As mentioned above, there is no sex difference when body water is used as a reference standard.

Acute blood loss produces a distortion of these fundamental relations of body composition. The autoregulatory responses evoked by hemorrhage reveal some of the cybernetic mechanisms that maintain a normal volume of blood under a variety of external challenges.

Plasma-Volume Refilling

Size of Bleed

The events described here occur after venous bleeds, over a period of twenty to forty minutes, of 10 to 20 per cent of blood volume. These have been carried out in unanesthetized normal subjects lying supine, conscious and co-operative on a bed-type scale. The subjects have been aware of the purposes of the experiment, have entered into the work of their own free will and in many cases have been scientific collaborators. The subjective symptoms of this bleed have usually been minor. In 1 subject with a bleed of 22 per cent of blood volume there was a typical bradycardia, with syncope and a transient asystole, but without changes in the configuration of the QRS complexes. As more discriminating methods for hemodynamic study are used, delicate hemodynamic adjustments become evident that escape observation when clinical monitoring of vital signs is the only criterion.

Rates and Volumes of Plasma-Volume Refill

The curve of plasma-volume refill is a slope of decreasing magnitude convex upward. The initial rates of refill, during the first two hours, range as high as 90 to 120 ml. per hour (1.5 to 2.0 ml. per minute). The fastest maintained rates are those during the first six to ten hours, and during this period, the average is 40 to 60 ml. per hour, or 0.6 to 1.0 ml. per minute. After this the rate of refill rapidly decreases, reaching a stable value for the new plasma volume evident at thirty to forty hours. The average rate for the whole period is a figure of little significance since it depends on the total volume to be achieved and is an overall mean for a constantly changing rate; a typical figure is 33 ml. per hour.

Of greatest interest is the fact that the plasma-volume refill achieves a volume that almost exactly equals the total volume of shed blood, and then ceases. The amount of new plasma entering the circulation is thus equal to the *sum of the volume of plasma and erythrocytes withdrawn.* These rates and volumes of refill have been consistent in human subjects of both sexes, with bleeds of this size. To-

*The interstitial phase of extracellular water is the fluid lying outside the plasma volume but not within cells. The term as used in body composition is not strictly synonymous with "interstitial fluid" as employed by physiologists to mean merely the small coating of water outside the capillary but not in the lymph.

tal volume has been restored at the expense of erythrocyte concentration, and it is the reciprocal change in the cell:plasma ratio that permits the point-to-point calculation of plasma-volume refill.

Red-Cell Volume

Once the bleed has occurred, the changes in red-cell volume are so slow as to be negligible during the thirty to forty hours involved with refill. Measurements of initial and final red-cell volume as corrected for measured withdrawals check out perfectly.

Red-cell synthesis starts immediately, as shown by an increase in reticulocytes, but the average resynthesis rate is very slow, being approximately 15 to 50 ml. per day.

Variations in cell size would interfere with the sequential measurement of plasma volume by reciprocal of the hematocrit; this has not been evident on repeated separate plasma-volume measurements, or observations of the relation between the hematocrit and hemoglobin concentrations. Red-cell size appears to remain constant, probably because total osmolality is unchanged.

Ions and Protein

No significant changes are evident in concentration of sodium, chloride or bicarbonate after hemorrhage of this magnitude. Although total solute (or osmolality, as indicative of total solute strength) might not be expected to be changed after a bleed, it is widely accepted that the plasma-volume refill results in a fall in total protein concentration (or colloid osmotic pressure). This is thought to result particularly from dilution of albumin, this protein moiety being responsible for about 70 per cent of the oncotic pressure of plasma. It therefore comes as a surprise to find that, in man, depending to some extent on oral or intravenous fluid intake, there is only an extremely transient or slight hypoalbuminemia after hemorrhage, and in many situations even this is not evident. The homeostatic limits within which iso-oncotic relations can be maintained after blood loss are unknown; in this range of bleed at 10 to 20 per cent of the blood volume, at least one refill period seems to be accomplished without loss of colloid osmotic pressure.

