THE INFLUENCE OF DIETARY DEPLETION OF THIAMINE, RIBOFLAVIN AND NIACIN ON THE INDUCTION OF GLIOMATA IN MICE

J. M. BRIDGES

From the Department of Pathology, Queen's University, Belfast

Received for publication September 18, 1959

THE first report of the successful production of gliomata by implantation of pellets of chemical carcinogens into the brains of experimental animals was that of Seligman and Shear (1939); they implanted 1·5 mg. pellets of methylcholanthrene in the right cerebral hemisphere of 20 C_3H mice and of these animals 11 subsequently developed gliomata. These results were confirmed by Zimmerman and Arnold (1941) who later showed that benzpyrene (Arnold and Zimmerman, 1943) and dibenzanthracene (Zimmerman and Arnold, 1943) also had a local carcinogenic action when implanted intracerebrally. These workers (Zimmerman and Arnold, 1944) also investigated the response of various strains of mice to intracerebral implantation of methylcholanthrene and showed that the C_3H strain reacted more readily than did the other strains studied.

In 1945 Russell showed that the implantation of pellets of methylcholanthrene into the brains of rats fed a standard diet led to the development of gliomata in some 35 per cent of animals and that these animals survived the implantation for an average period of 372 days. He further showed that if following the intracerebral inoculation the rats were rendered deficient in thiamine, riboflavin and niacin then those animals which developed gliomata survived the implantation for an average period of only 270 days. It was in an attempt to establish if a similar phenomenon occurred in C_3H mice and, if so, which vitamin deficiency was responsible that the following experiments were undertaken.

It is well known that patients with malignant disease, including gliomata, show a high globulin-albumen ratio and accordingly the serum of mice carrying intracerebral carcinogen was studied electrophoretically in the hope that some pattern would emerge which would enable the diagnosis of glioma to be made before the animal's death.

MATERIALS AND METHODS

The mice used in these experiments were all of the C_3H strain and were aged between 50 and 70 days at the time of beginning the experiments.

The diet prepared for the animals consisted of protein 19 per cent, fat 5·5 per cent, carbohydrate 65 per cent and in the control diet vitamins A, B, C and D were added in the amounts recommended by Morris (1944).

The mice were divided into 5 equal groups, one was fed the control prepared diet, one an identical diet except that thiamine, riboflavin and niacin were excluded and 3 groups in which only one of the above vitamins was excluded. Those animals in which it was desired to induce vitamin deficiency were initially fed a diet completely lacking in the particular vitamin and when the animals were in an obvious deficiency state sub-optimal amounts of that vitamin were added to the diet.

Pellets of pure methylcholanthrene weighing approximately 1·3 mg. were implanted into the right cerebral hemispheres of 150 mice according to the method described by Seligman and Shear (1939). These animals were equally divided amongst the 5 dietary groups and each group was further sub-divided into 2 sub-groups. One sub-group (*a*) was put on the dietary regime 4 weeks before the intracerebral implantation; in the other the dietary regime was instituted 2 weeks after the intracerebral implantation. Five control groups of 10 animals which did not have the carcinogen implanted were put on one of the above dietary regimes to assess the effect of each deficiency *per se*.

The method of electrophoresis used was the hanging strip method with barbiturate buffer at pH 8·6. Blood for the electrophoresis was obtained by puncturing the conjunctival sinus with fine glass tubing. The serum was allowed to separate at a constant voltage of 180 volts for 16–20 hr. and after fixing and drying the paper was stained with Lissamine Green.

