XXXVIII. A STUDY OF THE DISTRIBUTION OF NITROGEN IN THE PROTEINS OF TUMOURS AND NORMAL TISSUES.

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INTRODUCTORY.

A review of the literature dealing with the chemical aspects of the cancer problem will demonstrate how little serious attention, relatively speaking, this important subject has received at the hands of the biochemist.

The scattered facts which constitute our present knowledge of the subject have been ably collated by Wells [1914] in his text-book of Chemical Pathology.

Sufficient may be gathered from a perusal of his review to show that the chemist has been unable to demonstrate satisfactorily that the cancer cell is in any way strikingly different from the cell of the corresponding normal organ.

The proteins which enter into the composition of tumours have been investigated by several workers. Bang [1903] studied in particular the nucleoproteins of tumours, and was able to show that the nucleoprotein which he isolated from a lymphosarcoma was in all probability identical with the normal and characteristic nucleoprotein of lymph-glands.

Bergell and Dörpinghaus [1905] made a study of the amino-acids present in tumours, but they were unfortunately obliged to use for this investigation a somewhat mixed material from various sources. Upon analysis, using the "ester-method" of Fischer, they found that the amino-acids alanine, glutamic acid, aspartic acid and phenylalanine were apparently present in a higher proportion than is usually found in normal tissues; while the yield of leucine, glycine and tyrosine was somewhat lower.

The analyses of Neuberg [1906], however, gave results which led him to express the view that the amounts of the amino-acids leucine, glutaminic acid, tyrosine and glycine found in tumour proteins were very much of the same order as those found in the normal organs.

Bergell [1907] investigated the proteins of human carcinomata and also of mouse cancers. Using the quantitative nitrogen-distribution method of Hausmann [1900] he obtained much higher values for the diamino-acid fraction in all cases of cancer than in normal. Thus, in human carcinoma he found that 40 % of the total nitrogen was present in the form of diamino-acid nitrogen, the figure for normal tissues being about 29 %. He also obtained similar results in the analysis of the mouse tumour proteins, and concluded that the diamino-acids played an important rôle in the cancer cell.

Blumenthal [1907] after having reviewed all the previous work, and also that of Wolff [1905], who found a relatively greater proportion of albumin than globulin in the press-juices of several tumours, as compared with normal tissues, concluded that important differences exist in the chemical nature of normal and malignant cells. Abderhalden and Medigreceanu [1910] examined a series of primary tumours of the liver in cows, several mouse carcinomas, and a sarcoma, quantitatively for the three amino-acids tyrosine, glycine, and glutaminic acid. Their figures show good agreement and indicate that the proportion of these substances present in tumours is not abnormal.

Wells [1914] also inclines to the view that the proteins of tumours are probably identical with, or at any rate closely similar to, those of normal organs. He explains the results of Bergell, to which allusion has been made, on the grounds that the majority of cancerous tissues are very cellular, and are therefore rich in nuclear material, and he quotes in support of this opinion the work of Petry [1899], who found that a carcinoma of the mammary gland contained a much larger proportion of nucleoprotein than did the normal gland.

We can now see that the majority of the work that has been reviewed agrees in regarding the proteins that enter into the composition of malignant growths as very similar in composition to those present in the normal organs, although we cannot consider many of the results as satisfactory from the point of view of quantitative analysis.

Recently a paper appeared by Kocher [1915], in which this author claims to have demonstrated that the amounts of the hexone bases, arginine, histidine, and lysine present in tumour proteins are approximately double those found in normal tissues. He used the method of analysis introduced by Kossel and Kütscher [1901], with the subsequent modifications recommended by

Osborne, Leavenworth and Brautlecht [1909], Steudel [1903], and van Slyke [1914]. One cannot but regard his analytical data as being somewhat insufficient, for he has only submitted five tumour proteins to analysis, and has then compared the results of these analyses with figures published some time previously by Wakeman [1908] for the diamino-acid content of normal organs. The actual tumours examined by Kocher were of a somewhat miscellaneous nature, namely, a metastatic deposit in the liver secondary to a primary carcinoma of the stomach, a lymphosarcoma, an endothelioma, a primary carcinoma of the larynx and another secondary deposit in the liver from a primary carcinoma of the ileum.

Shortly before the appearance of Kocher's paper an examination along very similar lines was begun in this Institute as part of a detailed investigation into the chemical consideration of the cancer problem.

This examination consisted of a study of the distribution of nitrogen in the proteins of tumours, as compared with normal tissues, by the method of van Slyke.

One particular type of tumour was chosen at the outset for more or less exhaustive study, as it was realised that in order to be justified in drawing conclusions from the results obtained, it was necessary to compare examples of variations of one type of tumour—for instance the slow and rapidly growing forms—with one another, as well as with the normal organ.

The actual growth selected for study was cancer of the breast, and the reasons for this choice were several. Firstly, it is possible to obtain tumours of this variety with comparative ease, secondly, there are a large number of forms of cancer of the breast which vary in their rate of growth considerably, while for a third reason, it is possible to obtain specimens of pathological breast tissue in a condition which is regarded by many as being one of the so-called "pre-cancerous" states. On the publication of the summary of Kocher, however, the study was extended to an experimental neoplasm of the chicken which is receiving considerable attention in this Institute, namely, the Rous sarcoma.

From the summary of the paper by Kocher one is led to assume that this author regards the high values for the hexone bases in tumours as being in some way responsible for the abnormal rate of growth of these tissues, particularly in the case of the diamino-acid lysine.

It was therefore felt that this point could only be decided by an investigation of a tumour that could be controlled experimentally, and accordingly the neoplasm referred to above was chosen.

It is necessary to say that although many points remain to be decided before we can classify this particular tumour as a sarcoma, yet, for many reasons it was felt that its characters sufficiently resemble a malignant growth to justify such a study.

Indeed it would be out of place to discuss here the evidence on either side of the question as to whether the growth is one of the group of diseases known as the infective granulomata, or whether it is a form of cancer, for it is hoped that a certain amount of light on that point will be shed by the researches which are being made in this Institute at the present time.

By carefully adjusting the conditions under which this growth is propagated it is possible to vary to a considerable extent its virulence and its rapidity of growth. Originally the tumour was discovered by Rous [1910] in a hen of the Plymouth Rock strain, and for some time it was only possible to carry on the tumour by inoculating grafts when the host was a pure bred chicken of that particular breed; other strains apparently possessed a natural form of immunity. Passage of the tumour through a very large number of generations has, however, had the effect of decreasing the immunity enjoyed by the other breeds to some extent, so that if injected into a fowl of the Minorca or White Leghorn variety growth of the graft will sometimes be obtained, although usually its rate will be slow. The rate of growth in its natural host, the Plymouth Rock chicken, is generally very rapid, although even in these cases the rate is greatly influenced by the age and state of health of the host.

By a selection of suitable conditions it is therefore possible to control to some extent the rate of growth of this neoplasm; it being borne in mind that in all probability many of the conditions applied are not artificial or unnatural ones, but ones that in many cases do bear an important part in influencing the growth of human cancer. There is, unfortunately, one drawback to the use of this particular tumour for such a study as was intended, that being that it is a growth which simulates a true sarcoma in being a proliferative condition of the connective tissue of the fowl; it is therefore difficult to obtain a normal chicken tissue with which to make an adequate comparison. This point was, however, duly taken into consideration, together with the arguments in favour of using the growth, with the result that it was decided that the results of the analyses of the human tumours would serve as a control to some extent in this respect.

EXPERIMENTAL.

Methods of Analysis.

The proteins were in every case examined by the nitrogen distribution method of van Slyke [1911], utilising the modifications in the treatment of the phosphotungstate precipitate introduced by the same author at a later date [1915]. On arrival in the laboratory the tissues were immediately freed from all foreign tissue and finely minced in a small hand-mincing machine. The proteins of the minced tissue were then coagulated by boiling, a small quantity of dilute acetic acid being added to the boiling water. The coagulated protein was filtered off, washed with water containing a small trace of acetic acid, pressed and dried. Where necessary, traces of residual protein in solution in the filtrate were recovered by precipitation with alcohol, and added to the main portion.

The dried protein was then rendered fat-free by continuous extraction in a Soxhlet extractor with ether, after which the fat-free product was ground to a fine state of division and passed through a fine mesh sieve.

In most cases approximately 3 g. of the resulting greyish white powder was hydrolysed with 30 cc. of 20 % hydrochloric acid until hydrolysis was complete, as shown by determinations of the amino-nitrogen, the treatment of the resulting solution being then carried out along lines almost identical with those devised by van Slyke [1911].

