THE EFFECTS UPON THE BLOOD OF THE TUFNELL METHOD AND THE CALCIUM SALTS IN THE TREATMENT OF AORTIC ANEURISM.

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The following studies constitute a partial report of investigations which have been undertaken with the hope of determining the exact effects upon the blood of the current treatments of aortic aneurism. Drs. Pepper and Tyson placed at my disposal three such cases, one of whom died of rupture before the studies were completed, so that the report comprises but two cases.

The capillary and venous blood of the patients was first studied before any treatment was instituted, the natural volume of their urine noted, and the renal elimination of calcium accurately determined. The patients were then placed under treatment and the same determinations again carried out. In each case two sets of determinations were carried through both before and during treatment, with the exception of the estimations of urinary calcium and of the coagulation time of the capillary blood, which were performed every three days while the investigation was in progress.

The treatment consisted of rest, a dry diet, restriction of liquids, regular venesections (every 18 days), and the internal administration of potassium iodide grm. i and calcium chloride grm. ii daily. The use of a dry scant diet, restriction of liquids, and venesections are old therapeutic ideas, employed especially by Valsalva and Albertini; it is, however, due to the advocacy of Tufnell that they have held sway in modern practice. These measures have been held to reduce the volume of the circulating blood and to increase its coagulability. It cannot be believed today that therapeutic venesection more than momentarily diminishes the volume of the circulating blood, so

quickly is the amount abstracted replaced by fluids from the tissues; in fact it is not improbable that the lymph current may more than replace the volume withdrawn by the bleeding. For the rest there is more definite evidence. Withdrawal of all drink or profound catharsis soon leads to an oligohydræmia. I have, however, been able to find no studies tending to show that the degree of limitation of fluids practised in cases of aneurism has actually caused such an inspissation of the blood. Tufnell's own regimen did not include over 375 cc. of liquid in the food and drink, which under ordinary circumstances would not furnish over 300 cc. of urine. Such a regimen is extremely distressing. In the cases here reported it was not possible to reduce the patient's liquids below the urinary equivalent of 400 to 500 cc. per diem, and even that was very irksome. Whether or not a reduction of the water in the circulating blood renders it more coagulable is entirely an open question; I know of no experimental work tending to show that simple oligohydræmia *per se* is accompanied by an increase in the coagulable properties of the blood. Potassium iodide was administered for the relief of pain, without expectations of any action upon the blood. The employment of calcium salts to heighten the coagulating properties of the blood, so warmly advocated by Wright, is based upon the now generally admitted fact that calcium bears in some way an integral relation to the molecule of fibrin.

The methods employed by me were as follows: The calcium in the urine was estimated by weighing as calcium oxide after precipitation as calcium oxalate, according to established procedures. The specific gravity of the blood was determined by accurate weighing in a picnometer of a capacity of about 12 grm. of water. The calcium in the blood was estimated by evaporating about 75 cc. of blood to dryness; the residue was then incinerated, the ash dissolved in hydrochloric acid, diluted and neutralized; the phosphates were then removed by the addition under heat of ferric chloride in the presence of acetic acid and sodium acetate; the calcium in the filtrate was then precipitated as the oxalate, reduced to the oxide by heat and weighed.

The volume of plasma and corpuscles was determined by the Bleibtreu method. In this method two or more portions of blood are mixed in

different known proportions with an isotonic * saline solution, the cells allowed to sedimentate, and the nitrogen of the clear plasma in the different mixtures estimated by the Kjeldahl method or the specific gravity determined. Since the respective quantities of blood and saline solution are known, and since the dilution occurs (theoretically) in the plasma alone, the volume of the plasma, and from it that of the corpuscles, is calculated according to the following formula:

