CLI. AVITAMINOSIS B IN RELATION TO TUMOUR GROWTH.

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(Received July 30th, 1930.)

THE observation that arginine increased the growth of a transplantable tumour [Gilroy, 1930, 1] formed the basis for further work in which it was shown that thyroxine was antagonistic to arginine in this respect [Gilroy, 1930, 2], and it was suggested that the inhibitory action of thyroxine might be due to an increase in protein catabolism which involved both neoplastic and somatic tissue and reduced the amount of arginine available for the tumour.

It was evident that a more direct method of depleting the organism of arginine would be to reduce its intake, but a difficulty was immediately encountered, as it was found that all foods which were relatively devoid of arginine were also deficient in vitamin B. This fact was of considerable interest, since it had been suggested that vitamin B was conducive to tumour growth [Burrows, 1926, 1, 2; Caspari and Ottensooser, 1929], but according to Thies [1929] avitaminosis had no effect upon the growth of mouse tumours, while Jackson and Krantz [1929] found that normal liver contained more vitamin B than malignant tissue, from which they concluded that the suggested correlation between a high vitamin B content of organs and malignancy could not be substantiated. In view of the high arginine content of foods reputed to be rich in vitamin B it seemed possible that it was in reality this constituent of the diet which acted as a growth stimulant, and that, if arginine were still present in a vitamin B-deficient diet, this would explain the apparently irreconcilable facts that, while a diet rich in vitamin B accelerated tumour growth, one in which vitamin B was deficient did not exert an opposite effect.

A comparison of the arginine content of caseinogen before and after subjection to the standard process for destruction of the vitamin B complex showed that no alteration had been effected by this treatment, as estimated by Sakaguchi's reaction [1915]. In view of the observation [Reader, 1929], that one of the factors (Reader's third rat factor, vitamin B₄), was inactivated if a yeast extract was autoclaved in an alkaline medium, crude caseinogen was treated by this method, but the arginine was not reduced by the process, although it might have been racemised by the high temperature to which it had been exposed.

It therefore seemed possible that vitamin B-deficiency was inimical to tumour growth only if it affected the general health of the animal and that,

in the experimental animal, tumour tissue might show relatively high powers of survival in virtue of the high arginine content of vitamin-deficient diets in so far as standard preparations were concerned.

Experiments were carried out to test the effect of such diets upon tumour growth, with and without injections of arginine, and to observe any correlation between loss of body weight and tumour growth. The technique and material were similar to those previously employed [Gilroy, 1930, 1], and the deficient diets were supplied by Messrs British Drug Houses Ltd. (Exp. 1), and Messrs Glaxo (Exp. 2); the caseinogen, untreated and treated for destruction of the vitamin B complex, was supplied by Messrs Glaxo, also the cod-liver oil used in Exp. 2.

Exp. 1 (Section A). 68 albino mice, 36 males and 32 females, received implants from the same tumour and were divided into 6 groups and treated as follows:

Males	Group 1	Group 2	Group 3
36	Supplemented with wheat germ (10 %) and baker's yeast. Cod-liver oil	Arginine injections 0.3 cc. of a 10 % solution daily. Cod-liver oil	No addition to diet, except cod-liver oil
Females			
32	Ditto	Ditto	Ditto

(*Section B.*) 65 mice, mixed coloured stock, 30 males and 35 females, received implants from two tumours and were divided into 6 groups as before, each group representing both tumours.

The result of this experiment is shown in Table I.

Table I.

		Days since implantation					
		10		16		22	
Exp. 1		Tumour size	Body-weight % loss or gain	Tumour size	Body-weight % loss or gain	Tumour size	Body-weight % loss or gain
Section A	Group 1						
	M.	18.5	+5.8	81.5	+10.1	163.9	+33.9
	F.	20.0	+2.6	69.1	+5.3	116.1	+19.2
	Group 2						
	M.	20.9	-6.1	50.0	-15.4	68.5	-9.4
	F.	13.5	-5.6	30.0	-11.9	50.1	-21.8
Group 3	M.	15.8	-3.1	27.7	-11.7	67.2	-22.2
	F.	23.3	-4.0	63.3	-6.1	84.9	-13.5
Section B							
Group 1	M.	12.0	+1.9	31.9	+8.5	84.9	+13.8
	F.	17.8	+5.7	73.0	+6.4	126.5	+15.9
Group 2	M.	30.4	-3.3	125.4	+0.6	203.2	-8.5
	F.	9.8	-9.1	23.1	-13.7	62.1	-9.9
Group 3	M.	13.7	-6.9	25.7	-11.6	63.2	-10.5
	F.	7.5	±0.0	14.0	-5.1	30.0	-10.3