One difficulty encountered was in the estimation of arginine, for it was found that to obtain reliable duplicate titrations was a matter of great uncertainty when the process of van Slyke was used. Plimmer [1916] has also had occasion to draw attention to this fact, and has suggested several slight modifications which undoubtedly improve the accuracy of the process.

Throughout the course of this research it has been the practice to estimate the total nitrogen of the diamino-acids fraction in a separate portion of the solution of the bases, and not in the residual liquid remaining after the determination of the arginine. Plimmer also recommends this procedure. The other improvements mentioned in his paper, namely, the use of a more dilute alkali for the hydrolysis of the arginine, and the distillation of the last traces of ammonia resulting from this hydrolysis by removing the condenser water and continuing the boiling for some twenty minutes, were adopted during the latter part of this work.

TABLE I.
Distribution of Nitrogen in the Proteins of Human Tumours.
Percentages of total nitrogen.

No. of case	Age	Weight of tumour g.	Approximate time of growth*	Characteristics of the tumour	Amide N	Humin N	Mon-amino N of filtrate	Non-amino N of filtrate	Arginine N	Histidine N	Lysine N	Cystine N	Total N of diamino acids
331A	63	33	24 months	Scirrhous carcinoma of breast, slow growing, with hyaline degeneration of stroma and vacuolation of the protoplasm	4.42	2.24	52.20	11.80	9.45	7.50	12.39	0.70	30.04
331B	—	32	—	Rapidly growing metastatic deposits in the axillary glands from Case 331	4.72	2.47	53.30	3.75	8.40	8.10	16.44	0.96	33.90
347	63	81	6 "	Scirrhous carcinoma of breast, slow growing but glandular type, somewhat fibrous	5.35	1.72	57.80	11.90	10.21	7.98	4.26	1.55	24.00
342A	41	48	12 "	Scirrhous carcinoma of breast, slow growing with much fibrosis	3.86	2.15	50.80	12.45	9.18	6.27	14.55	—	29.90
343A	52	25	12 "	Scirrhous carcinoma of breast, slow growing	4.36	2.02	52.30	10.62	16.75	1.64	11.41	—	29.80
343B	—	42	—	Secondary deposits from glands of axilla from Case 343, actively growing	4.75	1.97	59.00	4.10	12.00	7.90	9.60	—	29.50
384A	57	56	6 "	Scirrhous carcinoma of breast, more cellular than above cases	5.87	2.84	54.90	7.40	9.51	5.77	12.72	—	28.00
384B	—	24	—	Very active metastatic deposits in glands of axilla from Case 384	3.17	2.96	59.20	4.00	9.31	7.10	14.49	—	30.90
387A	52	29	13 "	Scirrhous carcinoma of breast, fairly cellular and more active	6.10	2.22	51.80	8.20	11.85	6.29	11.91	1.15	31.20

387B	—	20	—	Rapidly growing secondary growths in glands from axilla from Case 387	5.03	2.50	52.90	3.42	13.10	8.00	13.67	1.03	35.80	
18A	45	39	14	”	Carcinoma of breast, alveolar type, but not rapidly growing	4.90	2.02	55.55	8.72	8.15	7.10	12.55	—	27.80
18B	—	19	—	”	Metastatic deposits in axillary glands from Case 18	6.19	2.49	57.80	6.37	7.90	8.80	11.60	—	28.30
332	30	87	—	”	Cystic mastitis, much fibrosis, no evidence of malignancy	4.57	2.57	56.89	12.89	8.69	7.23	4.80	1.28	22.00
333	19	102	24	”	Cystic mastitis, much fibrosis, no evidence of proliferation of glandular tissue	3.01	3.09	51.10	12.43	8.91	6.44	13.55	—	28.90
349	47	84	3 weeks	”	General fibrosis of breast, no proliferation of glandular elements	3.74	1.39	52.70	13.70	10.75	6.86	9.20	1.20	28.01
353	49	180	3 months	”	General fibrosis of breast, no malignancy	3.47	1.26	51.36	11.55	13.95	6.25	10.70	1.10	32.00
20	54	60	24	”	Myxosarcoma of breast, slow growing	4.99	1.63	53.75	9.19	12.15	6.76	10.49	0.61	30.01
97	72	25	6	”	Adenocarcinoma of breast, fairly rapidly growing	5.76	2.29	58.80	3.83	10.90	7.00	9.90	1.60	29.40
103A	36	47	6-7	”	Encephaloid carcinoma of breast, extending rapidly	4.19	2.42	55.40	3.03	11.10	7.75	16.24	—	35.09
103B	—	39	—	”	Rapidly growing secondary deposits in glands from axilla from Case 103	5.62	3.32	53.10	2.85	12.30	8.70	14.20	—	35.20
110	58	600	36	”	Fibromyoma of uterus ...	5.21	1.62	52.50	10.00	9.45	7.70	12.25	—	29.40
16	20	900	6	”	Exceedingly rapidly growing lymphosarcoma of cervical glands	4.82	4.03	50.30	2.10	11.90	8.90	16.27	1.13	38.20

* A rough approximation of the duration of growth of the tumour derived from the observations of the patients. It must be borne in mind, however, that considerable growth may have occurred before a tumour makes its presence apparent, while there is no means of ascertaining at what stage involvement of the axillary glands occur.

TABLE II.
Distribution of Nitrogen in the Proteins of the Rous Sarcoma.
Percentages of total nitrogen.

No. of case	Weight of tumour g.	Time of growth days	Characteristics of tumour	Amide N	Humin N	Mon-amino N of filtrate	Non-amino N of filtrate	Arginine N	Histidine N	Lysine N	Cystine N	Total N of diamino acids
5	32	30	Slow growing, somewhat fibrous	...	4.98	5.48	45.98	14.39	8.10	11.30	0.98	34.77
117	39	36	Somewhat fibrous small central area of muroid degeneration	...	6.35	4.97	45.60	10.92	10.30	12.68	0.90	34.70
125	75	75	Very slowly growing, much fibrosis	...	6.25	6.25	51.30	11.94	7.59	11.05	0.82	31.40
12	32	40	Firm, slightly fibrotic tumour	...	4.04	5.52	43.10	11.50	11.22	13.13	0.85	36.70
3	45	42	Firm tumour, with area of central necrosis	...	6.32	6.15	48.50	11.50	9.10	10.28	0.77	31.65
90	90	30	Fairly rapidly growing tumour, with central area of muroid degeneration	...	4.78	4.03	47.90	10.34	9.77	13.80	0.99	34.90
91	78	30	Firm, steadily growing tumour	...	5.15	1.11	48.30	11.41	10.11	12.75	1.13	35.40
133	83	21	Firm, rapidly growing, area of central necrosis	...	5.85	3.24	—	11.62	9.72	12.45	0.91	34.70
25	43	18	Fairly rapidly growing tumour, much central necrosis and muroid change	...	3.98	4.95	52.40	10.72	10.00	11.90	0.78	33.40
100	70	30	Firm tumour, with some slight necrosis	...	4.68	3.66	47.70	10.44	10.12	13.48	0.66	35.70
101	68	27	Firm, solid, steadily growing tumour	...	6.43	3.43	48.00	10.41	10.60	13.63	1.06	35.70
72	108	18	Rapidly growing tumour, much necrosis of a myxomatous type	...	6.81	6.49	47.20	10.56	8.20	13.88	0.76	33.40
96	165	18	Very rapidly growing, no necrosis	...	6.81	6.60	50.75	11.00	10.52	10.49	0.89	32.90
139	136	36	Firm, very rapidly growing tumour	...	5.15	5.23	47.20	13.05	14.35	8.78	0.82	37.00
94	200	21	Rapidly growing, much central necrosis	...	6.43	5.72	50.01	12.81	8.05	10.63	0.91	32.40
94A	—	—	Metastasis in liver from 94	...	6.28	4.82	52.70	12.55	9.28	8.99	0.58	31.40
94B	—	—	Metastasis in lungs from 94	...	5.52	5.19	49.40	11.85	8.91	11.62	0.62	33.00
77	215	15	Very rapidly growing tumour, much central necrosis	...	5.68	4.93	46.50	11.16	12.82	12.15	0.64	36.77
60	307	51	Rapidly growing tumour, extensive central myxomatous degeneration	...	4.70	4.28	48.50	10.30	9.54	15.84	0.82	36.50

TABLE III.

Distribution of Nitrogen in Proteins of Normal Organs. Percentages of total nitrogen.