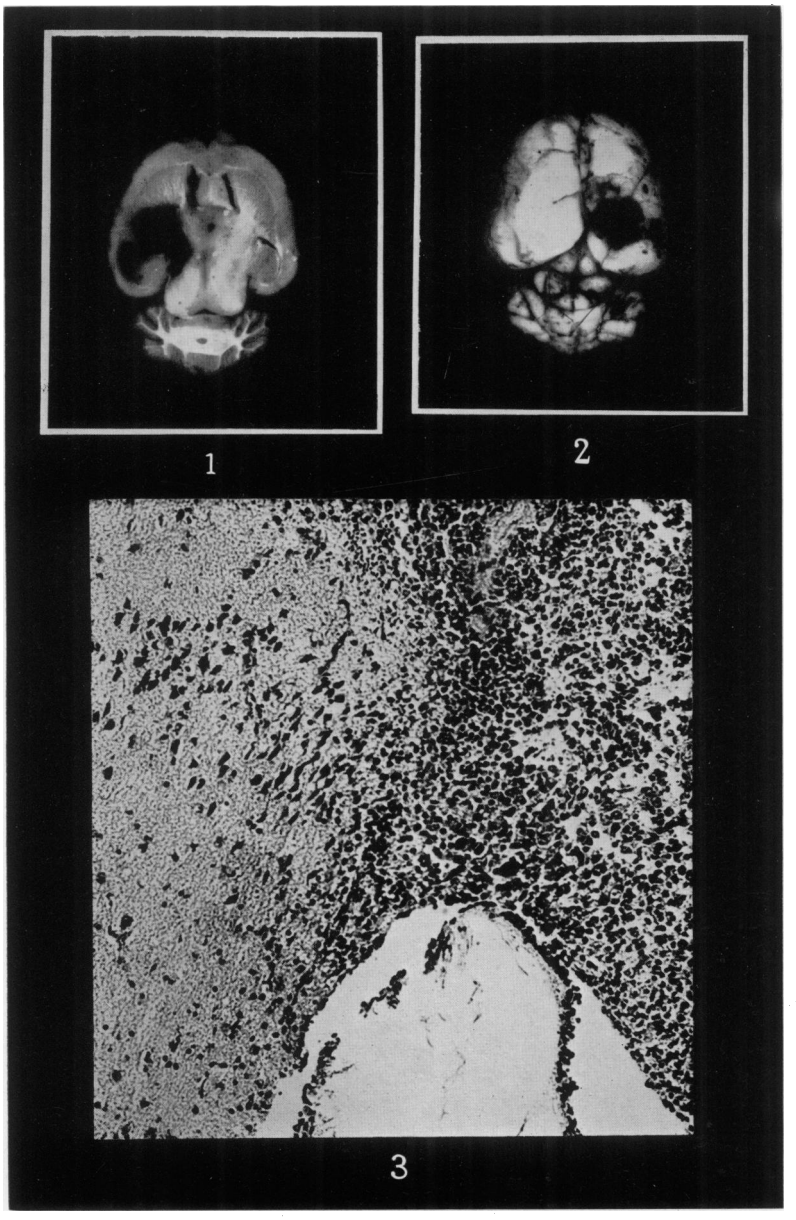
RESULTS

Effect of the dietary deficiency alone

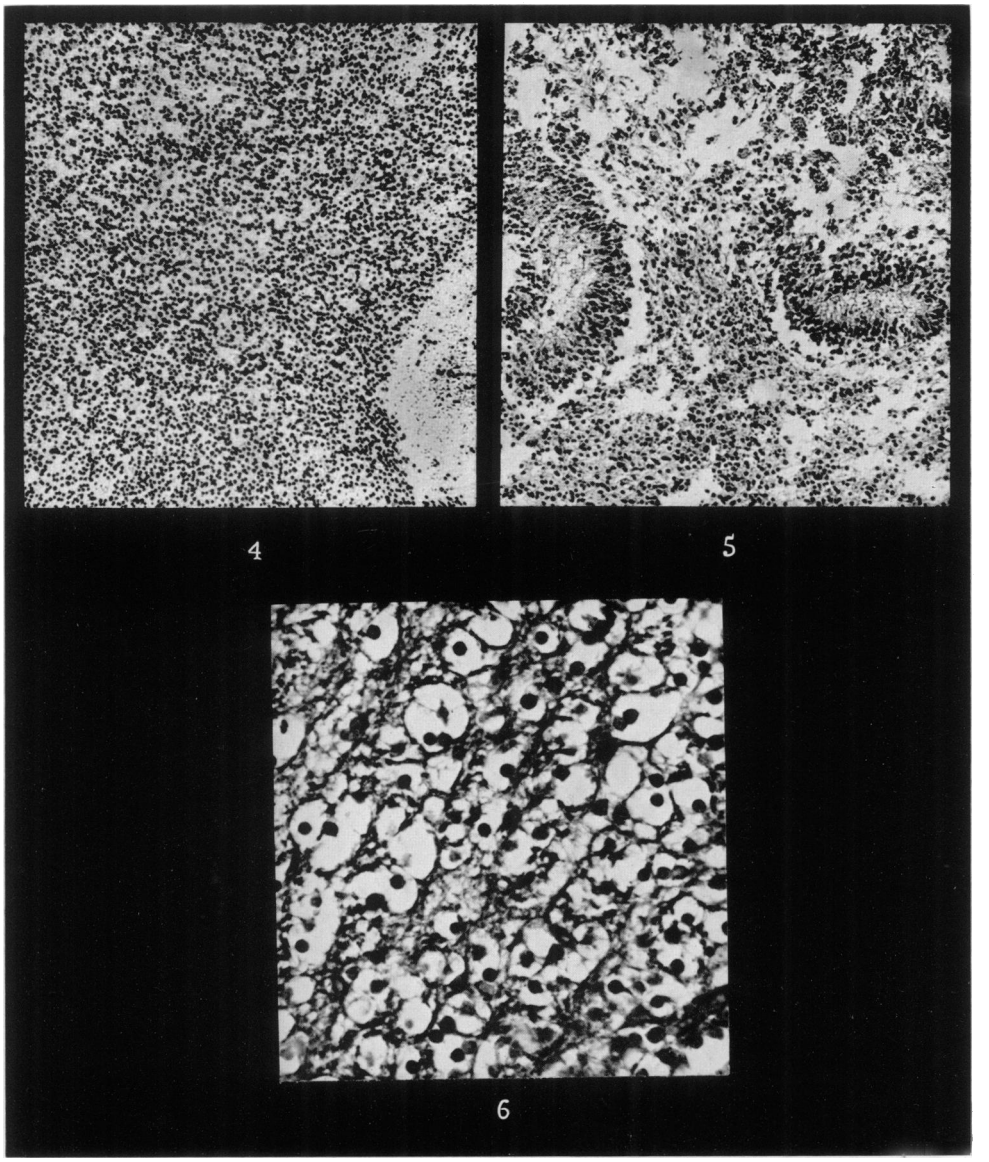
Animals fed the prepared diet with all added vitamins showed no difference in weight from animals fed the standard laboratory diet. Independent of the main experiment 2 male and 4 female mice were fed the complete prepared diet for 6 weeks and then mated. Reproduction and lactation in these females were quite normal. Those animals which were deprived of thiamine showed an initial slight gain in weight lasting 18–20 days and this was followed by a rapid loss of weight so that 30 days after institution of the thiamine-deficient diet the average weight had fallen by 40 per cent. At this stage 4 μg. of thiamine per animal per day was added to the diet. On this regime there was an immediate improvement in the general condition of the animals and their weight gradually increased to 70 per cent of their original weight at which level it was maintained for the duration of the experiment (28 weeks). Animals deprived of riboflavin showed a similar but less rapid response and after 36 days the average weight had fallen by 36 per cent: at this stage 1·5 μg. per animal per day was added to the diet and this was found sufficient to maintain the animals at 70 per cent of their original weight. Animals which were completely deprived of dietary niacin did not show any loss of weight or condition throughout the 28 weeks of this experiment: animals which had been deprived of dietary niacin for 10 weeks were mated and reproduction and lactation were quite normal. Harris and Raymond (1939) found that rats on a diet deficient in niacin continued to thrive and from the above results it would appear that niacin is not an essential dietary requirement for C_3H mice (on the above basic dietary regime). Animals on the dietary regime deficient in thiamine, riboflavin and niacin reacted initially as did those deprived of thiamine alone but at the stage of initial depletion thiamine was sufficient to improve their condition for only a short time and then riboflavin also had to be added to the diet. The amounts of thiamine and riboflavin necessary to maintain the animals on the triple deficiency diet at 70 per cent of their original weight were the same as those needed for the animals on a single deficiency.

