CCXV. THE PHOSPHATIDE AND CHOLESTEROL CONTENTS OF NORMAL AND MALIGNANT HUMAN TISSUES[1].

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PHOSPHATIDES and cholesterol are invariably constituents of animal tissues. They play a part in fat metabolism [MacLean and MacLean, 1927], and may be to some extent storage materials, but the work chiefly of Mayer and Terroine has shown that this is true only to a limited extent, and that they tend to maintain constant their quantities in any one tissue.

It is natural, then, that a rôle in regulating protoplasmic structure and function has been attributed to them. Of the modes of approach only one will be discussed here, which is derived from the facts that lecithin and cholesterol lower the interfacial tension between oil and water [Corran and Lewis, 1924; Okunev, 1928], and are emulsifying agents. Lecithin favours a dispersion of oil in water and cholesterol a dispersion of water in oil, the two being in this sense antagonistic, as shown by Corran and Lewis.

Similar action may well take place in the cytoplasm, particularly in cell-membranes. Corran and Lewis have expressed the view that a cell of which the contents show a high lecithin-cholesterol ratio is likely to be more permeable to water-soluble substances than a cell with a low ratio. This view receives some support from the fact that lecithin (or substances derived from it) favours haemolysis and cholesterol inhibits it *in vitro*. Too much lecithin presumably makes the membrane too labile for continued existence, while cholesterol counteracts the effect. Such effects may also be the reason for the retarding action of lecithin and the accelerating action of cholesterol on the growth of animal tumours found by Robertson and Burnett [1913], Moravek [1927] and Rondoni [1930].

The lipin content of a cell may, on the view of Corran and Lewis, influence its supply of building materials and metabolites.

This may permanently affect its general activities. In this way variation in lipin content might be one of many causal factors in the varied cell-differentiation that takes place in embryonic development, and might remain

[1] This investigation was undertaken on behalf of the Liverpool Medical Research Organisation (Director: Prof. W. Blair Bell, University of Liverpool).

in the adult cell as an expression of the variation in development. Cells having in some respects higher metabolism or more complex structure would be expected to have the higher lipin ratio.

An alternative view can be suggested on functional grounds. In the resting cell permeability to water-soluble substances may not be a limiting factor in maintenance of structure and metabolism. The growing cell, or more active resting cell, may require greater permeability to obtain its supplies. Hence the growing cell, or resting cell of high metabolism, may show a high lecithin-cholesterol ratio.

Yet little evidence can be offered that insufficient permeability hinders the mammalian cell from obtaining supplies.

The possible consequences of the antagonistic emulsifying properties can be developed in another way, not involving permeability. Although lipins probably tend to concentrate in cell-membranes, most of the material will usually remain in the interior of the cell, and may there share in regulating the state of aggregation of protoplasm. Lecithin is likely to favour fine dispersion in their aqueous medium of the protein-lipin colloids of protoplasm, and cholesterol is likely to tend to aggregate them. Finer dispersion will probably be accompanied by greater hydration. It may thus be argued that a high lecithin-cholesterol ratio will be found in cells of high water-content, high lability and high enzymic activity—since fine dispersion gives more available internal surface. These characteristics in a general way are those of young and growing cells, which have a high water-content and a high metabolic activity, and which must possess a labile structure to be able to undergo the rearrangements that precede cell-division.

Some evidence can be put forward in favour of the rôles attributed above to lipins. From a general colloid-chemical point of view their peptising effects are not likely to be restricted to oil-water systems, and work such as that of Handovsky, Lohmann and Bosse [1925] and of Theorell [1930] shows that there is very close association between lipins and serum or plasma proteins; Theorell finds that the less easily salted out the protein is, the higher is the phosphatide-cholesterol ratio of the associated lipin. Likewise, *in vitro* they affect the activity of enzyme extracts[1].

In the present paper are presented analyses of human tissues which, as far as they go, and in conjunction with already published data, tend to confirm the view that growing cells, in particular malignant cells, have a high phosphatide-cholesterol ratio. This is a finding not restricted to such cells; and there are other findings that theory does not predict. No exact and simple confirmation can be expected: factors other than lipins are concerned in

[1] Tsuneyoshi [1927] finds that lecithin increases and cholesterol diminishes (1) the consumption of oxygen by tissue powder in presence of succinic acid, and (2) the oxidation of glycine on charcoal. Eichholtz [1924] finds that lecithin promotes catalase action in serum. Cholesterol retards peptic action [Dörle, 1923]. However lecithin sometimes retards enzymic activity, as in the case of tryptic digestion [Hagihara, 1924; Standenath, 1925].

growth and metabolism. In addition, the material analysed seldom contains only one type of cell; our methods do not distinguish lecithin from other phosphatides, which may have quite different surface effects; nor is the capillary effect of cholesterol esters clear.