In section A treatment with arginine was discontinued on day 16. Tumour size is expressed as cross-sectional surface area, in mm.²

From this experiment it seemed that a vitamin B-deficient diet retarded the growth of tumours in proportion to the loss of body weight, if the per-

centage loss of weight in groups 2 and 3 of both sections was compared with the gain in group 1. Arginine only increased the rate of tumour growth in Section B, in which the tumours grew twice as fast in mice injected with arginine as in uninjected mice. The reason for this difference between the sections was not apparent, unless relative insusceptibility to this dietary deficiency is a characteristic of coloured stock.

It was difficult to form an opinion as to the effect on body weight in the later stages of the experiment, on account of the varying size of tumours in the several groups, especially in Section B. In Section A it seemed that the percentage loss in mice injected with arginine exceeded that of uninjected mice on a similar diet, and that males suffered more than females if tumour size were taken into account. It also appeared that after injections were stopped the weight of the males improved while that of the females decreased, but the increase in the males was correlated with a high mortality rate; treatment was not stopped in time to prevent deaths increasing from one to five in the last few days of the experiment.

It seemed unlikely that this result would always accompany the injection of arginine, as it had never before had such an effect¹, but the sex distinction was interesting, and it was decided to repeat the experiment on unimplanted mice, in order that the results should not be obscured by variations in tumour size.

From the data obtained in Exp. 1 it seemed possible that coloured stock was less susceptible to arginine injections than were albinos, and the former were therefore used in Exp. 2 to determine whether the apparent toxicity of arginine on a vitamin B-deficient diet could be confirmed.

Exp. 2 (Table II). 115 young mice, average weight 10–14 g. were divided into 4 groups, the largest being placed in group 3, in order that these should have if anything an advantage over the controls. The 4 groups were treated as follows:

Group 1.	17 males	...	...	Average weight	11.5 g.
	11 females	...	...	"	10.2 g.
Diet supplemented with wheat germ and yeast as in Exp. 1. No injections.					
Group 2.	18 males	...	...	Average weight	11.7 g.
	11 females	...	...	"	10.8 g.
Diet supplemented with wheat germ and yeast as in Exp. 1. Arginine injections.					
Group 3.	18 males	...	...	Average weight	14.3 g.
	11 females	...	...	"	11.0 g.
No additions to diet. Arginine injections.					
Group 4.	18 males	...	...	Average weight	11.9 g.
	11 females	...	...	"	10.4 g.
No addition to diet. No injections.					

The result of this experiment is shown in Table II.

¹ It might be correlated with the observation of Hartwell [1928] that edestin requires more vitamin B than does caseinogen or egg-albumin for normal metabolism.

Table II.

Day	Group 1		Group 2		Group 3		Group 4	
	M.	F.	M.	F.	M.	F.	M.	F.
1	—	—	—	—	—	—	—	—
3	+ 2.6 x	- 9.1	- 2.6	+ 5.5 x x	- 3.5	+ 10.9	- 5.8	- 6.7 x
5	+ 7.8	+ 14.0 x x x	+ 4.2	+ 12.0	± 0.0 x	+ 10.0	- 8.4 x	- 7.7
7	+ 13.8	+ 34.3 x	+ 5.1	+ 14.0	- 2.8	+ 6.4	- 5.8 x x x	- 8.7 x
9	+ 14.0 x	+ 40.0	- 1.7	+ 24.0 x x	- 2.1	+ 1.9	- 1.7 x x x	- 19.6
11	+ 14.8	+ 47.0	+ 2.6 x x	+ 43.5 x	- 2.1	+ 1.9	- 1.7	- 12.5
13	+ 14.8	+ 44.3	± 0.0	+ 43.5	- 11.1	- 8.1	- 2.5 x	- 15.4
15	+ 16.5	+ 45.7	± 0.0	+ 44.4	- 14.7	- 9.1	- 9.2	- 17.3
17*	+ 20.9	+ 48.6	+ 0.3	+ 47.2	- 16.1	- 8.1 x x	- 9.2	- 19.2
19	+ 20.9	+ 52.8	+ 3.4	+ 47.2	- 12.6	- 8.1	- 6.7	+ 1.9

The figures with prefix + or - represent change in weight.