It is thus evident that albumin not previously in the plasma volume is now appearing within it. Significant weights of albumin can now be shown to be present in the short-term volume distribution of albumin tracers that were not there before the bleed. The amounts involved vary, of course, with the size of the bleed, but bleeds of this magnitude summon 20 to 50 gm. of albumin. There is little evidence that the liver can synthesize this much albumin in such a short time. This albumin increment, newly appearing in the circulation during the first four to six hours, therefore, is interpreted as being due largely to the ingress of preformed albumin into the plasma volume, from some site outside the plasma volume. It is also possible that hemorrhage constitutes an endocrine stimulus to increased rate of albumin synthesis in the liver over the remaining thirty-six hours of plasma-volume refill. This would yield a two-phase system for albumin reconstitution of plasma oncotic pressure: first the movement of preformed albumin from the hepatosplanchnic area into the circulation, possibly by way of the thoracic duct[9]; and a second phase dominated by the synthesis of new albumin in the liver.

The period of refill is thus characterized for almost its entire duration by a normal plasma albumin concentration. This is not true of the globulin components. If the individual is given water by mouth starting at eight hours after his bleed the final globulin concentration is significantly lower than normal by the termination of the refill period. This suggests that the production of globulin cannot keep up with loss or that its partition between intravascular and extravascular sites is modified after hemorrhage. That the latter is the case is indicated by the fact that, with the subject fasting and thirsting throughout the entire refill period, globulin concentrations are significantly higher than normal by the end of refill. The concentration increment for other solutes is not proportionately altered, suggesting that this hyperglobulinemia is not a simple dehydration effect.

Bulk rates for movement of water, salt and protein across the capillary are much slower than the normal resting exchange rates calculated from the dynamic exchange of body constituents by diffusion across the capillary, as measured by isotopic technics in the steady state. The early isotopic measurements of Flexner, Gellhorn and Merrell[10-12] suggested that the first steep component of the deuterium-disappearance slope was a function of capillary permeability. This interpretation was cast into doubt by the subsequent work of Edelman,[13] who showed that the two predominant rates concerned in the early distribution slope of deuterium were due to its penetration into two different areas of body cells termed "fast" and "slow," as regards their permeability to water. In neither slope was the capillary rate discernible, since it is so very rapid. These data only served to accentuate the extreme rapidity with which water and ions pass the capillary under conditions of normal steady-state kinetics. The volumes concerned in diffusion and kinetic exchange must approximate that of the total plasma water in each pass through the capillaries, or in each minute. The rates of bulk filtration, reabsorption and lymph flow documented by Pappenheimer[14] and recently reviewed by Landis and Pappenheimer[15] are slower, but still very impressive, approximating 8000 ml. per twenty-four hours.

These rates of diffusion, filtration and reabsorption have little evident bearing on the rate of bulk movement of water, salt and protein after hemorrhage, except that they involve the same anatomic sites in the capillary. Renkin[16] and Pappenheimer[14] postulate two sorts of transcapillary passage for two ranges of molecular size. One, involving the main wall of the capillary itself, is concerned with water and small molecular-weight solutes and is a process of porous sieving. The other, involving a more distal site in the capillary or postcapillary venule, is a process of passage across the capillary wall by vesicle formation, pinocytosis or cytopempsis. This mechanism is considered as more active, possibly energy requiring, and of importance for molecular species having a weight over 10,000. It is thus conceivable that net flow for water and small species might be in one direction while albumin transport was in another; as described below, this is indeed the case under certain circumstances after hemorrhage in man.

Although the flow of water and salt (and possibly protein) across the capillary is not considered an energy-requiring reaction the system cannot be thought of as being devoid of energy, since the impulse of cardiac output and the maintenance of normal microcirculation are essential for normal refill.

Participation of Cell Water

This volume-regulatory response of transcapillary refill represents a transaction between the plasma volume and the interstitial fluid. Several pathways and anatomic sites may be involved, as already mentioned, including the liver or gut lymph, thoracic-duct lymph and peripheral capillary exchange.