Experimental.

The tissues examined were removed at operations in Liverpool. The author is indebted for them principally to Prof. W. Blair Bell, Mr M. M. Datnow and Miss G. Griffith. Histological examinations of representative pieces of each tissue were made by Mr M. M. Datnow.

To avoid chemical change, tissues were minced and immersed in alcohol or acetone as soon as possible; this was complete in from 1 to 3 hours after excision for all but five specimens, which were kept overnight in a refrigerator.

The analytical methods have been described in an accompanying paper [Jowett and Lawson, 1931]. Mr E. W. Lawson assisted in the analytical work. Estimations were carried out in duplicate (with the exception of estimation of dry weight), and frequently several times when agreement was not good.

Tissues were sometimes kept some time in alcohol, and extracts were usually kept in chloroform for a considerable time, before the analyses were complete. Serious changes are unlikely to occur under these conditions.

Discussion of analytical results.

The analytical data that have been obtained are given in Table I. The tissues are seldom composed almost entirely of one type of cell. Some of the malignant tissues are diluted with fibrous tissue; one specimen of malignant tissue and some of the muscular tissues are diluted with fat[1]. Comparisons are made more difficult, but a number of conclusions can nevertheless be drawn.

According to many workers, for instance Cramer [1916], the water-content of young and of malignant tissues is higher than that of resting tissues, but in the present data this tendency seems usually to be obscured for malignant tissue.

In the present work, malignant tissues and normal epithelial tissues show on the whole higher contents of phosphatides and of cholesterol than do fibromyomata and normal muscle.

Of the seven specimens of malignant tissue, those containing the highest proportion of malignant cells have definitely the highest content of phosphatides and cholesterol, and tend to show the highest ratio of phosphatides to free cholesterol.

The data for cholesterol show a number of discrepancies, free cholesterol being sometimes found higher than total cholesterol, but nevertheless the conclusion may be drawn fairly definitely that of the tissues analysed malignant

[1] In Table I (last column), fat has been calculated by subtracting from the total lipoids the phosphatides and cholesterol; the figure will include fatty acids bound to cholesterol.

tissues are the only class in which the proportion of cholesterol in the ester form is considerable, averaging nearly 40 %.

Chorionic villi, according to Bell [1930], share many of the properties of malignant tissue. Of the two specimens analysed, the one obtained at an early period of pregnancy and containing a large proportion of syncytium should show these characteristics more than that at a later period, which

Table I.

No.	Tissue	% dry weight	% on dry weight of: Total lipoids	Phosphatides	Cholesterol: Free	Cholesterol: Total	Phosphatides / Free cholesterol	% cholesterol as esters	% fat in total lipoids
	Malignant:								
11 A	Carc. kidney (all malignant)	22·3	29·89	11·70	1·238	2·026	9·45	39	54
15 A	Sarc. uterus (all malignant)	17·5	19·51	8·98	1·667	2·64	5·4	37	40
3 A	Carc. omentum (¾ growth)	18·65	17·42	9·12	1·545	2·23	5·9	31	35
2 A	Carc. breast (40 % growth)	20·7	27·95	5·58	0·827	1·389	6·75	40	75
8	Carc. caecum (half fibrous tissue)	15·15	13·46	5·51	1·113	1·759	4·95	36	46
9 A	Carc. breast (half fibrous tissue)	20·1	14·71	5·36	1·138	2·096	4·7	46	50
14 A	Carcinoma (growth ¼, blood clot ¾)	12·5	9·22	4·06	1·053	1·208	3·9	13	43
	Fibromyomata uteri:								
5 B	All healthy growth	21·0	4·66	2·71	0·544	0·619	5·0	12	29
10 A	All healthy growth	24·5	3·38	1·395	0·320	0·286	4·4	?	50
7 A	Hyaline degeneration of half growth	19·5	2·84	1·45	0·247	0·344	5·9	28	37
16 B	Healthy growth, but chiefly muscle	20·5	7·48	5·93	0·753	0·605	7·9	?	13
	Epithelial tissues:								
5 C	Normal ovary	19·0	11·04	8·25	0·727	0·835	11·35	13	17
11 B	Normal kidney	20·2	17·58	8·04	1·522	1·587	5·3	4	45
17	Chorionic villi, 10–12 weeks	11·94	16·33	8·24	1·407	1·492	5·8	6	40
13	Chorionic villi, 7 months	15·5	10·90	5·10	1·133	1·101	4·5	0	43
	Uterine muscle (healthy):								
5 A	—	21·2	6·55	3·30	0·749	0·851	4·4	12	37
7 B	With a little mucosa	19·3	4·97	3·04	0·606	0·636	5·0	5	36
10 B	—	19·7	7·38	2·97	0·818	0·595	3·6	?	51
15 B	With a little mucosa	21·4	8·65	3·77	0·788	—	4·8	—	45
16 A	—	18·2	8·38	5·42	0·936	0·935	5·8	0	24
	Muscle:								
2 B	Pectoral	25·9	33·74	2·66	0·537	0·717	4·95	25	90
2 C	Pectoral	24·9	29·24	2·13	0·615	0·901	3·5	33	90
9 B	Striated, pectoral	27·1	25·19	2·84	0·291	0·242	9·8	?	88
14 B	Leg	15·8	11·03	5·74	1·042	0·843	5·5	?	40
14 C	Leg	22·5	7·38	2·83	0·264	0·235	10·7	?	59
6 B	Bowel muscle, ⅕ mucosa	17·1	23·85	3·40	0·660	0·628	5·15	?	83

