ON THE BIOCHEMICAL MECHANISM OF GROWTH.

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In the following investigations, as in previous ones (1–5), the mechanism of growth has been studied as it presents itself in the growth of transplantable neoplasms. These neoplasms when propagated experimentally exhibit the phenomenon of growth in the simplest form. The growth of many tumours consists in a simple autonomous multiplication of cells, freed from the accessory phenomena which often accompany growth in the higher animals, such as progressive differentiation as it occurs in the foetus, functional activity, correlation between different tissues and organs and so on. It is important to realise that this property of growth is not the feature which distinguishes tumour cells from normal adult cells. All normal cells possess the property of growth, although in a varying degree, and their rate of growth is not necessarily less than that of the cells of the neoplasm. The formation of lymphocytes in the lymph nodes and of sex cells in the testis are perhaps the most obvious instances of active growth processes taking place in the adult organism. That the newly formed cells are dispersed and secreted, or excreted, so that there is no increase in the mass or size of these organs, is merely an incident which does not deprive these phenomena of their fundamental character of growth processes. Many transplantable tumours show indeed a rate of growth exceeding that of normal tissues although not approaching that of foetal tissues. But there are also tumours which grow very slowly, so that their rate of growth if measured, according to Minot(6), by the mitotic index would certainly fall below that of the more actively growing normal tissues. The fundamental difference between a normal cell and the cell of a neoplasm lies, as has been repeatedly pointed out in papers from this laboratory, in the property of unlimited growth which is exhibited by the latter. This problem of the ageing and senility of the cell which leads to the death of the normal cell, while the cancer cell escapes—for it is not altogether absent in the cells of a neoplasm which show phases of senescence

and rejuvenation—is a problem by itself. Here we are dealing with the more general problem—Why and how does a cell grow? or as it should be formulated more correctly,—Why do some cells grow more quickly than others living under the same nutritional conditions?

In a previous investigation this problem had been attacked(1) by determining the retention of nitrogen by a young animal (rat), and then observing the effect of implanting into this animal cells (a neoplasm) which grew more rapidly than the host. In confirmation of other experimental evidence, it was found that the growth of the neoplasm did not interfere with the continued retention of nitrogen by the host. An unexpected result was obtained when the fate of the retained nitrogen was further analysed(2). It was then found that with equal amounts of nitrogen the neoplasm had built up a larger mass of protoplasm than the host. This was confirmed by an analysis of the protein content of the fresh tissues of the neoplasm and in the host. Weight for weight the former contained less protein than the latter, while the amount of nitrogenous metabolites was as great or slightly greater in the case of the tumour cells. In other words, the rapidly growing cells of a neoplasm build up living protoplasm more economically with reference to protein than the more slowly growing cells of the host.

If the cells of a rapidly growing neoplasm contain less protein it is obvious that the deficit must be made up by other cell constituents. It was thought at first that lipoids, fats, or carbohydrates might account for the difference, but this supposition was not confirmed by chemical or histo-chemical observations(3). It seemed also possible to assume that the protein of the cells of the neoplasm differed from that of normal tissues in being much poorer in nitrogen. This however was negatived by the fact that the nitrogen content of the dried normal tissues and of the tumour did not show any marked difference. The observations of Medigraceanu and Abderhalden(7) on the analysis of the proteins isolated from transplantable tumours also show that no such gross chemical differences exist.

There remained then only the inorganic constituents of protoplasm, water and inorganic salts, to account for the diminished protein content of the rapidly growing cells of the rat tumour. An estimation of the water content of these cells and a comparison with the water content of the liver, kidney and muscle of the host and of normal rats showed indeed that the protoplasm of the tumour cells is much richer in water than that of the normal tissues mentioned above.

This raised the question whether a high water content was a property common to all cancerous protoplasm but absent from the protoplasm of normal cells. If that could be established, it would show an interesting difference in the composition of normal and cancerous protoplasm. Such a difference, however, whatever its significance might be, would not help us to understand the biochemical mechanism of growth. For, as has been indicated above, the phenomenon of growth is exhibited in a varying degree by normal cells as well as by cancerous cells and the variations of the rate of growth in both types of cells are considerable. If, on the other hand, the biochemical mechanism of growth were related to the water content of a cell in such a way that a rapidly growing cell builds up protoplasm with relatively more water and relatively less of the complex organic substances (proteins, lipoids, etc.) than a cell showing a slower rate of growth, the water content of a cell whether normal or cancerous should vary parallel with its rate of growth.

