SCIENCE

Friday, July 30, 1915

CONTENTS

MSS. intended for publication and books, etc., intended for review should be sent to Professor J. McKeen Cattell, Garrison-on-Hudson, N. Y.

EXPERIMENTAL AND CHEMICAL STUDIES OF THE BLOOD WITH AN APPEAL FOR MORE EXTENDED CHEMICAL TRAINING FOR THE BIOLOGICAL AND MEDICAL INVESTIGATOR[1]

Before beginning my address let me say that I feel it to be a very great honor to have been asked to deliver the first Mellon Lecture under the auspices of the Society for Biological Research of this university. The establishment of a lectureship of this character is a great encouragement to men of science. It affords additional opportunity to bring to the attention of a wider public the recent results of scientific investigation as well as to emphasize again a truth which can not be too often repeated, that science constitutes a sure and lasting part of the intellectual treasure which mankind possesses.[2]

I have ventured to take as the subject of my address some recent experimental and chemical studies of the blood. In order to give my subject a proper setting I must, first, refer briefly to the history of blood-letting, and to make clear its relation to pressing medical problems, I shall in the hour discuss the interaction of the blood and the organs of internal secretion.

The overwhelming significance of the blood to all people in all times is shown in folk sayings, in tradition and in literature. The expressions, "the life of flesh is in the blood," "tainted blood," "blood will tell," "blood oath," "blood brother," all suggest how nearly blood has been held to be synon-

[1] The first Mellon lecture, delivered in the Assembly Hall of the Mellon Institute, Pittsburgh, on February 27, 1915.

[2] Ostwald,

ymous with life. It was an ancient Celtic custom to emphasize the inviolability of a treaty by having it written with the blood of both clans mixed in one vessel.

In the earlier systems of medicine, as those of Asiatic countries, of Egypt and of Greece, alterations in the composition of the blood were held to be of great significance. In Hippocratic medicine the right admixture of the four humors, the blood, phlegm, yellow bile and black bile, constituted health, while wrong proportions or distribution caused disease. This humoral theory of disease, variously modified down to our own time, has always fitted in well with the practise of blood-letting, or making running issues, and with other depletory measures.

Blood-letting seems, however, to antedate all systems of medicine and to have been one of the earliest therapeutic procedures applied by primitive races. Leeches have been used for this purpose since the earliest times in Asiatic countries, especially in India, and let no one suppose that their use has been discontinued in our day. Dr. Shipley, the master of Christ's College, Cambridge, writing in the *British Medical Journal*,[3] tells us that the Allies and Germans are now fighting on some of the best leech areas of Europe, and goes on to state that the traffic in leeches probably reached its height in the first half of the nineteenth century, that, for instance, in the year 1832, 57,500,000 of these annelids were imported into France, 60,000 to 80,000 leeches a day frequently leaving Strassburg for Paris, having been shipped overland from Hungary *via* Vienna. So great was the demand that the artificial cultivation of leeches was taken up in various countries and became a profitable industry. And now a new use for leeches has arisen. Certain glands surrounding the oral end of the digestive canal of this annelid secrete a remarkable substance which keeps blood from coagulating and which has been named hirudin. This substance is much used in our laboratories to keep the blood of man and animals in the fluid state. Leeches have thus become an article of commerce quite aside from their employment as depleting agents, and the demand is constantly growing. We are at present greatly hampered by our inability to obtain them from Europe, as their importation has practically ceased since the outbreak of the war.

It is not my purpose to attempt to give a history of blood-letting, even in abstract; the history of the subject is practically coextensive with the history of medicine itself. I must therefore content myself with a few selections from historical writing which will demonstrate to you that the influence of this method of treating disease has been paramount since long before the time of Hippocrates, whose writings furnish one of the earliest prescriptions for blood-letting, beginning with the direction to

> Bleed in the acute affections, if the disease appears strong, and the patients be in the vigor of life, and if they have strength.

In the latter part of the twelfth century, when universities as we now know them were coming into existence, there originated in the School of Salernum the "Regimen Sanitatis Salerni" or "Code of Health," a poem written in Latin hexameter verses and giving the medical notions of the day, as derived from the Arabic writers in regard to blood-letting, diet and personal hygiene. The high value placed on the "Regimen" may be seen from the fact that it passed through some 240 different edi-

[3] No. 2813, November 28, 1914, p. 917, and No. 2814, December 5, 1914, p. 962. These papers contain much valuable information concerning the medicinal leech, as also the curious history of exotic leeches which in certain eastern countries constitute a serious menace to the life of men and animals.

tions and was translated into all the known languages.[4] In general praise of blood-letting the poem[5] says

Bleeding the body purges in disguise,
For it excites the nerves, improves the eyes
And mind, and gives the bowels exercise,
Brings sleep, clear thoughts, and sadness drives away,
And hearing, strength and voice augments each day.

Other verses give directions as to what months are proper and what improper for bleeding, tells what diseases are benefited by blood-letting and in what quantities blood should be drawn, and the effect of age and other circumstances.