It has long been suspected that cell water does not remain a passive spectator, viewing this traffic among its neighbors. Evidence for loss of potassium after hemorrhage has been observed by several students of the subject,[17] and from this loss of potassium, and in the absence of any observable concentration change, it has been deduced that cell water participates in this adjustment.[18] To study this, it was essential to view sequential changes in equilibrated radiobromide decay slopes, in the absence of renal function. A series of studies was therefore carried out in the dog with ureters ligated, employing the equilibrated radiobromide slope to search for evidence of dilution as new cell water was mobilized after isotonic volume reduction.[19-21] Unexpectedly, the results in the dog showed that a single small hemorrhage, massive multiple hemorrhages, severe hypotension and multiple trauma, with or without cortisone, were all ineffective in mobilizing cell water. Only under the circumstance of the administration of aldosterone to the adrenalectomized dog was it possible to demonstrate the withdrawal of water from cells. The dog experiments had many limitations as compared with those in man, particularly the fact that we were only observing changes in the first four to eight hours. Over the long term, catabolic responses produced by trauma and hemorrhage unquestionably mobilize cellular salt and protein, not so much as a part of the response to hemorrhage, as an adjustment to the injury as a whole, occupying several days or weeks.

Transferring these data from dog to man, there are no exactly comparable experiments. Nonetheless, the evidences are strongly in favor of the view that in man, likewise, hemorrhages of this size do not evoke any net movement of water from body cells to extracellular fluid. None of the characteristically extracellular ions are diluted during the plasma-volume refill. The amount of "new" potassium appearing in the extracellular phase or urine after hemorrhage is very small (10 to 20 milliequiv.) and can be calculated as being equivalent to about 60 to 150 ml. of cell water. This is no more than one would expect in a fast of this duration. The changes in albumin and globulin may reflect some alteration in the distribution of protein as between cells and extracellular fluid; mobilization of liver glycogen in response to the accompanying fast is likewise a transaction between cells and extracellular fluid; as evidence now stands, the alteration in body-cell mass is no greater than that accounted for by the movement of these solutes themselves, and the interstitial phase remains depleted by its losses into plasma volume.

Return to the Steady State

Thus, at the close of the period of transcapillary refill, with plasma volume returned to a figure above normal and blood volume normal, body compositional relations reveal a considerable increase in the PV:IF ratio from the normal resting value of 0.230 to values as high as 0.350: the plasma volume has been refilled at the expense of interstitial fluid, and there is a downward distortion of the relations of red-cell to body-cell mass. If water and salt are provided by mouth in liberal quantities the interstitial volume reduction is short lived.

The events of the next few days are devoted to a restoration of interstitial-fluid volume to normal by ingestion and retention of water and salt, and a gradual restoration of PV:IF ratio to the vicinity of 0.230; the events of the next six weeks are devoted to a return of the red-cell mass to normal by red-cell resynthesis; as this occurs, there is a *pari passu* dispersal and disposal of plasma-volume components so that at the end of the entire period, the normal cell:plasma ratio is restored, and that of the blood volume to the rest of body composition returns to normal.

Evidences of Endocrine Participation

Adrenal Cortex

Attention was turned first to the measurement of cortisol and its metabolites in the urine; no changes

were seen with hemorrhages of this type, size and rate in normal adult subjects. The same was true for the urinary excretion of 17-ketosteroids. As methods for the measurement of free and conjugated cortisol in the plasma became available, they were applied to this problem, and again only minor changes were seen with hemorrhages of 500 to 750 ml. carried out in the supine position, and from a venous source. The threshold hemorrhage, above which increases in plasma concentration of glucocorticoids are regularly seen in man, is still unknown, and considering the many other endocrine effects of hemorrhage, it is of some interest that here is a "stress" in which the glucocorticoids usually remain unmoved.[18,22]

It became evident, in the work of our laboratory as early as 1954, that such a hemorrhage as this produced an immediate and drastic effect on the sodium:potassium ratio in the urine. This reduction in ratio resulted almost completely from a reduction in sodium excretion, the increase in potassium excretion over the short term being negligible. The initial reduction in urinary sodium:potassium ratio was often evident before the completion of the hemorrhage, and excretion of sodium usually failed to return to normal until the period of plasma-volume refilling was complete; in several balance studies carried out at about that time, it was noted that in some persons a strongly positive sodium balance lasted as long as four days after a hemorrhage of this magnitude.