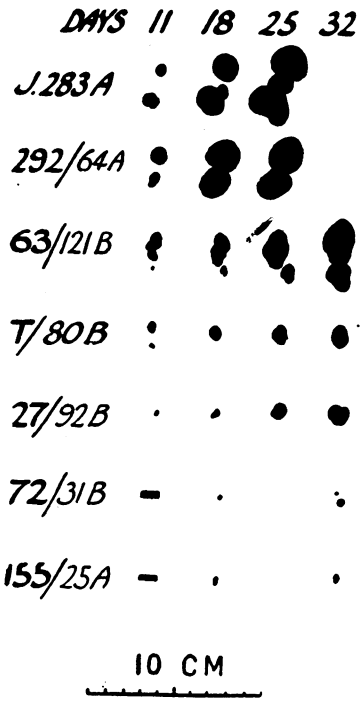

Fig. 1. The figure represents graphically the relative rate of growth of the different strains of mouse carcinomata with which this paper deals. The figure has been obtained by charting at equal intervals the size of typical tumours belonging to each strain.

It was decided, therefore, to investigate the water content of a number of different strains of transplantable neoplasms showing great differences in their rate of growth and to compare the results with the water content of different normal tissues and organs. The following eight strains of mouse carcinomata were taken on the advice of Dr J. A. Murray as representing great differences in their rate of growth: 63, Jensen, 292, 206, T, 27, 72, 155. Dr Murray, who has had these strains under observation for many years, grouped them as follows:

Very rapidly growing strains: Jensen, 292, 206, 63.

Very slowly growing strains: 27, 72, 155.

Strain of intermediate rate of growth: T.

The great differences of growth which exist between these different tumour strains can be seen from Fig. 1. A good idea of these differences in the rate of growth can be formed from the fact that a fortnight after subcutaneous transplantation of a piece of tumour tissue weighing about two centigrams, the rapidly growing tumour may have attained a weight of between two and five grams and threaten to break through the skin, while after the same period the slowly growing tumours are not visible and are barely palpable.

In the first series of observations the water content was estimated at irregular intervals after transplantation whenever any tumour belonging to one of the strains was being transplanted. The water estimations were carried out by heating the tissue in a weighing bottle in a toluol bath, which gave a constant temperature of 107°, until constant weight was obtained. With the amounts of tissue used (0·5 to 1·0 grm.) this required about 36–48 hours. The tumour was freed as much as possible from necrotic tissue by dissection. This can be done satisfactorily when the necrosis is central, as it usually is when it is due mainly to the size of the tumour being so great that the growth of the stroma is unable to keep pace with it. It is, however, not possible when the necrosis is more diffuse owing to inherent changes in the tumour cells themselves or when the central necrosis has become so extensive that only a thin layer of healthy tissue remains at the periphery. The occurrence of this miliary necrosis therefore introduces a fallacy in this series of observations which will be dealt with later. Another fallacy would be the occurrence of cysts filled with a watery fluid, but such strains were excluded from these observations. In the strains which had been selected the occurrence of such cysts is rare.

TABLE I. *Analysis of Mouse Carcinomata. I series.*

Tumour strain	Generation	Weight of tumour in grams	Days after transplantation	Necrosis	H_2O %
Jensen	280 A	0·8	14	–	83·5
	279 A	1·6	14	–	83·1
	242 B	3·0	21	+	82·3
	278 A	1·6	14	–	84·1
	277 A	2·7	14	–	84·2
	243 D	0·6	10	–	83·0
292	62 B	4·6	26	–	84·8
	63 B	4·1	22	++	84·6
	64 B	1·0	27	+	84·7
	65 B	1·2	28	+	83·4

TABLE I (*continued*)

Tumour strain	Generation	Weight of tumour in grams	Days after transplantation	Necrosis	H_2O %
206	173 B	3·0	16	+	84·4
		0·8		+	83·7
		0·6		+	83·0
63	116 C	5	32	++	83·2
	117 B	5	30	+++	82·1
	118 D	1·3	14	+++	82·2
T	76 A	0·6	101	+	80·7
	77 A	3·5	49	+	82·2
	78 A	1·5	45	++	82·5
27	85 D	3·4	69	+++	81·0
		1·7	44	+	81·6
	90 A	2·3	56	+	79·1
		3·0	56	+	79·4
		3·1	56	−	80·4
		3·4	44	−	81·7
	91 A	1·9	58	+	80·6
72	29 A	2·4	70	−	78·4
		3·0	70	−	80·0
	30 A	8·0	97	+	79·4
	31 A	5·9	88	−	80·0
155	21 B	2·5	147	−	79·9
	22 B	3·4	168	+	81·5

The results are given in Table I. The presence of visible necrosis which is usually central and well defined is indicated by the sign +. The extent of visible necrosis is indicated by the number of crosses, "+++" signifying an extreme amount of necrosis and consequently some difficulty in obtaining by dissection healthy tissue free from necrosis.

