tinctly double. The H.A.B. was in the fifth space, 6 in. from the middle line. At the apex the first sound was reduplicated; there were no murmurs. The systolic blood pressure was on an average 170 mm. Hg. *Post mortem* the pericardium was normal, the heart weighed 24 oz., and the muscle substance was firm. The arteries showed little or no degeneration although the left heart was chiefly enlarged; mitral and tricuspid rings were dilated. The tracings from H.A.B. and three arteries show the same time relations throughout. The possibility of the second wave being of dicrotic origin is negatived by this, and by the fact that the second wave reached the superficial temporal artery before the second sound was audible.

The tracing shown in Fig. 7, A, was taken by means of a light straw lever from the jugular vein. It shows the single auricular impulse preceding the double beat of the carotid. The tracing excludes the possibility of a double auricular beat.

Fig. 8 is from a case of severe mitral stenosis of rheumatic origin in a lad, aged 21. He was admitted with marked cyanosis and dropsy, and a large wavy double heart impulse over the fifth to seventh spaces extending 7 in. from the middle line. The blood pressure varied from 90 to 117 mm. for different beats, the pulse being irregular. Originally the pulse was interpreted as dicrotic, but more careful examination proved it to be of the bisferient type, for the second sound was audible an instant later than the upstroke of second wave, also the dicrotic wave is apparent in the last half of each curve.

The type of *pulsus bisferiens* described in the last two cases is uncommon, and is worthy of more attention. No account of it has been found in the literature; possibly it has been mistaken for dicrotism. The type is very different from that depicted in an earlier part of this contribution, and it is notable that in both cases it was unassociated with arterial sclerosis. The cases strongly support the hypothesis of the *deux temps* beat of the ventricle, advocated by D'Espine⁴; it is indeed the only rational interpretation of the tracings. As two cases alone have been met with, it is not possible to draw any further conclusions as to its origin or prognostic significance. Its recognition is of importance in that it is indistinguishable from a dicrotic pulse by palpation.

In conclusion, there appears only one constant relationship with the *pulsus bisferiens*, and that, a greatly dilated and hypertrophied left ventricle; there appear to be two types, the one associated the other unassociated with arterio-sclerosis.

REFERENCES.

- ¹ Broadbent, *The Pulse*, London, Paris, etc., 1890, pp. 26 and 200.
- ² Walter Broadbent, *BRITISH MEDICAL JOURNAL*, January, 1899, i, p. 75.
- ³ Clarke, *Lancet*, December, 1894.
- ⁴ D'Espine, *Revue de Médecine*, 1882.
- ⁵ Galabin, *Journal of Anatomy and Physiology*, vol. viii and vol. x; *Med.-Chir. Trans.*, vol. lix, 1876, p. 361.
- ⁶ Lewis, *Lancet*, September 15th, 1906, ii; *Practitioner*, February, 1907; *Journ. of Anat. and Physiol.*, January, 1907; *Journ. Phys.*, 1906, p. 391.
- ⁷ Mackenzie, *The Study of the Pulse*, Edinburgh and London, 1902.
- ⁸ Mahomed, *Medical Times and Gazette*, 1872, i and ii; 1873, ii; *Guy's Hospital Reports*, 1879.
- ⁹ Marey, *La circulation du sang*, Paris, 1881; *Physiologie Expérimentale*, Tome i to iii, Paris, 1875.
- ¹⁰ Morrison, *Lancet*, 1894, ii, p. 1532.
- ¹¹ Steell, *The Sphygmograph in Clinical Medicine*, Manchester, 1899; *Lancet*, 1894, ii, p. 1206; *Medical Chronicle*, 1888.

ON THE PART PLAYED BY THE CALCIUM SALTS IN THE BLOOD AND TISSUES,

WITH SPECIAL REFERENCE TO THEIR INFLUENCE IN REGARD TO THE FEMALE GENITAL FUNCTIONS; TOGETHER WITH A DESCRIPTION OF A SIMPLE METHOD OF QUANTITATIVE ANALYSIS.

By W. BLAIR BELL, M.D., B.S.LOND.,
ASSISTANT GYNAECOLOGICAL SURGEON, ROYAL INFIRMARY, LIVERPOOL.

[PRELIMINARY COMMUNICATION.]

A YEAR ago I made a short communication,¹ based entirely on clinical observation, in which I expressed my belief that the uterus took an active part in the genital functions of the female, and was not merely a passive receptacle for the fertilized ovum. I then stated that I looked upon the uterus as an organ whose glands secreted some substance which, for the want of a better or more accurate name, I called "uterin," and which, when in excess, was excreted, producing in some way the menstrual cycle. I further stated that "uterin" circulated in the blood, and produced many of the phenomena common to women, such as the manifestations of the menopause.