$$N^2 \frac{s^2}{b^2} - N^1 \frac{s^1}{b^1} = (N^1 - N^2)\, x.$$

N^1 = nitrogen of mixt. 1. N^2 = nitrogen of mixt. 2. b^1 and b^2 = volumes of blood in the dilutions. s^1 and s^2 = volumes of solution in the dilutions. Technically this procedure is accurate. In principle, however, it is not accurate unless the diluting fluid have the same osmotic density as the plasma of the particular blood under consideration, otherwise there will be an exchange between the cells and the plasma.† Whether this exchange is always in one direction or not has not been determined; so far as clearly demonstrated, the exchange seems to be from the cells into the plasma. The extent of this exchange is certainly not marked; the amount of nitrogen involved is so small in comparison to the quantity of nitrogen in the native plasma that the method with its acknowledged error remains much more accurate than those of sedimentation and centrifugation. The error was minimized, I believe, by diluting the blood with small quantities of saline solution to which potassium oxalate was added to prevent coagulation, and then quickly removing the cells by centrifugation so that only a few minutes elapsed from the time the blood was drawn from the vein until the plasma was transferred to the Kjeldahl digestion flasks. The diluting fluid employed was a 4 per cent oxalate solution in a 0.9 per cent NaCl solution. The first mixture was of large quantity, since it also served for the estimation of the fibrin, usually 4 cc. of the solution to about 96 cc. of blood; the second mixture was usually in the proportion of 2 parts of the solution to 25 parts of blood. I have often

* The word "isotonic" is here employed in its exact sense, related to osmotic density.

† If the osmotic density of the plasma of the particular blood be determined, preferably by the estimation of the reduction of the freezing point, and the saline diluting fluid made to correspond thereto, the result will be accurate. In the use of such solutions whose osmotic density corresponds to that of human plasma on an average (as 0.91 per cent. sodium chloride or 1.42 per cent. sodium sulphate) there will in the individual cases probably always be some exchange between the cells and the diluted plasma.

made smeared preparations of the corpuscles at the moment when the diluted plasma was drawn off for nitrogen analysis and found the corpuscles as a rule of normal appearance and staining reactions. At the end of an hour, however, the appearances of the red cells began to be altered, and these alterations progressed as time elapsed, and corresponding thereto later analyses of the plasma have always given higher quantities of nitrogen. I likewise made comparative determinations with the hæmatokrit, both with diluted and undiluted blood, and the results convinced me that the Bleibtreu method amply repays in accuracy the labor it involves.

From the plasma-nitrogen the amount and percentage of proteids in the plasma may be calculated, and in connection with the volume of plasma the amount of albumin in the plasma of 100 cc. of blood may be easily determined. These factors are of value, since any alteration in the quantity of water in the plasma would at once affect the quantity of albumin. There is in normal plasma a small quantity of non-proteid nitrogenous substances. In my cases the plasma has on an average contained azotized substances not precipitable by tannic acid corresponding to 0.060 N in 100 cc. of plasma, and that amount has been regularly deducted from the plasma-nitrogen before converting it into terms of albumin.

The fibrin was estimated by the recent method of Kossler and Pfeiffer.* A known quantity of venous blood, about 100 cc., is rendered non-coagulable by the addition of from 4 to 6 cc. of a 4 per cent solution of potassium oxalate in 0.9 per cent NaCl solution. The cells are immediately removed by centrifugation and the plasma-nitrogen determined by the Kjeldahl method (this also serves as mixture 1 of the Bleibtreu method). To 20 cc. of the oxalate plasma are then added 3 cc. of a 2 per cent calcium chloride solution. Spontaneous coagulation occurs rapidly; following the complete separation of the fibrin and the serum, the nitrogen of the serum is determined by the Kjeldahl method. Since the volume of plasma, the oxalate dilution of the blood and the calcium dilution of the oxalate plasma are known, the difference between the plasma-nitrogen and the serum-nitrogen with consideration of the aforementioned factors represents the nitrogen of the fibrin in the native volume of blood, the formula being as follows:

$$Nf = \frac{v}{v - v'}\left(Np - Ns\,\frac{p + c}{p}\right).$$

Nf = fibrin nitrogen in 100 cc. blood. v = volume of oxalate plasma

* Kossler and Pfeiffer, *Centralbl. f. innere Med.*, xvii (1896), 1.

(Bleibtreu method). v^1 = amount of oxalate solution in 100 cc. of oxalate blood. Np = nitrogen in 100 cc. of oxalate plasma. Ns = nitrogen in 100 cc. of the diluted serum. p = quantity of oxalate plasma in the artificial coagulation. c = quantity of calcium solution in the same. Since we do not know the formula for the molecule of fibrin, the findings must be expressed in terms of nitrogen. This method is very accurate, though technically quite difficult.

