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SOME OBSERVATIONS ON HÆMALKALIMETRY IN MALIGNANT DISEASE.

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The alkalinity of the blood has long been known, but the fact of its clinical significance has only been recognised comparatively recently. Since this recognition the blood in many conditions, both physiological and pathological, has been tested and its reaction estimated quantitatively and qualitatively.

The methods employed have varied greatly, not only as to the means of determining the reaction but also as to the part of the blood used, whole blood, plasma, Serum, and laked blood have all been tried, the results obtained being naturally very diverse. The earliest attempts at estimating the reaction were chiefly qualitative, the most important of which may be mentioned shortly.

a. KUHNE'S METHOD (I) The blood was dialysed through filter paper into water, the water was then tested with litmus paper.

b. LIEBREICH'S METHOD (2). Slabs of Plaster of Paris soaked with neutral litmus were prepared, the blood was dropped on to a slab and a few seconds after the corpuscles were washed away and the colour of the stain remaining gave the reaction.

c. ZUNTZ'S METHOD (3). Glazed litmus paper soaked in a solution of Sodium Chloride was wet with the blood, this was almost immediately washed away with salt solution and any change in the colour of the paper was noted.

It will be noticed that in each of these three methods an attempt was made to get rid of the corpuscles which owing to their colour interfered with the reading considerably.

The quantitative methods are much more numerous, many of them being only slight modifications of ones previously described. One of the earliest was that of Zuntz (I868) (4). Special dilutions of phosphoric acid were used with neutral litmus, the blood being estimated titrametrically. Lassar (I874) (5) used the same method substituting tartaric for phosphoric acid. Landois (6) in I885, introduced a method suitable for clinical work, a Lassar solution of tartaric acid and a saturated solution of sodium sulphate were

The Nos: in Brackets refer to the list of references on page NoI6.

mixed in varying proportions. Small quantities of blood were mixed with these fluids in turn,using capillary tubes, and the results tested with the finest litmus paper. Von Jaksch (I887) (7) modified this process slightly, he drew blood by wet cupping and mixed definite quantities of it very rapidly with the test fluids in glasses, the resulting mixtures were tested with blue and red litmus papers till the neutral one was found.

Haycraft and Williams (I888) (8) used glazed litmus paper containing different strengths of oxalic or some other acid. The blood was dropped on these papers in turn left for tenseconds and then wiped off, the first one showing a blue colour being taken as the index. Drouin (I892) (9) wrote a Thesis on the reaction of the blood, and he advised the use of Landois' method, substituting oxalic for tartaric acid. In I895 Kraus (IO) took steps to prevent the coagulation of the blood by defibrinating it, this was then mixed with ten volumes of a one per cent solution of sodium chloride and allowed to stand in a narrow glass while the corpuscles fell to the bottom, the supernatant fluid was then titrated against I/IO hydrochloric acid, litmus being the indicator.

In all the above methods,except the last mentioned one, the whole blood was employed, the presence of the cells as has already been noted, giving rise to difficulties in reading the indicator In addition to Liebreich and Zuntz, Landois and Drouin tried to escape the difficulty, they diluted the blood with solutions of various salts, and then suspended pieces of litmus paper in the dilutions so that the fluid part alone might creep up by capillarity, this was not satisfactory either, because the cells ascended also and gave rise to the same difficulty

Von Limbec and Steindler (I895) (II) pointed out how fallacious the results obtained by estimating the plasma were, because a varying quantity of alkali got into the fluid from the corpuscles and so destroyed the possibility of any trustworthy figures being recorded. These same observers made a series of estimations of the serum exuded after spontaneous coagulation. The highest result in their series was in a case of carcinoma of the rectum, but no special attention seems to have been drawn thereto. In I897, Wright (I2) published a method suitable for

clinical purposes in which he tested the serum exuded by spontaneous coagulation. The serum having been allowed to stand for a definite number of hours, in order to come to an equilibrium, was then titrated against various dilutions of normal sulphuric acid and the mixtures tested with very sensitive litmus paper. Wright pointed out that after shedding, the alkalinity of the serum changes rapidly but comes to an equilibrium in anything between three and twentyfour hours. The serum was selected by him , as against the whole blood, because the red cells did not interfere with the result and also because the serum is more important clinically as it is the fluid which comes in contact with the cells themselves.