With the description of aldosterone as the electrolyte-active component of the adrenocortical secretion, it became evident that this alteration in urinary excretion of cation was probably traceable to alterations in aldosterone concentration in body fluids. The work of Bartter[23-25] soon showed this to be true. He demonstrated that isotonic volume reduction produced by phlebotomy was one of the most potent stimuli to an increase in the excretion of aldosterone in the urine. These data, based on hemorrhage in normal man, and using bioassay for the aldosterone determinations, have been of key importance in the development of current concepts of volume regulation after hemorrhage. Several other workers have devoted an effort to determine which areas of the body are most sensitive to this reduced volume, postulating some difference between volume-sensing mechanisms and strictly pressure-sensing or baroreceptor mechanisms. Areas in the brain, the peripheral circulation and the liver have been postulated. The most consistent data, however, have pointed to the right side of the heart and the kidney. Farrell and his co-workers[26-28] have shown that the muscle of the right atrium contains stretch receptors responsive to acute volume overload and, by implication, volume reduction. This interesting finding has an aspect of clinical utility, now widely used, through the monitoring of central venous pressure, not only for high-volume overloads but likewise for the discrimination of low-volume states. Recent evidence by Zimmermann et al.[29] suggests that the left atrium may be of particular importance in regulating water metabolism (see below) in contrast to the right atrium, which may be more concerned with salt changes.

Renin

The kidney, itself, also appears to harbor a sensing site. The data of Ganong and his co-workers at the University of California[30-32] have demonstrated in the dog that the presence of an intact kidney is more important than the presence of an intact pituitary gland in activating the secretion of aldosterone after isotonic volume reduction by hemorrhage in the dog. This localization of the kidney as another volume-sensitive site has occurred *pari passu* with the demonstration of the endocrine significance of the juxtaglomerular apparatus and the macula densa, its high content of renin, its hyperplasia in renal-artery stenosis and the increased concentrations of blood angiotensinase in renal ischemia.[33] In addition, there is good evidence that angiotensin has a direct effect on the adrenal cortex, stimulating an increased secretion of aldosterone without the necessary intermediacy of any higher centers.[34-37]

The current work of Edelman[38-41] has now carried this activation chain one step farther by showing that aldosterone apparently acts within the nucleus of cells concerned with sodium transport and that its mode of action involves DNA-primed synthesis of RNA; this ribonucleic acid in turn is assumed to control the production or activity of some enzyme concerned with active sodium transport.

Adrenal Medulla

Direct evidence for the involvement of adrenal medullary hormones in hemorrhages of this magnitude is still lacking in man. Technics for such measurements generally depend upon adrenal-vein catheterization since peripheral blood levels change only with very massive stimulation.[42,43] In man, no one has yet reported any extensive studies of catechol amine concentrations in the blood, catechol end products in the urine or secretory rates, using minor hemorrhage as the only stimulus. Turning to the dog, a series of studies carried out in our laboratory and involving direct catheterization of the renal vein merely corroborated what had been suspected for almost fifty years — namely, that an acute isotonic volume reduction is an immediate and potent stimulus to the production of epinephrine from the adrenal medulla, and of norepinephrine, not only from the adrenal medulla but also from neural synapses throughout the body. The magnitude

of this stimulus can be adduced by the fact that the adrenal vein-concentration of these substances increases tenfold to fiftyfold within seconds after the induction of the hemorrhage, and by the fact that this large increase is immediately and promptly returned to normal with the restoration of the shed volume. These hormones have a very short life in the circulation; reduction in their production, therefore, is reflected immmediately by reduction in the blood concentration. There are few endocrine responses more spectacular than the reduction in blood concentration of catechol amines that results from the restoration of blood volume to normal. This occurs without reference to shock or prolonged tissue ischemia with anaerobiosis. The response of the adrenal medulla to a brisk hemorrhage in the dog, amounting to 10 to 30 per cent of blood volume, is an immediate rise; with restoration of the shed blood the medullary activity ceases abruptly.