Acute disease, or only so in part,
Demands bloodletting freely from the start.
In middle age, bleed largely without fear,
But treat old age like tender childhood here.

In the latter part of the middle ages blood-letting was carried to great excess. During this period astrology strongly influenced medical thought, and physicians made diagnoses and bled their patients according to the position of the planets, constellations and single stars (horoscopic medicine). For their greater convenience semi-popular calendars were even prepared with illustrations such as the so-called venesection manikins of Johann Nider von Gemünd (1470) and of Stoeffler (1518) with directions as to the vein to be opened for the cure of each malady.

In the sixteenth and seventeenth centuries we come upon heated controversies between the upholders of the Hippocratic and of the Arabian theories of blood-letting. By the former method it was thought that the vein to be opened should be as near as possible to the diseased part—in order that the "foul and stagnant" blood might be directly removed from the inflamed area ("derivation"). On the other hand, the doctrine elaborated by the Arabians taught that blood should be taken from a vein remote from the inflamed part, for instance, in inflammation of the lungs and pleura, from the arm or even the foot of the opposite side, with the idea that this process ("revulsion") prevented good blood from accumulating in the diseased part.

This latter doctrine was in the ascendancy in European countries in the sixteenth century, but the learned Pierre Brissot (1478–1522), basing his opinion on his own large clinical experience in Paris in 1514, when an acute affection of the lungs was prevalent, revived the Hippocratic method of bleeding and thus started the famous Brissot-venesection controversy in which most of the great men of the century, including Vesalius, took part. The importance attached to the controversy at the time is shown in the fact that the opponents of Brissot induced the French Parliament to forbid the practise of his method, and their attacks were so bitter as virtually to drive him from Paris and his professorship. Haeser[6] informs us that the quarrel assumed such violence that when the University of Salamanca took sides with Brissot, the Emperor Charles V., who was called on to render a "decision" in the matter was assured that the new false doctrine was no less dangerous than the heresy of Luther.

While Brissot was anything but a "therapeutic nihilist" as to breeding, and held firmly to the doctrine that the "foul blood" of the inflamed area should be removed, some of his followers rejected bleeding altogether in acute disease of the lungs and pleura (the pleuritis of that day). Their moderation was looked upon as little less than heretical, and toward the end of the sixteenth century we find Leonardo Botallo, a Piedmontese, an eminent prac-

[4] Garrison, "The History of Blood-letting," *N. Y. Med. Jour.*, March 1 and 8, 1913.

[5] Professor John Ordronaux's translation.

[6] *Geschichte der Medicin*, II., p. 64.

titioner of his time, chief physician to Charles IX., advising venesection to the limit, regardless of the nature of disease, the age or condition of the patient. Blood-lettings of three to four pounds each repeated as often as four or five times were advised, says Haeser, and this historian adds that the explanation of this "Vampyrismus" is probably to be found in the circumstance that Botallo lived in northern Italy, where diseases of an inflammatory character were prevalent and more especially that in his experience as an army surgeon he encountered only patients of the most robust type. Botallo, in defending his practise said,

the more foul water is drawn from a well, the more good water can flow in to replace it.[7]

An ardent follower of Botallo was Riolan the younger, who falls back upon Hippocrates and Galen and lays down the rule that one must take away as much blood as possible in every disease. As an adult is judged to have about thirty (!) pounds of blood,[8] the tapping of half this amount, or fifteen pounds, in the course of fourteen days would be about the right amount to take, says Riolan. Guy Patin (1602–1672), himself an ardent bleeder and purger, informs us that Bovard, body physician of Louis XIII., bled that monarch forty-seven times, gave him 312 clysters and prescribed emetics and purges, 215 times, all in one year.

A little later, that able and credulous Belgian mystic and follower of Paracelsus, J. B. Van Helmont (1578–1644), an iconoclast in general, called by his admirer Haeser "the fist of the seventeenth century" went so far as to condemn venesection entirely. To him is attributed the often-quoted phrase "a Bloody Moloch presides in the chair of medicine."

Also as holding that in place of excessive blood-letting should be substituted therapeutic procedures ("alterantia") and change of diet, stands the genial and talented Franciscus de le Boë (Sylvius) (1614–1672), one of the leading medical authorities of the seventeenth century and one of the earliest defenders of Harvey's doctrine of the circulation, who taught at Leyden that abnormal fermentations in the fluids of the body cause disease, a variant of the ancient humoral doctrine. In Chapter XX. of his "New Idea" (translated by Richard Gower, London, 1675), entitled "On the Motion of Blood through the Lungs Affected," he shows his good sense and his caution when he says

A Plethora of Blood is soon and safely *cur'd*, by a sufficient *Emptying* of it by *opening a Vein;* whether it be together and at once, or by repeted turns, according to the peculiar nature and strength of the *Sick*. For there are many who can not bear to have much taken away together, but soon fall into a Swouning; by which feeling none can at any time receive any good, I had rather that it should be prevented, as often as may be, and every Cure be done securely rather than rashly, seeing it often happens to those rash Blood-Letters, that they educe Life together with Blood.

