CCVI. INVESTIGATIONS OF BLOOD-SERUM LIPOIDS IN CANCER AND OTHER CASES.

I. THE MEAN MOLECULAR WEIGHT OF THE FREE AND COMBINED ACIDS IN THE BLOOD-SERUM LIPOIDS OF CANCER AND OTHER SUBJECTS.

By HAROLD HAYDEN BARBER.

II. ULTRAVIOLET ABSORPTION MEASUREMENTS.

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I. THE MEAN MOLECULAR WEIGHT OF THE FREE AND COMBINED ACIDS IN THE BLOOD-SERUM LIPOIDS OF CANCER AND OTHER SUBJECTS.

THE work described in the following section is an examination of the method published by von Noël [1931] as a means of diagnosing cancer from the chemical analysis of serum "fat". The underlying principle on which the method is based is the assumption that in cases of cancer, although the depot fat remains of normal composition, the serum fat will show the presence of acids of a mean molecular weight lower than that normally obtained.

The reason put forward in support of this view [Dannmeyer & Seel, 1931] is based on the well-known theory of Warburg *et al.* that there is an increased formation of such substances as lactic, pyruvic and acetoacetic acids as a result of deficient oxidation of carbohydrate in the diseased tissue cells.

The serum lipoids actually employed are those obtainable by extraction of the dried serum with ether followed by extraction with an alcohol-ether solution containing 2% ethyl alcohol. For the chemical analysis the several ether extracts are mixed with the alcoholic ether extracts and the total residue examined; but in the present work, in those cases where the physical method described in Part II was employed, the alcoholic ether extract was the fraction supplied for the physical examination, and this solution on its return was added to the ether extract for the chemical tests.

The chemical examination consists simply of the determination of the acid value and the ester value of the extract, and from the results of these two determinations the "Krebszahl" [von Noël, 1931] of the specimen is calculated. This "cancer number" is based on the assumption that the normal depot fats have a mean equivalent weight of 280, so that for saponification 1 ml. of 0.1 *N* alkali solution is equivalent to 28 mg. of fat. If the number of ml. of 0.1 *N* alkali solution required to saponify 100 mg. of the unknown sample of fat is multiplied by 28, the

resulting figure (the Krebszahl) will be very near to 100 if the fat is normal depot fat, but will be greater than 100 if fatty acids of lower molecular weight preponderate.

The Krebszahl is given by the expression

$$\text{Krebszahl} = \frac{100 (a + b) 28}{\text{wt. of substance in mg.}},$$

where a = ml. of 0.1 N solution to neutralize free acids and b = ml. of 0.1 N solution to saponify the neutralized fat.

It is also assumed that there is no significant variation in the proportion of the unsaponifiable fraction in the cancer and non-cancer specimens.

An interesting fact not mentioned by von Noël is the simple numerical relationship between the Krebszahl and the ordinary saponification value; the latter, based on a molecular weight for KOH of 56.1, is almost exactly twice (2.004) the former.

From the results of the examination of over 80 blood samples from normal, cancer and other cases, von Noël suggested a critical value of 130 for the Krebszahl, all values above this were claimed to be characteristic of cancer, whereas normal cases and cases of tuberculosis, diabetes, arteriosclerosis, benign tumour etc. gave values below 130.

In order to test this claim more than 40 specimens of blood from cancer and other cases have been examined by the above technique, and in order further to simplify the conditions the majority of the non-cancer cases selected were as near to normal as possible whereas many of the cancer cases were in an advanced stage of disease.

With the exception of the cases A, B and C, the blood specimens were kindly supplied by Mr F. C. Hunt from the Nottingham General Hospital, and with the above exception the details in Table I were also furnished by him.

The specimens were taken by venepuncture practically all after an overnight fast of 15 hours.

EXPERIMENTAL.

The blood specimens were defibrinated by means of a stick in the test-tube used for receiving the sample. They were then transferred to the laboratory, centrifuged in graduated 15 ml. tubes and a definite volume of the serum removed by means of a pipette. To the sample of serum in a small beaker, twice the volume of saturated zinc sulphate solution was added and, after mixing, the contents were poured on to a weighed amount of A.R. anhydrous sodium sulphate contained in a porcelain dish. The weight in grams of sodium sulphate was equal to four times the volume in ml. of the original serum. This proportion was found to cause the resulting paste to solidify when the dish was left overnight in a desiccator.

