XLIX. THE EFFECT OF ARGININE UPON THE BODY WEIGHT OF MICE INJECTED WITH THYROXINE, AND BEARING THE TUMOUR M 63.

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IN an earlier paper [Gilroy, 1930, 2], the hypothesis was advanced that thyroxine might modify the growth rate of neoplastic tissue, and in the case of a transplantable tumour of the mouse, M 63, this assumption was to a great extent confirmed. The suggestion that thyroxine exerted an inhibitory effect by inducing the catabolism of tissue protein also received support, for it was possible to cancel the effect characteristic of thyroxine if arginine was administered during the same experimental period, the results suggesting the maintenance of normal equilibrium in relation to supply and demand.

It remained to be decided whether the action of thyroxine was primarily upon the tumour, or whether the latter was only affected indirectly owing to some alteration of the metabolism of the host. The injection of 0.1 mg. of thyroxine daily for 12 days did not induce any toxic effects, but when the experiment was extended to 18 days 7 mice died out of a total of 47, from which it appeared that the threshold of toxicity had then been reached.

The records of weight showed an average gain which corresponded very closely with increase in tumour mass, and there was no evidence of any loss of body weight, although it seemed probable that some reduction in the latter would have occurred. The work of Abderhalden and Wertheimer [1928], and Romeis [1923], has shown that in spite of the relative insusceptibility of rats and mice towards thyroxine, a loss of weight is induced by the repeated administration of small amounts. It was evident that if total weight remained stationary, or diminished, while the tumour continued to grow, a reduction in body weight must have occurred. Observations were therefore confined to a determination of the number of mice bearing tumours of increasing size in which total weight either remained stationary or diminished.

EXPERIMENTAL.

The technique of implantation was described in an earlier paper [Gilroy, 1930, 1]. Treatment consisted in the daily injection of 0.1 cc. of a 0.1 % solution of thyroxine, or of 0.3 cc. of a 10 % solution of arginine; in one group

both substances were injected, but the arginine was injected in the morning and the thyroxine at night. The total duration of the experiment was 12 days, the first injections being given 10 days after implantation; in certain groups treatment was reversed halfway through the experimental period, and the data are therefore given in every case for the 16th and 22nd days after implantation.

Table I shows the number of mice in which weight was gained, remained stationary, or diminished, expressed as a percentage, the actual numbers being given in brackets below.

Table I.

No. of mice	Weight increased		Weight stationary		Weight decreased	
	Day 16	Day 22	Day 16	Day 22	Day 16	Day 22
27	Arginine	Thyroxine	Arginine	Thyroxine	Arginine	Thyroxine
	89.0 (24)	55.6 (15)	7.4 (2)	22.2 (6)	3.7 (1)	22.2 (6)
27	Thyroxine	Arginine	Thyroxine	Arginine	Thyroxine	Arginine
	55.6 (15)	89.0 (24)	14.8 (4)	7.4 (2)	29.6 (8)	3.7 (1)
16	Arginine + thyroxine		Arginine + thyroxine		Arginine + thyroxine	
	87.5 (14)	94.0 (15)	— (0)	6.0 (1)	12.5 (2)	— (0)
18	Arginine	Arginine	Arginine	Arginine	Arginine	Arginine
	89.0 (16)	94.5 (17)	— (0)	— (0)	11.0 (2)	5.5 (1)
18	Thyroxine	Thyroxine	Thyroxine	Thyroxine	Thyroxine	Thyroxine
	72.2 (13)	83.5 (15)	— (0)	5.5 (1)	27.8 (5)	11.0 (2)
20	Untreated	Untreated	Untreated	Untreated	Untreated	Untreated
	95.0 (19)	75.0 (15)	— (0)	10.0 (2)	5.0 (1)	15.0 (3)

Owing to the small numbers involved no statistical significance can be attached to the results, but from the data presented certain inferences may be made. In a group of 27 mice injected with thyroxine for 6 days, 12 lost weight, while of 27 mice injected with arginine only 3 lost weight in the same number of days. Among 18 mice 8 lost weight when treatment with thyroxine was continued for 12 days and again only 3 out of 18 mice lost weight when injected with arginine for a similar period of time. When both arginine and thyroxine were injected for 12 days 3 out of 16 mice lost weight; this compares favourably with a loss of weight in 6 out of 20 in the group receiving no treatment at all.

Under ordinary circumstances, with normal stock, the injection of arginine does not appear to have any effect upon the body weight of mice, but it seems that if for any reason an undue strain is thrown upon the system of the animal, the tendency to lose weight can be compensated by the administration of this amino-acid. Thus the growth of a tumour absorbs certain nutritive substances at the expense of the tissues of the host; thyroxine increases the

demand for such materials by increasing general metabolism, and in both cases arginine seems capable of balancing the tissue loss. It may be inferred that arginine either exerts some protective action against the influence of thyroxine, or facilitates the immediate reconstruction of tissue protein.