The time of coagulation was determined by Wright's method. A dozen glass tubes of $\frac{1}{4}$ mm. dimensions are supported in glass or rubber cases and suspended in water kept at body temperature. An assistant noting the time, the numbered tubes are successively employed as follows: from a deep puncture in the finger the blood is drawn one-third the length of the tube, which is then removed from the finger and the column of blood drawn up so that it occupies the middle third of the tube, which is then replaced in its warm case. After a lapse of about two minutes (a preliminary orientation is always advisable) the tubes are successively blown out and the coagulation point noted, the time of which can then be calculated from the records. The range of error in this method is fully forty seconds, that is, it may be 20 seconds to either side of the given result. From studies on normal male adults I have found the time of coagulation with this method to range from 1.5 to 3 minutes, the average being a little lower than Wright's figures, 2.5 minutes. It may not be amiss to say that this seemingly simple method requires not a little practice if constant results are to be obtained.

The results of the observations are presented in the tables on pages 380-1.

X in case Brown indicates the beginning of the treatment, XX indicates the withdrawal of the calcium, XXX its resumption. X in case Griffith indicates beginning of treatment; XX indicates its rigorous enforcement, since the urine gave evidence of neglect of orders.

The average output of urine of Brown under voluntary conditions was always over 1100 cc.; under the Tufnell regimen it averaged 440 cc. Griffith's urine naturally fluctuated from 950 to 1300 cc.; under the treatment the average daily output was 525 cc. Prior to beginning treatment both patients were upon the same diet and drinking water, yet Brown eliminated by the urine only about 0.200 grm. $Ca_3(PO_4)_2$, while Griffith eliminated over 0.750 grm., a further illustration of the wide individual variations in urinary calcium elimi-

nation so often noted by different observers. In the case of Brown, despite the dry diet, the administration of calcium increased markedly the urinary elimination of that element, often to twice or thrice its natural quantity. Upon withdrawal of the drug (at XX) the quantity in the urine fell at once, and again rose promptly when its administration was resumed (at XXX). In the case of Griffith, the administration of calcium without strict enforcement of the dry diet (from X to XX) caused a considerable increase in its urinary elimination. But when the drinking was rigidly reduced the urinary elimination of calcium fell below the quantities previously eliminated while upon a normal diet without treatment. Even thus, however, the quantities eliminated were greater than in the case of Brown. It seems clear from these figures and from what is well known regarding the effect of the free use of water upon the absorption and urinary elimination of calcium salts, that were it held of prime importance to saturate the body with these salts, abundance and not restriction of water would be indicated.

That the ingestion of calcium affects the quantity in the circulating blood is suggested but not demonstrated by my analyses. Before the treatment, in the case of Brown, the quantities were 0.0172 and 0.0158 grm., during the treatment 0.022 and 0.0179 $Ca_3(PO_4)_2$ in 100 cc. blood; in the case of Griffith, before treatment, 0.018 and 0.0191 grm., during treatment 0.0146 and 0.015 grm., thus inclining to agree with the urinary elimination. It is clear, however, that so many other factors are concerned in this question that no conclusion can be established on this point.

The specific gravity of the blood in both cases was not materially affected by the treatment—1.0534, 1.051 before treatment, and 1.053, 1.052 during treatment, in the case of Brown; 1.060, 1.0607 before treatment, and 1.0598, 1.0609 during treatment, in that of Griffith. During the course of the treatment in both cases the number of red cells and the percentage of hæmoglobin diminished somewhat. Since the estimation of the specific gravity of the blood by weighing is much more accurate than the clinical methods of Thoma and Fleischl, I am compelled to look upon the apparent reductions either as

erroneous or as due to peripheral conditions. Both the constancy of the specific gravity and the reductions in the results of the clinical blood examinations show that in these cases no diminution in the water of the blood was effected by the treatment.

The same result is clearly shown by the percentages of the volume of plasma in the volume of native blood. These were in the case of Brown, 61.75 and 64 per cent before treatment, and 66 and 64 per cent during treatment; in the case of Griffith, 57.4 and 56.8 per cent before treatment, and 57 and 57.2 per cent during treatment. Any oligohydræmia would result in a diminution of the volume-percentage of the plasma, and since in the case of Brown the variations were in the direction of hydræmia and in the case of Griffith the results were constant, it is again clear that in these cases no oligohydræmia was effected by the treatment.