The sign x indicates a death.

* Indicates arginine injections stopped.

DISCUSSION.

It may be inferred from Exp. 1 that the general debility induced by a diet deficient in vitamin B is accompanied by a reduction in the rate of tumour growth, and that growth is unaffected if the health of the host is not impaired.

The results obtained in Exp. 2 showed that coloured stock was not, as suggested by the results of Exp. 1, resistant to a deficient diet, but the observation that in Exp. 1 males suffered more than females was confirmed in Exp. 2. The percentage loss in males was double that of females, the actual values corresponding closely to those for the same period in Exp. 1. The males receiving arginine injections and also wheat germ and yeast in their food only maintained their weight; females on the same diet showed the same percentage increase as uninjected mice.

It is evident that the mortality rate is an important factor in such an investigation; deaths invariably reduce the recorded loss, or increase the gain. This may explain the relatively great increase in weight among females in both groups receiving wheat germ and yeast, as compared with males on a similar diet. For the same reason it is probable that the males on an unsupplemented diet and not injected would have shown a greater loss of weight if the mortality had not exceeded that of the corresponding injected group, and from this standpoint it might be argued that the injections in reality exerted a beneficial effect upon the males, only one death occurring in this group compared with eight in uninjected males. Since both percentage increase and mortality rate were similar in the two groups of females on a diet supplemented with wheat germ and yeast it appears that arginine was without effect in modifying their weight, but in males injections of arginine

appeared to prevent an increase in weight, and this was not due to a differential mortality as an equal number of deaths occurred in both injected and uninjected males. It is to be noted that the inclusion of wheat germ and yeast in the diet increases the total arginine intake, but that the introduction of vitamin B enables females on such a diet to metabolise it without any ill effects, although males under such conditions can only just maintain their weight.

The sudden acceleration in loss of weight which occurred at the end of the second week of the experiment may have been due to final exhaustion of stores of vitamin B in the body of the animal. Before this date the injected mice looked decidedly less healthy than those which were not injected, and were far less active. Termination of the experiment was necessitated by the development of sores on the backs of the males; this did not precede the rapid loss of weight, and was undoubtedly due to daily injections in emaciated animals; it did not occur in the females of either group.

The comparative good health of the females precludes the possibility that the arginine was contaminated with some toxic substance, or that the injections introduced an element of sepsis.

Owing to the variations in mortality in the several groups it is impossible to draw any definite conclusion as to the influence of arginine upon the body weight of mice on a diet deficient in vitamin B, but the results may perhaps be of some interest as a contribution to the study of avitaminosis in relation to the metabolism of purines, and the effect of vitamin B-deficiency upon the rate of tumour growth.

SUMMARY.

1. The growth of a transplantable tumour (M 63) in mice on a diet deficient in vitamin B is only affected if the general health of the animals is impaired.

2. It is suggested that the high arginine content of foods rich in vitamin B accounts for the fact that diets rich in vitamin B stimulate tumour growth. The arginine content of caseinogen does not appear to be reduced by the process employed for destruction of the vitamin B complex, and this may explain the observation that vitamin B-deficient diets do not retard the rate of tumour growth.

3. Evidence was obtained that arginine may exert a toxic effect in mice on a vitamin B-deficient diet, especially in the case of males, but a differential mortality rate makes it difficult to come to any conclusion on this point. If wheat germ and yeast are added to such a diet the effect of arginine injections is only detrimental in the case of males.

I wish to acknowledge my indebtedness to the Medical Research Council for a grant which made this investigation possible, also to Professor Crew for scientific hospitality, and his encouragement and advice. I am indebted to Messrs Daren, Ltd., for the gift of the wheat germ used in the experimental diets.

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