I. *Human.*

Organ	Remarks on organ	Amide N	Humus N	Monamino N of filtrate	Non- amino N of filtrate	Arginine N	Histidine N	Lysine N	Cystine N	Total N of diamino- acids
Pancreas ...	Normal	5.52	4.50	53.27	3.63	11.30	8.82	10.91	0.97	32.00
Kidney ...	"	5.05	3.24	59.30	5.10	11.95	5.89	7.00	1.01	25.85
Spleen...	"	5.18	3.82	47.62	4.88	12.50	13.50	11.00	1.10	38.10
Cardiac muscle	"	5.67	1.55	52.95	5.90	9.38	10.20	12.50	0.82	32.90
Skeletal muscle	"	6.18	2.29	58.90	4.41	11.05	2.72	13.60	0.80	28.17
Liver ...	"	6.12	3.32	53.85	4.35	10.50	8.20	11.76	1.34	31.80
Cardiac musculo	"	6.35	3.60	53.20	5.00	10.00	10.00	9.25	0.80	30.05
Kidney ...	"	6.05	3.57	52.80	5.28	11.45	8.60	9.25	1.10	30.40
Skeletal muscle	"	6.42	2.30	57.00	4.20	11.30	5.79	12.71	0.90	30.70
Pectoral muscle	Removed with breast No. 103	5.42	2.46	58.30	4.20	11.85	4.88	10.41	0.85	27.99
Breast...	Normal, but slightly fibrotic	5.50	2.50	57.17	6.40	10.10	4.66	12.47	—	27.23
" ...	"	5.10	2.85	56.20	6.00	10.02	2.81	13.38	—	26.21

II. *Chicken.*

Lungs ...	Normal	7.02	4.54	51.20	7.40	11.25	7.22	11.11	1.32	30.90
" ...	"	6.58	3.82	50.70	6.70	12.20	8.42	10.98	1.40	33.00
Liver ...	Stunted chick ¹	7.49	4.40	56.50	3.58	9.09	8.10	11.24	1.27	29.70
" ...	Normal	8.19	5.56	54.80	4.95	9.15	8.00	9.25	1.30	27.70
" ...	"	8.17	5.52	55.20	4.00	9.45	7.44	9.45	1.16	27.50
Pectoral muscle	Stunted chick ¹	6.08	2.20	57.90	4.75	10.88	5.24	10.82	0.86	27.80
" "	Normal	6.55	1.77	57.85	4.36	11.10	9.71	8.89	0.90	30.60
" "	"	6.72	1.75	56.15	4.55	11.10	7.20	10.74	0.91	29.95
" "	Stunted chick ¹	6.47	3.42	59.50	5.08	8.60	7.40	9.37	0.88	26.25
Cardiac muscle	Normal	6.64	3.34	54.00	5.80	11.40	7.72	7.63	0.85	27.60
" "	"	6.02	3.17	58.05	5.10	9.99	8.00	8.36	0.90	27.25
" "	"	10.57	5.25	67.95	7.35	7.35	2.72	5.03	0.90	16.10
Brain ...	"									

¹ Undersized chicks exhibiting signs of malnutrition through being reared from an early age indoors.

TABLE IV. *Analytical Data for*

No. of case	Total N vol. 100 cc. N in 5 cc. cc. 0.1 N acid	Monamino-acid fraction vol. 500 cc.								
		Total N	Amide N vol. 90 cc. cc. 0.1 N acid	Amide N	Humin N vol. 90 cc. cc. 0.1 N acid	Humin N	N in 25 cc. cc. 0.1 N acid	Total N in filtrate	cc. N gas from 10 cc.	Amino N of filtrate
331A	{15.00} {15.15}	0.4228	12.00	0.0187	6.20	0.0096	{8.40} {8.30}	0.2600	6.62, 19.5° C., 762 mm.	0.2103
331B	{14.80} {14.90}	0.4160	12.60	0.0196	6.65	0.0103	{7.70} {7.55}	0.2370	7.09, 19° C., 750 mm.	0.2220
347	{12.40} {12.20}	0.3440	11.80	0.0184	3.80	0.0059	{7.60} {7.80}	0.2400	6.26, 19.5° C., 762 mm.	0.1985
342A	{13.60} {13.65}	0.3810	8.40 (<i>k</i>)	0.0147	4.60	0.0082	{6.70} {6.85}	0.2380	5.49, 16.5° C., 743 mm.	0.1935
343A	{12.85} {13.00}	0.3612	10.10	0.0157	4.70	0.0073	{7.30} {7.20}	0.2270	5.92, 17.5° C., 762 mm.	0.1890
343B	{13.30} {13.50}	0.3750	13.30	0.0178	4.70	0.0073	{7.70} {7.55}	0.2370	7.06, 19° C., 751 mm.	0.2210
384A	{15.30} {15.10}	0.4258	16.10	0.0250	7.80	0.0121	{8.55} {8.50}	0.2680	7.20, 15° C., 762 mm.	0.2340
384B	{9.30} {9.45}	0.2630	5.30	0.0082	5.00	0.0078	{5.30} {5.30}	0.1660	4.97, 16.5° C., 743 mm.	0.1656
387A	{17.75} {17.75}	0.4973	19.45	0.0303	7.10	0.0110	{9.75} {9.45}	0.2980	8.20, 16.5° C., 743 mm.	0.2570
387B	{15.00} {15.00}	0.4200	13.60	0.0212	6.80	0.0105	{7.60} {7.60}	0.2370	6.82, 15° C., 762 mm.	0.2220
18A	{14.20} {14.40}	0.4004	12.60	0.0196	5.20	0.0081	{8.20} {8.30}	0.2570	6.82, 15° C., 762 mm.	0.2220
18B	{5.55} {5.60}	0.1568	6.25	0.0097	2.50	0.0039	{3.25} {3.24}	0.1008	2.89, 24° C., 766 mm.	0.0906
332	{14.60} {14.80}	0.4120	12.10	0.0188	6.80	0.0105	{9.40} {9.20}	0.2900	7.20, 14.5° C., 762 mm.	0.2340
333	{12.40} {12.40}	0.3470	5.20 (<i>m</i>)	0.0104	5.40	0.0107	{5.30} {5.70}	0.2202	4.20, 13.5° C., 764 mm.	0.1770
349	{16.90} {16.90}	0.4740	11.40	0.0176	3.90	0.0066	{10.20} {9.95}	0.3145	7.92, 19° C., 750 mm.	0.2490
353	{16.20} {16.25}	0.4540	10.10	0.0157	3.60	0.0057	{9.10} {9.25}	0.2865	7.49, 16.5° C., 743 mm.	0.2340
20	{11.80} {11.90}	0.3332	10.65	0.0166	3.40	0.0054	{6.70} {6.70}	0.2090	5.72, 24° C., 766 mm.	0.1785
97	{8.80} {8.95}	0.2492	9.20	0.0143	3.60	0.0057	{5.00} {5.00}	0.1560	4.66, 24° C., 766 mm.	0.1460
103A	{16.35} {16.05}	0.4540	12.20	0.0190	7.10	0.0110	{8.55} {8.45}	0.2650	7.91, 18.5° C., 765 mm.	0.2520
103B	{16.00} {15.85}	0.4452	16.10	0.0250	9.50	0.0148	{8.00} {8.00}	0.2490	7.48, 18.5° C., 765 mm.	0.2370
110	{17.50} {17.70}	0.4930	16.40	0.0256	5.10	0.0080	{9.90} {9.85}	0.3080	8.13, 18.5° C., 765 mm.	0.2590
16	{15.80} {15.60}	0.4400	13.60	0.0212	11.45	0.0177	{7.50} {7.30}	0.2310	7.05, 21.5° C., 760 mm.	0.2220

(*k*) Estimations carried out on 80 cc.(*m*) Estimations carried out on 70 cc.

Proteins of Human Tumours.