EXPLANATION OF PLATES.

FIG. 1.—Spontaneous haemorrhage in right cerebral hemisphere.
FIG. 2.—Astrocytoma right cerebral hemisphere showing terminal haemorrhage.
FIG. 3.—Astrocytoma arising at edge of pellet which is also surrounded by normal brain.
FIG. 4.—Astrocytoma which is very cellular but shows little anaplasia.
FIG. 5.—Ependymoma showing rosette pattern.
FIG. 6.—Oligodendroglioma showing boxing of the nuclei.



Bridges.



4

5

6

The only outstanding autopsy findings in the dietary control animals were in 2 animals which had been on thiamine-deficient regime ; these animals survived the onset of the dietary regime by 10 and 13 weeks respectively and both showed large intracranial haemorrhages (Fig. 1). Intracranial haemorrhage was also noted in 2 animals on the triple deficiency diet which had had intracerebral implants. The haemorrhages were at the site of the implant and had occurred 12 and 14 weeks after the pellet had been inserted. Careful histological study failed to show any evidence of neoplasm at the edges of these haemorrhagic foci.

In none of the dietary control animals nor in those animals on the various dietary deficiencies which had had the carcinogen implanted but did not develop tumour, except for the 4 showing intracranial haemorrhage, was any histological abnormality noted in the brain.

Tumours produced and effect of dietary regime on tumours produced

Of the 150 animals with methylcholanthrene implants 27 developed gliomata and 34 meningeal sarcomata ; all these tumours arose at the site of the implantation.

From the majority of the gliomata the carcinogenic pellet was recovered intact and in those animals which did not develop gliomata only a very slight glial reaction occurred round the pellet. The gliomata varied in size up to 1 cm. diameter and the majority were the seat of recent haemorrhage (Fig. 2). The most striking histological feature was the very close resemblance to the human gliomata (Fig. 3, 4, 5, 6) and all the typical histological patterns of the human gliomata were encountered. This very close histological resemblance of the experimentally produced gliomata and the human gliomata has previously been noted by Seligman and Shear (1939) and Zimmerman and Arnold (1941 and 1943). The 27 gliomata were classified on a histological basis as Glioblastoma multiforme 4, Astrocytoma 11, Oligodendroglioma 2, Ependymoma 4 and mixed tumours 6.

Table I shows the incidence of the gliomata in the various dietary groups. From this Table it is clear that the dietary deficiencies induced did not alter the incidence of the gliomata nor was the incidence altered by instituting the dietary regime before or after the implantation of the carcinogen.

Table II shows the interval elapsing between the implantation of the carcinogen and death in those animals which developed gliomata ; 4 gliomata were discovered when mice which had developed an abnormal albumin-globulin were killed and they are not considered in this Table. The results obtained show that those animals which were chronically deficient in thiamine whether alone or in combination with riboflavin and niacin and which died as a result of a glioma did so significantly earlier than did animals with glioma which were either on a complete diet or on a dietary regime deficient in only riboflavin or niacin.

The meningeal sarcomata showed no difference in incidence in the various dietary groups. There was no significant difference in the survival time of animals with meningeal sarcomata in the various dietary groups, the average figure for the various groups being : complete diet 223 days, diet deficient in thiamine 217 days, diet deficient in riboflavin 206 days, diet deficient in niacin 218 days and diet deficient in all 3 vitamins 237 days.

