CLXXXII. FURTHER OBSERVATIONS UPON THE CORRELATION BETWEEN THE ARGININE AND VITAMIN B CONTENT OF DIETS, AND THE EFFECT OF THYROXINE UPON TUMOUR GROWTH,

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IN an earlier communication reference was made to the difficulty of finding a diet for mice in which there would be a natural deficiency of arginine, but not of vitamin B [Gilroy, 1930, 3]. It had been shown previously [Gilroy, 1930, 1, 3], that arginine, administered by hypodermic injection, accelerated the growth of a transplantable tumour of the mouse (M 63), and it therefore appeared that a diet in which there was a relative deficiency of this amino-acid should have a depressing effect upon the rate of tumour growth.

Making use of Sakaguchi's [1915] reaction for combined guanidine, a comparison was made of the arginine content of various cereals, and it was found that the diet which most nearly fulfilled this requirement consisted of a proprietary preparation of barley, with rice starch, lettuce, and cod-liver oil. The probability that this diet lacked amino-acids other than arginine, and also vitamin B, made it desirable to include in any investigation of its effects upon tumour growth, unimplanted as well as tumour-bearing mice, in order that the former could be used to examine the efficacy of any additions to the diet in correcting a deficiency if loss of weight occurred. Synthetic diets were avoided, as it had been noticed that animals seldom eat such food in sufficient amount, and it was hoped that this important variable might be eliminated by using a diet which would be consumed in quantities comparable with those eaten by the controls. It was the object of this investigation to compare the growth rate of tumours in mice kept on this diet with that of tumours whose hosts were given standard food, and secondly to examine further the effect of thyroxine upon the rate of tumour growth [Gilroy, 1930, 2]. It seemed possible that animals receiving thyroxine might compensate for an augmented metabolism by consuming more food than untreated controls, and that its effect upon tumour growth was essentially a problem of nutrition. In the previous series of experiments all the mice received approximately equal rations and it

was proposed in the present investigation to give each group as much as they would eat, and to observe the actual quantity consumed at different stages of the experiment.

The technique and material were similar to those previously employed [Gilroy, 1930]. The rice starch was supplied by Messrs British Drug Houses, Ltd., and Engelberg's cod-liver oil was used in the special diet.

Exp. 1. 162 mice were implanted from four tumours, and distributed into sections so that each section included only implants from one tumour; the sections were then subdivided into groups for dietetic and other treatment, as follows:

	Diet	No. of mice
1. Barley, rice starch, lettuce, cod-liver oil		27 males 11 females
2. Ditto + injections of thyroxine, 0.1 cc. of 0.1 % solution daily, on and after day 10		25 males
3. Brown bread, flaked maize, and lettuce		38 males 14 females
4. Ditto + injections of thyroxine as in group 2		37 males 10 females

The mice were not placed on special diet until six days after implantation. The result of this experiment is shown in Table I.

Table I.

The figures denote cross-sectional surface area of tumours in mm.²
(group average). Death of a mouse is indicated by the sign +.

Group	% takes	Days since implantation						
		10	13	16	19	22	25	28
1	76.5	8.6	15.5 + +	30.3	40.7	61.6 + +	74.5	81.4
2	80.0	10.6	21.5	35.8 +	52.3 + + +	59.0 + + +	68.7 +	45.1 + + +
3	79.0	12.3	30.9	65.9	94.9	135.0 + +	183.9 +	240.9
4	85.0	24.4	40.6	62.3	91.6	140.8 + + +	175.7 + + + +	236.9

The percentage of takes was not affected, as an interval of 6 days elapsed between the date of implantation and the exhibition of a special diet, but on the 10th day the average size of tumours in groups 1 and 2 was less than that of groups 3 and 4, a difference which may be attributed to diet. Before the commencement of treatment with thyroxine on the 10th day the mice in groups 3 and 4 were re-distributed in order that those bearing the largest tumours on that date should be allocated to group 4 which was to receive thyroxine. Previous experience had indicated that very large tumours did not yield to treatment and this point required confirmation; it also appeared that the inclusion of mice bearing large tumours in the control group must give the latter an initial advantage. The difference in average size of tumours in groups 3 and 4 was not therefore due to any treatment. The mice were weighed in

groups every 3 days when measurements were taken of the tumours, and the results are shown in Table II.

Table II.