The water content of the normal tissues of the mouse is given in Table II. The tissues were taken from normal animals of the same

TABLE II. *Analysis of Normal Mouse Tissues.*

Tissue	H_2O % Average values	Ash % Average values calculated for	
		Dried tissue	Fresh tissue
Liver	72·3*	5·25	1·4
Muscle (skeletal) ...	75·8†	4·9	1·2
Heart	77·9	4·9	1·1
Kidney	77·2	6·0	1·4
Spleen	78·8	7·9	1·6
Testis	83·0	7·5	1·3

* Individual estimations varied from 73·6 % to 71·2 %.
† Individual estimations varied from 77·7 % to 73·8 %.

age, about eight weeks, as those used for transplantation. The animals had been kept in the laboratory for at least a month. With such animals which are kept under good and equal nutritional conditions the water content in different individuals is fairly constant for most tissues except the liver and skeletal muscle, where considerable variations occur. The figures given in the body of Table II represent therefore averages of four or six estimations for each tissue. In the case of the liver and skeletal muscle the extremes of the individual estimations are also given for the reasons just stated. The values obtained for normal tissues of the mouse agree well with the results obtained by Medigraceanu(8) in this laboratory. According to the same author there is no essential difference between normal animals and tumour-bearing animals of the same age as regards the water content of normal tissues, provided of course that accidental factors such as sepsis, cachexia, etc., were excluded. The liver, however, forms an exception as it has a slightly higher water content in tumour-bearing animals. The water content of tumours was not determined by Medigraceanu.

Inspection of Tables I and II shows:

1. *Differences between different tumour strains.* The highest water content (about 83–84·5 %) is exhibited by the rapidly growing strains, Jensen, 292, 206, while the lowest water content (79–80 %) is shown by the slowly growing strain 72. Next in order to strain 72 as regards the water content comes strain 155 (80–81·5 %), which also grows very slowly, and strain 27 with a water percentage of 79·1 to 81·7. An intermediate position is taken by strain T (about 81–82 % H_2O) and the more quickly growing strain 63 (about 82–83 % H_2O).

2. *Differences between the water content of different normal tissues.* These require no further comment beyond emphasising the exceptional position of the testis, the water content of which is very much higher than that of any other normal tissues.

3. *Differences between the water content of normal tissues on the one hand and the various carcinomata on the other hand.* If we except the testis, the water content of all the tumours is above that of the normal tissues, the lowest values of the slowly growing tumour strains just approaching the water content of the spleen (79 %). The water content of the testis (83 %) is above that of the slowly growing strains and is about equal to that of strains with a fairly rapid rate of growth such as strains 63 and Jensen.

4. *Differences between the water contents of tumours belonging to*

the same strain. There are minor differences superimposed upon the more marked differences existing between strains with very different rates of growth. These minor differences are due partly to the fallacy introduced by the varying amount of necrosis to which reference has already been made. They may, however, be related to other factors. As has been shown in communications from this laboratory certain tumour strains (Jensen and 292 for instance) show considerable fluctuations in their rate of growth at different times. Again there are sometimes differences in the rate of growth even between different individual tumours of the same generation. How far these minor differences are related to differences in the rate of growth has not been investigated in detail, since a clearer relationship between these two phenomena can be established by comparing the behaviour of strains as a whole. But as far as these observations go it can be said that these minor fluctuations in water content run parallel with fluctuations in the rate of growth in the same strain (compare, for instance, Jensen 283 A and 284 A with Jensen 243 D and 280 A) or even in the same generation (see for instance the three tumours of strain 206, generation 173 B). This point comes out even more clearly in the second series of observations and will be referred to there.