Diamino-acid fraction vol. 50 cc.								
N in 5 cc. cc. 0.1 N acid	Total N of bases	cc. N gas from 5 cc.	Amino N of bases	Arginine N in 10 cc. cc. 0.1 N acid	Argin- ine N	g. BaSO ₄ from 5 cc.	Cystine N	Total N re- covered
8.30 8.20	0.1300	11.90, 15° C., 765 mm.	0.0763	2.60	0.0405	0.0009	0.0030	0.4183
9.10 9.10	0.1410	12.22, 15° C., 765 mm.	0.1018	2.30	0.0350	0.0012	0.0040	0.4079
5.40 5.20	0.0825	6.15, 15° C., 765 mm.	0.0397	2.25	0.0350	0.0016	0.0053	0.3468
6.55 6.65	0.1145	10.20, 17° C., 750 mm.	0.0729	2.00	0.0350	—	—	0.3754
6.90 6.80	0.1070	8.90, 15° C., 765 mm.	0.0581	3.90	0.0605	—	—	0.3570
7.10 7.10	0.1105	8.75, 15° C., 765 mm.	0.0570	2.90	0.0450	—	—	0.3726
7.65 7.70	0.1200	11.30, 15° C., 765 mm.	0.0725	2.60	0.0405	—	—	0.4251
5.20 5.20	0.0810	7.80, 15° C., 765 mm.	0.0510	1.60	0.0245	—	—	0.2630
9.90 9.85	0.1545	14.12, 22° C., 760 mm.	0.0889	3.80	0.0590	0.0017	0.0057	0.4828
9.65 9.70	0.1510	13.90, 22° C., 760 mm.	0.0873	3.52	0.0550	0.0013	0.0043	0.4180
7.20 7.10	0.1110	10.60, 21° C., 753 mm.	0.0669	2.10	0.0325	—	—	0.3957
2.85 2.85	0.0444	4.00, 15.5° C., 765 mm.	0.0258	0.80	0.0124	—	—	0.1587
5.70 5.85	0.0905	6.90, 17° C., 750 mm.	0.0436	2.30	0.0357	0.0016	0.0053	0.4098
4.85 5.00	0.1000	5.94, 17° C., 750 mm.	0.0509	1.55	0.0310	—	—	0.3113
8.50 8.50	0.1325	11.25, 15° C., 765 mm.	0.0724	3.35	0.0510	0.0017	0.0057	0.4712
9.30 9.20	0.1445	11.50, 15° C., 765 mm.	0.0772	4.10	0.0635	0.0015	0.0050	0.4524
6.50 6.35	0.1000	8.70, 21.5° C., 757 mm.	0.0546	2.65	0.0405	0.0006	0.0020	0.3310
4.70 4.70	0.0730	5.95, 21.5° C., 757 mm.	0.0389	2.20	0.0272	0.0012	0.0040	0.2490
10.22 10.20	0.1590	15.70, 22° C., 758 mm.	0.0972	3.25	0.0505	—	—	0.4541
10.00 10.20	0.1570	14.25, 22° C., 758 mm.	0.0894	3.55	0.0550	—	—	0.4458
9.20 9.40	0.1450	13.20, 18.5° C., 765 mm.	0.0818	2.97	0.0465	—	—	0.4866
10.70 10.85	0.1680	16.05, 21° C., 753 mm.	0.1061	3.40	0.0525	0.0015	0.0050	0.4379

TABLE V. *Analytical Data for*

No. of case	Total N vol. 100 cc. N in 5 cc. cc. 0.1 N acid	Total N	Amide N vol. 95 cc. cc. 0.1 N acid	Amide N	Humin N vol. 95 cc. cc. 0.1 N acid	Humin N	Monamino-acid fraction vol. 200 cc.			
							N in 25 cc. cc. 0.1 N acid	Total N in filtrate	cc. N gas from 10 cc.	Amino N of filtrate
5	15.65	0.4385	14.60	0.0215	16.30	0.0240	19.65	0.2320	17.00, 20°, 752	0.2020
117	16.00 (a)	0.4480	18.25	0.0284	14.30	0.0222	19.00	0.2360	15.70, 18.5°, 770	0.2037
125	14.90	0.4178	17.70	0.0261	17.70	0.0261	8.65 (c)	0.2550	8.98, 18°, 766	0.2133
12	14.30	0.4010	11.00	0.0162	15.05	0.0222	18.10	0.2140	14.60, 20°, 752	0.1730
3	14.30	0.4010	17.20	0.0254	16.80	0.0247	7.60 (c)	0.2240	6.35, 17.5°, 764	0.1940
90	14.50	0.4070	13.20	0.0195	11.10	0.0164	18.75	0.2210	16.10, 16°, 754	0.1950
91	15.50	0.4348	15.20	0.0224	3.25	0.0048	20.10	0.2360	17.30, 16°, 754	0.2100
133	16.90 (b)	0.4730	16.80	0.0277	9.30	0.0153	—	—	—	—
25	16.05 (a)	0.4495	11.70	0.0179	14.30	0.0223	17.55	0.2365	18.85, 18.5°, 770	0.2355
100	13.60	0.3820	12.10	0.0179	9.50	0.0140	17.35	0.2040	15.20, 18°, 750	0.1819
101	14.40	0.4040	17.50	0.0258	9.30	0.0137	18.10	0.2140	16.20, 18°, 750	0.1935
72	14.50	0.4070	18.60	0.0274	17.90	0.0264	7.20 (c)	0.2120	6.28, 17.5°, 764	0.1920
96	14.80	0.4150	19.20	0.0283	16.80	0.0274	7.80 (c)	0.2300	6.88, 17.5°, 764	0.2100
139	14.80	0.4150	14.50	0.0214	14.70	0.0217	18.10	0.2140	16.50, 20°, 752	0.1960
94	15.80	0.4425	19.30	0.0285	17.10	0.0253	8.20 (c)	0.2410	7.45, 19.5°, 757	0.2220
94A	15.47	0.4335	18.40	0.0272	14.10	0.0208	8.35 (c)	0.2460	7.68, 20°, 757	0.2290
94B	14.40	0.4040	15.10	0.0223	14.20	0.0209	7.40 (c)	0.2180	6.65, 18°, 754	0.1990
77	16.90 (a)	0.4730	17.20	0.0268	15.00	0.0233	19.35	0.2410	16.92, 18.5°, 771	0.2200
60	22.20	0.6210	19.80	0.0292	18.08	0.0266	28.35	0.3350	24.00, 20°, 752	0.3070

(a) Volume of liquid carried on for analysis 90 cc.

(b) Volume of liquid carried on for analysis 85 cc.

(c) Volume of monamino-acid fraction 500 cc.

In spite of these improvements in the technique of this particular estimation, it has been found difficult to obtain concordant results. The figures for arginine are therefore not regarded as being more than approximately accurate. There is reason to believe that the values given for this amino-acid are lower than they should be, and consequently those for histidine are somewhat high.

Corrections for the solubilities of the phosphotungstates of the basic amino-acids have not been applied as results of comparative value were alone required.

The human tumour tissues utilised in this work were in all cases obtained from the Pathological Department of the Cancer Hospital. The majority of these cases were specimens removed at operation, and were in all cases available for analysis from 6 to 12 hours subsequently. One or two of the tissues were obtained post-mortem, and in these cases the time elapsing between death and the treatment of the tumour tissue was usually somewhat longer.

Proteins of Rous Sarcoma.

Diamino-acid fraction vol. 50 cc.

N in 5 cc. cc. 0.1 N acid	Total N of bases	cc. N gas from 5 cc.	Amino N of bases	Arginine N in 10 cc., cc. 0.1 N acid	Arginine N	g. BaSO ₄ from 5 cc.	Cystine N	Total N re- covered
10.30	0.1520	13.50, 18.6° C., 752 mm.	0.0805	4.30	0.0632	0.0015	0.0043	0.4295
10.00	0.1558	13.65, 18.5° C., 771 mm.	0.0883	3.15	0.0490	0.0015	0.0040	0.4424
8.90	0.1310	12.00, 18° C., 750 mm.	0.0715	3.40	0.0500	0.0012	0.0034	0.4382
10.05	0.1475	12.10, 18.5° C., 756 mm.	0.0727	3.30	0.0462	0.0012	0.0034	0.3999
9.60	0.1290	11.60, 19° C., 761 mm.	0.0700	3.30	0.0462	0.0011	0.0031	0.4031
9.65	0.1415	13.85, 19° C., 761 mm.	0.0837	2.85	0.0420	0.0014	0.0040	0.3984
10.40	0.1535	14.39, 19° C., 761 mm.	0.0869	3.37	0.0496	0.0017	0.0048	0.4167
9.95	0.1640	13.45, 17.5° C., 766 mm.	0.0919	3.35	0.0591	0.0017	0.0043	—
9.60	0.1490	12.80, 18.5° C., 772 mm.	0.0829	3.10	0.0483	0.0013	0.0035	0.4257
9.28	0.1632	13.10, 18.5° C., 772 mm.	0.0804	2.72	0.0400	0.0009	0.0025	0.3991
9.78	0.1440	13.63, 18.5° C., 772 mm.	0.0837	2.86	0.0422	0.0015	0.0043	0.3975
9.20	0.1356	13.45, 19.5° C., 760 mm.	0.0810	2.92	0.0430	0.0011	0.0031	0.4014
9.30	0.1376	12.40, 19.5° C., 760 mm.	0.0745	3.10	0.0456	0.0013	0.0037	0.4233
10.40	0.1535	12.30, 19.5° C., 752 mm.	0.0732	3.67	0.0541	0.0012	0.0034	0.4106
9.70	0.1435	13.10, 19° C., 745 mm.	0.0772	3.85	0.0568	0.0014	0.0040	0.4383
9.20	0.1355	11.50, 19° C., 745 mm.	0.0678	3.70	0.0545	0.0009	0.0025	0.4295
9.00	0.1330	12.38, 19° C., 745 mm.	0.0730	3.25	0.0479	0.0009	0.0025	0.3942
11.18	0.1740	14.55, 18.5° C., 767 mm.	0.0938	3.40	0.0528	0.0011	0.0030	0.4651
15.40	0.2270	23.40, 19.5° C., 752 mm.	0.1392	4.35	0.0641	0.0018	0.0051	0.6178