TABLE I

Dietary group		Number of gliomata	Number of fibrosarcomata
All vitamins added	A	3	3
	B	1	5
	Total	4	8
Deficient in thiamine, riboflavin and niacin	A	3	4
	B	3	3
	Total	6	7
Deficient in thiamine	A	3	3
	B	3	2
	Total	6	5
Deficient in riboflavin	A	4	4
	B	3	4
	Total	7	8
Deficient in niacin	A	2	3
	B	2	3
	Total	4	6

The incidence of intracranial tumours in the various dietary groups. Sub-group A refers to those animals in which the dietary regime was instituted prior to the intracerebral implantation and B to those animals in which the intracerebral implant preceded the institution of the dietary regime.

TABLE II.

	All vitamins added		Deficient in thiamine, riboflavin and niacin		Deficient in thiamine		Deficient in riboflavin		Deficient in niacin	
	A	B	A	B	A	B	A	B	A	B
Interval in days between implantation of pellet and death of animal	249	—	151	182	199	176	230	241	281	292
	291	—	197	206	209	204	292	272	317	—
	321	—	—	239	—	269	322	300	—	—
	—	—	—	—	—	—	330	—	—	—
Average	287	—	174	209	204	216	293	271	299	292
Average in group as a whole	287		195		211		284		297	

Showing the interval between implantation of the carcinogen and death in animals which developed gliomata.

Electrophoretic studies

The electrophoretic patterns obtained were generally satisfactory as regards the separation of the two main protein fractions, *i.e.* globulin and albumin, but the separation of the various globulin fractions was very inconstant. The Albumin/Globulin (A/G) ratios in 100 stock C_3H mice aged between 50 and 300 days were calculated and found to be between 0·75 and 1·35 with a mean reading of 1·08. No variation in the A/G ratio with increasing age was noted. The A/G ratio in the control dietary deficient animals failed to show any significant deviation from

normal even when the animal was in an acute deficiency state. The serum from 11 animals which became suddenly ill and were proven at autopsy to have gliomata was studied. The A/G ratio in these animals varied from 0·35 to 0·55 with a mean reading of 0·5. The A/G ratio in animals with meningeal sarcomata was also found to be consistently low.

The serum of 20 mice with methylcholanthrene implants was studied weekly. Of these animals, 2 which were on the thiamine-deficiency regime, 2 on the riboflavin deficiency and 1 on the thiamine, riboflavin- and niacin-deficiency regime all developed gliomata. Assuming that a reading of 0·6 or below represents an abnormal A/G ratio then an abnormal A/G ratio developed in each of these 5 animals at approximately the same interval after the intracerebral implantation. However, the duration of life following the development of the abnormal A/G ratio was much longer in those animals deficient in riboflavin than in those deficient in thiamine (Table III).

TABLE III

Dietary group	Interval in days between carcinogenic implantation and development of abnormal A/G ratio	Interval in days between carcinogenic implantation and death	Interval in days between development of abnormal A/G ratio and death
Dietary group deficient in riboflavin	154	238	84
	230	322	92
Average	192	280	88
Dietary group deficient in thiamine .	133	175	42
	164	210	46
Dietary group deficient in thiamine, riboflavin and niacin	189	238	49
Average	162	207	46

Showing intervals between implantation of carcinogen, development of abnormal A/G ratio, and death in 5 animals which developed gliomata.

DISCUSSION

In these experiments mice which developed gliomata subsequent to the intracerebral implantation of methylcholanthrene and which were fed a complete prepared diet survived the implantation by an average of 287 days. Mice with similar tumours but fed a diet deficient in thiamine, riboflavin and niacin survived the implantation of the carcinogen by only 195 days. These results agree well with those reported by Russell (1945) who, however, used rats. In examining the survival time of mice with gliomata which were fed a diet deficient only in thiamine or riboflavin or niacin it becomes clear from these experiments that the vitamin deficiency responsible for the shortened survival time was thiamine.

