THE REACTION OF THE BLOOD SERUM IN MALIGNANT DISEASE.¹

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IN 1905 and 1906, Moore, Alexander, Kelly and Roaf (1905²) published the result of their investigation into the acidity of the gastric secretions in malignant disease situated in any part of the body. Their results, since confirmed by Palmer, showed a practically constant reduction, if not complete absence, of the acidity in their cases, and in seeking a possible cause therefor they found the blood serum in these diseases to be more alkaline than normal. In 1906 Moore and Wilson (1906³) described the modification of Wright's method of measuring the alkalinity of the serum which I have used in the present research (see below), 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-amido-azo-benzol showed a striking increase as compared with healthy subjects and with non-malignant hospital patients; (c) the "basic reactivity" to dimethyl-amido-azo-benzol of the inorganic salts of the serum, after removal of the proteins by incineration, showed a small but distinct increase.

OBJECTS OF THIS INVESTIGATION.

This work suggested that inquiry into the following points might prove of interest:—

1. If the "basic reactivity" of the whole serum to dimethyl-amido-azo-benzol 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 indication as to whether the increased alkalinity is due to some action on the

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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 of the serum in the following cases was practically that described by Moore and Wilson (1906³).

Solutions necessary.—Dilutions of normal sulphuric acid ranging from $\frac{N}{8}$, $\frac{N}{7.5}$, $\frac{N}{7}$, $\dots$, $\frac{N}{3}$. To each 100 c.c. of these dilutions, 8 drops of a 1 per cent. alcoholic solution of dimethyl-amido-azo-benzol were added.

Apparatus necessary.—Wright's glass capsules for collecting the blood, glass capillary pipettes, watch glasses, and an opaque white glass slab for mixing on. 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 each day before using.

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. 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 twenty-four 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 (Wright, 1897⁵).

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 a mark made on it with a blue pencil about 2 c.m. from the point. 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 farther 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 on to 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, and the dilution of acid which last gave the yellow colour is taken as the index. If, on the other hand, 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, and 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 non-malignant cases $\frac{N}{7}$ will be found suitable.

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 12.30 p.m. and

1 p.m., just before dinner and three hours after the previous meal. The capsules were left for twenty-four hours, and were then centrifugalised and the test done.

RESULTS OBTAINED.

The results obtained by this method are given in the following tables, which show that the alkalinity of normal blood is definitely less than in cases of malignant disease both before and after operation.

TABLE I.—*Normal Cases.*

Number.	Sex.	Age.	Alkalinity expressed as a Fraction of Normal.
1 . . .	F.	26	0·166 normal.
2 . . .	M.	22	0·182 „
3 . . .	M.	24	0·182 „
4 . . .	F.	23	0·182 „
5 . . .	M.	26	0·166 „
6 . . .	F.	25	0·166 „
7 . . .	F.	22	0·154 „
8 . . .	F.	20	0·166 „
9 . . .	M.	25	0·166 „
			Average, 0·170 „

TABLE II.—*Non-malignant Hospital Cases.*

Number.	Sex.	Age.	Disease.	Alkalinity expressed as a Fraction of Normal.
1 . .	M.	25	Psoas abscess	0·182 norm
2 . .	M.	48	Compound fracture of femur . .	0·166 „
3 . .	M.	51	Fistula in ano	0·166 „
4 . .	M.	38	Compound fracture of arm . .	0·182 „
5 . .	F.	42	Old compound fracture of leg . .	0·182 „
6 . .	F.	60	Fractured spine	0·182 „
7 . .	F.	33	Gastric ulcer	0·154 „
8 . .	F.	23	Pott's disease	0·166 „
			Average,	0·172 „

TABLE III.—*Malignant Cases previous to Operation.*

Number.	Sex.	Age.	Disease.	Alkalinity expressed as a Fraction of Normal.
1 . .	F.	43	Carcinoma uteri	0·222 normal.
2 . .	F.	50	„ uteri	0·200 „
3 . .	F.	57	„ mammæ	0·222 „
4 . .	F.	56	Paget's nipple	0·250 „
5 . .	F.	43	Carcinoma uteri	0·250 „
6 . .	M.	34	„ recti	0·200 „
7 . .	M.	53	Epithelioma of tongue	0·222 „
8 . .	M.	64	„ of tongue	0·222 „
9 . .	M.	50	Recurrent carcinomatous glands	0·200 „
10 . .	F.	45	Carcinoma uteri	0·200 „
11 . .	M.	52	„ recti	0·200 „
12 . .	M.	46	Epithelioma of tongue	0·222 „
13 . .	F.	34	Carcinoma of sigmoid	0·222 „
14 . .	M.	41	„ of cæcum	0·200 „
15 . .	F.	53	„ uteri	0·200 „
16 . .	F.	45	„ recti	0·200 „
17 . .	F.	42	„ uteri	0·200 „
18 . .	M.	53	„ of neck	0·200 „
19 . .	F.	51	„ mammæ	0·182 „
20 . .	F.	34	„ of colon	0·200 „
21 . .	M.	64	„ of œsophagus	0·200 „
Average,				0·210 „

Case No. 19 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.