In the preceding pages we have spoken of the rate of growth of different tumours, and in doing so the size or weight of a tumour has been taken as the measure of its rate of growth, as is usually done in experimental cancer research. As a rough indication, this method is adequate. But for a more exact comparison a number of factors which give rise to fallacies must be excluded. If a neoplasm consisted only of a solid group of cells which continued to multiply, then the weight or size of the tumour at any given time would be a true indication of its rate of growth. But some tumours grow in cysts, others grow not only by multiplication of cells but also by deposition of material (fibres, mucin cartilage) between the cells (*e.g.* many sarcomata), or of fat within the cells (*e.g.* lipomata). In such cases increase in weight or size is not a true measure of growth[1]. Such tumours have therefore

[1] A detailed discussion of this point would go beyond the scope of this paper. It can only be indicated here that, from the point of view of the mechanism of growth, a distinction must be drawn between two types of growth. In the one represented by the growth of solid carcinomata, and, in the case of normal tissues, of organs such as the testis or lymph nodes, there is a new formation of living protoplasm in the cell which leads to the new formation of living cells. In the other case represented by the growth of connective tissue tumours and of normal tissues such as bone and cartilage this process of the new formation of living cells is accompanied and complicated by a process akin to secretion, which leads to the deposition of material between the newly-formed cells.

been excluded from our observations which refer only to solidly growing adenocarcinomata of the mouse. But there is another factor which introduces an error, namely the occurrence of necrosis. If we find, for instance, that two different tumours have reached the weight of 2 grms. within 20 days from an initial dose of 2 centigrms. we can conclude that these tumours have the same rate of growth provided that both tumours are free from necrosis. If one of them was markedly necrotic, the weight would cease to be an accurate means of comparing the rate of growth of these tumours and one would have to conclude that the necrotic tumour must have a greater rate of growth than the non-necrotic tumour, without however being able to make a numerical comparison, since in the former only the remaining healthy fraction of tumour cells continues to build up new protoplasm while in the latter all the cells do so.

In order to obtain results capable of a numerical comparison a second series of observations was carried out with the same strains of tumours. In this series the difficulties and fallacies referred to above were avoided or excluded by adopting the following plan. In each strain several tumours of about the same size and the same age after transplantation were used so that average values could be calculated. In order to avoid necrosis the tumours were taken as soon as possible after transplantation, whenever they had formed sufficient tissue for an analysis. This would require about 14 days in the rapidly growing strains (292, J, 63), and from three to four weeks in the strains of slower growth (T, 27, 72). Under these conditions it is possible to deduce the relative rate of growth in the case of the rapidly growing strains directly from the weight of the tumours. But it is not possible to compare directly the quickly growing strains with the slowly growing strains in this way, as the tumours of the latter strains had to be allowed to grow at least one or two weeks longer. The relative rates of growth of the various strains were therefore determined by taking the average square sizes of all the tumours as obtained by charting at the same period after transplantation (12 days).

The results obtained in this second series are given in Table III. The figures given there under the heading "growth index" indicate the average of the relative sizes of the tumours, using an arbitrary unit as the measure. That this is a fairly accurate method of comparing the rate of growth is shown by a comparison of the growth index thus obtained with the average weights in the case of the quickly growing tumour. They will be found to vary in about the same ratio, except

TABLE III. *Analysis of Mouse Carcinomata. II series.*

Tumour strain and generation	Days after transplantation	Weight of tumours in grammes: Single	Weight of tumours in grammes: Average	Growth index	H_2O Percentage: Single	H_2O Percentage: Average	Ash Percentage calculated for: Dried tissue	Ash Percentage calculated for: Fresh tissue	Average
		1·7			86·0		5·4	0·75	
		1·5			84·7		5·3	0·81	
292 69 A	13	1·7	**1·9**	**12**	86·9	**85·7**	7·9	1·03	**0·89**
		2·9			85·3		5·9	0·87	
		1·7			85·5		6·8	0·99	
		3·5			85·1				
(*a*) 283 A	14	3·5	**2·9**	**20**	84·3	**84·8**	8·3	1·3	**1·1**
		3·8			85·2				
		1·0			84·5		6·1	0·9	
J									
		2·0			84·4				
(*b*) 284 A	13	1·0	**1·5**	**11**	84·2	**84·4**	7·9	1·2	**1·2**
		1·9			84·4				
		1·0			84·7		8·0	1·2	
		0·4			83·3				
		0·4			83·1		6·4	1·1	
63 121 B	13	0·6	**0·47**	**3·7**	83·4	**83·6**			**1·15**
		0·4			84·4		7·8	1·2	
		0·6			83·9				
		0·4			84·2				
(*a*) 79 A	27	0·8	**0·65**	**1·5**	82·7	**82·6**	5·5	0·97	
		0·5			82·1				**0·95**
		0·9			81·4		5·0	0·93	
T									
		0·3			82·1				
(*b*) 80 B	21	0·4	**0·4**	**1·8**	82·9	**82·9**	5·1	0·88	**0·88**
		0·4			83·8				
		0·8			80·6				
		0·4			80·5		6·3	1·2	
(*a*) 92 B	24	0·4	**0·5**	**0·75**	80·8	**80·4**			**1·2**
		0·5			81·3		5·4	1·2	
		0·5			78·6				
27									
		0·4			79·8				
(*b*) 88 D	31	0·4	**0·37**	**0·33**	79·9	**79·4**	6·8	1·4	**1·4**
		0·3			78·6				
		0·6			81·6				
72 31 B	29	0·3	**0·38**	**0·3**	81·2	**81·0**	7·4	1·4	**1·4**
		0·4			81·2				
		0·2			80·2				