It was found somewhat difficult to obtain accurate information as to the length of time during which these tumours had been growing—a point of some importance in this investigation—and it has therefore been necessary to rely on the approximate data furnished by the patients themselves. In this respect I must state my indebtedness to the Medical Staff of the Cancer Hospital for their kindness in placing at my disposal the clinical records of the cases examined. For all but three of the normal human tissues I must express my gratitude to Dr E. H. Kettle, Assistant Pathologist to St Mary's Hospital, London. They were obtained from normal subjects who had been the victims of fatal accidents.

The Rous tumours were obtained by experimental inoculation of small grafts of the tumour made in the pectoral muscle of a series of fowls. The subsequent development of the graft was then closely watched and all the particulars of its growth carefully noted. By this means it was possible to gain possession of much valuable data with regard to the growth of each individual tumour which it was seldom possible to get in the human cases.

The full particulars of the cases are not reproduced in entirety, but the points of actual importance with regard to each tumour are given in the tables so that they are in the best position for purposes of comparison.

The normal chicken organs were taken from healthy adult hens of the Plymouth Rock strain. Included in the tables of the analyses of these normal chicken organs will be seen several which are described as being taken from stunted chicks; these chicks were ones which had been reared from an early age in this Institute and which had become stunted before reaching the adult age [Drummond, 1916].

DISCUSSION OF RESULTS.

From a consideration of the analytical data recorded in the tables (I-VI), it is quite apparent that this research has entirely failed to corroborate the conclusions of Kocher. That this is so will be more easily realised if the following table is surveyed. It contains the average figures for each group of analyses made in this study compared with the averages given by him, from which he draws his conclusions.

TABLE VII.

Average Values for Distribution of Nitrogen in Proteins of Normal and Cancer Tissues.

	Amide N	Humin N	Mon- amino N of filtrate	Non- amino N of filtrate	Arginine N	Histidine N	Lysine N	Cystine N	Total N of diamino- acids
Human tumours ...	4.73	2.42	54.24	7.90	10.09	7.09	12.04	1.12	30.54
Normal human tissues	5.82	3.09	55.88	4.94	11.13	7.86	9.83	0.93	30.71
Chicken tumour ...	5.58	4.84	48.44	5.87	11.50	9.96	12.04	0.83	34.38
Normal chicken organs	7.20	3.73	55.62	5.11	10.13	7.26	9.40	1.05	27.70
Normal organs (a) ...	—	—	—	—	6.07	1.78	5.68	—	13.52
Human tumours (b)	—	—	—	—	12.42	4.86	11.23	—	28.48

(a) consists of the averages of a series of analyses of normal tissues made by Wakeman [1908], and quoted by Kocher [1915], for purposes of comparison with the results of the analyses of five tumour proteins made by the latter author, which are averaged at (b).

The normal tissues which are included in the average (a), however, include widely diverse organs taken from man, dog, ox, and horse.

It is also at once apparent that the great error made by Kocher was in not carrying out control estimations upon normal human tissues himself, for his results for the partition of nitrogen in the tumour proteins are of very much the same order as those which have been obtained during the course of this research. His figures for the percentages of arginine and histidine are probably more correct than the ones given in this communication, because in several cases his determinations were controlled by the isolation of these

TABLE VI.

Analytical Data for Proteins of Normal Human Tissues.