An instance of lavish blood-letting in a medical crisis may be found in the experience of that adventurous spirit, Thomas Dover, to whom we owe the much used "Dover's powder." In 1708, Dover, then forty-eight years old, set out on a privateering expedition and was given command of a ship, the *Duke,* while his superior, Captain Woodes-Rogers took command of the other ship of the squadron, the *Duchess.* The three years' voyage of these buccaneers is of interest historically because,

touching at the island of Juan Fernandez, they took on board Alaxander Selkirk, who had lived alone on the island for four years and four months, and whose story was to develop in the skilful hands

[7] Bauer, *Geschichte der Aderlässe,* Gekrönte Preisschrift, Munich, 1870, p. 139.

[8] Bauer, *loc. cit.*

of Defoe into that of the immortal "Robinson Crusoe."[9]

In Dover's "Ancient Physicians Legacy to his country" we find the following interesting passages:

When I took by Storm the two Cities of Guaiaquil, under the Line, in the South Seas, it happen'd, that not long before, the Plague had raged amongst them. For our better Security, therefore, and keeping our People together, we lay in their churches, and likewise brought thither the Plunder of the Cities.

. . . In a very few days after we got on board, one of the Surgeons came to me, to acquaint me, that several of my men were taken after a violent Manner, with that Langour of Spirits, that they were not able to move. I immediately went among them, and, to my great Surprise, soon discerned what was the Matter. In less than Forty-eight Hours we had in our several Ships, one Hundred and eighty Men in this miserable condition.

I order'd the Surgeons to bleed them in both Arms, and to go round to them all, with Command to leave them bleeding till all were blooded, and then come and tie them up in their Turns. Thus they lay bleeding and fainting, so long, that I could not conceive they lost less than an hundred Ounces each Man.

If we had lost so great a Number of our People, the poor Remains must infallibly have perished. . . .

We had on board Oil and Spirit of Vitriol sufficient, which I caused to be mixed with Water to the Acidity of a Lemon, and made them drink very freely of it; so that notwithstanding we had one hundred and eighty odd down in this most fatal Distemper, yet we lost no more than seven or eight; and even these owed their Deaths to the strong Liquors which their Mess-Mates procured for them . . . Now if we had had Recourse to Alexipharmicks, such as Venice Treacle, Diascordium, Mithridate, and such-like good-for-nothing Compositions, or the most celebrated Gascoin's Powder, or Bezoar, I make no Question at all, considering the heat of the Climate, but we had lost every Man.

Of non-medical literature the Satire of Gil Blas, written early in the eighteenth century, but in reality giving a picture of seventeenth-century excesses in blood-letting, is worth citing.

Dr. Sangrado is called in to prescribe for a gouty old canon, and he at once sends for a surgeon and orders him to "take six good porringers of blood in order to supply the need of perspiration." The surgeon was ordered to return in three hours and take as much more and to repeat the evacuation the next day. The patient was "reduced to death's door in less than two days," and, the notary being summoned to make the will, seized his hat and cloak in a hurry when he learned from the messenger, Gil Blas that Dr. Sangrado was the physician. "Zooks," cried he, "let us make haste, for the doctor is so expeditious that he seldom gives the patient time to send for notaries; that man has choused me out of a great many jobs."

But the misuse of bleeding continued in the centuries following, and at no time was the practise more abused than in the latter part of the eighteenth or even in the first five decades of the past century. French and Italian authorities, especially, were great believers in blood-letting. Broussias (1772–1832) is said to have used 100,000 leeches in his hospital wards in one year. This physician and his follower, Bouilland, actuated by false theories of the cause of fevers, recommended the bleeding of a patient 10 to 12 and even 20 times in the course of treatment.

But more and more the opponents of general and excessive bleeding made headway in their respective countries. Many are the names that might here be named, as Pinel, Andral, Louis in France, Dietl the pupil of Skoda and Wollstein the professor of veterinary medicine in Vienna, Mezlar, Rademacher, von Pfeufer and others in Germany, Marshall Hall[10] and later Sir

[9] "Chronicles of Pharmacy," Wootton, II., p. 130.

[10] "While Marshall Hall favored Venesection, he was one of the earlier and important members of

William Jenner, Sir William Gull, Bennet and others in England, Strambio, Angelo, Meli in Italy, and Jackson in our own country, and many others in all of these countries.

But what finally led to the entire abolition of bleeding after the middle of the past century was not so much the opposition of clinicians who failed by its use to abort pneumonia ("the queen of inflammations," as Dietl calls it) or some other acute disease, but the rise of new theories of disease, based on discoveries of fundamental importance. The rise of the cell-theory and of cellular pathology, the discovery of bacteria and their connection with the inflammatory process of the infectious diseases, the appearance of hydrotherapy, the expectant medicine of the school of Skoda and Oppolzer, new and quicker methods of obtaining the effects of drugs, as by means of the hypodermic syringe, the discovery of new hypnotics, of the analgesics and anesthetics, altered the views of medical theorists and practitioners alike and inevitably led to the downfall of the theories on which venesection had been based.