Antidiuretic Hormone

That the kidney after hemorrhage is not under the influence of aldosterone and catechol amines alone became evident in our own work about 1955, in studying the effect of various types of surgical trauma on renal clearance of water. In these experiments subjects were given a steady slow infusion of 5 per cent dextrose and water, and hemorrhage then begun. By measuring glomerular filtration rate and total urine solute excretion, we could calculate the effect of such stimuli on the ability of the kidney to clear free water (that is, to excrete water in the bladder urine over and above that available from the tonicity of plasma ultrafiltrate).

A hemorrhage of this size immediately abolishes positive free-water clearance and produces a long negative free-water clearance that persists as long as the blood volume remains reduced. The effect of this on the final urine osmolality depends upon water ingestion, water reabsorption in the distal tubule, aldosterone-mediated reabsorption of sodium bicarbonate, the weight of filtered urea and the presence or absence of other solute loads. Glomerular filtration rate is little changed by such bleeds. In untreated hemorrhage the osmolality of bladder urine is generally high, but not uniformly so. Of far greater uniformity is the negative free-water clearance, observed with a variety of urine osmolalities and showing a peak value only with high glomerular filtration rates. If water loading is excessive in the posthemorrhagic period (either by mouth or by vein) a dilution of all serum constituents is thus readily produced, being most notable with regard to the serum sodium concentration or its chemical partner, total osmolality. This hyponatremia results from the ingestion of new exogenous water during a period of negative free-water clearance, is readily mimicked by water loading during vasopressor administration and cannot be interpreted as due to an anomalous ingress of cell water into the circulation or of extracellular sodium into body cells: it is a typical effect of antidiuretic hormone.

Data are now abundant from several laboratories to indicate that these findings, initially interpreted as due to increases in secretion or release of antidiuretic hormone from the hypothalamus and posterior pituitary, were indeed due to that mechanism.[44-51] As bioassay methods for the detection of antidiuretic hormone have become available, their application to the hemorrhage problem has shown that isotonic volume reduction in the form of hemorrhage is one of the most potent stimuli to antidiuretic activity, just as it is to the production of aldosterone, likewise acting, at least in part, via cardiac receptors.

Further detailed study of the effects of hemorrhage of this type on renal and endocrine function remains for the future. Effects on glomerular filtration rate are inconstant; there is every reason to believe that if the supine subject immediately after hemorrhage were to be brought to the standing position, drastic effects on renal and endocrine function would become evident. A fall in the systolic pressure, a change in the peripheral pulse contour and a sharp reduction of glomerular filtration rate would presumably ensue. Studies employing the Valsalva maneuver as an additional circulatory challenge after hemorrhage demonstrate that in the posthemorrhagic state there is marked sensitivity of the circulation to any further decrement in peripheral flow[52] even though blood pressure and cardiac output are normal.

Thus, hemorrhage stimulates a variety of endocrine mechanisms most of which are pressor, and several of which are importantly involved in certain classes of patients suffering from essential hypertension, hypertension with renal-artery stenosis or hyperaldosteronism. The possibility, therefore, arises that some patients develop essential hypertension because of an unusually sensitive volume-regulatory mechanism stimulated by minor blood losses — for example, in the menses.

Erythropoietin

It is of interest that the kidney is involved in one further endocrine response to hemorrhage. This relates to the resynthesis of red cells. Since the initial description by Jacobson and his co-workers[53] of a humoral substance concerned with an increase in erythropoiesis in the bone marrow in anemic animals, there has been controversy over the exact nature and site of production of erythropoietin. Much evidence suggests that a large fraction is produced in the kidney. Evidence from totally nephrectomized patients indicates that in the absence of both kidneys, normal blood volume can be main-

tained; a sluggish reticulocyte response to hemorrhage would be expected. In the hemorrhages of the type considered here, there are early and readily measurable increases in plasma concentration and urine excretion of erythropoietin activity. It is noteworthy that some hypertensive patients and some patients suffering from hyperaldosteronism also have polycythemia.[54] We are indebted to Dr. Louis Plzak[55,56] for these studies on erythropoietin.