Tissue	Total N vol. 100 cc. N in 5 cc. cc. 0.1 N acid	Total N	Amide N vol. 90 cc. cc. 0.1 N acid	Amide N	Humin N vol. 90 cc. cc. 0.1 N acid	Humin N	Monamino-acid fraction vol. 500 cc.					Diamino-acid fraction vol. 50 cc.										Total N re- covered
							N in 25 cc. cc. 0.1 N acid	Total N in filtrate	cc. N gas from 10 cc.	Amino N of filtrate	N in 5 cc. cc. 0.1 N acid	Total N of bases	cc. N gas from 5 cc.	Amino N of bases	Arginine N in 10 cc. cc. 0.1 N acid	Arginine N	g. BaSO ₄ from 5 cc.	Cystine N				
Pancreas 1 ...	$\begin{pmatrix} 14.15 \\ 14.30 \\ 15.00 \end{pmatrix}$	0.3980	14.10	0.0219	11.50	0.0179	$\begin{pmatrix} 7.30 \\ 7.30 \\ 8.70 \end{pmatrix}$	0.2265	7.20, 20° C., 751 mm.	0.2125	$\begin{pmatrix} 8.15 \\ 8.20 \\ 7.00 \end{pmatrix}$	0.1270	$\begin{pmatrix} 10.90 \\ 11.00 \\ 8.00 \end{pmatrix}$	19.5° C., 763 mm.	0.0700	2.90	0.0450	0.0011	0.0037	0.3933		
Kidney 1 ...	$\begin{pmatrix} 15.00 \\ 15.10 \\ 15.95 \end{pmatrix}$	0.4220	13.60	0.0212	8.80	0.0137	$\begin{pmatrix} 8.70 \\ 8.70 \\ 7.50 \end{pmatrix}$	0.2707	22° C., 758 mm.	0.2500	$\begin{pmatrix} 8.00 \\ 8.00 \\ 6.95 \end{pmatrix}$	0.1090	$\begin{pmatrix} 8.00 \\ 7.75 \end{pmatrix}$	20° C., 764 mm.	0.0547	3.25	0.0505	0.0012	0.0040	0.4046		
Spleen 1 ...	$\begin{pmatrix} 15.95 \\ 16.10 \\ 14.40 \end{pmatrix}$	0.4480	14.90	0.0232	9.95	0.0155	$\begin{pmatrix} 7.50 \\ 7.60 \\ 7.50 \end{pmatrix}$	0.2350	22° C., 758 mm.	0.2190	$\begin{pmatrix} 7.10 \\ 7.05 \\ 6.80 \end{pmatrix}$	0.1750	$\begin{pmatrix} 11.20 \\ 11.30 \\ 8.40 \end{pmatrix}$	19.5° C., 763 mm.	0.0928	3.62	0.0560	0.0015	0.0049	0.4487		
Cardiac muscle 1	$\begin{pmatrix} 14.40 \\ 14.15 \\ 14.40 \end{pmatrix}$	0.4000	14.70	0.0227	3.95	0.0062	$\begin{pmatrix} 7.50 \\ 7.50 \\ 8.25 \end{pmatrix}$	0.2350	22° C., 758 mm.	0.2110	$\begin{pmatrix} 6.75 \\ 7.55 \\ 7.65 \end{pmatrix}$	0.1315	$\begin{pmatrix} 8.50 \\ 13.60 \\ 12.20 \end{pmatrix}$	19.5° C., 763 mm.	0.0862	2.42	0.0375	0.0010	0.0033	0.3954		
Skeletal muscle 1	$\begin{pmatrix} 14.40 \\ 14.55 \\ 15.00 \end{pmatrix}$	0.4050	16.10	0.0250	6.00	0.0093	$\begin{pmatrix} 8.25 \\ 8.30 \\ 7.85 \end{pmatrix}$	0.2575	8.06, 20° C., 751 mm.	0.2380	$\begin{pmatrix} 7.55 \\ 7.65 \\ 8.10 \end{pmatrix}$	0.1180	$\begin{pmatrix} 12.20 \\ 12.30 \\ 10.40 \end{pmatrix}$	19.5° C., 763 mm.	0.0772	2.87	0.0446	0.0010	0.0033	0.4098		
Cardiac muscle 2	$\begin{pmatrix} 15.00 \\ 15.00 \\ 12.95 \end{pmatrix}$	0.4200	17.20	0.0267	9.70	0.0151	$\begin{pmatrix} 7.90 \\ 7.90 \\ 6.80 \end{pmatrix}$	0.2445	20° C., 751 mm.	0.2240	$\begin{pmatrix} 7.10 \\ 7.15 \\ 8.10 \end{pmatrix}$	0.1260	$\begin{pmatrix} 10.40 \\ 10.44 \\ 10.40 \end{pmatrix}$	19.5° C., 763 mm.	0.0665	2.70	0.0420	0.0010	0.0033	0.4123		
Liver 2 ...	$\begin{pmatrix} 12.95 \\ 12.90 \\ 14.50 \end{pmatrix}$	0.3620	14.20	0.0221	7.70	0.0120	$\begin{pmatrix} 6.80 \\ 6.75 \\ 7.60 \end{pmatrix}$	0.2100	6.42, 20° C., 764 mm.	0.1940	$\begin{pmatrix} 7.15 \\ 7.10 \\ 7.95 \end{pmatrix}$	0.1115	$\begin{pmatrix} 10.40 \\ 10.40 \\ 10.30 \end{pmatrix}$	20° C., 764 mm.	0.0663	2.45	0.0380	0.0014	0.0047	0.3556		
Kidney 2 ...	$\begin{pmatrix} 14.50 \\ 14.60 \\ 17.30 \end{pmatrix}$	0.4075	15.80	0.0246	9.40	0.0146	$\begin{pmatrix} 7.60 \\ 7.60 \\ 9.55 \end{pmatrix}$	0.2360	20° C., 764 mm.	0.2145	$\begin{pmatrix} 6.75 \\ 6.75 \\ 7.95 \end{pmatrix}$	0.1239	$\begin{pmatrix} 10.30 \\ 10.20 \\ 14.30 \end{pmatrix}$	21° C., 764 mm.	0.0653	3.04	0.0466	0.0013	0.0044	0.3991		
Skeletal muscle 2	$\begin{pmatrix} 17.30 \\ 17.30 \\ 15.20 \end{pmatrix}$	0.4840	20.00	0.0312	7.10	0.0111	$\begin{pmatrix} 9.55 \\ 9.55 \\ 8.65 \end{pmatrix}$	0.2960	9.32, 20° C., 751 mm.	0.2760	$\begin{pmatrix} 9.60 \\ 9.55 \\ 7.95 \end{pmatrix}$	0.1485	$\begin{pmatrix} 14.30 \\ 14.00 \\ 11.30 \end{pmatrix}$	20° C., 764 mm.	0.0889	3.50	0.0545	0.0013	0.0044	0.4868		
Pectoral muscle 3	$\begin{pmatrix} 15.20 \\ 15.20 \\ 14.00 \end{pmatrix}$	0.4260	14.80	0.0231	6.70	0.0104	$\begin{pmatrix} 8.65 \\ 8.50 \\ 8.00 \end{pmatrix}$	0.2660	8.25, 20° C., 764 mm.	0.2490	$\begin{pmatrix} 7.95 \\ 8.00 \\ 6.80 \end{pmatrix}$	0.1240	$\begin{pmatrix} 11.30 \\ 11.40 \\ 10.10 \end{pmatrix}$	20° C., 764 mm.	0.0722	3.25	0.0505	0.0011	0.0037	0.4235		
Breast 4 ...	$\begin{pmatrix} 14.00 \\ 13.85 \\ 14.00 \end{pmatrix}$	0.3900	13.80	0.0215	6.30	0.0098	$\begin{pmatrix} 8.00 \\ 8.02 \\ 7.85 \end{pmatrix}$	0.2480	7.40, 20° C., 764 mm.	0.2230	$\begin{pmatrix} 6.80 \\ 6.80 \\ 6.60 \end{pmatrix}$	0.1060	$\begin{pmatrix} 10.10 \\ 10.00 \\ 10.39 \end{pmatrix}$	21° C., 764 mm.	0.0644	2.53	0.0394	—	—	0.3853		
Breast 5 ...	$\begin{pmatrix} 14.00 \\ 14.00 \end{pmatrix}$	0.3925	12.85	0.0200	7.20	0.0112	$\begin{pmatrix} 7.85 \\ 7.90 \end{pmatrix}$	0.2446	7.35, 20° C., 764 mm.	0.2210	$\begin{pmatrix} 6.60 \\ 6.60 \end{pmatrix}$	0.1030	$\begin{pmatrix} 10.39 \\ 10.30 \end{pmatrix}$	21° C., 764 mm.	0.0660	2.53	0.0394	—	—	0.3788		

Normal Chicken Tissues.

Lungs 3 ...	13.80	0.3860	*18.40	0.0272	*11.90	0.0175	7.70	0.2260	6.50, 20° C., 772 mm.	0.1975	$\begin{pmatrix} 8.15 \\ 8.10 \\ 9.40 \end{pmatrix}$	0.1195	$\begin{pmatrix} 11.30 \\ 11.40 \\ 12.60 \end{pmatrix}$	19.5° C., 762 mm.	0.0684	2.94	0.0434	0.0016	0.0051	0.3902
Lungs 2 ...	14.88	0.4160	18.60	0.0274	10.80	0.0159	8.10	0.2385	6.93, 20° C., 770 mm.	0.2105	$\begin{pmatrix} 9.40 \\ 9.25 \\ 7.80 \end{pmatrix}$	0.1370	$\begin{pmatrix} 12.60 \\ 12.40 \\ 11.30 \end{pmatrix}$	19.5° C., 762 mm.	0.0758	3.43	0.0508	0.0018	0.0058	0.4188
Liver 1 ...	13.65	0.3820	19.50	0.0287	11.40	0.0168	7.80	0.2295	7.11, 20° C., 772 mm.	0.2160	$\begin{pmatrix} 7.60 \\ 7.60 \\ 6.00 \end{pmatrix}$	0.1135	$\begin{pmatrix} 11.30 \\ 11.30 \\ 8.30 \end{pmatrix}$	18° C., 742 mm.	0.0670	2.35	0.0346	0.0015	0.0049	0.3885
Liver 2 ...	11.55	0.3240	18.10	0.0267	12.30	0.0181	6.60	0.1940	5.85, 20° C., 770 mm.	0.1775	$\begin{pmatrix} 6.25 \\ 6.25 \\ 6.00 \end{pmatrix}$	0.0900	$\begin{pmatrix} 8.40 \\ 8.30 \\ 8.35 \end{pmatrix}$	19° C., 762 mm.	0.0506	2.00	0.0295	0.0013	0.0041	0.3288
Liver 3 ...	11.70	0.3270	18.25	0.0269	12.30	0.0181	6.60	0.1940	5.95, 20° C., 772 mm.	0.1810	$\begin{pmatrix} 6.25 \\ 6.25 \\ 5.40 \end{pmatrix}$	0.0900	$\begin{pmatrix} 8.30 \\ 8.35 \\ 7.50 \end{pmatrix}$	17.5° C., 772 mm.	0.0505	2.10	0.0310	0.0012	0.0038	0.3290
Pectoral muscle 1	10.20	0.2860	11.80	0.0174	4.27	0.0063	6.05	0.1780	5.45, 20° C., 772 mm.	0.1655	$\begin{pmatrix} 5.40 \\ 5.40 \\ 9.55 \end{pmatrix}$	0.0795	$\begin{pmatrix} 7.45 \\ 7.45 \\ 12.30 \end{pmatrix}$	17.5° C., 772 mm.	0.0461	2.11	0.0311	0.0008	0.0025	0.2812
Pectoral muscle 2	15.42	0.4320	19.20	0.0283	4.90	0.0072	8.80	0.2595	8.25, 20° C., 770 mm.	0.2500	$\begin{pmatrix} 9.55 \\ 9.55 \\ 9.00 \end{pmatrix}$	0.1405	$\begin{pmatrix} 12.50 \\ 12.50 \\ 13.80 \end{pmatrix}$	17.5° C., 772 mm.	0.0765	3.26	0.0479	0.0012	0.0038	0.4355
Pectoral muscle 3	15.90	0.4455	20.30	0.0299	5.30	0.0078	9.20	0.2705	8.25, 20° C., 772 mm.	0.2500	$\begin{pmatrix} 9.10 \\ 9.10 \\ 3.90 \end{pmatrix}$	0.1335	$\begin{pmatrix} 13.80 \\ 13.80 \\ 4.55 \end{pmatrix}$	18° C., 742 mm.	0.0834	2.60	0.0383	0.0013	0.0041	0.4417
Cardiac muscle 1	7.85	0.2195	9.60	0.0142	5.10	0.0075	4.80	0.1415	4.30, 22° C., 771 mm.	0.1302	$\begin{pmatrix} 3.90 \\ 3.90 \\ 7.70 \end{pmatrix}$	0.0575	$\begin{pmatrix} 4.60 \\ 4.60 \\ 9.10 \end{pmatrix}$	18° C., 742 mm.	0.0280	1.10	0.0250	0.0006	0.0019	0.2207
Cardiac muscle 2	14.45	0.4050	18.20	0.0268	9.10	0.0134	8.20	0.2420	7.20, 20° C., 772 mm.	0.2185	$\begin{pmatrix} 7.55 \\ 7.55 \\ 4.80 \end{pmatrix}$	0.1120	$\begin{pmatrix} 9.30 \\ 9.30 \\ 7.20 \end{pmatrix}$	19° C., 761 mm.	0.0565	3.14	0.0461	0.0011	0.0038	0.3942
Cardiac muscle 3	9.30	0.2605	10.70	0.0158	5.60	0.0082	5.60	0.1647	4.97, 20° C., 770 mm.	0.1510	$\begin{pmatrix} 4.85 \\ 4.85 \\ 2.82 \end{pmatrix}$	0.0710	$\begin{pmatrix} 7.25 \\ 7.25 \\ 3.70 \end{pmatrix}$	18.5° C., 762 mm.	0.0436	1.77	0.0260	0.0007	0.0022	0.2597
Brain ...	9.30	0.2605	18.70	0.0276	9.30	0.0137	6.00	0.1765	—	—	$\begin{pmatrix} 2.82 \\ 2.85 \end{pmatrix}$	0.0416	$\begin{pmatrix} 3.70 \\ 3.75 \end{pmatrix}$	18.5° C., 762 mm.	0.0225	1.30	0.0191	0.0007	0.0022	0.2594

