LXVI. THE INFLUENCE OF ARGININE UPON THE GROWTH RATE OF A TRANSPLANTABLE TUMOUR IN THE MOUSE.

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RESEARCH in nutrition has shown that the various amino-acids differ among themselves in respect of their relative importance in relation to growth in the immature animal, and to maintenance of weight in the adult [Mitchell and Hamilton, 1929].

The results of Ackroyd and Hopkins [1916] provided evidence that arginine and histidine are interconvertible in the body, and that the presence of one or the other is essential for the processes of growth; that arginine is necessary for the maintenance of weight had been demonstrated by Geiling [1917] in the mouse. Confirmation of the indispensability of histidine was obtained by Rose and Cox [1924], but they reported that arginine, as a dietary constituent, was without effect on weight; similar results were recorded by Rose and Cook [1925]. The results obtained by Stewart [1925] were in agreement with the latter in respect of histidine, but with regard to the significance of arginine they were intermediate between the results of Ackroyd and Hopkins, and those of Rose and Cox. From these more recent investigations it appeared that arginine and histidine were not mutually replaceable in metabolism, and that arginine was definitely inferior to histidine in its ability to maintain weight, or as a promoter of growth. Owing to variations in the amount of food assimilated the interpretation of feeding experiments must often be a matter of difficulty, and the criterion of the capacity of a substance to induce growth is its effect upon the animal as a whole. It may be assumed that the amino-acid requirements of the various organs and tissues of the body differ in accordance with differences in specific function, and with the chemical composition of their structural units, the cells of which they are composed. It has been suggested that histidine is necessary for nuclear synthesis [Rose and Cook, 1925], and the researches of Kossel [1928] indicate that arginine may be an important constituent of nuclear substance as in the case of sperm. Edlbacher and Merz [1927], have shown that the arginase content of tumours exceeds that of most normal tissues, and it may be noted that the arginine content of neoplastic tissue, as estimated by Sakaguchi's reaction [1915] is also relatively high. Therefore it appears that arginine is present in considerable amount in tumours, and

that such tissues are well equipped for its catabolism, but unless they are capable of synthesising this amino-acid they must obtain it from their host; if arginine were present in excess of the nutritive requirements of the latter it would be available for the purpose of nuclear synthesis and cell proliferation, and this should be manifested by an increase in the rate of tumour growth.

It was the object of this investigation to determine whether arginine had any influence upon tumour growth-rate, and whether it was distinct from other amino-acids in exhibiting this effect.

MATERIAL.

The mice used for the experiments were ordinary, healthy, unselected stock, representing a mixed population; they were 2-3 months old at the time of implantation, and an approximately equal number of males and females were included in each experimental group. They were all fed alike on a diet of brown bread, flaked maize, and lettuce (when available). It may be of interest to note in connection with diet that the arginine content of brown bread, as estimated by Sakaguchi's reaction, is quite considerable, and both treated and control groups were given as much as they would eat.

The tumour was a transplantable mouse carcinoma, for which I am indebted to Mr J. J. M. Shaw, of the Department of Surgery, Edinburgh University. This tumour (M 63), is palpable about ten days after implantation, often earlier, and attains a large size in a period of three weeks. It produces no metastases, and therefore the mice remain perfectly well.

METHOD.

Implantation was performed by means of an implantation needle, strict aseptic precautions being observed.

The amino-acids¹ were administered by hypodermic injection at some distance from the site of implantation.

Exp. I. Implantation was carried out as described, and after ten days all mice bearing palpable tumours on that date were segregated, divided into groups, and treatment was commenced. This consisted in the injection of 0.3 cc. of a 10 % solution of the amino-acid allotted to the group, and treatment was repeated every 2 days until the termination of the experiment 22 days after implantation. On each day of treatment caliper measurements were taken of the tumours, and transferred direct to mm. squared paper; in this way it was an easy matter to measure the cross-sectional area of tumours of irregular shape. At the end of the experiment, size, *i.e.* cross-sectional area, was estimated, and from the tabulated results the mean was calculated for each group showing the increase during the experimental period. The ratio of the mean values on the 10th and 22nd days respectively was taken as an index of the rate of growth.