TABLE IV.—*Inoperable Malignant Cases.*

Number.	Sex.	Age.	Disease.	Alkalinity expressed as a Fraction of Normal.
1 . .	F.	56	Carcinoma recti	0·200 normal.
2 . .	M.	56	Epithelioma of mouth	0·250 „
3 . .	M.	46	„ of jaw	0·200 „
4 . .	F.	40	Carcinoma uteri	0·250 „
5 . .	F.	50	„ mammæ	0·222 „
6 . .	F.	45	Recurrent carcinoma mammæ	0·222 „
7 . .	M.	53	Carcinoma of neck	0·200 „
8 . .	F.	44	„ uteri	0·182 „
9 . .	F.	40	Recurrent carcinoma mammæ	0·200 „
10 . .	M.	67	Carcinoma recti (colotomy)	0·154 „
Average,				0·208 „

The cases in Table IV. were neither very advanced nor of very long duration, but on account of their site, tissues involved, and other factors they were deemed inoperable. Case No. 10 was estimated

just twenty-four hours after a colotomy had been done under a general anæsthetic, and unfortunately opportunity did not arise for repeating the test. Possibly this low alkalinity was due to some acid intoxication. Bainbridge (1908¹) remarks that no estimations of the alkalinity of the blood have been made in cases of post-anæsthetic 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 anæsthetic.

TABLE V.—*Advanced Cases of Malignant Disease.*

Number.	Sex.	Age.	Duration.	Disease.	Alkalinity expressed as a Fraction of Normal.
1	M.	71	1 year	Carcinoma recti	0·200 normal.
2	F.	45	10 months	„ uteri	0·200 „
3	M.	53	15 „	„ of neck	0·182 „
4	M.	72	3 „ (?)	Epithelioma of mouth	0·182 „
5	M.	65	3 „	Carcinoma of antrum	0·200 „
6	M.	50	12 „	„ of superior maxilla . .	0·182 „
7	M.	61	6 „	„ linguae	0·166 „
8	F.	74	8 „	„ of ethmoid	0·154 „
9	F.	61	6 years	Epithelioma of face	0·166 „
10	F.	79	21 months	„ of cheek	0·166 „
Average,					0·179 normal.

All these cases were very advanced both as to the local condition and as to the metastases. The patients themselves were in most instances markedly cachectic. Cases 9 and 10 had very extensive spreading ulcers involving a considerable portion of the face. Case 3 is the same patient as Case 7, Table IV.; during the four months which intervened between the two examinations the growth had advanced markedly and the emaciation of the patient had much increased. It is recognised that the alkalinity of the serum is diminished in the state of cachexia, more especially 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.

TABLE VI.—*Cases of Sarcoma.*

Number.	Sex.	Age.	Locality.	Alkalinity expressed as a Fraction of Normal.
1 . . .	M.	31	Mediastinum	0·222 normal.
2 . . .	F.	35	Abdomen	0·182 „
3 . . .	M.	12	„	0·222 „
4 . . .	F.	44	Axilla	0·200 „
5 . . .	M.	37	Testicle	0·200 „
				Average, 0·205 normal.

TABLE VII.—*Cases where Diagnosis was Doubtful.*

Number.	Sex.	Age.	Disease.	Alkalinity expressed as a Fraction of Normal.	Real Nature of Disease.
1	M.	46	Gumma or epithelioma of tongue (?)	0·222 normal	Epithelioma.
2	F.	58	Distended gall-bladder . .	0·166 „	Simple.
3	F.	34	Pyo-salpinx or carcinoma of sigmoid	0·200 „	Carcinoma.
4	F.	37	Possible deciduoma malignum .	0·166 „	Simple.
5	M.	33	Carcinoma of abdomen . .	0·166 „	Tubercle
6	M.	62	Enlarged prostate . . .	0·182 „	Simple.
7	M.	53	Swelling in neck . . .	0·200 „	Malignant.
8	M.	41	Swelling in appendix region .	0·200 „	„
9	M.	64	Stricture of œsophagus . .	0·200 „	„
10	M.	53	Carcinoma of prostate (?) . .	0·166 „	Tubercle.
11	M.	31	Tumour of mediastinum . .	0·222 „	Sarcoma.
12	F.	44	Swelling in axilla . . .	0·200 „	„
13	F.	51	Cyst of breast (septic) . .	0·182 „	Malignant.

These cases in which the diagnosis was doubtful were estimated either before operation or before the diagnosis was settled by the microscope or by the progress of the case. It will be noted that all the cases in which the end point was reached with acid of at least $\frac{N}{5}$ strength eventually turned out to be malignant, and five out of the six in which only $\frac{N}{6}$ or $\frac{N}{5\cdot5}$ acid was required were simple.

TABLE VIII.—*Malignant Cases before and after Operation.*

Number.	Sex.	Age.	Disease.	Alkalinity expressed as a Fraction of Normal.	
				Before.	After.
1	M.	53	Epithelioma of tongue . .	0·222 normal.	0·222 normal.
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 . . .	0·182 "	0·182 "
6	M.	52	Carcinoma recti . . .	0·200 "	0·200 "
7	F.	53	" mammae . . .	0·200 "	0·200 "
8	M.	45	Epithelioma of lip . . .	0·200 "	0·200 "
Average,				0·215 "	0·212 "

Case 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. Case 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 examined, 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 of the liver with jaundice were tried. The serum was of a bright yellow colour, and on titrating with $\frac{N}{8}$ H_2SO_4 with dimethyl-amido-azo-benzol 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-amido-azo-benzol.

Summary of Averages.

9 normal cases . . .	0·170 normal
8 non-malignant hospital cases . .	0·172 "
21 operable malignant cases . . .	0·210 "
10 inoperable malignant cases . .	0·208 "
10 advanced malignant cases . . .	0·179 "
5 cases of sarcoma . . .	0·205 "

CONCLUSIONS.

1. The alkalinity of the serum to dimethyl-amido-azo-benzol in malignant disease shows a marked increase as compared with that in healthy cases and in non-malignant hospital cases.

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

3. In very advanced cases of carcinoma with marked cachexia the average alkalinity is hardly above normal.

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 their presence.

6. In *early* 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 0.182 resulted from the test, that degree of the alkalinity being occasionally found in early malignant and also in non-malignant cases.

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