The substances which are known to modify the effect of thyroxine upon metabolism fall into two groups; the first of these, exemplified by ergotamine [Abderhalden and Wertheimer, 1927], may be described as specific in their action; the second are non-specific and are represented by the carbohydrates. Arginine, being an inert substance, is clearly not to be included in the first category but it is possible that in the present instance arginine functioned as a precursor of sugar. That this might occur is shown by the studies of amino-acid metabolism in diabetes, from the results of which Dakin [1923], concluded that "arginine is the only amino-acid with more than five carbon atoms which can furnish glucose freely," the intermediate step being the formation of ornithine by the action of the enzyme arginase. It is therefore possible that under certain circumstances arginine may be glycogenic, and it is well known that a diet rich in sugar-forming materials can counteract the influence of thyroxine upon metabolism [Abderhalden and Wertheimer, 1926], while protein accentuates its effects.

Although arginine may serve as a source of sugar to the organism if need arises, this does not elucidate its action in accelerating the rate of tumour growth. It was shown by Cioffari and Piccaluga [1926], that a diet rich in carbohydrate did not have a stimulating effect, and in a previous series of experiments [Gilroy, 1930, 1], it was observed that amino-acids such as glycine and alanine were without any influence in increasing the rate of tumour growth, although these can be completely converted into glucose in the diabetic organism [Ringer and Lusk, 1910]. That arginine is an important constituent of tumours was shown by Kocher [1915], who found that it accounted for 12.4 % of the total protein nitrogen of neoplastic tissue. The specific importance of arginine in this connection was disputed by Drummond [1916], who pointed out that the diamino-acid content of any tissue was correlated with its nuclear content, and not with its rate of growth. It would seem, however, that the formation of nuclear material must involve the assimilation, or synthesis, of those substances which enter into its constitution, otherwise growth by cell division could not occur.

The alleged indispensability of arginine in general metabolism was questioned by Rose and Cox [1924], and Rose and Cook [1925], but more recently Scull and Rose [1930], have demonstrated in the case of rats the possibility of a synthesis of arginine in the body. It seems not unlikely that a synthetic mechanism may be provided by a reversal of the normal hydrolytic action of arginase, for it is known, from the experiments of Wasteneys and Borsook [1925], that proteolytic enzymes are also capable of synthesis. The high arginine content of tumours may perhaps be thus explained, since arginase has been shown by Edlbacher and Merz [1927], to be present in neoplastic

tissue in considerable amount. A tissue thus equipped would not be dependent upon preformed arginine for nuclear synthesis and would to this extent more readily attain optimum conditions for growth.

SUMMARY.

126 mice were implanted with the carcinoma M 63, and 10 days later were divided into groups, 88 mice receiving injections of thyroxine in doses of 0.1 mg. daily for a period of 6 or 12 days. In certain groups arginine was injected coincidentally with thyroxine; the remainder were either injected with arginine alone, or were given no treatment at all.

The mice were weighed individually three times during the experiment and the results obtained indicated that arginine prevented a loss of weight which thyroxine would otherwise have induced.

As a high carbohydrate diet is known to modify the influence of thyroxine upon metabolism it is suggested that, in this instance, arginine was utilised for the formation of sugar, the latter being derived from ornithine, but reasons are advanced for considering that the glycogenic function of arginine could not be responsible for increasing the rate of tumour growth.

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REFERENCES.

- Abderhalden and Wertheimer (1926). *Pflüger's Arch.* **213**, 328.
— — (1927). *Pflüger's Arch.* **216**, 697.
— — (1928). *Pflüger's Arch.* **219**, 594.
Cioffari and Piccaluga (1926). *Tumori*, **12**, 387.
Dakin (1923). *Oxidations and reductions in the animal body*, pp. 64–71 (London, 2nd edition).
Drummond (1916). *Biochem. J.* **10**, 473.
Edlbacher and Merz (1927). *Z. physiol. Chem.* **171**, 251.
Gilroy (1930, 1). *Biochem. J.* **24**, 589.
— (1930, 2). *Biochem. J.* **24**, 1181.
Kocher (1915). *J. Biol. Chem.* **22**, 295.
Ringer and Lusk (1910). *Z. physiol. Chem.* **66**, 106. Cited by Mitchell and Hamilton, *The biochemistry of the amino-acids* (1929).
Romeis (1923). *Biochem. Z.* **135**, 85; *Amer. Chem. Abst.* **17**, 2751.
Rose and Cox (1924). *J. Biol. Chem.* **61**, 747.
— and Cook (1925). *J. Biol. Chem.* **64**, 325.
Scull and Rose (1930). *J. Biol. Chem.* **89**, 109.
Wasteneys and Borsook (1925). *J. Biol. Chem.* **62**, 633, 675; **63**, 563.