Since that time I have been following out the lines of investigation then suggested, and although in this preliminary communication I can only indicate briefly some

of the results obtained, I shall describe fully the method by which I have been able to demonstrate them, and point out in what directions these investigations open up possibilities for further research in branches of medicine other than that in which I am specially interested. Much of this is already in hand, and will I hope be carried out by those who are more competent to deal with it than I am.

It was only after much thought that an explanation of the probable nature of the substance secreted by the uterine glands dawned upon me. Finding no clue from a chemical consideration of the phenomena associated with the function, I turned my thoughts to comparative physiology, and there at once I found the explanation I was seeking—the oviducts of birds secrete calcium salts. Why should not those of the higher animals do likewise?

The more I thought the matter over the more it seemed to fit in with many of the hitherto inexplicable phenomena to be daily observed by any one dealing with women. To take but one example: During lactation, when the woman is giving away to her infant large quantities of lime salts in her milk, the menstrual function is normally in abeyance.

So far I had only the hypothesis, and in my attempt to obtain definite proof I was confronted with a serious obstacle: There was no known method for estimating small quantities of calcium salts in the blood, secretions, and tissues. At least a litre of blood would be necessary for incineration and analysis; even then only a very skilful chemist could be relied upon to give an approximately accurate estimate.

It was necessary, however, to have a method at once simple and accurate that could be used for clinical investigation. I have therefore devised the following, whereby in about half an hour I can calculate the amount of calcium in any solution, in however small a quantity it may be present.

Method as Applied to Blood.

A small capsule (Fig. 1) containing 250 c.mm. of oxalic acid (1 in 30 aqueous solution) sealed at the end of the stem is taken to the bedside of the patient whose blood is to be examined.

N.B.—In Fig. 2 the drawing should show a longer stem between the rubber ball and the largest bulb. This stem should also be marked to indicate the 500 c.mm. level.

When all is ready the stem is broken at the mark A, care having been taken first to shake all the oxalic acid solution into the bulb, and the finger or lobe of the patient's ear pricked; 100 c.mm. of blood are then drawn into a graduated pipette (Fig. 2), and immediately emptied into the oxalic acid by passing the fine stem of the pipette down the neck of the capsule. The stem (or neck) of the capsule is then sealed. The contents on shaking become uniformly chocolate-coloured, and the blood is laked. After ten minutes this mixture is ready for further dilution, but if necessary the final processes may be left over for some hours, although the sooner they are carried

out the better, for if left until the following day the heavy sediment of pigment may vitiate the result.

When the examination is to be continued, the capsule, after the fluid has been well shaken down into the bulb, is broken at the mark B, and 250 c.mm. of the following mixture added to the oxalic acid and blood:

Acetic acid (1 per cent.) 95 parts.
Glycerine ... 5 "

This is allowed to stand for another ten minutes, a rubber cap being placed over the broken end of the stem to prevent evaporation. The capsule is then thoroughly shaken, and 100 c.mm. are withdrawn and immediately mixed in another capsule with 500 c.mm. of distilled water previously placed there. It is of this last mixture that the examination is made.

A drop is placed on an ordinary Thoma haemocytometer plate, which must be carefully examined microscopically before each experiment to see that it is clean, and the crystals of calcium oxalate are counted and noted for each square. At least 250 squares should be counted.

I have found that a standard aqueous solution of 1 in 5,000 calcium chloride, which is practically equivalent to a solution of 1 in 10,000 CaO, gives 0.59 crystals per square. I have therefore taken as my standard that an average of 1 crystal per square is equivalent to a 1 in 6,000 solution of CaO.*

In ordinary individuals of middle life I find the normal, as estimated from this standard, varies from 1 to 4,000 to 1 to 5,000 of CaO in the blood.

The count is a perfectly easy matter, as the above dilutions result in a faintly-coloured solution affording a background against which the crystals show up well. A few hints as to details of technique are necessary.

Tap water must, of course, be avoided entirely in all washings throughout the whole of the procedures, and only reliable distilled water used. I have had several calculations entirely upset by impure distilled water. The greatest delicacy and accuracy are required in the measuring of the quantities; for to obtain an accurate estimate accurate dilutions are necessary. The special pipette (Fig. 2) which I use has three bulbs and a fine stem; it is accurately marked for all the necessary quantities.† (Three separate pipettes may be used if desired; they have the advantage of cheapness.) If the blood clots in the pipette it can be cleaned out with a strand of fine silkworm gut.