Thus, there are two masses of specialized tissue lying on each side of the spine, closely related embryologically and anatomically; both are concerned with the regulation of blood volume, and are stimulated strongly by an isotonic volume reduction such as that seen in venous hemorrhage. These tissues include the zona glomerulosa of the adrenal cortex, the adrenal medulla, the glomerulotubular excretory axis of the kidney, the afferent and efferent arteriolar systems of the glomerulus, the juxtaglomerular apparatus of the kidney and finally some as yet unidentified renal tissue that governs the production of erythropoietin.

Modification of Plasma-Volume Refill — Plasma Dispersal and Disposal

Intentional modification of the normal homeostatic sequence has been limited in our laboratories to only a few of the many products of pharmacy or blood bank that have been offered for the treatment of patients after hemorrhage.

Norepinephrine has been known for some time to force plasma out of the circulation when administered to normal subjects; there is a rise in hematocrit and a fall in plasma volume.[57,58] It is of remarkable interest that norepinephrine shows this same property, though intensified to some extent, when administered to normal volunteer human subjects who have been bled.[5] If it is given as a slow infusion over a four-hour period starting four hours after the bleed, there is a rise in hematocrit that is due solely to the loss of plasma from the circulation; when the infusion of norepinephrine is stopped, there is a very abrupt fall of hematocrit as plasma-volume refilling is suddenly resumed and returns back to its normal and expected curve. The clinical corollaries of this for surgeons or anesthetists employing norepinephrine in patients after blood loss are too obvious to bear further discussion.

Angiotensin is thought to act at a slightly different site in the vascular tree. Studies of angiotensin infusion after hemorrhage[59] demonstrate that angiotensin likewise drives plasma out of the circulation though not in as large a volume as norepinephrine, and also with the difference that plasma begins to re-enter the circulation again even during the latter phases of the angiotensin infusion.

Fasting-thirsting, or conversely the liberal administration of food and fluid by mouth, introduces marked changes in the plasma-volume refill curve and in protein concentration. Early in our experience, when one of these subjects took a large drink of water an hour or so after the hemorrhage and produced a very dramatic fall in his hematocrit, it became evident that the posthemorrhagic state biases the distribution of recently ingested fluid and salt toward the plasma volume, so that new fluid entering the body water favors an increase in the PV:IF ratio to an extent greater than the normal partition of 0.230.

Study of this postulate was carried out in a series of experiments as yet unreported, in which infusions of balanced salt solution were given during the plasma-refill phase.[60] The results were of remarkable interest. They showed that a recent hemorrhage indeed biases the body water distribution of infused balanced salt solution so that almost twice as much of it remains in the plasma volume as would be expected in a normal resting individual.

In such experiments it is also possible to quantify the dispersal rate of infused saline solutions out of the circulation; we have found that with total infusion rates of approximately 8.33 ml. per minute (2000 ml. in four hours), the outward dispersal rate has a mean value of 5.0 ml. per minute., leaving a net plasma increment of about 3.33 ml. per minute. Thus, by saline infusion during the plasma-refill phase, the "tidal current" of net fluid movement across the capillary has been reversed from one of plasma-volume refilling to one of outward dispersal.

One might expect that such a brisk outward dispersal of water and low-molecular-weight crystalloids would inhibit the entry of protein into the circulation. Quite the contrary, calculation of the total circulating albumin before and after the saline infusion demonstrates a clear increase: the net flow of water and salt in one direction was quite consistent with ingress of albumin in the opposite direction. Indeed, the subjects who maintained good hydration by the saline infusion had higher albumin concentrations at the terminus of the experiment than those who were kept fasting and thirsting! The latter, as mentioned previously, had higher globulin concentrations.

The interpretation of such findings is greatly facilitated by the concepts of Pappenheimer and of Renkin, already referred to, that the passage of water, ions and small crystalloids occurs by one set of mechanism in one anatomic area, while the passage of macromolecules by cytopempsis may be energy-requiring, and occurs in a different portion of the capillary, at the postcapillary venule. In the saline-infusion-after-hemorrhage experiment there is such clear evidence of a simultaneous net movement of macromolecules in a direction opposite to that pursued by water and salt that a two-channel theory is quite attractive. The thoracic duct could provide

an additional source of albumin-rich lymph during refill.[9]

It is further of significance that the late phase of plasma-volume refilling (from approximately four to forty hours) occurs without the stimulus of any detectable hemodynamic residual. One might postulate in this connection that the early events are hemodynamic in origin, obeying Starling's Law, that the movement of protein into the circulation occupies a different segment of the circulation from the movement of water and salt, and, finally, that the late phase of plasma-volume refilling is due entirely to the synthesis of new albumin in the liver, with its inevitable osmotic consequences as it enters the circulation. This slow response, returning total plasma albumin to normal, bears all the earmarks of a response to some endocrine stimulus; is "albuminopoietin" to be the next addition to the endocrine family?