During a period of study of six and a half years, from 1884 to 1891, as a student of chemistry and medicine in several of the larger medical centers of Germany, Austria and Switzerland, I never once saw a patient bled in clinic or hospital. The procedure may have been employed now and then, but so little stress was laid upon it that it was not thought worth while to demonstrate it to the young men who walked the wards.[11]

Bleeding did not disappear, however, from the world. The common man, especially in Germany and France, still held firmly that benefits did follow the use of the wet cup, the lancet and the leech. Tenaciously the old practises were upheld. If physicians refused to bleed, there was always the barber surgeon, fully competent, as in teeth pulling, to give relief. I remember that in my boyhood in Ohio the practise of blood-letting in the spring of the year was in vogue among the farm laborers from southern Germany. After their return from a visit to their barber surgeon in the town the scarified backs were exhibited as a special favor, and irrefutable arguments advanced in respect to the benefits of bleeding either to ward off disease or to improve nutrition. Was it not true and known to all stock-breeders that the domestic animals could be fattened by judicious bleeding at certain fixed intervals?

And it appears now that the common man was right, after all. An empirical method of treatment which has been practised by nearly all races since before the day of Hippocrates almost certainly contains a basis of truth. This is now admitted, and physicians are again saving lives by the judicious and timely use of blood-letting. Says the experienced Sir T. Lauder Brunton:

> Blood-letting not only relieves symptoms, but may save the patient's life, as in engorged conditions of the right heart, whether due to mitral incompetence or pulmonary affections.[12]

the profession to throw doubt upon indiscriminate bloodletting." D'Arcy Power, "Dr. Marshall Hall and the Decay of Bloodletting," *The Practitioner,* 1909, Vol. 32, p. 320.

[11] See also F. de Havilland Hall, The Westminster Hospital Reports, Vol. XVII., p. 1, 1911, who make the following statement in a clinical lecture on bloodletting: "To such an extent had bleeding been discarded that during my student days at St. Bartholomew's Hospital, I never heard of a patient being bled, so that I was quite taken back, when shortly after I was appointed House Surgeon at a country hospital, the Senior Surgeon came to me to be bled. Indeed, in 1892 when I requested a member of the Surgical Staff at St. Bartholomew's to bleed a patient for me, he told me that this was the first time he had ever been called upon to perform phlebotomy."

[12] On the use of leeches in relieving overdistension of the right heart, in cases of pneumonia, see

In puerperal eclampsia, to mention but one more instance, we also have a condition which is generally strikingly benefited by blood-letting.[13]

Venesection, then, will probably never again be entirely excluded from medicine, as it was during the last quarter of the past century, nor need we fear that the practise will be again misused.

PLASMAPHERESIS

But venesection, like all therapeutic procedures, has certain drawbacks which prescribe limits to its use, and these drawbacks are inherent in the very composition of the blood and in the nature of the circulatory apparatus. As is known to all the oxygen-carrying power of the blood resides in the red corpuscles, or erythrocytes, which constitute about 36 per cent. of the volume of the blood. These erythrocytes, like other cellular constituents, can be built up only slowly in the body by the hemapoietic, or blood-building, organs. It is apparent, therefore, that the bad effects of overbleeding, as formerly practised, must be due mainly to the loss of these cellular elements. Common experience has shown that the loss of too much blood is either immediately fatal, or else is followed by a prolonged illness, recovery from which is often doubtful.

Reflecting on these drawbacks I conceived the idea that the main objection of blood-letting could be obviated by the speedy return into the body of the red and the white corpuscles instead of throwing them away as hitherto has been our custom.

D. B. Lees, *Lancet*, February 25, 1911. Also for cases in which blood-letting (either by venesection or by means of leeches) may be advantageously employed, see F. de Havilland Hall, Westminster Hospital Reports, Vol. 17, p. 1, 1911.

[13] See Zweifel, "Zur Behandlung der Eklampsie," *Centralbl. f. Gynäkologie*, 1895, No. 46. Alexander Strubell, "Der Aderlass, Eine Monographische Studie," Berlin, 1905.

The only thing that would be removed from the blood of a person bled in this way would be its fluid part—the plasma. If this method were found to be practicable the value of bleeding would be enhanced and its field of application extended. Such a method, if successful, would appear to be advantageous for the patients, not only in those instances in which venesection is performed, admittedly with good results, but would also open the way for the withdrawal of fluid when it is desired to decrease the volume of blood in the vascular apparatus or to remove excess of deleterious substances, or where bleeding has hitherto been contraindicated because of the danger of reducing the oxygen-carrying capacity of the blood, as, for example, in aneurism, or in cardiac decompensation with a low blood count.