Next morning the solid mass was transferred to a glazed porcelain mortar and roughly powdered by hand. The pestle was then clamped in an automatic grinding device, electrically driven, which ground the mass to a fine powder in 30 min. The powder was transferred to a 750 ml. conical separating funnel fitted with a plug of fat-free cotton wool in the apex, and the contents were covered with dry ether and left overnight. (The ether used throughout this work was of B.P. (sp. gr. 0.720) quality which had been dried over sodium wire and distilled from further sodium.) The following morning the solution was run out of the funnel into a flask, the last runnings being blown out by means of dry oxygen-free compressed nitrogen. The pasty residue in the funnel was then shaken with a

second quantity of ether which was immediately blown out into the first extract. The residue was dug out of the funnel into the mortar, a little more (about 10%) anhydrous sodium sulphate added, and the whole reground for half an hour. The powder was then returned to the funnel, covered with ether and again allowed to stand overnight. Next day the previous day's procedure was repeated. On the third morning of the ether extractions the first ether extract only was added to all the previous extracts.

Thus if $a \equiv$ addition of sodium sulphate and grinding and $b \equiv$ addition of ether and running and blowing off, then the total steps are

$$a, b, b; \quad a, b, b; \quad a, b.$$

The total ether extract thus obtained measured about 350–400 ml.

The mass in the funnel was then treated with an alcoholic ether mixture containing 2% of ethyl alcohol according to the following scheme:

$$c; \quad a, c, c; \quad a, c, c,$$

where $c \equiv$ addition of alcoholic ether followed by running and blowing off, the funnel again being left overnight after each further grinding.

The total alcoholic ether extract, of about the same volume as the total ether extract, was distilled to about 25 ml., and in the cases where the absorption spectrum was investigated was passed on to L. A. Woodward for the work described in Part II of this paper. After its return the alcoholic ether extract was added to the similarly concentrated ether extract and the total mixture used for the chemical examination. This procedure was followed in all cases whether the physical examination was carried out or not.

In von Noël's original paper [1931] the steps in the extraction process are not very clearly described and the above method of procedure is that given in a private communication from him.

The total extract, concentrated to a volume of about 15 ml., was then filtered through a small paper into a 50 ml. tared Erlenmeyer flask and the solvent removed by blowing dry oxygen-free nitrogen on to the surface of the filtrate. The flask was then heated to constant weight in a vacuum drying-oven at 37°. All weighings were carried out to five places of decimals in grams on a Kuhlmann microbalance. The residue was obtained as a colourless or very pale yellow crystalline mass.

The acid and ester values were then determined in the following manner.

The fat was quantitatively dissolved in 15 ml. of a neutralized ether-alcohol solution consisting of 2 volumes of alcohol to 1 volume of ether, immediately transferred to a 100 ml. flask and directly titrated with $N/10$ sodium hydroxide solution from a microburette using phenolphthalein as indicator. The ester value was determined on the neutralized mixture by adding 20 ml. of an ether-alcohol potassium hydroxide solution freshly prepared in the following way. To 20 ml. of $N/2$ alcoholic potassium hydroxide solution were added 60 ml. of absolute alcohol and 40 ml. of dry ether giving a solution of $N/12$ concentration. The saponification was carried out by heating the flask for half an hour on an electrically heated water-bath with frequent shaking and, after cooling, by back-titrating the contents with $N/10$ hydrochloric acid solution. Two blank titrations were carried out, each on 15 ml. of the neutral ether-alcohol solution heated simultaneously with the samples under test.

The results obtained from 41 specimens are given in Table I.

Table I.