The plasma-nitrogen, plasma-albumin (factor 6.38) and quantities of albumin in the plasma of 100 cc. of blood were quite constant in the case of Brown, except for the second estimation, in which all were noticeably low. The patient at that time was suffering from an attack of influenza, but since that was before the beginning of the treatment, and since the two later determinations under treatment agreed with the earlier one prior to treatment, the absence of changes is clearly shown. In the case of Griffith the results were constant, except in the third estimation, in which they were high. The causes of this are obscure, since the percentage-volume of the plasma and the specific gravity were not altered. That the nitrogen and albumin of the plasma should vary constantly with the volume of plasma and the specific gravity of the blood is, however, not to be expected, since so many other factors are concerned in the latter.

The fibrin-nitrogen was not increased by the treatment beyond the range of error of the method. The only notable variation was the reduction observed at the time of the second estimation of Brown (before treatment), 0.0593, when suffering from the attack of influenza already mentioned. It is, therefore, clear that the treatment in these two cases did not increase the quantity of fibrin in the native volume of blood. The gross phenomena of clotting were in all cases

closely observed in the blood drawn apart for the estimation of calcium; in no case was the clot larger or firmer, nor did it retract or contract more quickly than normal.*

The time of coagulation as determined by Wright's method varied in the two cases. In Brown it was noticeably reduced during treatment below the figures noted previous to treatment, and reduced beyond the range of error of the method. During the six days when the calcium was withdrawn no differences in the time of clotting could be noted. In the case of Griffith, although there were marked variations both up and down, in general the time of clotting was not shortened. I believe, therefore, that in these two cases no acceleration in the process of clotting was effected by the use of calcium, since in Griffith, who best absorbed his calcium, no changes were noted, while in Brown the acceleration persisted while the drug was withheld. No inferences can be drawn respecting the influence of the Tufnell treatment upon the time of coagulation, since the results in the two cases do not agree.

The action of the calcium salts upon coagulation is and must remain purely empirical until the chemical relations of the process are better understood. Having apparently demolished the older views, Lilienfeld and his followers have in turn had grave doubts thrown upon their own by Pekelharing, Hammarsten and others,† and only thus much appears definitely known: that under certain circumstances, under the influence of several ill-understood factors, blood coagulates, and the product fibrin contains calcium and a proteid, plus how much more we know not, in a molecule of undetermined quantity. Under such circumstances discussion of the relations of calcium salts to blood coagulation under pathological circumstances would be profitless.

* Realizing that terms of nitrogen do not convey to the general reader a clearly defined idea of quantity, I have converted the figures of nitrogen into terms of fibrin in order to make the results more tangible, using as a basis of calculation the formula of Schmiedeberg; this formula, however, can be accepted only in a provisional sense. The quantities of fibrin thus determined read as follows: Brown, before treatment, 0.464 and 0.355; during treatment, 0.433 and 0.475 grm.; Griffith, before treatment, 0.457 and 0.450; during treatment, 0.478 and 0.466 grm.

† For example, Hammarsten, *Zeitschr. f. phys. Chem.* xxii, 333, and Cramer, ibidem, xxiii, 74.

While it seems clear that in every point respecting the *modus operandi* of this mixed treatment for aneurism and its influence upon the blood the result was entirely negative, I wish most definitely to state that under the treatment both patients improved. Brown received but slight betterment, the dilatation seemed simply held in check. Griffith, however, improved markedly; the tumor and the physical signs and symptoms receded progressively until he was finally discharged with no symptoms and no signs except dulness beneath the manubrium and a faint bruit. Brown was kept constantly in bed. Griffith was allowed the liberty of the hospital and its grounds, so that in his improvement rest played no role.

TABLE I.—CASE, BROWN.

X indicates beginning of treatment; XX, withdrawal of calcium; XXX, its resumption.