* Volume analysed 95 cc. in all this series.

substances in the form of their picrolonates. It will be noticed that his values for arginine are somewhat higher, and those for histidine slightly lower than have been found in the cases recorded here; this, however, is explainable by the error which it is believed exists in the determinations of the first-named substance.

The averages given in the last table are only of value to indicate whether any striking difference exists between tumour tissues and normal tissues from the point of view of the present study, for they are not in many cases strictly comparable; thus, for example, it is not permissible to draw any definite conclusion from the comparison of the amino-acid partition of the proteins of a sarcoma and of normal voluntary muscle, for we are dealing with two totally different types of tissue. We must therefore draw our conclusions as far as possible from the comparison of the normal and malignant cell of the same type.

Owing to the great difficulty of obtaining many of the specimens one would like to examine, the possibilities of carrying this out are very restricted, and only in the case of one organ—the breast—has this ideal comparison been to some extent possible. Even in this case the ideal has, unfortunately, not been realised to the extent hoped for, owing to the difficulty in obtaining certain of the material, particularly the normal gland (Table VIII).

TABLE VIII.

Average Values for the Distribution of Nitrogen in the Proteins of Normal and Pathological Conditions of the Mammary Gland.

		Amide N	Humin N	Mon- amino N of filtrate	Non- amino N of filtrate	Arginine N	Histidine N	Lysine N	Cystine N	Total N of bases
Normal	...	5.30	2.67	56.68	6.20	10.06	3.73	12.92	—	26.72
General fibrosis	...	3.94	2.08	53.01	12.64	10.58	6.69	9.56	1.19	27.72
Slow growing scirrhus	...	4.49	2.03	53.30	11.69	11.42	5.84	10.65	1.10	28.46
Rapidly growing tu- mours	...	5.36	2.35	55.29	6.23	10.30	5.78	12.06	—	30.30
Secondary deposits in glands	...	4.91	2.62	55.88	4.08	10.50	8.10	13.33	—	32.60

Coming to a more detailed study of the various groups of analytical data we will first turn to the results obtained in an examination of the normal and pathological mammary gland.

Only two normal breasts have been examined, owing to the difficulty mentioned, and in both these cases there was present a slight degree of fibrosis.

Fibrosis of the breast when extensive may, and often does, give rise to consequences which make the removal of the gland advisable; in fact the condition is regarded by many as being one of the so-called "pre-cancerous" states. If this view is adopted one may fairly compare results obtained in the analysis of such a case with the normal breast on the one hand, and with the case of the fibrous scirrhus carcinoma of the breast on the other. Four examples of this excessive fibrosis of the breast have been investigated. The average percentage of nitrogen present in the form of diamino-acids is 27.72, a figure that is slightly higher than the corresponding value of 26.92 for the normal gland. The large amount of fibrous tissue present in the cases of so-called chronic mastitis is to some extent reflected in the abnormally high value for the proline group of amino-acids, which averages 12.64 % of the total nitrogen, as compared with the figure 6.2 % in the case of the protein of the normal gland.

A similarly high percentage for the nitrogen present in this form is also recorded in the analysis of the uterine fibromyoma, another structure very rich in fibrous tissue. In the examples of the slow growing scirrhus carcinomata of the breast, of which there are five, we have a tissue which besides showing a certain degree of proliferation of the glandular elements, also possesses a considerable increase in the amount of fibrous stroma. Both these facts are distinctly traceable in the analytical results; thus the diamino-acid nitrogen percentage of 28.13 is a higher figure than that recorded for either the normal gland or for the mastitic condition, while the comparatively high figure for the proline group of amino-acids denotes a larger amount of the proteins of fibrous tissue than is normal. Taking now the more rapidly growing tumours of the organ under examination, examples of which are the encephaloid carcinomata, the adenocarcinoma, and the myxosarcoma, we find that the percentage of the diamino-acids is somewhat larger, 30.92 % of the total nitrogen being present in this form, whilst the proportion of the non-amino-acid of the filtrate is considerably lower.

Once again both these results are in accordance with the fact that we are now dealing with the more cellular type of tumour, that is one richer in nuclear material and relatively poorer in connective tissue stroma.

As is well known, malignant tumours of the breast are particularly prone to form metastatic deposits in the lymphatic glands of the axilla, and it is probable that in some cases the rate of growth of these secondary deposits is relatively much more rapid than that of the primary growth, especially in the case of the slow growing scirrhus tumours, a result that

is possibly due to what may be called the greatly improved environment of the secondary foci.

Six specimens of such secondary deposits have been analysed, and in each case it is possible to compare these analyses with those of the primary growths from which they are derived. The results are uniform in nature, while their average will show that their greater rate of growth, and therefore their greater content of cellular matter, is portrayed in both the high figure of 32.36 % for the diamino-acid nitrogen, and the low value of 4.08 % for the proline fraction.

The other tumours which have been analysed have given results of both interest and importance. Case No. 16 is particularly interesting as being an exceedingly rapidly growing lymphosarcoma of the cervical glands. Occurring in a young man of twenty, it attained a very large size and weighed over 900 g. in the unusually short space of six months. Microscopically the tumour was seen to be an extremely active one, containing a very large amount of nuclear substance, with little supporting tissue, and this observation is well borne out by the analytical data. The percentage of the total nitrogen present in the form of diamino-acids in this tumour is the highest recorded in the whole series of analyses, namely, 38.20. It is also of considerable interest to compare this analysis with the only analysis made upon a normal lymph-gland, that of the spleen, when it will be seen that in this normal organ we have an almost identical figure for this determination, namely, 38.10 %. Results such as these should have much weight in effectively disposing of the theory that in cancer tissues we have an abnormally high proportion of the diamino-acids.

Turning now to the analytical data recorded for the proteins of the Rous sarcoma and normal chicken tissues, we find that they are very similar in nature to those we have just considered. As has been already remarked, we are not strictly justified in drawing comparisons between these two types of tissues because no normal example of the connective tissue of the fowl has been examined; for it must be remembered that the neoplasm under consideration is one of connective tissue origin.

From the average figures of analysis it will be seen that there is a distinctly higher proportion of the diamino-acids present in the proteins of the tumours of this series than in the normal chicken tissues. This is in all probability due to the much more cellular character of the tumours, for microscopically the growth is a richly cellular structure possessing a very large amount of nuclear substance.