Ref. no.	Age	Sex	Clinical diagnosis	Cancer (C), non-cancer (N)	Vol. of serum ml.	Wt. of "Fat" mg.	Vol. of N/10 NaOH to neutralize ml.	Vol. of N/10 KOH to saponify after neutralization ml.	"Krebszahl"
1	22	F	Chronic appendicitis	N	14.5	86.69	7.58	1.26	285
A	60	M	Carcinoma of rectum with colostomy. Columnar type	C	8.5	77.72	7.32	0.60	285
B	47	M	Bronchitis and asthma	N	8.5	44.85	3.08	0.50	224
C	24	M	Neurasthenia	N	10.5	53.20	5.23	0.55	304
15	63	F	Malignant left ovarian cyst. Secondary deposits in liver, ascites	C	5.0	57.07	4.20	0.57	234
16	20	F	Acute appendicitis	N	6.5	39.05	2.97	0.33	165
19	48	M	Carcinoma of colon	C	10.5	80.65	6.35	0.93	253
20	46	M	Acute appendicitis	N	8.5	91.00	5.43	1.33	208
21	18	M	T.B. pleural effusion	N	8.3	56.46	5.02	1.05	301
22	43	M	Gastric ulcer	N	6.1	68.10	4.76	1.40	253
23	52	M	Functional dyspepsia	N	7.6	47.20	2.76	1.07	227
24	62	M	Carcinoma of rectum	C	6.4	57.10	4.84	1.09	291
25	64	M	Carcinoma of rectum	C	9.5	28.00	2.50	0.39	289
26	70	M	Carcinoma of colon	C	8.8	81.95	7.75	1.03	300
27	53	F	Advanced carcinoma of cervix uteri	C	8.5	41.34	1.91	0.93	192
28	57	F	Columnar celled carcinoma of rectum	C	7.0	51.60	1.48	1.32	152
30	24	M	Hydrocele	N	6.8	33.00	2.12	0.88	255
31	41	M	Acute nephritis	N	8.4	42.20	0.98	1.02	133
32	57	M	?Gastric ulcer	N	12.2	31.80	0.80	0.99	296
33	70	M	Epithelioma of tongue	C	7.0	21.61	0.87	0.91	231
34	69	F	Epithelioma of tongue	C	7.4	31.60	1.54	1.37	258
35	40	F	Carcinoma. Radical amputation right breast	C	9.5	32.39	0.62	0.91	132
36	19	M	Acute mastoiditis	N	9.5	28.37	1.08	1.36	241
37	45	F	?Left hemiplegia. Cerebral tumour	N	7.5	41.10	1.02	1.44	168
38	49	F	Large inoperable carcinoma of left breast. Blood sample, 27. x. 33	C	7.5	38.42	0.77	2.60	246
39	39	F	Caesarian section	N	4.0	14.45	0.56	0.42	190
40	59	F	Fracture dislocation of head of left humerus	N	4.0	16.90	0.65	0.58	204
51	48	F	Advanced carcinoma of cervix uteri	C	13.0	34.00	0.79	1.10	156
52	See No. 38		Now large actively growing mass of carcinoma of left breast. Radium in Oct. 1933 (sample, 20. ii. 34)	C	8.0	22.51	0.46	0.63	136
53	46	F	Acute pyelitis	N	9.0	39.40	0.82	1.48	164
54	38	F	Concussion and abrasions 8 days previously	N	10.0	19.09	0.24	0.53	113
55	30	F	?Cholecystitis	N	7.0	20.49	0.58	0.66	169
56	36	F	Carcinoma of cervix uteri rapidly growing	C	7.6	22.11	0.29	1.03	167
57	16	M	Acute appendicitis	N	7.0	13.92	0.28	0.35	127
58	62	M	Epithelioma of tongue	C	5.6	10.18	0.27	0.26	146
59	35	M	?Malignant disease of left lung	C	7.8	29.66	0.55	1.31	176
60	24	M	Calculi in left ureter	N	6.5	17.28	0.32	0.42	120
61	60	M	Advanced carcinoma of colon	C	7.5	14.65	0.41	0.34	143
62	63	M	Cerebral haemorrhage. W.R. neg.	N	7.7	23.77	0.31	0.42	86
63	21	M	Chronic suppurative otitis media	N	6.6	14.28	0.35	0.34	135
64	60	M	Epithelioma of palate	C	6.5	22.99	0.37	0.51	107

Average Krebszahl for 19 cases of Cancer=205.

Average Krebszahl for 22 cases other than Cancer=199.

DISCUSSION.

Two facts which emerge from the foregoing tables of results are (a) The "Krebszahlen" have a somewhat higher average value than the figures given by von Noël [1931] and (b) there is no indication, from the cases examined, of a higher "Krebszahl" generally characterizing cancer.

The saponification value of the serum lipids is an analytical figure of such a general nature and the lipid fraction itself under the conditions of isolation must of necessity contain so many different substances ranging from free acids of 3 or 4 carbon atoms to complex glycerides and cholesteryl esters, that the figures obtained can reveal only a very general average composition. The fact that the saponification value of such a complex mixture does not show any significant

differences in value characteristic of one single pathological condition is not therefore remarkable. In a further private communication von Noël reports that he has personally found that an increased amount of bile acids in the blood stream tends to give a false value on account of their high molecular weight.