	No. of mice		Weight of mice		Average weight		% loss or gain
	Day 7	Day 28	Day 7	Day 28	Day 7	Day 28	
Special diet; no injections	8	7	155.0	107.5	17.2	14.5	- 15.7
	10	6	165.0	82.5			
	9	6	166.0	100.5			
	11	9	166.0	116.5			
Special diet + thyroxine	7	2	120.0	24.0	17.7	12.6	- 28.8
	8	3	140.0	41.5			
	10	5	182.0	60.5			
Normal diet; no injections	13	13	224.0	254.0	16.0	20.7	+ 29.4
	9	8	161.5	169.5			
	16	14	213.0	251.5			
	14	13	235.5	276.5			
Normal diet + thyroxine	17	12	308.0	315.0	19.5	25.1	+ 28.8
	20	19	396.5	484.0			
	10	9	186.0	206.5			

It appeared to be of interest to compare these figures with the data obtained in previous experiments [Gilroy, 1930, 2], in which thyroxine and arginine were injected separately or together, the mice all receiving the same quantity and quality of food. The weights of these mice are shown in Table III.

Table III.

Injections	No. of mice in group	Weight of mice		Average weight		% loss or gain
		Day 10	Day 22	Day 10	Day 22	
Thyroxine daily	17	378.1	397.9	22.2	23.4	+ 5.4
Arginine substituted on 6th day	16	390.9	439.2	24.4	27.4	+ 12.6
Arginine and thyroxine daily	26	559.4	638.8	21.5	24.5	+ 13.9
Thyroxine daily and arginine on alternate days	6	125.3	132.5	20.9	22.1	+ 5.8
No injections, similar diet	26	557.3	627.4	21.4	24.1	+ 12.6

The diet which caused such rapid loss of weight in groups 1 and 2 of Exp. 1 was also given to 40 unimplanted mice, a further group of 12 mice being given for comparison a diet deficient in vitamin B. The mice were divided into groups and treated as follows:

Exp. 2.

Group 1. 12 adult males.

Diet as in exp. 1, groups 1 and 2.

Arginine injected (0.3 cc. of a 10 % solution daily) 9th–15th day.

Gelatin added to diet on day 15.

Group 2. 12 adult females.

Diet as in group 1.

Gelatin added to diet on day 15.

Group 3. 16 immature males.

Diet as in group 1.

Raw wheat germ* added to diet on day 15.

Group 4. 12 adult males.

Vitamin B-deficient diet (Glaxo).

Wheat germ added to diet on day 15.

* The wheat germ was the gift of Messrs Daren.

DISCUSSION.

It is evident that the experimental diet was not deficient in arginine only, since injections of this amino-acid increased rather than checked the loss of weight (group 1), and the subsequent addition of gelatin to the diet produced no improvement in this group and only a temporary weight increase in group 2. From this it appeared that the diet lacked some important constituent other than an amino-acid, it having been shown by Downey [1924] that gelatin is supplementary to pearl barley in this respect. The amount of food consumed was at first quite comparable with that of controls on normal diet, the appetite of the mice only failing when a considerable loss of weight had already occurred. After the addition of gelatin to the diet the food consumption again rose for a few days, and this may perhaps account for the temporary improvement in group 2, in which the average weight at this time was further increased by the death of three of the smallest mice.

The addition of wheat germ to the diet produced more permanent results: it is possible that this corrected a deficiency in tyrosine or tryptophan which would not be supplied by gelatin¹ [Mitchell and Hamilton, 1929]. The barley used in the experimental diet gave a negative result when tested for these amino-acids, using the Adamkiewicz, Liebermann, Millon and xanthoproteic tests, whereas the wheat germ gave a positive reaction to the last of these. It had been found in earlier experiments that male mice on a standard vitamin B-deficient diet injected with arginine lost weight more rapidly than did un-injected mice [Gilroy, 1930], and it is to be noted that in the present experiment also injected males showed a greater loss of weight, and subsequently failed to respond to dietary additions which benefited the uninjected mice. The diet included lettuce, which would contain water-soluble accessory food factors, but it was shown by Sherman [1929], that lettuce is not growth-promoting even in large amounts. From these considerations it seems probable that the introduction of vitamin B in the wheat germ was the deciding factor, rather than the correction by the same means of an amino-acid deficiency; in this case the suggestion would be justified that diets which are naturally deficient in arginine are also deficient in vitamin B. The inhibition of tumour growth in mice on this diet was similar to that induced by avitaminosis [Gilroy, 1930, 3], but this result is of no significance as it would probably accompany a state of severe malnutrition, whatever its cause might be. The mortality from thyroxine on this diet was very considerable; in one group it was so high that the data on tumour growth could not be utilised at all.