in the case of strain 292 where the weight of the tumours gives a higher rate of growth than is indicated by the figure given under "growth index." No reference is made to necrosis in Table III because under the conditions adopted this factor is negligible. The table also contains analyses of the ash of the various tumour strains. This will be referred to later.

The results obtained in this second series of observations which confirm those of the first series can be expressed graphically (see Fig. 2). This figure shows at a glance the parallelism which exists between the rate of growth of the various tumour strains and the respective water content of the protoplasm of the tumour cells. It shows also that such a parallelism exists even in different generations of one and the same strain, whenever these different generations exhibit differences in their rate of growth. In representing graphically the differences in the water content of the protoplasm of normal tissues the figure further demonstrates very clearly the exceptionally high water content of the testis which approaches that of a rapidly growing neoplasm, while the spleen approaches in this respect a slowly growing tumour and the other normal tissues fall below that level. No attempt has been made to compare the relative rate of growth of these normal organs as such a comparison could only be made by determining the mitotic index for the various tissues. Our knowledge of the function of the testis and of its histological appearance is sufficient to establish it as the normal tissue which is *par excellence* the normal organ of growth.

It is an interesting fact that the cells which are most susceptible to the action of *X*-rays and radium are those that are richest in water. This is especially striking when one considers the normal tissues, where the spleen and the testis take such an exceptional position with regard to their susceptibility to the influence of these agents. This correlation suggests that the sensitiveness of a cell to these rays is dependent on the water content of its protoplasm, and since water is known to be a good absorbent for *X*-rays and radium rays it is easy to see why that should be so. From this point of view a systematic investigation of the absorbent power of the various tissues would be of interest.

Ash of normal and cancerous tissues. The factors which determine the water content of the cell are so far as is known the osmotic force exerted chiefly by the inorganic salts, and the force of imbibition of the colloid constituents of protoplasm. The differences in the water content of the various tissues which have been observed may therefore be due to changes in these two factors acting either separately or