An artificial light is much better than daylight for distinguishing the crystals, and the diaphragm must be almost closed.

For counting, a magnification of 450 diameters should be used. Only those crystals which can be definitely seen should be counted. The fine adjustment should be moved to and fro in order to get a bright speck of light followed by a dark spot.

In blood solutions the crystals are very evenly distributed, are usually of uniform size, and do not lodge in the lines of the haemocytometer plate; they are, too, until they have been on the plate for some little time, continually undergoing "Brownian" movements. This is especially the case if the plate is not completely covered with the drop. They should not be counted, therefore, until they have been on the plate for a few minutes.

In aqueous solutions the crystals are rather more uneven, not being held so well in suspension, and tend to collect in the lines. Only those should be counted which are clearly discernible, and are not fixed in the lines in such a way as to prevent them from giving the bright and dark spot on focussing, and render their identity uncertain.

The above remarks indicate that crystals should be counted in a thicker medium than water, and I am now making experiments in this direction. Chemists will, no doubt, adopt this suggestion, if they feel disposed to estimate dilute solutions by this method, and avoid pure aqueous solutions, which, although they give rise to some difficulty and would be apt to lead those who were inexperienced into error, can be estimated as I have stated above, if sufficient care be taken.

There are two other points. In albuminous solutions, and especially with the use of glycerine (vide second solution above), clumping of the crystals does not occur unless the mixture be allowed to stand too long before examination.

In very strong solutions (1 in 1,500 and stronger) the crystals are sometimes uneven in size. This difficulty may be surmounted by diluting the liquid to be examined four or more times with normal saline solution before adding it to the oxalic acid. This can be effected in the three-bulb pipette. An allowance, of course, should be made in the final calculation, for the same quantity (100 c.mm.) of the diluted fluid is used as though it had not been diluted.

* The atomic weight of one molecule of calcium chloride (CaCl_2) is 111; that of CaO, 56. This latter is, therefore, practically half the molecular weight of CaCl_2 . But each molecule contains the same weight of Ca, and so a 1 in 5,000 solution of CaCl_2 is equivalent to a 1 in 10,000 solution of CaO as regards the amount of Ca present. Consequently, if the above solutions give an average of 0.59 (which we may take as 0.6) crystals per square, a 1 in 6,000 solution of CaO will give an average of 1 crystal per square. When we speak of the calcium content of the blood as being 1 in 4,000 or 1 in 5,000, we mean one part by weight of CaO to so many thousand parts by weight of blood, whatever the latter number may be.

† The apparatus required can all be obtained from Mr. Hawksley, 357, Oxford Street, London, who has made it for me.

This is the method recently alluded to by Sir James Barr,² and I believe it to be accurate in all respects. I hope, however, to improve the technique still further, and perhaps place the normal calcium index at 1. At present, if an average of one crystal per square indicates a solution of 1 in 6,000 of CaO, the normal calcium index is an average of 1.5 (1 in 4,000) to 1.2 (1 in 5,000). The dilutions now used, however, are so simple that I am not inclined to alter them, since the calculation is a very straightforward matter, the formula being $\frac{\text{calcium index}}{6,000} = \text{CaO content}$.

It will occur to every one that the crystals might be estimated in bulk in a capillary tube by centrifugalization; but this, in dealing with blood and similar fluids, introduces so many complications in the way of obviating the deposit of pigment and white corpuscles, not to mention the difference of "packing" of the crystals according to the revolutions, time employed, and density of the medium, that I have not thought it advisable at present to do otherwise than estimate the calcium by the simple method above described, which can be carried out without laboratory assistance.

It remains, however, to prove that the objects which are counted consist of calcium oxalate in a crystalline form.

This is a somewhat difficult matter owing to the small size of the objects and the small quantity available.

The following facts are therefore submitted as forming a conclusive chain of evidence on this point:

1. When in excess many are of the definite "envelope" type, while the rest assume the recognized polymorphous (dumb-bell or irregular octohedral) shapes.
2. A comparison (with a magnification of 880 diameters) of these crystals with those obtained from an aqueous solution of calcium chloride of a strength similar to that in the blood gave an identical result as regards the appearance and number of the crystals.
3. The crystals do not stain.
4. On standing for twenty-four hours the crystals "clump," forming definite crystalline masses, which are easily recognized.
5. The crystals are insoluble in acetic acid (20 per cent.), but are soluble in hydrochloric acid.
6. With a small quantity of crystals obtained (from hydrometra fluid) and dissolved in hydrochloric acid, the typical flame coloration was observed, but there was not a sufficient quantity for spectrum analysis.
7. As far as can be ascertained, oxalic acid or the oxalates alone produce these crystals in the blood.
8. By adding to the blood taken an equal quantity of calcium chloride solution equivalent to a solution of calcium oxide, 1 in 5,000, a corresponding increase of similar crystals was observed.