Exp. II. In this experiment the tumours used for implantation were known to be of lowered vitality (they were derived from tumours which had recurred after removal, and when implanted into fresh hosts had been found to exhibit a slow rate of growth, and a reduction in the usual percentage of "takes").

Two amino-acids were tested in this experiment for their ability to restore the vitality of this debilitated tissue. Treatment was begun 2 days before implantation, and continued every second day as in *Exp. I.*, and caliper measurements were taken at the same intervals as before.

Special precautions were taken to ensure the uniformity of the implanted tissue in its distribution among the various groups, the following procedure being adopted; the mice were divided into

¹ Supplied by Messrs British Drug Houses, Ltd.

three main groups, two treated, and one control, and each of these was subdivided into four small groups, *A, B, C, D*. One tumour was used to implant all the "*A*" groups, another for *B*, etc. In addition to this the mice were implanted in strict rotation as follows: mouse 1, Group I *A*; mouse 1, Group II *A*; mouse 1, Group III *A*; then mouse 2 in each group as in the first round.

As an extra check upon the usual measurements all the tumours were weighed at the close of the experiment, the mean weight was calculated, and the ratio of each treated group to the control was compared with the ratio obtained by measurement of cross-section. The results will be found in Table III, Exp. II.

The results of these two experiments made it appear almost certain that arginine had a definite effect upon the rate of tumour growth; the variation in the growth potentiality of apparently healthy tumours observed in earlier work, however, had made it desirable to limit the number of animals used in each experiment, in order that the implanted tissue should be derived, as far as possible, from the same source. Consequently it seemed advisable to apply to the results the method described by Fisher [1928] for the determination of the significance of the difference of the means of small samples; the tabulated results therefore include the "*t*" and "*P*" values used in this method. According to Fisher [1928] we may "draw a conventional line at 0.05," i.e. if the value is at, or below, this figure the difference may be held to be significant, and would not have occurred by chance, and one may place "a high degree of confidence in the results" if $P = 0.01$.

The choice of amino-acids was limited in accordance with the degree of their solubility in water. The low solubility of tyrosine prohibited its use for hypodermic injection; a considerable mortality attended the use of cystine for the same reason, and cysteine was substituted as a result. It was obviously impossible to test the guanidine radical of arginine separately [Barger, 1914], but sodium valerate was included in the series. Glutamic acid was given a separate trial in view of it being a constituent of glutathione; lysine was not tested.

*An example of experimental data from which
the results were calculated.*

(Treatment begun 10 days after implantation of M 63.) Surface area of tumours as estimated by caliper measurements in mm.²

Arginine:	Days since implantation						Ratio of size, day 10 : 22
	10	12	14	16	18	22	
1	16	64	106	154	250	527	1 : 33.0
2	60	118	193	290	332	614	1 : 10.2
3	64	126	166	274	443	714	1 : 11.1
4	96	119	205	254	254	419	1 : 4.3
5	96	156	198	212	260	303	1 : 33.1
6	10	42	58	173	237	430	1 : 43.0
7	23	57	105	175	207	242	1 : 10.5
8	8	24	75	175	260	353	1 : 44.7
9	7	28	36	96	143	216	1 : 30.8
10	4	6	74	131	176	246	1 : 61.5
11	6	6	131	180	210	437	1 : 72.9
12	6	8	137	183	225	257	1 : 32.1
Mean	33.0	62.8	123.6	174.7	249.0	396.0	1 : 29.7

After estimation of the probable error the mean values were respectively 33.0 ± 6.6 , and 396.0 ± 29.5 .

	Days since implantation						Ratio of size, day 10 : 22
	10	12	14	16	18	22	
Controls:							
1	11	17	32	32	57	80	1 : 7.3
2	44	66	102	137	196	288	1 : 6.5
3	56	79	121	180	197	257	1 : 4.9
4	5	15	19	39	40	49	1 : 9.9
5	33	67	79	102	159	179	1 : 5.4
6	50	66	99	217	372	497	1 : 9.9
7	13	48	57	82	114	184	1 : 14.1
8	13	16	31	58	74	157	1 : 12.2
9	7	21	21	33	84	265	1 : 37.8
10	47	82	113	113	119	277	1 : 6.0
11	14	24	31	40	51	84	1 : 6.0
12	60	143	171	291	291	336	1 : 5.6
Mean	29.0	46.2	69.8	100.3	146.1	221.0	1 : 10.4

After estimation of the probable error the mean values were respectively 29.0 ± 4.4 and 221.0 ± 24.4 .