During the last ten years a number of methods have been devised, some new, but most of them modifications of previous ones. Strauss (I3) Engel (I4) and Salkowski (I5) tried using laked blood and got much higher degrees of alkalescence than were obtained by the methods already quoted . Moore and Wilson (I6) in I906 published a series of observations on Na emalkalimetry in obtaining which they used a modification of Wright's method . The results obtained with litmus paper as the indicator were not sufficiently accurate,partly on account of the difficulty of reading the indicator and partly because "neutrality to litmus does not correspond to any definite point in the titration of carbonates and phosphates" (Moore and Wilson). The indicators phenolphthalein and dimethyl-amido-azo-benzol(called di-methyl hereafter for the sake of brevity) were therefore used instead. "Phenolphthalein gives neutrality with carbonates exactly at the point where bicarbonate is present, and with phosphates at the point where the secondary phosphate ($M_2H.PO_4$) alone is present, while dimethyl changes colour at a point where all the carbonate is neutralised and when phosphate is present as primary phosphate (MH_2PO_4)" (Moore and Wilson)

It is not difficult to see that the results obtained by the above alkalimetric methods are not measures of the true alkalinity of the fluid tested, but are what Moore calls "the total or potential alkalinity". The blood is only an alkaline fluid in the sense that it contains bases which are joined with the very weak acids, and as such it has the faculty of yielding up these bases to weak acids, this is

what occurs when the fluid is tested with indicators. An indicator is really an acid substance which possesses the power of combining with bases to form a distinctly coloured salt. When an acid is added to the blood and the result tested with an indicator, what is being determined is the amount of acid necessary to combine strongly with the bases already present in weak combination and so prevent the indicator from withdrawing them.

In order to estimate the "active alkalinity" of the blood at a given moment, the concentration of the hydrogen and hydroxyl ions must be determined, but owing to the exceedingly small variations in the ionic concentration which are compatible with the physiological and pathological cell life, the physical methods at present known fail, hence the titration methods still remain. Incomplete and inaccurate though the results of the above methods are, yet the figures obtained seem to fall somewhere near the right points, and the results obtained therefrom are of considerable clinical interest and importance.

In I905 (I7) and I906(I8) Moore, Alexander, Kelly and Roaf, published the result of their investigation into the acidity of the gastric secretions in malignant disease situated in any part of the body. They found a practically constant reduction if not complete absence of the acidity in their cases and in seeking a possible cause therefore they found the blood serum in these diseases to be more alkaline than normal. In I906 Moore and Wilson (I9) described the method of clinical Haemalkalimetry already mentioned, and with it were given the results of their observations on the alkalinity of the whole serum and of the inorganic Salts of the serum in malignant disease. Stated shortly their results were as follows:- (a) The acidity of the whole serum to phenolphthalein did not show any definite alteration. (b) The alkalinity of the whole serum to dimethyl showed a striking increase as compared with healthy subjects and with other nonmalignant hospital patients. (c) The "basic reactivity" to dimethyl of the inorganic salts of the serum, after removal of the proteins by incineration, showed a small but distinct increase. The term "reactivity" was used by Moore and Wilson in these papers to denote the results obtained by titration with acids and indicators, which as have already been shown, are not indications of the true neutral point,

alkalinity or acidity.

-.OBJECTS OF THIS INVESTIGATION.-

The perusal of the reports of Moore and Wilson's work on the reaction of the serum in malignant disease, suggested that enquiry into the following points might prove interesting and possibly profitable.

I. If the "basic reactivity" of the whole serum to dimethyl is so constantly increased in malignant disease, could its estimation be made use of clinically as a means of differential diagnosis in doubtful cases?

2. Whether the basic reactivity increases with the duration and severity of the disease.

3. Whether after operation and removal of the growth, there is any reduction in the alkalinity or not. This would give an answer to the question as to whether the increased alkalinity is due to some action on the part of the cancer cells, or whether the growth of the cancer is favoured by the state of the serum.

-.METHOD EMPLOYED.-

The mode of procedure followed in estimating the alkalinity in the hereafter cited cases was practically that described by Moore and Wilson (I9). The alkalinity of the whole serum to dimethyl has been estimated in all the cases, the acidity to phenolphthalein was not tested as the results therewith lack definition. The estimation of the "reactivity" of the inorganic salts of the serum would have been done had facilities therefore presented themselves.

-.TECHNIQUE.-

SOLUTIONS NECESSARY.- Dilutions of normal Sulphuric Acid ranging from $\frac{N}{8}$ $\frac{N}{7\cdot5}$ $\frac{N}{7}$ . . . $\frac{N}{3}$. To each I00cc of these dilutions 8 drops of a I% alcoholic solution of dimethyl were added (2I).