It would seem too that even in cases where super-normal amounts of such relatively strong acids as lactic or acetoacetic acid are formed, if the acidosis is compensated the technique under discussion would not reveal their presence in any quantitative manner. An alternative possibility that the acids in question could be present as esters, thus revealing themselves by an enhanced ester value, seems somewhat improbable.

It is suggested therefore, on purely theoretical grounds, that the detection and estimation of the relatively strongly dissociated acids generally considered to be characteristic of the abnormal glycolysis of pathogenic tumours, is far from quantitative when attempted by measurement of the acid or saponification value of serum lipoids.

II. ULTRAVIOLET ABSORPTION MEASUREMENTS.

INTRODUCTION.

The measurements described in this Part were made with the object of testing the claim of certain previous workers [Dannmeyer *et al.*, 1931] to have obtained a method of diagnosing cancer by observation of the shape of the ultraviolet absorption curve of an extract from blood serum. The procedure of these workers was therefore followed as closely as possible. The work is intimately connected with that described in Part I of this paper, since the explanation put forward by the previous workers for the reported differences between the absorption curves of cancer and non-cancer cases is similar to that for the differences between the chemical "Krebszahlen", reference to which has been made above. Actually, however, the chemical results of Part I lend no support to the corresponding conclusions of the previous workers; but this fact does not preclude the possibility that absorption measurements, which are inherently capable of giving individual information about the different species present rather than an average result for the whole system, might provide a reliable diagnostic criterion.

EXPERIMENTAL.

The measurements were made with a special type of photoelectric spectrophotometer designed and constructed by the writer for the purpose. A description of this instrument and method and of its accuracy has already been published elsewhere [Woodward, 1934]. As in the measurements of the previous workers, a double quartz monochromator was used, so that the illumination of the liquid under investigation was very nearly monochromatic. The source of light was a mercury vapour arc and determinations were made at the following wave-lengths: 313.0, 302.3, 296.8, 289.4, 280.2, 275.6, 265.3, 253.7, 248.1, 240.0 $m\mu$. Where the mercury line in question consists of a close assemblage of components not separable by the monochromator, the wave-length given is an average reckoned with due regard to the relative intensities of the components. Under the conditions of experiment the maximum error in the determination of extinction coefficients is about 0.5%; except for the highest and lowest values, for which it is about 1%.

Owing to the volatility of the solvent (ether + 2% alcohol) difficulties were encountered in using absorption vessels of the customary type, i.e. consisting of a tubular glass distance-piece with optically worked ends upon which are pressed quartz end-plates. With such vessels it was found impossible to prevent the formation in the liquid of a bubble, the presence of which gave rise to errors in the measurements. The trouble was overcome by using fused silica one-piece absorption cells (Hilger Type D) in which the plane end-plates are adhered on to the tubular part of the cell by a special process without cements. These vessels, the filling tubes of which were closed during the experiments by means of cork stoppers previously washed with ether, proved entirely satisfactory. Two exactly similar cells were used, one for the solution under investigation and one for the solvent. The thickness of the liquid layer was exactly 20 mm. Measurements were carried out at room temperature.

The blood-serum extract investigated was the ether-alcohol extract referred to above in Part I. The concentration of this solution, as first extracted, naturally varied from case to case. In order therefore to facilitate the absorption determinations and to ensure more easily comparable results, the concentration was always adjusted by trial (either by adding solvent or by carefully evaporating down) until the observed absorption lay in the range most suitable for measurement. After the determinations the extract was returned to H. H. Barber for the chemical examination described in Part I.