contains little syncytium. Actually the younger has a greater water-content, a higher lipin content and a higher phosphatide-cholesterol ratio. Neither specimen contains an appreciable amount of cholesterol esters[1].

Associated with growing tissues we therefore appear to have a high lipin content and a high phosphatide-cholesterol ratio, but these are not restricted to such tissues. In the present data normal ovary[2] and normal kidney have

[1] Bienenfeld [1912] found a large proportion of bound cholesterol in early placentae, but her extraction appears to have been incomplete.

[2] The tissue analysed contained approximately: lutein tissue 2 parts, corpora albicantes 4, and ovarian stroma 11.

a high lipin content; ovary and two specimens of muscle have a very high phosphatide-cholesterol ratio.

That these quantities have nevertheless a definite significance for growing tissue of an abnormal nature can be shown in another way, by comparing abnormal tissue with the neighbouring tissue from which it may have arisen. Theoretically, the comparison should probably be made between the abnormal cell and the type of normal cell from which it arose. Practically, we are restricted to a comparison with neighbouring tissue of the same general type as that from which the abnormal may have arisen. Comparisons of this kind are given in Table II.

Table II. *Comparisons between growths and normal tissue.*

No.	Tissue	% on dry weight: Phosphatides	% on dry weight: Free cholesterol	Phosphatides / Free cholesterol	% cholesterol bound	% fat in total lipoids
11 A	Carc. kidney	11·70	1·238	9·45	39	54
11 B	Normal kidney	8·04	1·522	5·3	4	45
15 A	Sarc. uterus	8·98	1·667	5·4	37	40
15 B	Uterine muscle	3·77	0·788	4·8	—	45
5 B	Uterine fibromyoma	2·71	0·544	5·0	12	28
5 A	Uterine muscle	3·30	0·749	4·4	12	37
7 A	Uterine fibromyoma	1·45	0·247	5·9	28	37
7 B	Uterine muscle	3·04	0·606	5·0	5	36
10 A	Uterine fibromyoma	1·395	0·320	4·4	?	50
10 B	Uterine muscle	2·97	0·818	3·6	?	51
16 B	Uterine fibromyoma	5·93	0·753	7·9	?	13
16 A	Uterine muscle	5·42	0·936	5·8	0	24

The two malignant tissues are fortunately not admixed with normal tissue; of the four fibromyomata, one is degenerating and one is admixed with normal muscle. In all cases the abnormal tissue shows a higher ratio of phosphatide to free cholesterol than the normal tissue. Malignant tissue shows also a higher phosphatide content than the normal, but for the innocent growths the converse is usually true for both phosphatides and cholesterol. If the data for the fibromyomata and corresponding normal tissues be examined, it will be seen that regularities, which may be extended to the proportion of free fat in the lipoids, emerge only if each fibromyoma is compared with the neighbouring normal muscle removed at the same operation. If fibromyomata and uterine muscle are compared indiscriminately as classes, regularities are much less evident.

This suggests tentatively that abnormal tissue retains much of the character of the normal tissue it arises from; a suggestion in agreement with the well-known pathological fact.