APPARATUS NECESSARY.- Wright's glass capsules for collecting the blood, glass capillary pipettes, watch glasses, an opaque white glass slab for mixing on. The pipettes can be quite easily drawn over a bunsen burner,using 5/I6" tubing, the same applies to the capsules, only a smaller tubing is more convenient for them. On account of the alkali present in the glass all tubing etc

employed in the process must be carefully washed with strong hydrochloric acid,rinsed repeatedly with distilled water and dried in an oven at 120°C in order to drive off any residual acid. The glass slab and watch glasses should be similarly washed with distilled water and dried before using each day.

COLLECTING THE BLOOD.- Owing to the occurrence of the "alkaline wave" following the ingestion of food, blood must not be collected until three hours after a meal and if possible always at the same hour of the day. The subject for examination should not have had any violent exercise just previous to having his blood taken. A bandage is tied round the wrist just sufficiently tight to constrict the superficial veins, but not tight enough to cause a marked venous congestion as the reaction of the blood is apt to be altered thereby. The thumb is cleansed and pricked with a suitable pricker and a sufficient quantity of blood collected in a glass capsule the ends of which are then sealed in a flame. The capsule is put in an upright position and left for twentyfour hours in order to allow the clot to contract and squeeze out the serum and also to allow the serum to come to an alkaline equilibrium. Care must be taken in filling the capsule that the blood remains at one end of it, as the serum is apt to be opalescent and coloured if the blood is allowed to spread over the rest of the surface..

METHOD OF TITRATION.- The capsule is first of all centrifugalised in order to drive the clot to the extreme end of it, and leave the clear serum superimposed, a nick is made in the capsule with a file, above the level of the fluid, and the top broken off. The bottles having been well shaken small quantities of the mixtures of acid and indicator are poured into watch glasses. A suitable pipette is then selected and mark made on it with a blue pencil about 2cms from the point. The titration is greatly facilitated by the use of an ordinary rubber teat on the top end of the pipette. The pipette is dipped into the serum and the fluid allowed to run up to the mark, it is then withdrawn, and by means of the rubber teat the serum is drawn a short distance further up the tube, thereby introducing a small quantity of air at the point. The pipette is next dipped into one of the

watch glasses containing the mixture and this is allowed to run up to the mark, the air bubble being between the two columns of fluid acting as a separator and index. The contents of the tube are then blown out onto the slab and mixed thoroughly by being aspirated in and out of the capillary tube several times. If a yellow coloured fluid results a stronger acid is taken until an orange red is given, then the dilution of acid, which last gave the yellow colour is the index. If instead a pink or orange colour results from the first titration, then a weaker acid is used until a yellow colour free from red is given. The first dilution of acid which gives the yellow colour is the index.

A convenient dilution to commence at in undoubted malignant cases is $\frac{N}{6}$ and in doubtful and nonmalignant cases $\frac{N}{7}$ will be found suitable. A new pipette should be used for each test, the trouble of making new ones being considerably less than that of cleaning and drying the old ones.

As far as possible all the following estimations were carried out under exactly similar conditions, the great majority of the patients were in bed, and all of them were bled between I2-30 p.m. and I.p.m., just before dinner , and three hours after the previous meal. The capsules were left for twentyfour hours, and were then dentrifugalised and the test done.

-.T A B L E. I.-

. N O R M A L C A S E S

NUMBER	SEX	AGE	ALKALINITY TO DIMETHYL EXPRESSED AS A FRACTION OF NORMAL
I	F	26.	0·I66 Normal
2	M	22.	0·I82 :
3	M	24.	0·I82 :
4	F	23.	0·I82 :
5	M	26.	0·I66 :
6	F	25.	0·I66 :
7	F	22.	0·I54 :
8	F	20.	0·I66 :
9	M	25.	0·I66. :

AVERAGE ..0·I70 normal

-.T A B L E. I I.-

NONMALIGNANT HOSPITAL CASES

NUMBER	SEX.	AGE	DISEASE	ALKALINITY TO DIMETHYL EXPRESSED AS A FRACTION OF NORMAL
I	M	25.	Psoas Abscess	0·I82 Normal
2	M	48.	Comp: Fract: of Femur	0·I66 :
3	M	5I.	Fistula in Ano	0·I66 :
4	M	38.	Comp: Fract: of Arm.	0·I82 :
5	F	42.	Old Comp: Fract: of Leg	0·I82 :
6	F	60.	Fractured Spine.	0·I82 :
7	F	33.	Gastric Ulcer	0·I54 :
8	F	23.	Pott's Disease.	0·I66 :

AVERAGE .. 0·I72 normal

-.T A B L E. I I I.-

MALIGNANT CASES PREVIOUS TO OPERATION.