The quantity actually measured was the ratio of the intensity I_0 of the beam incident upon the solution under investigation to the intensity I of the transmitted beam. The simultaneous use of a comparison vessel containing pure solvent introduced full correction for reflection and for absorption by the vessel and solvent. The results are expressed as extinction coefficients ϵ , defined by the equation

$$\epsilon = \frac{1}{d} \log_{10} \frac{I_0}{I},$$

in which d is the thickness of the liquid layer in cm. In our case $d = 2.000$. It is to be noted that the concentration does not appear in this equation. Assuming the validity of Beer's Law, the quantity ϵ would have to be divided in each case by the molecular concentration of the solution in order to obtain the strictly comparable quantity, the molecular extinction coefficient. Actually no account is taken of the small differences in the concentrations of the extracts studied. This means that the whole set of ϵ -values for any particular extract is multiplied by a certain small concentration factor, as compared with the set of values for another extract. Since, however, we are not concerned with absolute values, but only with the relative shapes of the respective absorption curves, this circumstance is of no importance. It should be mentioned also that the extinction coefficient ϵ used here differs from the quantity α used by the previous workers in that the equation defining ϵ contains a logarithm to the base 10, whereas the exactly analogous equation defining α contains a logarithm to the base e . This introduces a constant conversion factor which, however, is again of no significance since we are concerned with relative, and not with absolute, values.

The results of the determinations for 20 cases are given in Table II. The first column gives the reference number of the case and the second the clinical diagnosis, C denoting cancer and N non-cancer. For further details of the cases see Table I in Part I, in which the same reference numbers are used. The rest of Table II contains the observed values of ϵ in columns headed by the respective wave-lengths expressed in $m\mu$.

Table II.

Ref. no.	Clinical diagnosis	Observed values									
		313.0	302.3	293.8	289.4	280.2	275.6	265.3	253.7	248.1	240.0
35	C	0.158	0.215	0.249	0.310	0.390	0.435	0.518	0.599	0.668	—
36	N	0.213	0.248	0.266	0.330	0.386	0.414	0.468	0.574	0.639	0.754
37	N	0.161	0.205	0.246	0.287	0.344	0.376	0.461	0.663	0.762	0.91
38	C	0.179	0.291	0.360	0.468	0.578	0.644	0.785	0.96	1.01	—
39	N	0.154	0.227	0.281	0.373	0.508	0.595	0.800	1.08	1.17	—
40	N	0.132	0.207	0.269	0.383	0.545	0.648	0.889	1.15	—	—
51	C	0.106	0.155	0.190	0.256	0.360	0.421	0.545	0.749	0.894	—
52	C	0.107	0.165	0.201	0.260	0.343	0.396	0.546	0.813	0.97	—
53	N	0.093	0.158	0.205	0.291	0.422	0.513	0.726	1.09	—	—
54	N	0.100	0.132	0.160	0.207	0.265	0.306	0.382	0.560	0.690	0.892
55	N	0.096	0.139	0.169	0.221	0.310	0.368	0.504	0.747	0.91	—
56	C	0.092	0.137	0.166	0.219	0.295	0.348	0.483	0.726	0.854	—
57	N	0.094	0.140	0.176	0.241	0.339	0.403	0.553	0.818	0.974	—
58	C	0.089	0.128	0.158	0.207	0.276	0.323	0.416	0.584	0.706	—
59	C	0.097	0.122	0.142	0.179	0.226	0.245	0.276	0.310	0.354	0.456
60	N	0.085	0.110	0.130	0.162	0.203	0.225	0.257	0.324	0.387	0.554
61	C	0.101	0.137	0.161	0.201	0.259	0.282	0.313	0.382	0.466	0.703
62	N	0.092	0.135	0.160	0.196	0.239	0.271	0.332	0.448	0.487	0.579
63	N	0.092	0.122	0.145	0.178	0.227	0.250	0.302	0.391	0.465	0.609
64	C	0.118	0.167	0.202	0.261	0.348	0.393	0.503	0.683	0.810	1.08

The procedure in carrying out the determinations was to start with the longest wave-length and work through progressively to the shortest. In a large number of cases, however, values measured at the beginning of a run were re-determined afterwards. The agreement obtained was always within the limits of experimental error given above.

DISCUSSION OF RESULTS

Dannmeyer *et al.* [1931] express their results in the form of absorption curves in which the measured absorption coefficients α are plotted against wave-lengths. They reproduce 10 of these curves, 5 for cases of cancer and 5 for non-cancer, the examples being chosen as being "especially characteristic" (p. 652). They claim that the form of the curve in any given case furnishes a diagnostic test for the presence or absence of cancer. The test is actually effected either (a) by purely visual inspection of the curve or (b) by a formal method of analysis. As far as (a) is concerned, it is stated to be "clearly apparent" from the 10 examples reproduced that the curves of the cancer show "an essentially more uniform course" (*einen wesentlich einheitlicheren Verlauf*, p. 652) than those of the non-cancer cases. The present writer is unable, however, to perceive this qualitative difference by visual inspection of the curves in question. As the test appears to be so indefinite for examples chosen as especially characteristic, it is presumably even more so in average cases.