Difference of mean day 10 = 4.0 ± 0.3 .

Difference of mean day 22 = 175.0 ± 3.2 .

The value of t on day 10 = 0.3.

The value of P on day 10 = 0.9.

The value of t on day 22 = 3.2.

The value of P on day 22 = 0.01.

Experiment I.

Table I.

Group	No. in group	Size on 10th day			Size on 22nd day		
		Difference of mean	t	P	Difference of mean	t	P
Arginine	12						
Untreated	12	+ 4.0 ± 0.3	0.30	0.90	+ 175 ± 3.2	3.2	0.01
Glycine	10						
Untreated	7	± 0.0	0.30	0.70	- 28 ± 0.6	0.7	0.50
Alanine	12						
Untreated	7	+ 12.0 ± 1.3	1.00	0.30	+ 5 ± 0.09	0.1	1.00
Cystine	6						
Untreated	7	± 0.0	1.00	0.30	- 25 ± 0.3	1.4	0.20
Glutamic acid	10						
Untreated	7	+ 2.0 ± 0.7	0.60	1.00	- 14 ± 4.9	0.2	1.00
Arginine	4						
Untreated	7	+ 5.0 ± 0.7	0.14	0.90	+ 192 ± 4.2	3.4	0.01
Sodium valerate	11						
Untreated	6	- 8.0 ± 4.1	0.40	0.70	+ 54 ± 0.5	0.6	0.50
Arginine	4						
Untreated	6	+ 23.0 ± 0.7	0.90	0.40	+ 122 ± 2.1	1.6	0.10

The comparison of arginine and cystine is of value in view of the importance which has been attached to glutathione in connection with tumour growth. From the results obtained it would appear that neither cystine nor glutamic acid, a constituent of glutathione, has any appreciable effect upon the rate of tumour growth.

The results of the second experiment showed that histidine could not replace arginine as a stimulant of growth. It appeared to exert a certain effect upon the percentage of successful implantations, when tested on devitalised tissue, the number of failures being greater in the case of arginine than histidine, but

Table II. *Ratio of surface area of tumours ten and twenty-two days after implantation.*

Group	Ratio. Group mean	Difference of mean	P
Arginine	29.7 ± 14.3		
Untreated	10.4 ± 6.0	+ 19.5 ± 0.84	0.01
Glycine	17.9 ± 6.8		
Untreated	20.2 ± 6.3	- 2.3 ± 0.17	0.70
Alanine	15.1 ± 7.1		
Untreated	20.2 ± 6.3	- 5.1 ± 0.36	0.40
Cystine			
Untreated	21.4 ± 4.4	+ 1.2 ± 0.63	0.90
Glutamic acid	19.4 ± 7.5		
Untreated	20.2 ± 6.3	+ 0.8 ± 0.05	1.00
Sodium valerate	9.8 ± 2.2		
Untreated	11.8 ± 3.5	+ 3.0 ± 0.11	0.15
Arginine	15.0 ± 7.8		
Untreated	11.8 ± 3.5	+ 8.2 ± 0.07	0.15
Arginine	52.9 ± 5.7		
Untreated	20.2 ± 6.3	+ 32.0 ± 2.60	0.01

Table III. *Comparison of the effect of arginine and cystine, or cysteine, the latter being substituted for untreated controls.*

Group	No. in group	Size on 10th day			Size on 22nd day		
		Difference of mean	t	P	Difference of mean	t	P
Arginine	4	± 0.0	0.2	0.80	+ 217 ± 3.1	2.50	0.04
Cystine	6						
Arginine	8	+ 1.0 ± 0.16	0.1	0.80	+ 49 ± 0.8	0.90	0.35
Cysteine							

less in arginine-treated mice than in the untreated controls. The exact percentages of failures were, arginine 37.5 %, histidine 26 %, untreated 50 %. These figures are very high for implantation failures in the tumour M 63.

Experiment II.

Table I.