In the work now going on in my laboratory, we are still in the stage of experimentation and study, but our experiments on animals have proved the feasibility of the method. With the skilful cooperation of my colleagues, Drs. Rowntree, Turner, Marshall and Lamson, a considerable number of experiments on animals have already been made. Our procedure, in a word, is the following. Blood is withdrawn freely from an animal and is prevented from clotting by addition of leech extract; Locke's fluid in equal volume is then added to the blood, and the mixture is sedimented in the centrifugal machine until the corpuscles have settled out in the flasks. The supernatant plasma is then drawn off and replaced by Locke's fluid, the corpuscles are stirred up and the new mixture is returned to the animal. By repeating this process it has been learned that blood-letting can be carried out repeatedly, without endangering the life of an animal, provided only that the cellular elements of the blood are returned. We have named the procedure plasmapheresis.

It is apparent that when blood-letting is practised in the usual way there is always the risk of greatly reducing the oxygen-carrying capacity of the blood through loss of red corpuscles, but in our experiments the fluid of the blood can be withdrawn in large quantities without affecting this capacity, as far as we can determine at the present moment. Just how large quantities of plasma can be withdrawn without permanent injury can not at present be stated. In certain cases very large amounts have been successfully removed in experiments extending over several days. We have actually withdrawn from a dog by repeated bleedings in a *single* day, a volume of blood more than twice that contained in the body, with no apparent injury, by our method of returning the corpuscles after each bleeding. How far this exceeds the quantity of blood that may be safely removed from a dog at one time without return of corpuscles is seen when we recall that the loss at one time of 60 to 70 per cent. of the animal's blood is quickly fatal.

It may yet be possible to attach an electrically controlled centrifugalizing apparatus directly to the blood vessels of an animal and tap off a desired quantity of the fluid part of the blood while directing the stream of corpuscles back into the body (or vice versa), the whole apparatus being analogous in a way to the modern cream separator.

It has been our purpose in our recent experiments to find the limits to which the plasmapheresis may be carried and to learn what pathological changes ensue when the procedure is carried to a point beyond which life is endangered. With the form of Locke's solution now employed by us, we have in the course of five days carried the removal of plasma to a point where the total volume of blood withdrawn from the body equals at least five times that ordinarily contained in the body. In this experiment the limit of the procedure was probably reached, as the animal was very nearly lost during the last bleeding; only the speedy return of the sedimented corpuscles saved the dying animal. Unfortunately, one can not conclude from these most successful experiments that similar or even markedly lower quantities can always be removed without danger. We have recently carried out a large number of experiments with a view to determine the safe limits of plasmapheresis both as to quantity per day and total quantity of blood withdrawn, but unfortunately these experiments are vitiated by an error which has only recently been discovered. It has been found that the imported hirudin which we are now using is strongly toxic. This was not the case with the product which we ourselves manufactured and which was used in our earlier experiments. Further experiments will have to be done, therefore, to settle this question.

Some interesting results have been obtained by studying the chemical changes during plasmapheresis. Since the method consists essentially in replacing the plasma of the blood by a saline solution, it is natural to find a decrease in the soluble proteins of the blood. While not as rapid as a purely mathematical calculation based on the amounts drawn off and returned would indicate, if the vascular system were regarded as a vessel of given capacity to be washed out, the decrease is considerable. In three days the soluble proteins have been reduced to about one third their original value, after which there is a slight rise as the process is continued. Evidently, as was expected, there is a continual renewal of plasma proteins from the tissues.

In striking contrast to this, the non-proteid nitrogen of the plasma shows a slight *rise* on the first day in every case studied, and a tendency to rise, with some fluctuations, as the process is continued. This increase, which is chiefly due to urea, may be due either to an increase in nitrogenous katabolism or to a diminution of nitrogen excretion.

TABLE I

Plasmapheresis on Three Dogs for Several Days. Nos. 8 and 11 Three Days Each, No. 10 Two Days

Experiment No.	8	10	11
Weight before operation	9.6 kg.	12.8 kg.	8 kg.
(Estimated) blood volume	730 c.c.	960 cc.	600 c.c.
Total blood drawn	2,037 c.c.	2,046 c.c.	1,835 c.c.
Ratio of volumes	2.79	2.13	3.06
Weights, July 8	10.5 kg.	11.8 kg.	8.5 kg.
Weights, July 10	10.7 kg.	12.5 kg.	8.0 kg.
Weights, July 15	11.2 kg.	12.8 kg.	8.2 kg.
Blood count (millions) July 8	4.5	5.3	
Blood count (millions) July 9	4.6	5.9	6.5
Blood count (millions) July 13	4.2	5.5	5.1
Blood count (millions) July 16	5.2	6.2	5.8
Haemoglobin, July 8	52 %	74 %	
Haemoglobin, July 9	52 %	65 %	78 %
Haemoglobin, July 13	60 %	80 %	79 %
Haemoglobin, July 16	65 %	79 %	80 %
Weights, July 22	11.0 kg.	13.35 kg.	8.5 kg.
Blood count, July 22	4.1	5.4	5.3
Haemoglobin, July 22	72 %	80 %	75 %

Studies have also been made of blood pressure and blood counts. Plasmapheresis, like hemorrhage, causes a seemingly paradoxical increase in the number of red cells per unit volume of blood. This, which appears to be a general phenomenon accompanying temporary asphyxia, is being investigated in all its bearings by Dr. Lamson.[14] The blood pressure, which falls on bleeding, is restored to a satisfactory value on returning the corpuscles and, for a long period the two changes may nearly compensate each other. A slight downward tendency is noticed, however, as plasmapheresis is continued, and in the end a dangerously low point (about 50 mm.) will be reached on withdrawing amounts of blood considerably smaller than those taken at the start. The previous bleeding usually shows a fall to a point (from 60 to 80 mm.) which should be regarded as a warning, even though 100 mm. or more may be reached on reinjection.