In two cases we have data that may be used to answer in a preliminary way the question whether the composition of normal tissue situated close to a malignant growth differs from the composition of that situated further away. The data are given in Table III. In one case a carcinoma of the breast (2 A)[1]

[1] This specimen contained: healthy growth 6, fibrous tissue 4, leucocytes and necrotic tissue 3, and fat 2 parts.

is imbedded in pectoral muscle, and that near the growth (2 B) may be compared with that situated further away (2 C). In the other case a carcinoma (14 A) is surrounded by leg muscle, neighbouring (14 B) or further away (14 C). The specimens of muscle show no gross difference histologically, but

Table III. *Composition of tissue near a growth.*

	% on dry weight			Phosphatides / Free cholesterol
No.	Phosphatides	Free cholesterol	Total cholesterol	
2 A	5·58	0·827	1·389	6·75
2 B	2·66	0·537	0·717	4·95
2 C	2·13	0·615	0·901	3·5
14 A	4·06	1·053	1·208	3·9
14 B	5·74	1·042	0·843	5·5
14 C	2·83	0·264	0·235	10·7

examination of the figures shows some tendency for the normal muscle close to the growth to be more like the growth in composition than is the more distant muscle. Little more can be said with the limited data available, but the subject merits further investigation.

Previous work.

The results of previous work, some of it carried out by methods of doubtful value, will be briefly considered in relation to our findings.

The phosphatide contents of tissues tend to be higher in malignant tissues than in normal, in nervous tissue and glands than in muscle, and in young tissues than in old. In general, active tissues show higher contents than inactive. We may cite, for instance, the work of Enselme and Enselme [1927], who find more phosphatides in the malignant breast than in the normal gland. Decrease of phosphatide content on passing from young to older tissues is found for the testicle by Koch and Woods [1905], for the bone marrow by Glikin [1907], and the carcase of the young white rat by Sinclair [1930]. Sorg [1929] finds that in three kinds of rabbit muscle, the phosphatide content is greater in muscle of greater endurance capacity. According to Momigliano [1925], during involution of the human corpus luteum the phosphatide content decreases. Data are quoted by Bloor, Okey and Corner [1930] to show that the variation of phosphatide content in different animals parallels the intensity of metabolism; the content is, for instance, higher in the mouse than in man.

Bürger and Schlomka [1928] find that the cholesterol content of human skin decreases with increasing age, and Dam [1931] finds a decrease with age for the newly hatched chick. Burgheim and Joel [1931] find the cholesterol content of malignant growths greater than that of benign growths.

The ratio of phosphatides to cholesterol was found to be higher in a mouse tumour of rapid growth than in one of slow growth by Bullock and Cramer [1913–14]. In the corpus luteum of the sow, according to Bloor, Okey and Corner [1930], the phosphatide content parallels the physiological activity,

while the free cholesterol changes similarly but to a less extent. According to Iwano [1925] the lecithin-cholesterol ratio falls with age in transplanted rat tumours.

It cannot be pretended that indiscriminate comparison of different tissues shows any intelligible relations between lipin contents or lipin ratios and any individual structure or function as yet compared. The data of Osato and Heki [1930] on rabbit tissues may be used to illustrate the point. Their mean figures yield the following ratio of phosphatides to free cholesterol: heart muscle 21, liver 16, skeletal muscle 10·5, spleen 9·1, kidney 8·6, lung 6·7, and blood 3·8. It is understandable that heart muscle shows a higher value than skeletal muscle, but further interpretation is at present impossible. Muscle, liver and kidney have all different chemical functions, and a common factor showing a relation with the lipin ratios is yet to be found.

The relation of cholesterol esters to growth is not yet clear. The author finds much of the cholesterol in malignant tissue to be bound; but in transplanted tumours of mice and rats, according to Bullock and Cramer [1913–14], Iwano [1925], and Bolaffi [1929] there is little bound cholesterol. In placenta, according to the present work, there is little bound cholesterol, while Dam [1929, 1931] finds a large proportion in newly hatched chicks, which decreases in the first few weeks of life. Cholesterol esters accompany regression in the corpus luteum [Bloor, Okey and Corner, 1930; Momigliano, 1925]. However, Onizawa [1929] finds that the bound cholesterol varies more than the free cholesterol in tissues, and other data suggest the same. The conclusion may be due to greater experimental error, but yet it seems likely that cholesterol esters are fairly inert and constitute storage material.

Summary.

A discussion of the physico-chemical properties of lecithin and cholesterol leads to the conclusion that the lecithin-cholesterol ratio of growing cells, and probably other active cells, should be high.

A limited set of analyses of human tissues gives the following results. Pure malignant tissues have a higher phosphatide and cholesterol content, and tend to show a higher phosphatide-cholesterol ratio, than do malignant tissues admixed with normal tissue. Malignant tissues show a high proportion of bound cholesterol. Malignant and benign tumours show a higher phosphatide-cholesterol ratio than do the neighbouring tissues from which they may have arisen. Previous work has shown a definite parallelism in tissues between growth and activity and the phosphatide content.

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