Modification of plasma-volume refill by the infusion of high concentrations of low-molecular-weight crystalloids, such as mannitol, is of interest because cell water is now brought into play very clearly. These studies involved the infusion of 500 ml. of 20 per cent mannitol, with an osmolality of 1210 milliosmoles per liter. It would be expected that the infusion of such a concentrated solute at a molecular weight of 180 would move a large volume of water across the capillary into the plasma initially and very transiently. This would hasten the plasma "refill" process. Such a small molecular species itself would then cross into the interstitial phase, and from there again exert its osmotic attraction, withdrawing water from body cells prior to its excretion.

These mannitol experiments[61] demonstrated such a sequence and yielded the approximate rates by which water is brought into the circulation in response to mannitol, the mannitol molecule moves into the interstitial fluid, and cell water is then attracted into both. The maintenance of an osmotic gradient in the plasma is extremely transient, as new water is moved into the circulation very rapidly. Then, within half an hour, sodium dilution begins, and the dilution finally amounts to a volume of water almost exactly equal to that predicted from the osmolality of the infused solution, and leaves little question that cell water is being mobilized. The studies of Lilien et al.[62] indicate that the red-cell volume itself is the source of some of this water, the red cell being shrunken after the administration of hypertonic crystalloids of this type. Calculation demonstrates, however, that most of the water that is brought into the circulation must come from other sources, presumably muscle and viscera. The effect of hypertonic mannnitol in shrinking the brain has been noted by neurosurgeons for many years[63]; presumably, no tissue is immune to this simple osmotic challenge. Analogous to the finding with balanced salt infusions is the fact that the extracellular-fluid increment produced by mannitol (in withdrawing water from body cells) is larger and is maintained longer in the posthemorrhagic state than it is in normal persons. This change probably has little to do with the precise osmotic activity of mannitol, but is traceable instead to the delay in excretion of water and sodium associated with the posthemorrhagic state. Although mannitol infusion produces an increase in total sodium excretion, the water:sodium relation of urine and plasma is such that prolonged infusion of mannitol will inevitably remove more water than sodium from the body. This produces the "anomalous sodium effect" of mannitol: an early brisk *hyponatremia* (due to the withdrawal of cell water), followed by the gradual production of *hypernatremia* as more water than sodium is excreted in the urine.

The administration of whole blood to subjects with a stabilized posthemorrhagic normovolemic anemia (twenty-four hours or more after bleed) produces a series of events that, though seen daily on the hospital wards, is of remarkable interest in body compositional research. The events produced are the exact inverse of plasma-volume refilling after hemorrhage. Here, the patient has first regained a normal blood volume because of the recent successful accomplishment of a plasma-volume refill. Then, he is given a large blood transfusion. This increment of whole blood, carrying the typical "blood-bank hematocrit" of about 37, is added to a blood volume that is normal or slightly high. The cells of the banked blood show some fragility with initial cell destruction, but the remaining cells live out their normal life-span; the plasma components are isotonic, there is a slightly elevated plasma potassium in the banked blood, and a notable excess of unbound citrate.

Upon receiving this material, the patient exhibits the sudden cutoff of any residual renal salt-and-water-conserving activity initiated by the recent hemorrhage; there is a diuresis of sodium and water. Although we have not measured the effects of this on free-water clearance, the characteristic changes in urine tonicity suggest that there is likewise a "cutoff" of antidiuretic effect. Given the hematocrit of the infused blood and that of the patient, as well as the volume of both, one can predict the "passive increment" in hematocrit that the recipient will exhibit upon receiving this load of more concentrated erythrocytes. This passive increment is observed at the close of transfusion. Over the next twelve to twenty hours (usually at night in hospitalized patients) the hematocrit continues to "coast" upward, and the patient's body weight (which has shown an increase equal to the weight of the infused blood) recedes down again as the outward dispersal of water and salt from the infused plasma into the extracellular fluid, and its subsequent disposal via the urine, grad-

ually return volume relations and total body water to normal.