The following tables give in condensed form some of the data to which reference has been made in the foregoing pages.

TABLE II

Chemical Analyses. Plasmapheresis for one day, onthree dogs

	Date					
	Dec. 21		Jan. 11		Jan. 18	
Weight of dog	15.1 kg.		11.9 kg.		7.1 kg.	
Blood volume estimated at 7.5%	1,132 c.c.		892 c.c.		532 c.c.	
Total volume bled and % of total blood	1,185 c.c. = 105%		1,150 c.c. = 129%		410 c.c. = 77%	
Number of bleedings	3		3		5	
Results of analyses, percentages	Before plasmapheresis	After plasmapheresis	Before plasmapheresis	After plasmapheresis	Before plasmapheresis	After plasmapheresis
Total protein of blood	22.27	25.23	25.15	25.32	17.34	17.19
Protein of plasma	6.62	3.63	6.59	3.17	6.28	3.68
Difference of above[15]	15.65	21.60	18.56	22.15	11.06	13.51
Blood counts, millions	9.0	11.7	11.8	12.9	6.6	7.5
Total non-proteid N	0.037	0.039	0.029	0.036	0.045	0.055
Urea nitrogen	0.016	0.019	0.011	0.017	0.016	0.023
Non-urea nitrogen	0.021	0.020	0.018	0.021	0.029	0.032
Aminonitrogen	0.0041	......	......	......	0.0048	0.0048

[14] "Polycythemia," P. D. Lamson, *Jour. Pharm. and Expt. Ther.*, VII., No. 1, July, 1915.

[15] Blood taken for analysis not included unless made up by equal amount of washed corpuscles from other dogs.

TABLE III

Continued Plasmapheresis on a Dog for Five Successive Days

Exp. No. 6, Jan. 22 to 26, inclusive. *A* before plasmapheresis, *B* after plasmapheresis.

Weight of dog 8.5 kg. Estimated blood volume (7.5 per cent.) = 640 c.c. Total blood removed in five days, 3,335 c.c. = 521 per cent. Analytical results in percentage of total blood.

	Date.				
	A Jan. 22	*B* Jan. 22	*B* Jan. 24	*B* Jan. 26	Feb. 19
Total protein of blood	19.28	19.31	16.81	15.83	11.78
Plasma protein	6.38	3.44	2.23	2.92	5.75
Difference of above	12.90	15.87	14.58	12.91	6.03
Blood count millions	8.5	8.5	7.5	6.5	3.5
Total non-protein nitrogen	0.035	0.040	0.037	0.042	0.030
Urea nitrogen	0.013	0.019	0.014	0.021	0.012
Amino nitrogen	0.0047	0.0056	0.0033	0.0059	0.0038

The results obtained in continued plasmapheresis are shown in Table III. The amount of blood taken was about one volume on each day. The first two columns of analytical results, obtained with samples taken at the beginning and end of the day's work, compare closely with those in Table I. The third column shows the results at the end of the *third* day's work, when the plasma protein reached the lowest value, 2.23 per cent. The fourth column gives the results at the end of five days of plasmapheresis, while the last column shows the results twenty-four days later. Here the plasma protein has gone up again nearly to its original value. The corpuscle protein, and consequently the total protein, also, are low, owing to the anemia.

VIVIDIFFUSION

I should like now to describe a second method for the study of the blood, and to state briefly some of the results that have already been obtained by its use. But first let me remind you that there are numerous constituents of the blood derived from various organs which are of the most vital significance to the economy and which are present in the blood in only minute quantity at any one time. Among these as yet unidentified substances, which nevertheless are certainly known to pass from one organ to others via the blood, are all of the so-called hormones, the active principles of the organs of internal secretion. Of these organs I shall presently speak more in detail.

Influence of Plasmapheresis on Blood Pressure

Mean Systolic Pressures in Millimeters of Mercury

Exp. No. 6, Jan. 22 to 26, 1915, inclusive.