Group	No. in group	Size on 10th day			Size on 22nd day		
		Difference of mean	t	P	Difference of mean	t	P
Arginine	15						
Untreated	12	+ 14.0 ± 0.4	0.1	0.40	+ 134.0 ± 4.3	0.5	0.05
Histidine	17						
Untreated	12	+ 18.0 ± 0.1	0.1	0.10	+ 38.0 ± 0.8	1.7	0.10

Table II.

Group	No. in group	Ratio. Group mean	Difference of mean	P
Arginine	15			
Untreated	12	10.5 ± 7.47	5.5 ± 0.06	0.10
Histidine	17			
Untreated	12	6.8 ± 4.7	1.8 ± 0.06	0.40

Table III.

Group	No. in group	Mean wt.	Difference of mean	<i>t</i>	<i>P</i>
Arginine	15	1.6 ± 0.27	0.9 ± 0.21	1.5	0.1
Untreated	12	0.7 ± 0.22			
Histidine	17	0.7 ± 0.14	0.0	0.1	0.9
Untreated	12	0.7 ± 0.22			

DISCUSSION.

From the tabulated results of Experiments I and II it can be seen that among the amino-acids tested for their effect upon tumour growth arginine alone induces a significant difference from the mean of the untreated control group on the 22nd day, as estimated by the value of *P*.

It is evident that if the size of tumours in a treated group exceeds that of the corresponding untreated group on the 10th day the former would be expected to exceed the latter on the 22nd day as well, apart from any treatment which the animals had received. In certain cases it may therefore appear that the difference of mean in the arginine-treated groups on the 22nd day was not so significant as it otherwise would seem. In order to assess the real value of the difference two points must be taken into consideration, of which one is the value of *P*. On the 10th day the *P* value indicates in every case that the difference in mean could be accounted for as the result of random sampling from a homogeneous population. The *P* value for the 22nd day, on the other hand, in three cases out of four of the arginine-treated groups is 0.05 or less, while the value for the groups treated with other amino-acids is in no case less than 0.1. The second point to be considered is the ratio of the sizes (cross-sectional area), on the 10th and 22nd days respectively within one and the same group. The mean ratio obtained after calculating the individual ratios in such a group can then be compared with its untreated control. The ratio will obviously be greater or less according to the mean size on the 10th day of the experiment unless the mean on the 22nd day is proportionately very great; since in some of the arginine-treated groups the mean was higher on the 10th day than it was in the controls the ratio might have been thus reduced, but in spite of this fact a markedly higher ratio occurs in the arginine-treated groups alone. The second experiment indicates that arginine and histidine have some slight effect in raising the percentage of "takes" in the case of debilitated tissue (as previously defined), but that only arginine has any appreciable influence upon its subsequent rate of growth. The difference in weight in these groups is not significant if judged by the value of *P*, but the mean weight is about 50 % greater in the arginine-treated group than in the untreated control, which agrees well with the difference in ratio where cross-sectional area was the criterion of size.

From this analysis of the data it seems justifiable to conclude that the influence of arginine upon tumour growth is real, and that it is specific in so far as a similar effect was not observed in the case of any other substance used in

this series of experiments. It is suggested that an increase in nuclear substance leading to an acceleration of the rate of cell division, may be the mechanism through which the observed increase in size is attained. Certain inferences concerning the biology of tumour growth suggest themselves, assuming this hypothesis to be correct, but the consideration of theoretical implications is beyond the scope of the present paper, and the facts are therefore recorded alone.

SUMMARY.

A comparison of the effects upon tumour growth of certain amino- and fatty-acids (arginine, histidine, glycine, alanine, cystine, cysteine, glutamic acid, and sodium valerate), showed that arginine was the only substance investigated which exerted a definite effect upon the rate of tumour growth.

In an experiment dealing with the implantation of "devitalised" tissue it was found that histidine appeared to increase the percentage of "takes." It had no effect upon the subsequent rate of growth, whereas arginine had a definitely stimulating effect.

I wish to express my thanks to Prof. Crew for the facilities provided for the prosecution of this research, and for much helpful criticism and advice, and I would also acknowledge my indebtedness to Dr Kermack, of the Royal College of Physicians, for his advice in the statistical treatment of the results.

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