McFarlane[64] and Beeken et al.[65] have studied the metabolism of albumin and have shown that its degradation is a slower process than these dispersal and disposal rates for water and salt. For this reason a concentration-increment of albumin is likewise recorded after transfusion, though it is transient. The red cells that are healthy and undestroyed, alone of the constituents of the infused blood, remain in the bloodstream and demonstrate, by their subsequent gradual concentration-increment, the loss of water, salt and protein components of the infused blood.

This "plasma dispersal and disposal" is an essential feature of the metabolism of transfused whole blood; it requires normal cardiac output and circulation, renal function and probably hepatic function for its accomplishment. When these organs are impaired, dangerous hypervolemia is persistent after blood transfusion in stabilized anemia. With a sharp increase in volume, the normal rates of plasma dispersal and disposal far exceed those of its physiologic counterpart, the transcapillary refill; outward flow of water and salt may achieve rates as high as 7.5 ml. per minute, and degradation of albumin may reach 1.0 gm. per hour.

Summary and Conclusions

Bodily changes in man, after venous hemorrhage of 500 to 1000 ml., are reviewed. Understanding and interpretation of these changes have been enlightened by recent data on the endocrine and visceral responses to isotonic volume-reduction.

Nonshock-producing venous hemorrhage is a sharp challenge to the autoregulation of body composition. It is followed by the net movement of water, salt and protein into the plasma. This plasma-volume refill, in man, occupies a period of twenty to forty hours. Its rate curve is one of constantly decreasing magnitude. Initial rates as high as 2.0 ml. per minute are observed. The final volume of refill accurately restores the blood volume to normal, the volume of new plasma equaling the sum of erythrocytes and plasma withdrawn. With completion of refill these events cease and a steady state is resumed; circulatory adequacy has been restored though body compositional ratios remain distorted.

During the period of refill there are readily demonstrable alterations in the renal handling of sodium and water, and in association with these visceral changes, an increase in the urinary excretion of aldosterone and blood level of antidiuretic hormone. There is an early increase in erythropoietin in both plasma and urine.

The initial distortion of body composition may be characterized as a lowering of the PV:IF ratio below its norm of 0.23; this gradually increases during refill until at completion the value is elevated, upwards of 0.35, depending upon the volume of the blood lost. Interstitial fluid is then restored to normal by the injection or ingestion of water and salt; with the completion of this process, volume-conserving activity appears to cease.

New albumin enters the circulation in large amounts; the most rapid rate of entry is during the first four hours, when it may achieve rates of 4.0 gm. per hour. Any dilutional hypoalbuminemia is therefore transient, and in some cases not observed at all.

Under circumstances of salt infusion during the refill phase, one can demonstrate a continued inward current of albumin despite a brisk dispersal of water and ions outward from the plasma volume. This finding favors the view that anatomic sites for the passage of water with small molecules, as opposed to macromolecules, are distinct in the capillary or postcapillary venule. The time-sequence of the appearance of new albumin in the circulation suggests that the early rapid phase involves the appearance of preformed albumin from extravascular sites. The subsequent slow ingress of new albumin at a much slower rate suggests the appearance of albumin newly synthesized in the liver.

The posthemorrhagic state modifies the pharmacology and metabolism of infused substances; studies on norepinephrine, angiotensin, mannitol, balanced salt and whole blood are reviewed.

Of notable importance in the autoregulation of the blood volume are the responses of two specialized masses of tissue lying on both sides of the spine and anatomically located in the adrenal glands and kidneys. These consist particularly of the zona glomerulosa and the medulla of the adrenal glands and in the kidneys, the glomerulotubular excretory apparatus, the renal arterial supply, the juxtaglomerular apparatus and the macula densa, and an unidentified cellular component responsible for the production of erythropoietin.

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