Having thus got my method, I was able to settle, as I believe, the question of the secretion of the uterine glands by an examination of the fluid in an artificial hydrometra. The fluid obtained from this source gave an estimate of CaO, 1 in 350! Since they will be published later, I need not here enter into the experiments made with reference to the blood of menstruating women and the menstrual fluid, it is sufficient to state they are of a highly interesting and confirmatory nature. Nor shall I discuss now the probable part the ovary plays in the cyclical event. A large number of experiments are at present proceeding to the complete elucidation of the part played by the calcium salts in this and other directions.

Taking a broad survey, I would enunciate the belief that there are three calcium periods in a physiological sense. First, the period of growth, when the young person or animal requires all the calcium salts obtainable for the formation and growth of the bony skeleton. Secondly, the period of reproduction, when a calcium equilibrium as regards growth is reached and an "excretion" of the excess occurs. This is most obvious in the reproductive processes of women, when large quantities of the calcium salts are first given up to the fetus and then for the formation of milk, during which the menstrual function is in abeyance. Some observations I have made seem to show that pregnancy is terminated when the fetus ceases to absorb (or receive) calcium salts from the mother's blood and a large accumulation occurs in her system, bringing about contraction of the uterine muscles. The third period is that of late life, when no calcium salts are required for the building-up processes or for those of reproduction. These salts then accumulate in the tissues, especially in the vessels (and it is interesting to note how

rare this is in women in middle life), atrophy occurs, and the individual gradually declines.

Apart from the physiological investigations above mentioned, observations are being carried out, at my suggestion, by the above method in the following directions:

The relation of the calcium salts to infective processes, acute and chronic, especially with regard to the temperatures, to the opsonic power of the blood and to the fibrosis which results from chronic toxæmias. This investigation will undoubtedly prove the value of calcium salts in certain types of pneumonia and similar affections.

The influence of menstruation on the opsonic power of the blood from this standpoint.

An estimation of the lime salts in the blood in bone diseases, such as rickets and osteomalacia.

The relation of the calcium salts to the thyroid secretion and their bearing on thyroid disease. Owing to the frequent occurrence of this disease in women and the relation often seen to the genital functions important results should be obtained.

The significance of the calcium salts in neuroses and in such diseases as spasmodic asthma and urticaria.

I am myself making a series of observations on the disorders of menstruation, on the neuroses of women, and on the diseases of pregnancy, such as vomiting, chorea, eclampsia, and thrombosis, many of which conditions seem undoubtedly to have a definite relationship to a disordered calcium economy on the part of the patient.

Also, in conjunction with Professor J. E. S. Moore, I am carrying out a clinical and experimental investigation into the influence the calcium salts may have in the onset of cancer and the cancer period.

I have much hesitation in putting forward this formidable list, on which some work has been done already, lest I should appear to overstate the importance of the subject. I should not now have had the courage to do so had I not had, and did I not still hope to have, the generous advice, co-operation, and assistance of my colleagues and others interested in these researches. In this connexion I should like to express my obligations to Sir James Barr, Professor Benjamin Moore, Dr. Grimsdale, and Dr. Pantland Hick.

REFERENCES.

¹ Liverpool Medical Institution, May 3rd, 1906, vide *Liverpool Medical-Chirurgical Journal*, July, 1906. ² BRITISH MEDICAL JOURNAL, March 23rd, 1907, p. 717: On the Use of Calcium Salts in Pneumonia.

FURTHER OBSERVATIONS ON THE ETIOLOGY AND PATHOLOGY OF PEPTIC ULCER.*

By FENTON B. TURCK, M.D.,

DIRECTOR OF THE TURCK INSTITUTE; PROFESSOR IN THE POST-GRADUATE MEDICAL SCHOOL, CHICAGO.

(From the Research Laboratory of the Turck Institute.)

INTRODUCTION.