In the hope that the characteristic difference between cancer and non-cancer absorption curves, if it exists, might be more apparent from the results of the present investigation, these results were plotted in the same manner; but visual inspection of the curves obtained reveals no characteristic differences such as might serve as basis for a diagnostic test for cancer.

It may be noted that for all our curves of ϵ against λ the slope is everywhere negative, whereas some of the curves reproduced by the previous workers show regions of positive slope in the same wave-length range.

Reproduction of our curves, which are plotted from the data given in Table II, is prohibited by considerations of space.

Though purely visual inspection is claimed by the previous workers to suffice for purposes of diagnosis, they also give an account of a formal method (b) whereby the absorption curve under consideration is analysed into a so-called "basic curve" (*Grundkurve*) and a number of superimposed "partial bands" (*Teilbanden*). The latter are claimed to be characteristically different in cancer cases, as compared with non-cancer cases. For the purpose of the analysis it is found expedient, instead of plotting the absorption coefficient α against the wave-length λ , to plot $\log \alpha$ against the reciprocal of λ^2 . The "basic curve" then becomes a straight line. From the four examples given there appears, however, to be a certain arbitrariness involved in deciding the exact position of this line. The "basic curve" having been drawn, its ordinates are subtracted from those of the original curve and the differences replotted as ordinates of a new curve which we may call the selective absorption curve. This is finally analysed into a number (as many as six in one of the examples) of superimposed "partial bands", the sum of whose ordinates at any value of λ is equal to the ordinate of the curve. Now it is clear that small experimental errors in the determination of the original total absorption curve become relatively much greater for the ordinate differences which are plotted to obtain the selective absorption curve, and also that the values of these differences are affected seriously by a small uncertainty in the position of the "basic curve". The number of known points on the selective absorption curve is limited, being equal to the number of wave-lengths at which the original absorption determinations were made, and the course of the curve is in general very irregular. Hence it appears that a certain degree of arbitrariness is necessarily associated with an analysis into a number of overlapping "partial bands", even though these bands are required to satisfy certain conditions.

These considerations would seem to detract from the applicability of this method of analysis to the absorption curve of such a complicated system as the serum extract investigated. Since, therefore, the object of the present investigation was to find out whether ultraviolet absorption measurements on the extract could furnish a diagnostic test for cancer, it was thought best (in the absence of a thoroughly unambiguous method of analysis) to confine attention to purely visual inspection of the form of the absorption curves obtained. As stated above, our results on 20 cases give no evidence of the possibility of a diagnostic test of the kind sought.

SUMMARY.

Part I.

From the results of the examination of 41 specimens of blood serum obtained from both normal and cancer cases, there does not appear to be any significant difference in the saponification values of the serum lipoids as isolated under the conditions laid down in the paper of von Noël [1931].

The "Krebszahlen" calculated in the prescribed manner do not show differences sufficiently characteristic to be of diagnostic value.

Part II.

The investigations of this part constitute an independent test of the claim of previous workers to have obtained a method of diagnosing cancer by the form of the ultraviolet absorption curve of an extract from blood serum. An account is given of ultraviolet absorption measurements made with a double quartz

monochromator and photoelectric spectrophotometer. Results are tabulated for 20 cases, 9 of which were clinically diagnosed as cancer and 11 as non-cancer. These results furnish no evidence for the possibility of the diagnostic method under test.

The work described in this paper was carried out at the instigation of Prof. H. L. Brose, to whom we are grateful for his continued encouragement. We wish to acknowledge our indebtedness to the Nottingham General Hospital for their co-operation in supplying the blood samples. Our thanks are due in particular to Mr F. C. Hunt, who was responsible for taking the samples and keeping the clinical notes of the cases. We should like also to thank Dr F. H. Jacob for his interest in the investigations. In addition we wish to express our gratitude to the Nottinghamshire Branch of the British Empire Cancer Campaign for grants covering the cost of the apparatus and part of the salary of one of us (H. H. B.) as biochemist. The work was carried out during the tenure by one of us (L. A. W.) of the Massey Scientific Fellowship at University College, Nottingham. We wish to express our thanks to Mrs Massey and Mrs Massey Stewart, whose financial generosity in creating the Fellowship made the work possible.

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