By the study of this particular neoplasm it was hoped that it would be possible to trace in the results the influence of the rate of growth upon the content of diamino-acids. From the summarised results given below, however (Table IX), it will be seen that this expectation has not been realised to the extent that was desired. Differences of a small nature do exist, and they are in every case in the direction which was expected, but it is probably due to the fact that the slowest growing tumour of this type is relatively rich in nuclear matter that they are not of a more striking order.

TABLE IX.

Influence of the Rate of Growth upon the Distribution of Nitrogen in the Proteins of the Rous Sarcoma.

Rate of growth ¹	Amide N	Humin N	Mon- amino N	Non- amino N	Arginine N	Histidine N	Lysine N	Cystine N	Total diamino N
Approx. 1 g. per day	5.57	5.67	46.89	8.00	12.05	9.26	11.68	0.86	33.83
From 2-4 g. per day	5.14	3.40	48.86	4.90	10.82	10.05	13.00	0.92	34.90
From 5-12 g. per day	5.93	5.38	48.38	4.66	11.48	10.58	11.96	0.81	35.79

¹ An approximation derived from the final weight of the excised primary growth and the period of time from the injection to the excision. The weight of tumour tissue in the bird in the form of metastatic deposits is not allowed for, although in some cases considerable.

From a general survey of the analyses as a whole, it must be acknowledged that there is more evidence to be derived from them in favour of the opinion that the higher proportion of hexone bases in malignant growths is dependent on the amount of nuclear substance present, than there is in support of the view, held by some, that this fact constitutes a specific property of the cancer cell.

Turning now to the individual amino-acids which have been estimated, it is felt that the accuracy of these estimations is not of a sufficiently high degree to warrant any definite deductions. On the other hand, if it is assumed that the errors of determination are of such a character that the results yielded by them may be used for a roughly comparative study, then one or two points of interest are noticeable. Firstly the values for lysine are higher in both the human and the chicken tumour proteins than they are in the normal tissue proteins.

Now it is quite true, as Kocher has remarked, that the recent work of Osborne and Mendel, and also of McCollum and Davis, has taught us the great importance of lysine for the growing animal, but it is hardly justifiable on these grounds alone to connect the high lysine content of tumours with their rapid growth.

On the one hand the increase of this particular diamino-acid in these cases—or indeed the increase of the diamino-acids generally—may merely represent the much greater activity of the cancer cell, expressed in its increased rate of cell-division and its consequent richness in nuclear substance; whereas on the other hand there is the view, apparently held by some, that one or more of these hexone bases acting as what Kocher calls a “growth-factor” may actually be the cause of cell-proliferation. The latter alternative seems altogether untenable, although at the same time it must be borne in mind that there seems every reason to believe that the growth of tumours is controlled by very much the same factors as influence normal growth.

These factors are many in number, for it is realised now that, in order to be adequate for growth, the diet of a young animal must be satisfactory from several standpoints. Thus, not only must the supply of protein in the diet be ample for the nutritive needs of the animal as regards its nitrogen intake, but it must also be satisfactory as regards its amino-acid make-up; while there is also the group of substances which are classed as “accessory growth hormones” which play such an important part in nutrition, but the nature of which we are at present quite unable to define.

All these factors, and no doubt many others, probably influence tumour growth to a greater or less extent, but it seems unwise to infer that any one of them, merely because it occurs in malignant growths to a greater extent than in normal tissues, is the underlying cause of an abnormal rate of cell-division.

Although the average results for all the tumours gives a higher figure for lysine than in the case of the normal tissues, this is not so when the analyses of the normal and pathological breast tissues are surveyed, and again no greatly increased amount of this amino-acid is present in the rapidly growing tumours as compared with the more slowly growing ones.

Of the other amino-acids, high figures for histidine will be observed in some cases, although it is realised that the doubtful accuracy of the arginine determinations has had the effect of yielding higher values for histidine than actually exist, in nearly every case.

Hopkins [1916] has recently demonstrated that this latter amino-acid, together with arginine, is to be regarded as of as much importance to the growing animal as is lysine. His studies have shown that either one of these two compounds can act as the precursor of the purines synthesised in the growing organism, so that they must both be of the greatest importance in the elaboration of nuclear substance.

We may on these grounds designate either histidine or arginine "growth-factors" with quite as much justice as Kocher has done in the case of lysine, although there is little evidence that they are markedly increased in cases of cancer.

The high figures for histidine, to which reference has been made, probably have their origin in at least two facts; firstly, there is a large amount of nucleo-protein in the product analysed, and secondly there is the presence of a certain amount of blood proteins in most of the tumours.

The Rous chicken sarcoma closely resembles the true rapidly growing sarcomata in being exceedingly vascular in character, so that all the growths examined contained a considerable amount of blood. Particularly in the case of the more rapidly growing examples was this so, for it was usual to find these tumours necrotic in the centre, and the cavity filled with extravasated blood and a thick mucoid fluid.

It is therefore conceivable that the high histidine figures may be partly due to the inclusion of a greater or less proportion of blood in the analysis.

Some evidence in favour of this explanation is given by the analysis of the spleen where a similarly high value for this particular determination is recorded.

Returning to a general survey of the results as a whole, it will at once be admitted that the conclusions put forward by Kocher have received no confirmation from this research. Seeking for an explanation of this, we find it in the reliance which this worker has placed on the analysis of normal tissues made by other workers.

Kocher's analyses of tumour proteins are in very good agreement with the analytical data recorded in this communication, as well as with those of Bergell [1907], so that it seems fair to assume that the analytical results for normal tissues which he quotes are inaccurate.

Below are tabulated as far as possible the available analytical figures given by various workers for some normal tissue proteins (Table X). It will be seen that the majority of the figures are in agreement in recording a higher percentage of diamino-acids in these tissues than is given by Wakeman.

In conclusion, it can be said that this research has yielded many results which support the view of Wells [1914], who holds that the cancer cell is very similar, if not identical, in chemical composition with the normal cell of the same type.

All the evidence points to the conclusion that the higher percentage of

diamino-acids which is often present in the proteins of malignant growths is due to the greater proportion of nuclear material which they contain, for similarly high figures for this fraction of the protein molecule are found in the analysis of those normal tissues which possess cells rich in nuclein, as for example, normal human pancreas and spleen.

A similar explanation is applicable to the high diamino-acid content of spermatozoa, which is quoted by Kocher as an example of a germinating tissue rich in hexone bases.

TABLE X.

Analyses of Proteins of Normal Tissues. (Percentage of Total N.)

Tissue	Amide N	Humin N	Mon-amino N	Arginine N	Histi-dine N	Lysine N	Total di-amino N	Observer
Muscle, chicken ...	7.45	2.73	59.85	—	—	—	30.00	Osborne and Heyl [1908]
Muscle, ox ...	5.50	2.58	64.40	—	—	—	27.23	Osborne and Jones [1909]
Muscle, ox ...	—	—	—	7.50	1.80	7.60	16.90	Koelker and Slemmons, quoted by Kocher [1915]
Muscle, chicken ...	6.63	1.76	61.55	11.10	8.45	9.81	30.27	Drummond
Muscle, human ...	6.30	2.30	62.25	11.20	4.26	12.65	29.90	„
Liver, human ...	—	—	—	6.24	1.88	6.07	14.19	Wakeman [1908]
Liver, human ...	6.12	3.32	58.20	10.50	8.20	11.76	30.05	Drummond
Liver, chicken ...	8.18	5.53	59.47	9.30	7.30	9.30	27.60	„
Liver, dog ...	—	—	—	8.32	2.31	4.81	15.44	Wakeman
Kidney, dog ...	—	—	—	4.19	2.51	6.05	12.75	„
Kidney, human ...	5.05	3.24	64.40	11.95	5.89	7.00	25.85	Drummond
Haemoglobin ...	5.24	3.60	59.90	7.70	12.70	10.90	31.30	van Slyke [1911]
Serum protein, ox ...	7.05	1.60	58.80	11.05	4.40	13.55	31.75	Hartley [1914]
Serum protein, horse	7.25	1.60	61.95	9.50	5.60	12.80	30.00	„

SUMMARY.

1. The distribution of nitrogen in a large number of the proteins of human and chicken tumours and normal tissues has been examined. A higher proportion of the diamino-acids generally exists in the case of the tumour proteins.

2. It has been shown that in all probability the diamino-acid content of a tissue varies directly with the amount of nuclear material present.

3. The more rapidly growing and cellular tumours therefore show a higher percentage of these substances than do the slowly growing ones.

4. There is no evidence from the analytical data recorded for the proteins of malignant tumours, which supports the view that the hexone bases are in any way responsible for an increased rate of cell-division.

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