Day of Expt.	Volume Bled, C.c.	Bleeding		Return	
		Before	After	Before	After
1st	250	208	165	...	202
	250	115	65	95	135
	170	140	65	85	115
2d	200				
	125	pressures not observed on this day.			
	170				
3d	195	130	...	...	110
	210	130	60	...	110
	200	110	55	70	110
	185	135	50	...	105
4th	205	135	100	110	135
	200	135	105	100	120
	200	115	60	80	105
	135	95	50	75	105
5th	180	...	105	100	110
	190	120	52	65	105
	175	100	50	47	95
				1 hr. later =	110

Our present methods of blood analysis give us little help when we endeavor to *isolate and identify* one of these elusive yet vitally important principles, not to mention other substances of the greatest interest arising in the intermediary stages of metabolism.

Pondering over this problem, it occurred to me that possibly one might construct an apparatus which could be attached to the blood vessels of a living animal and remove from the blood flowing through it all traces of these substances as fast as they are poured into it, without at the same time removing proteids or the indispensable cellular elements (erythrocytes, leucocytes, etc.) of the blood. Such an apparatus might conceivably be employed also in an emergency in certain toxic states in which the eliminating organs, more especially the kidneys, can not act rapidly enough to relieve the system.

An apparatus of this kind was constructed with the skilful assistance of Dr. Turner. Essentially, the method consists in connecting an artery or a vein of the animal by a cannula to an apparatus made of celloidin or other dialyzing membrane, in the form of tubes, immersed in a saline solution or serum, and providing for the return of the blood to the animal's body by another cannula attached to a vein. The tubes and cannulæ are filled completely before attachment with a saline solution which approximates in composition to the salt content of the serum of the animal. This is displaced into the body by the inflow of blood, when the circulation in the apparatus is established. The blood leaving the artery flows through a perfectly closed system and returns to the body within a minute or two without having been exposed to contact with the air or any chance of microbial infection, while the diffusible substances which it contains can pass out, more or less rapidly, through the walls of the tubes. Coagulation of the blood is prevented by injection of hirudin. We have named the process vividiffusion and the apparatus itself constitutes an "artificial kidney," as it were, but differs from the natural organ in that it makes no distinction whatever between the various diffusible constituents of the blood, permitting their escape from the celloidin tubes in a manner which is presumably proportional to their coefficients of diffusion. As you are well aware, the natural kidney does not ordinarily allow the sugar of the blood to escape into the urine, its excretory function is elective and discriminatory. The artificial kidney, as just stated, makes no such distinction. Sugar is eliminated in proportion to its presence in the blood equally with a waste product like urea. We have it in our power, however, to give to this vividiffusion apparatus a certain selective ability, at least in the sense that we can prevent any given substances, as sugar, glycocoll, and the like, from escaping from the blood, by the simple expedient of placing an equivalent quantity on the outer side of the celloidin tubes.

With this apparatus we have already separated from the blood a number of constituents which can not be obtained with equal ease by other methods. I shall not here enter into the details of the chemical methods employed in differentiating the various constituents of the dialysate, but will merely point out some of the results that we have obtained.[16] It has been found:

1. That the non-protein constituents of the blood can be accumulated in any desired quantity by our method, the quantity depending on the extent of the dialyzing surface of our apparatus and the number of experiments made.

2. That the rate of accumulation of various nitrogenous substances in the dialysate and their relative proportions in it do not differ very greatly from those in the blood.

3. That alanine and valine can be obtained in crystalline form; that histidine and creatinine can be shown by reactions to be present.

4. Quite recently it has been found by

[16] See *Jour. of Pharmacology and Experimental Therapeutics,* Vol. V., pp. 275–317 and 625–44.

Dr. Alice Rohde, working in my laboratory, that the ammonia-yielding substances of the blood can be divided into two classes by the vividiffusion apparatus; the one, comprised of diffusible substances only and giving off their ammonia rapidly and completely on the addition of sodium carbonate; the other, non-diffusible and therefore not escaping through our apparatus, and characterized by the property of losing their ammonia only very slowly on the addition of sodium carbonate.

5. By means of our method of vividiffusion we have also found that oxyacids circulate in the blood in noticeable proportion. Lactic acid and β-oxybutyric acid in particular have been identified as constituents of the diffusate.

6. From the residue from one of the processes employed, that known as the "ester distillation," I obtained a crystalline substance having the composition $C_7H_{12}N_2O_2$. Dr. Turner and I were finally enabled to identify this substance as α-isobutyl hydantoin (1. isobutyl 2.4 diketotetrahydroimidazol) first prepared by Pinner and Lifschütz[17] and later by Fritz Lippich[18] from valeraldehydecyanhydrin and urea, also by E. Koenigs and B. Mylo[19] from *dl*leucinamid and ethylchlorcarbonate. I suspect that other hydantoins are present in the fraction from which this particular hydantoin was isolated. As α-isobutyl hydantoin is the first of its class to be isolated from an animal fluid or tissue, one must be certain that the substance has not been formed as a by-product of the many chemical processes that are involved in obtaining it; in other words, one is obliged to prove conclusively that the substance in question really exists, as such, in the blood of the dog. For the present we can not offer this final proof. Dr. Turner, however, is now engaged in searching for hydantoins in the blood of the pig by a method that will remove the uncertainty that still attaches to the find as it now stands.