An analysis of the weights of implanted mice subsequently injected with thyroxine shows that increase in body weight is in general parallel with, and probably dependent in part upon, increase in tumour size. The effect of thyroxine upon tumour growth as shown by body weight could be modified

¹ Millon's reaction was positive for the sample of gelatin used (prepared by Messrs J. and G. Cox, Ltd.).

by injection of arginine which appeared to compensate for the effect of thyroxine partially or completely according to the amount administered (Table III); daily injections of arginine or the substitution of the latter for thyroxine halfway through the experimental period balanced the increased catabolism and gave values for weight corresponding with those of untreated controls. In the present experiment, the results of which are shown in Table II, the percentage increase in weight of mice on normal diet hardly differed in mice injected with thyroxine and in untreated controls, although arginine was not exhibited to counter its effects. The explanation of this apparently anomalous result is to be found in the larger quantity of food consumed by treated mice, which, 2-5 days after injections of thyroxine were commenced, developed an appetite far greater than that of the controls. It therefore appears that the injection of arginine or the assimilation of a greater quantity of food is equally effectual in preventing the tissue loss which thyroxine would otherwise induce. The administration of a diet rich in carbohydrate is known to counteract the destruction of tissue protein which would result from inanition [Mitchell and Hamilton, 1929], and a diet of brown bread and maize, if eaten in sufficient quantities, might be expected to exert a similar effect.

Although the curves of tumour growth shown in Fig. 1 appear to approximate in both groups on normal diet, the increase in tumour size in mice receiving thyroxine was in reality considerably less than in the untreated controls. In the group injected with thyroxine 6 mice bearing small tumours (*i.e.* not exceeding 120 mm.²) died in the last 6 days of the experiment, while only one mouse bearing a large tumour died. In the untreated group, on the other hand, 3 mice with very large tumours died during the same period and none died in which the tumours were small. A consideration of these facts will show the average weight and tumour size to have been less in mice receiving thyroxine, and greater in untreated mice at this stage of the experiment if mortality were taken into account, and that the curves from the 22nd to the 28th day therefore give an erroneous impression of the relative rate of growth.

In previous experiments thyroxine had not injured the health of the mice, and no deaths had occurred in the animals receiving it. The present experiment was continued for 18 days instead of 12 as before, and this may account for the higher mortality rate in injected mice. It is perhaps significant that of the 7 mice which died as a result of treatment only one had a large tumour, and this was originally much above average size (153 mm.² on the 10th day of the experiment). It may be inferred that in these a smaller dose would have sufficed, inducing in such individuals a response equivalent to that obtained by the dosage actually adopted in the case of a large number of mice, in which tumour growth was checked without serious detriment to the host. Similarly mice in which the rate of tumour growth was rapid might have tolerated a larger dose, as these individuals were evidently resistant to its effects. It is clearly impossible to disregard individual variations and yet produce in every animal just that degree of response which will prevent the further growth

of tumours without serious injury to the host. It seems possible that those individuals in which a response is obtained with relative ease are those in which a greater potential activity of the thyroid exists, and that this represents a diathesis which is inimical to the development of neoplastic growth.

SUMMARY.

Fifty-two male mice were implanted with tumours (M 63), and 5 days later were placed on a diet deficient in arginine. On this diet tumour growth was inhibited to an extent similar to that induced by a diet deficient in vitamin B. This was accompanied by steady loss of weight and a high mortality rate in 25 mice which were also injected with thyroxine.

Forty unimplanted mice were placed on a similar diet, and 12 mice were given for comparison a diet deficient in vitamin B. The addition of gelatin on the 9th day caused only a temporary recovery, while wheat germ induced a permanent increase in weight. Injections of arginine increased the loss of weight, a phenomenon only associated previously with a deficiency in vitamin B.

Seventy-five male mice implanted with tumours were injected with thyroxine daily from the 10th to the 28th day after implantation. A slight but definite inhibitory effect upon tumour growth was observed in spite of an increase in weight, the less marked effect in this second experiment being due to the larger quantity of food consumed by mice receiving thyroxine as compared with untreated controls. The results were comparable with those obtained when arginine was injected during the period when the mice received thyroxine in an earlier series of experiments.

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