NUMBER.	SEX.	AGE.	DISEASE.	ALKALINITY TO DIMETHYL EXPRESSED AS A FRACTION OF NORMAL
I	F	43.	Carcinoma Uteri	0·222 Normal
2	F	50.	Carcinoma Uteri	0·200 :
3	F	57.	Carcinoma Mammae	0·222 :
4	F	56.	Paget's Mipple	0·250 :
5	F	45.	Carcinoma Uteri	0·250 :
6	M	34.	Carcinoma Recti	0·200 :
7	M	53.	Epithelioma of Tongue	0·222 :
8	M	64.	Epithelioma of Tongue	0·222 :
9	M	50.	Recurrent Carcinomatous Glands	0·200 :
IO.	F	45.	Carcinoma Uteri	0·200 :
II.	M	52.	Carcinoma Recti	0·200 :
I2.	M	46.	Epithelioma of Tongue	0·222 :
I3.	F	34.	Carcinoma Sigmoid	0·222 :
I4.	M	4I.	Carcinoma Caecum	0·200 :
I5.	F	53.	Carcinoma Uteri	0·200 :
I6.	F	45.	Carcinoma Recti	0·200 :
I7	F	42.	Carcinoma Uteri	0·200 :
I8.	M	53.	Carcinoma of neck	0·200 :
I9.	F	5I.	Carcinoma Mammae	0·I82 :
20.	F	34.	Carcinoma of Colon	0·200 :
2I.	M	64.	Carcinoma of Oesaphagus.	0·200. :

AVERAGE ..0·2I0 normal

-.R E M A R K S.-

Case No I9 shows a lower alkalinity than any of the others, possibly the fact that the tumour which was cystic had burst some weeks previous to coming into Hospital and had been infected with pyogenic organisms may have influenced the reaction . There was no doubt as to the diagnosis on microscopic examination.

-.T A B L E. I V.-

INOPERABLE MALIGNANT CASES .

NUMBER	SEX.	AGE	DISEASE	ALKALINITY TO DIMETHYL EXPRESSED AS A . . FRACTIO N OF NORMAL
I	F	56.	Carcinoma Recti	0·200 Normal
2	M	56.	Epithelioma of Mouth	0·250 :
3	M	46.	Epithelioma of Jaw	0·200 :
4	F	40.	Carcinoma Uteri	0·250 :
5	F	50.	Carcinoma Mammae	0·222 :
6	F	45.	Recurrent Carcinoma Mammae	0·222 :
7	M	62.	Epithelioma of Neck	0·200 :
8	F	44.	Carcinoma Uteri	0·I82 :
9	F	40.	Recurrent Carcinoma Mammae	0·200 :
IO	M	67.	Carcinoma Recti (Colotomy)	0·I54 :

AVERAGE 0.208 Normal

-.R E M A R K S.-

These cases were not very advanced nor yet were they of very long duration, but on account of their site, tissues involved, and other factors they were deemed inoperable.

Case No IO was estimated just twentyfour hours after a colotomy had been done under a general anaesthetic and unfortunately opportunity did not arise for repeating the test. Possibly this low alkalinity was due to some acid intoxication. Bainbridge (20) remarks that no estimations of the alkalinity of the blood have been made in cases of post anaesthetic acetonuria, and as no note was made on the condition of the urine in this case after operation, nothing more than a suggestion can be made as to the possibility of this low reading being due to an acidosis following an anaesthetic.

-.T A B L E. V.-

ADVANCED CASES OF MALIGNANT DISEASE.