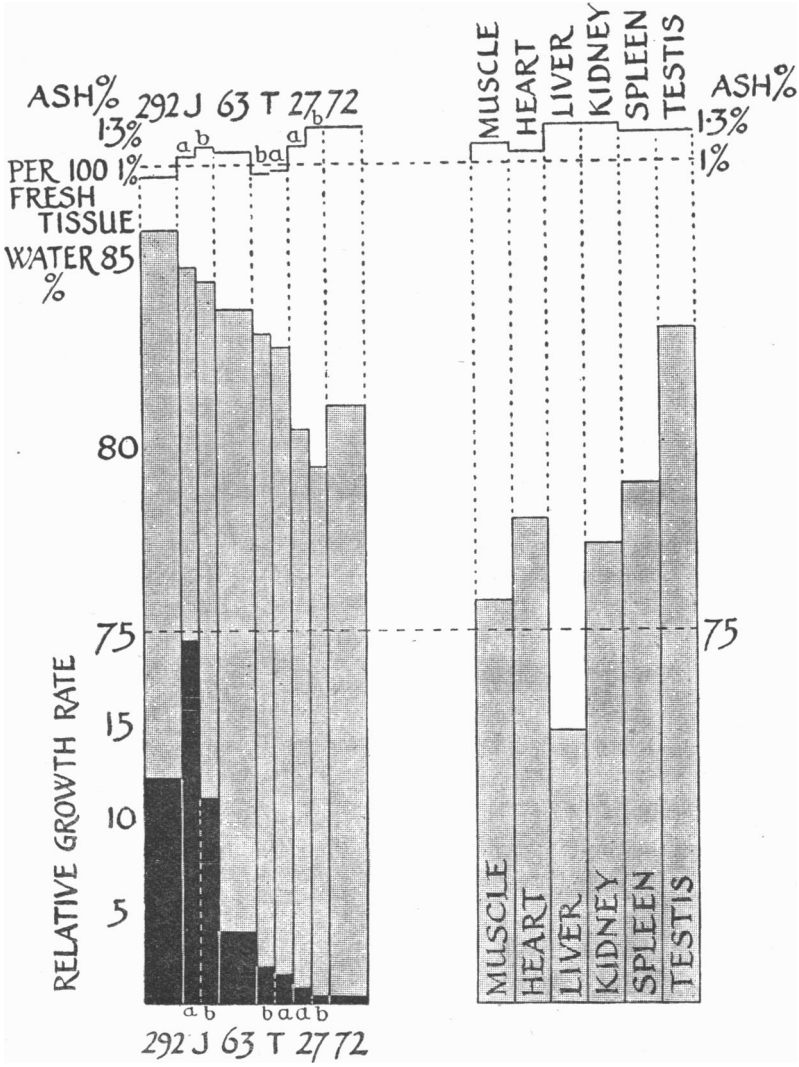

Fig. 2. The left half represents graphically the relative rate of growth (black columns), the water content (light columns) and the ash content of cancerous cells belonging to different tumour strains as given in Table III. The letters "a" and "b" applied to strains "J," "T" and "27" indicate two different generations in each of these strains. The right half of the figure represents the water content (light columns) and ash content of normal tissues of the mouse as given in Table II. For further explanations see text.

conjointly. If for some reason there was primarily an increase in the salt content of protoplasm it might lead to an inflow of water into the cell. If the facts described in this communication were explicable on this basis one would expect to find a relatively high salt content in the dried material obtained from rapidly growing cells and one should be able to establish a parallelism between the salt content of dried tissues and their rate of growth. It seemed of interest, therefore, to determine the ash content of the dried tissues. The ash contains, of course, in addition to the preformed inorganic salts of protoplasm also the oxidised inorganic elements which were present in protoplasm in organic combination, more particularly phosphoric acid derived from the phosphorus of the nucleo-proteins and of the phosphorised fats. Variation in the amount of nucleo-proteins will therefore produce variations in this amount of ash apart from differences due to preformed inorganic salts. For this reason the ash from tissues such as skeletal muscle or heart muscle which are relatively poor in nuclei and rich in cytoplasm is not directly comparable as a measure of the inorganic salts to the ash of glandular tissues such as the liver or kidney. The same factor will operate in the opposite direction in the case of a tissue like the spleen, where the cells are composed almost entirely of dense nuclei with comparatively little cytoplasm. It is in accordance with this *a priori* consideration that we find the lowest ash percentage in muscle and heart and the highest in the spleen. But this factor is not likely to produce great differences in the ash content of the various adenocarcinomata which have been considered, so that there it may be taken as an indication of the differences in the amount of inorganic salts present.

The ash estimations were carried out by incinerating the dried material in flat silica capsules, first over a flame and then over a blow-pipe until all visible carbon particles had disappeared. Heating was then continued until constant weight was obtained. The amount of ash obtained from 0·5–1·0 g. of fresh tissue is so small that in most cases the dried material from two or three estimations of the water content of the individual tumours had to be combined. The results (see Table III) show that the ash percentage of the dried material fluctuates irregularly in the different tumour strains and sometimes shows considerable variations even in different tumours of the same generation. There is therefore no indication of a parallelism between the ash content of the dried tissue and the rate of growth. One may conclude then that the greater water content of the rapidly growing

tumours is not due entirely, if at all, to a primary concentration within the cell of inorganic salts. The ash content of the fresh tissues has been calculated from these results and represented graphically in Fig. 2. It shows similar fluctuations which cannot at present be interpreted without an actual estimation of the individual inorganic salts present in the ash.

Summary and Conclusions.

1. The water content of different tissues, both normal and cancerous, varies with their rate of growth. It is highest in rapidly growing tissues, lowest in slowly growing tissues. This means that rapidly growing cells have the property of building up protoplasm with relatively less of the complex organic substances such as proteins, lipoids, etc., and relatively more water. This property is in itself an explanation of the biochemical mechanism of growth.

2. The variations in the water content of cells differing in their rate of growth are due to differences in imbibition.

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