THE present communication on the etiology of peptic ulcer presents two phases, developed by the author's experimental researches on animals. By feeding animals, principally dogs, with a certain strain of bouillon cultures of *Bacillus coli communis* for several months, spontaneous or induced, genuine peptic ulcers of the stomach or duodenum were found in every animal experimented upon, the ulcers causing death, either by perforation or hæmorrhage. Certain changes in the blood serum and cellular changes found indicated a general process characterised by diminution of the normal protective bodies, resulting in autoctolysis. The blood changes and general condition seemed to be produced by the absorption of products formed in the gastro-intestinal tract. Histological examinations of the induced peptic ulcers showed no round-cell infiltration or other evidences of a healing process.†

In the first series death occurred from perforation or hæmorrhage; in the second series the feeding of bouillon

of *B. coli* was stopped as soon as there appeared indications of the formation of the ulcer. Then allowing sufficient time to elapse for healing to take place, the animal was killed. On *post-mortem* examination we found round-cell infiltration and cicatrization of the incomplete ulcers, in various stages of healing, thus further showing the close relation between the feeding experiments with bouillon cultures of *B. coli communis* and spontaneous or induced peptic ulcers in animals.

Controls were made by observation on 271 dogs, 189 of which were healthy and were killed at the dog-pound, and the other 82 dogs that had died from various diseases—injury, poison, etc. On *post-mortem* examination no peptic ulcers were found in the stomach or intestines of any of these 271 dogs, showing that the occurrence of peptic ulcers both in the healthy street dogs and diseased animals is at least exceedingly rare.

HISTORICAL.

The peculiar type of the round peptic ulcer confined principally to the stomach and duodenum early led to the conclusion that the ulcers were the result of autodigestion of a portion of the mucous membrane rendered susceptible by some unknown cause. That the living healthy mucous membrane could not be digested was explained by Stahl as due to the protection offered by the "sensitive soul." Hunter's living principle displaced the idea of the "sensitive soul," and with the development of cellular pathology we acquired the idea of the vital resisting power of the tissues, which is yet used in pathological expressions. The vital resistance of the tissues is represented in more modern terms by Weinland, who holds that an antipepsin ferment residing in, and a part of, the gland cells protects them from destruction. It is easy to understand why so much experimental work has been done to produce a lessening of the resistance of the tissue by diminishing or destroying the vitality of local areas of the mucous membrane of the stomach. These experimental investigations, clinical observations, and *post-mortem* examinations may be grouped as follows:

(1) Attempts to produce ulcers by mechanical and physical injury, such as those by Ritter, Decker, Matthes, Schmidt, Korte, and others; (2) by chemical injury, such as employed by Roth, Matthes, Schmidt, and Ewald; (3) by inducing blood changes, thus lessening resistance, such as hæmoglobinaemia, by the methods employed by Silberman, Cohnheim, and Fütterer; (4) disturbances of the local circulation to conform to Virchow's idea of embolism and thrombi were worked out by Panum, Klebs, and Welti, Talma, Rindfleisch, and Müller; (5) injuries to nerves and nerve centres, such as accomplished by Schiff, Ebstein, Brown-Séquard, Vedora, Yzeren, Saitta, Ophüls, Roch, and Ewald; (6) local infection was studied by Cohnheim, Bottcher, and Nauwerck.

Ulcers were produced in many cases; but these artificial methods were either no other than would cause ulcer formation in any part of the body, or they were foreign to the conditions present in ulcer of the stomach in man.

EXPERIMENTS.

With some modifications I have carried on similar experiments in attempting to produce artificial ulcers, following out at first the methods of mechanical and chemical irritation, later taking up injections of toxins, then the question of metabolic disturbances, and, finally, the problem of the genuine induced peptic ulcer. Mechanical and chemical irritation failed to produce ulcers, even with such radical means as the feeding on mustard oil for fourteen months. The possibility of systemic disturbances, altered metabolism, impaired nutrition, being etiologic factors in the production of ulcer led to the experiment of confining animals in very close quarters for a long period of time. Of the 96 guinea-pigs and 36 rabbits used in this experiment, all the rabbits died, while 6 guinea-pigs survived nine months of confinement. Two genuine peptic ulcers were found in the 6 surviving guinea-pigs.

These induced ulcers, produced for the first time in the long series of experiments, suggested the possibility that systemic conditions were important factors; that alteration in the toxic state of the alimentary canal, with consequent changes in the blood, might play some rôle in the formation of the ulcer. To modify, then, the bacterial status of the alimentary tract, experiments were begun by

* Presented before the Section of Pathology and Bacteriology, British Medical Association, Toronto, August, 1906.

† The full report, with protocols, physiological examinations and micro-photographs, was presented at the International Medical Congress, Lisbon, 1906, and published in the *Journ. Amer. Med. Assoc.*, June 9th, 1906, vol. xlv, pp. 1753-1763.