NUMBER,	SEX.	AGE.	DURATION	DISEASE.	ALKALINITY TO DIMETHYL EXPRESSED AS A FRACTION OF NORMAL
I	M	7I.	I. Year.	Carcinoma Recti	0·200 Normal
2	F	45.	I0.Mos:	Carcinoma Uteri	0·200 :
3	M	55.	I5.Mos:	Carcinoma of Neck	0·I82 :
4	M	72.	3. Mos: ?	Epithelioma of Mouth	0·I82 :
5	M	65.	3. Mos:	Carcinoma of Antrum	0·200 :
6	M	50.	I2.Mos:	Carcinoma of Sup:Maxilla	0·I82 :
7	M	6I	6. Mos:	Carcinoma Linguae	0.I66 :
8	F	74.	8. Mos:	Carcinoma of Ethmoid	0·I54 :
9	F	6I.	6. Years	Epithelioma of Face	0·I66 :
I0.	F	79.	2I.Mos:	Epithelioma of Cheek	0·I66 :
				AVERAGE ..	0·I79 Normal

-.R E M A R K S.-

All these cases were very advanced both as to the local condition and also the metastases. The patients themselves were markedly cachectic in most instances. The last two cases Nos 9 and I0 were very extensive spreading ulcers involving a considerable portion of the face. It is recognised that the alkalinity of the serum is diminished in the state of cachexia,more especially of course in the various chronic acid intoxications, it seems therefore that the low readings found in these malignant cases associated with marked cachexia may be the result of reduced alkalinity of cachexia antagonising the increased alkalinity of malignant disease, and so producing a reading which is practically normal.

-.T A B L E. VI.

CASES OF SARCOMA.

NUMBER.	SEX.	AGE.	LOCALITY	ALKALINITY TO DIMETHYL EXPRESSED AS A FRACTION OF NORMAL
I	M	3I	Mediastinum	0·222 Normal
2	F	35.	Abdomen	0·I82 :
3	M	I2.	Abdomen	0·222 :
4	F	44.	Axilla	0·200 :
5	M	37.	Testicle.	0·200 :

AVERAGE .. 0·205 normal

-. T A B L E. VII.-

CASES WHERE DIAGNOSIS WAS DOUBTFUL.

NUMBER.	SEX.	AGE.	DISEASE.	ALKALINITY TO DIMETHYL EXPRESSED AS A FRACTION OF NORMAL	OPINION GIVEN & RESULT
I	M	46	Gumma or Epithelioma of Tongue ?	0·222 Normal	+ Epithelioma
2	F	58	Distended Gall Bladder	0·I66 :	- Simple
3	F	34	Pyo Salpinx or Carcinoma of Sigmoid	0·200 :	+ Carcinoma
4	F	37	Possible Deciduoma Malignum	0·I66 :	- Simple
5	M	33	Carcinoma of Abdomen?	0·I66 :	- Tubercle
6	M	62	Enlarged Prostate	0·I82 :	$\frac{-}{+}$ Simple
7.	M.	55.	Swelling in Neck	0·200 :	+ Malignant
8.	M.	4I.	Swelling in Appendix region	0·200 :	+ Malignant
9.	M.	64.	Stricture of Oesphagus	0·200 :	+ Malignant
IO.	M.	55.	Carcinoma of Prostate?	0·I66 :	- Tubercle
II.	M.	3I.	Tumour of Mediastinum	0·222 :	+ Sarcoma
I2	F	44.	Swelling in Axilla	0·200 :	+ Sarcoma
I3.	F.	5I.	Cyst of Breast (Septic)	0·I82 :	$\frac{-}{+}$ Malignant

-.R E M A R K S.-

These cases in which the diagnosis was doubtful, were estimated either before operation or before the diagnosis was settled either by the microscope or by the progressof the case. The signs + and - represent the opinion given as the result of the estimation, + meaning malignant - meaning nonmalignant and $\frac{-}{+}$ meaning that no definite opinion could be given on account of the doubtful figure obtained.

-.T A B L E. V I I I.-

-.MALIGNANT CASES BEFORE and AFTER OPERATION.-

NUMBER.	SEX.	AGE.	DISEASE	ALKALINITY TO DIMETHYL EXPRESSED AS A FRACTION OF NORMAL	
				BEFORE	AFTER
I	M	53	Epithelioma of Tongue	0·222 Normal.	0·222 Norma
2	F	43	Carcinoma Uteri	0·250 :	0·222 :
3	F	56	Paget's Nipple	0·250 :	0·250 :
4	F	57	Carcinoma Mammae	0·222 :	0·222 :
5	F	35	Sarcoma of Abdomen	0·I82 :	0·I82 :
6	M	52	Carcinoma Recti	0·200 :	0·200 :
7	F	53	Carcinoma Mammae	0·200 :	0·200 :
8	M	45.	Epithelioma of Lip	0·200 :	0·200 :
			AVERAGE ..	0·2I5 :	0·2I2 :

-.R E M A R K S.-

Case No 2 was the only one which showed any post operative change, but as this was the only one and the change was so slight, no great stress can be laid thereon, any one of the various causes of acidosis might have been responsible therefore.