7. Certain fractions of our dialysates, those derived from the so-called "phosphotungstic precipitate," have not yet been analyzed in detail, owing to the pressure of other parts of the problem; it is apparent, however, that we are dealing with an indeterminate number of substances, and it is more than probable that some hitherto unidentified constituents of the blood may here be found.

Half a year after we made our first communication[20] in which it was announced that we had separated from our dialysates several grams of amino-acid esters, Abderhalden[21] published a paper in which he describes the separation of some of the amino acids from large quantities of blood obtained from slaughter-houses. To secure the small amounts of amino acids needed for his identification tests this investigator was obliged to use at one time 50 or even 100 liters of beef blood. These large quantities of blood were worked up partly by dialysis, partly by precipitation methods which required the dilution of the blood by many volumes of water. The method of vividiffusion can be used in the most scantily equipped laboratory and has the great advantage of separating the diffusible substances from the proteids of the *circulating blood of living animals.* There can thus be no question here of secondary

[17] *Ber. d. d. chem. Ges.*, 20, p. 2,356 (1887).

[18] *Ibid.*, 41, p. 2,972 (1908).

[19] *Ibid.*, 41, p. 4,439 (1908).

[20] "On the Removal of Diffusible Substances from the Circulating Blood by Means of Dialysis," *Trans. Assoc. Americ. Physicians,* May, 1913. Also demonstration of our apparatus before the Pharmacological Section, Int. Med. Congress at London, August, 1913.

[21] *Zeitschr. f. physiol. Chemie,* Vol. 88, p. 478, December 23, 1913.

changes, such as may conceivably take place in shed and coagulated blood.

I come now to a newer application of the method of vividiffusion, one to which I alluded a few moments ago, namely its possible employment to abstract from the circulating blood certain hormones or products of internal secretion in amounts that will suffice for pharmacological study, if not for chemical analysis. This application is still in its very beginning, many difficulties yet remain to be surmounted, and I speak of it here only because it leads me quite naturally to a field of study which is of the greatest importance, a field which at present is ripe for the methods of the chemical explorer. I refer to the exploration of the organs of internal secretion, especially to the study by chemical methods of their specific products. In attempting this, a vividiffusion apparatus of the proper sort is attached to the veins of a particular organ, as the thyroid gland, and the diffusate thus obtained is studied by pharmacological and chemical methods. This diffusate must also be compared in respect to its pharmacological properties, at least, with both the arterial and the venous blood of the gland under investigation. But whatever may be the outcome of such studies, I hope to make it evident to you in what I am about to say that we are here dealing with matters of the greatest importance to biology and medicine.

JOHN J. ABEL
THE JOHNS HOPKINS MEDICAL SCHOOL

(*To be continued*)

THE RECENT ACTIVITY OF KILAUEA AND MAUNA LOA, HAWAII

THE volcanoes of Kilauea and Mauna Loa were both active during the past winter, furnishing the rather unusual spectacle of lava lakes within 22 miles of each other, but at a difference in altitude of practically 10,000 feet. The activity of Mauna Loa, as observed from Kilauea, lasted forty-eight days, from November 25, 1914, until January 11, 1915. At Kilauea, the first permanent open pool of magma was formed on October 3, 1914, and this pool increased in size and rose until a maximum height of 363 feet below the rim of the crater Halemaumau was reached on January 4, 1915. Since that time the lake has been slowly subsiding, with temporary rises and pauses. The activity of both volcanoes will be treated in some detail.[1]

The lava lake in Halemaumau, the crater of Kilauea, was visible from the autumn of 1907, which was six months after the cessation of activity on Mauna Loa, until the last of April, 1913. The maximum height reached during this long interval was 60 feet below the rim of the crater on January 1, 1912. During the autumn of 1912 the level of the lake was low, followed by a rise from November, 1912, until January, 1913. During January the lake sank; in February it was at about the same level as in the beginning of March, 1915; in March it sank almost out of sight after a brief rise on March 10; in April the lake was very low and very small, and finally went out.

During the summer of 1913, Halemaumau was an immense flat funnel of slide rock with the base at a depth of over 600 feet. The bottom of the pit was seldom seen on account of the dense fumes. Just before the time of the summer solstice, which reached a culmination on June 22, blowing noises were heard and a faint glow seen in the pit, indicating a slight rise of the lava. In the middle of July, the blowing noises recommenced and visible fire returned on July 23. An inner ring of fuming cones and a glowing chimney near the site of Old Faithful were seen. Once more the signs of activity ceased: the blowing noises did not recommence until the first of September, and a glowing cone near Old Faithful became visible September 24. The glow and the noises

[1] Weekly bulletins concerning the activity of the volcanoes, written by Professor T. A. Jaggar, Jr., and Mr. H. O. Wood, of the Hawaiian Volcano Observatory, are published by the Volcano Research Association. From these bulletins the data for the present paper, previous to the arrival of the writer at the volcano, are obtained. To Mr. H. O. Wood, and to Mr. Arthur Hannon, the writer is indebted for criticism.