	Quantity of urine in cc. per diem.	Urinary calcium $Ca_3(PO_4)_2$ in grm. per diem.	Blood calcium $Ca_3(PO_4)_2$ in grm. in 100 cc.	Sp. gr. of blood.	Percentage volume of plasma.	Plasma-nitrogen in 100 cc. plasma.	Plasma-albumin in 100 cc.	Albumin in plasma of 100 cc. blood.	Fibrin-nitrogen in 100 cc. blood.	Coagulation time in minutes and seconds.	Venesection.	Number of red blood corpuscles and percentage of hæmoglobin.*
	1040	0.1915	.....	.....	.....	.....	.....	.....	.....	3.10	.....	4820000, 85%
	1180	0.2164	0.0172	1.0534	61.75%	1.322 grm.	8.432 grm.	5.207 grm.	0.0776 grm.	2.50	300 cc.	4780000, 87
X	1070	0.2218	0.0158	1.051	64	1.165	7.433	4.757	0.0593	2.30	260	4620000, 83
	320	0.571	.....	.....	.....	.....	.....	.....	.....	2.30	.....	4750000, 80
	500	0.540	.....	.....	.....	.....	.....	.....	.....	1.55	.....	4620000, 80
	445	0.504	.....	.....	.....	.....	.....	.....	.....	2.15	.....	4780000, 85
	520	0.432	.....	.....	.....	.....	.....	.....	.....	2.09	.....	4730000, 80
	425	0.399	.....	.....	.....	.....	.....	.....	.....	2.04	.....	4760000, 82
	480	0.756	0.022	1.053	66	1.223	7.803	5.150	0.0725	2.18	250	4690000, 85
	425	0.416	.....	.....	.....	.....	.....	.....	.....	2.25	.....	4610000, 80
XX	420	0.246	.....	.....	.....	.....	.....	.....	.....	2.01	.....	4480000, 80
	380	0.185	.....	.....	.....	.....	.....	.....	.....	2.09	.....	4480000, 78
XXX	490	0.341	.....	.....	.....	.....	.....	.....	.....	2.11	.....	4580000, 75
	430	0.364	.....	.....	.....	.....	.....	.....	.....	2.18	.....	4520000, 78
	410	0.296	0.179	1.052	64	1.271	8.109	5.190	0.0795	2.05	270	4450000, 75

* Last two figures hæmoglobin %. Thoma-Zeiss and Fleischl apparatus.

TABLE II.—CASE, GRIFFITH.

X indicates beginning of treatment; XX, its rigorous enforcement.

	Quantity of urine in cc. per diem.	Urinary calcium $Ca_3(PO_4)_2$ in grm. per diem.	Blood calcium $Ca_3(PO_4)_2$ in grm. in 100 cc.	Sp. gr. of blood.	Percentage volume of plasma.	Plasma-nitrogen in 100 cc.	Plasma-albumin in 100 cc.	Albumin in plasma of 100 cc. blood.	Fibrin-nitrogen in 100 cc. blood.	Coagulation time in minutes and seconds.	Venesection.	Number of red blood corpuscles and percentage of hæmoglobin.*
	1160	0.860	....	....	....	....	....	....	....	2.15	....	5050000, 90%
	1240	0.889	0.018	1.060	57.4%	1.276 grm.	8.141 grm.	4.673 grm.	0.0765 grm.	2.20	260 cc.	4940000, 88
	1195	0.784	0.0191	1.0607	56.8	1.291	8.237	4.679	0.0752	1.55	250	4960000, 95
X	980	1.134	....	....	....	....	....	....	....	2.30	....	4910000, 88
	975	1.088	....	....	....	....	....	....	....	1.40	....	4930000, 85
	1210	0.972	....	....	....	....	....	....	....	2.02	....	4990000, 85
XX	580	0.652	....	....	....	....	....	....	....	1.59	....	4860000, 88
	550	0.720	....	....	....	....	....	....	....	2.10	....	4920000, 88
	560	0.380	0.0146	1.0598	57	1.351	8.619	4.913	0.080	2.08	225	4890000, 90
	525	0.535	....	....	....	....	....	....	....	2.08	....	4810000, 85
	400	0.498	....	....	....	....	....	....	....	2.12	....	4850000, 80
	625	0.671	....	....	....	....	....	....	....	2.50	....	4750000, 85
	490	0.574	....	....	....	....	....	....	....	1.59	....	4880000, 88
	460	0.560	0.015	1.0609	57.2	1.265	8.071	4.617	0.078	2.10	240	4830000, 85

* Last two figures hæmoglobin %. Thoma-Zeiss and Fleischl apparatus.