Case No 5 was being treated with injections of Coley's fluid,but the result was not good. Wherever the fluid was injected the tumour seemed to soften and grow smaller, but as the growth was diffused all over the peritoneum obviously every part of it could not be so treated. The period intervening between the operation and the second estimation varied from a fortnight up to three months, and in one or two cases the blood was examined several times during the interval but showed no change.

In addition to the above,four cases of carcinoma undergoing X Ray treatment were estimated, and although the local conditions were improving and any pain experienced was subsiding, yet the serum after two or three months showed no change.

Three cases of carcinoma in which the liver was the seat of growth giving rise to jaundice were tried. The serum was of a bright yellow colour and on titrating with $\frac{N}{3}$ H_2SO_4 + Dimethyl the resulting colour was still yellow. This might be due to the presence of the bile salts in

the circulation or possibly in part due to the bile pigment masking the colour of the Dimethyl.

-.SUMMARY OF AVERAGES.-

9. Normal Cases ..	..	0·I70 Normal
8. Nonmalignant Hospital Cases	..	0·I72 :
2I.Operable Malignant Cases	..	0·2I0 :
I0.Inoperable Malignant Cases	..	0·208 :
I0.Advanced Malignant Cases	..	0·I79 :
5. Cases of Sarcoma..	..	0·205 :

-.C O N C L U S I O N S.-

I. The alkalinity of the serum to dimethyl in malignant disease shows a marked increase as compared with that in healthy cases and in nonmalignant Hospital cases.

2. The difference between those suitable for operation and those slightly more advanced is very small.

3. Im very advanced cases of carcinoma with marked cachexia the alkalinity is only slightly above normal, case No 7, Table Vlll and case No 3, Table V, were the same patient, the interval between the examinations being four months, during which period the growth increased markedly and he became very emaciated, the alkalinity had fallen from 0·200 normal to 0·I82 normal in that time. This fall in the alkalinity in these cases is probably due to the patients' cachectic condition as mentioned above.

4. In cases of sarcoma the alkalinity is increased, but not quite so much as in the early carcinomatous ones.

5. Neither removal of the growths by operation nor treatment with X Rays seems to produce any appreciable alteration in the alkalinity of the serum. This fact tends to prove that the alteration in the serum favours the growth of malignant tumours rather than results from the growth.

6. In <u>early</u> cases of malignant disease an estimation of the alkalinity of the serum should be of diagnostic import, provided that none of the other known causes of increased or decreased alkalinity are present.

Judging from the above stated cases the only difficulty arose when the figure 0·I82 resulted from the test, that degree of the alkalinity being occasionally found in early malignant and also in nonmalignant cases.

-.R E F E R E N C E S.-

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2. Berichte der Deutschen chemischen Gesellschaft Band i, p.48. I868

3. Centralblatt für die medic Wissenschaften V53I, and 80I I867

4. Beiträge z Physiologie d.Blutes I868, p.I3

5. Archiv für die gesammte Physiologie Band IXVIII, I874

6. Réal Encyclopédie iii, 2nd edition, I885

7. Zeitschrift für klin med. XIII 350, I887

8. Proceeds of Royal Society Edinburgh, June I8, I888

9. Drouin Hémo-alcalimétrie et Hémo-acidimétrie.

I0. Archiv f. exp. Path. u. Pharmak. Bd. XV.

II. Centralblatt f. innere Medicin. No 27. I895

I2. Lancet Sept:I8th I897

I3. Strauss Zeitschr f. Klinische Medicin, XXX. 3I7. I895

I4. Engel Berliner Klin Wochenschr XXXV, 308, I898

I5. Salkowski Centralblatt fur die Medic. Wissenschaften XXXVI,9I3, I898

I6. Biochemical Journal June I906, Vol: I, No 7

I7. Proceeds of Royal Society I905, Vol:96, p.I38.

I8. Biochemical Journal June I906, Vol:1, No 6.

I9. Biochemical Journal,June I906, Vol:1, No 7.

20. Lancet March 28th, I908. p.9I5.

2I. The dilutions employed were prepared by Messrs Philip Harris & Co Ltd., Scientific Chemists, of Birmingham.