Russell has deduced from his work that the deficiency of thiamine and riboflavin altered the metabolism of the glial cells in such a way as to render these more suceptible to a carcinogenic stimulus. If this is so, then one would only expect that not only would the survival of such mice be reduced but that the incidence of gliomata in the deficient animals would be increased. No difference in the incidence of gliomata in the two groups was, however, noted in the present experiments, nor did Russell find such a difference. These results are, however,

open to a different interpretation; namely that the gliomata arise at approximately the same interval following the implantation of the carcinogen but that the dietary deficiency renders the animals more susceptible to the effects of the glioma. By whatever mechanism the thiamine deficiency causes the shortened survival of animals with gliomata it would appear to be specific to glial neoplasms, since in the present experiments those animals with meningeal sarcomata survived for an equal length of time irrespective of thiamine or riboflavin deficiency. A similar finding was noted by Russell.

In the present experiments on the effect of thiamine deprivation on the central nervous system the only abnormal autopsy finding in those animals which did not have intracerebral implants or did not develop gliomata subsequent to such implants was that 4 such animals showed large intracranial haemorrhages. Dunn, Morris and Dubnik (1947) noted spontaneous intracranial haemorrhage in 30 out of 50 thiamine-deficient C_3H mice and postulated that this was due to a defect in the capillary walls. The outstanding feature on macroscopic examination of the gliomata produced in these experiments, whether the animal was thiamine deficient or not, was that the vast majority of the gliomata had been the seat of terminal haemorrhage, and it would appear that mice with experimentally induced gliomata which are deficient in thiamine die sooner than similar animals on a normal diet because of the greater tendency for haemorrhage to occur into the tumour.

This view is supported by the results of the electrophoretic studies done (Table III). Three mice which were on a thiamine-deficient regime and developed gliomata were having their A/G ratio done weekly: the average interval between the intracerebral inoculation and the development of the abnormal A/G ratio in these animals was 23 weeks and they survived the development of the abnormal A/G ratio by $6\frac{1}{2}$ weeks. Similar figures for the 2 mice not thiamine deficient but in a similar state of inanition due to riboflavin deficiency were: abnormal A/G ratio developed at $27\frac{1}{2}$ weeks and was survived by $12\frac{1}{2}$ weeks. Although the small numbers in this experiment do not permit any firm conclusion to be drawn the results are nevertheless suggestive that the partial withdrawal of dietary thiamine in animals with intracerebral implants of methylcholanthrene does not unduly accelerate the development of gliomata but rather renders the animals more susceptible to the effects of such a tumour.

SUMMARY

The effect of dietary deprivation of thiamine, riboflavin and niacin on the survival time of mice with experimentally induced gliomata was studied.

The gliomata were produced by intracerebral implantation of methylcholanthrene.

It was found that mice which were receiving adequate dietary thiamine irrespective of riboflavin or niacin deficiencies and which developed gliomata survived the implantation of the carcinogen by 287 days whereas those which were deficient in thiamine and died as the result of gliomata did so 211 days after the implantation. The theory is put forward that this is not due to any altered susceptibility to carcinogenic stimulus of glial cells in animals deprived of thiamine, but that these animals die sooner because of the greater tendency for haemorrhage into the substance of the tumour to occur.

I wish to express my deep appreciation to Professor J. H. Biggart for his interest and advice, to Mr. D. McA. Mehaffey for taking the photographs, and to Mr. J. Davidson for his technical assistance.

REFERENCES

ARNOLD, H. AND ZIMMERMAN, H. M.—(1943) *Cancer Res.*, **3**, 682.
DUNN, T. B., MORRIS, H. P. AND DUBNIK, C. S.—(1947) *J. nat. Cancer. Inst.*, **8**, 139.
HARRIS, L. J. AND RAYMOND, W. D.—(1939) *Biochem. J.*, **33**, 2037.
MORRIS, H. P.—(1944) *J. nat. Cancer. Inst.*, **5**, 115.
RUSSELL, W. O.—(1945) *Cancer Res.*, **5**, 140 and 152.
SELIGMAN, A. M. AND SHEAR, M. J.—(1939) *Amer. J. Cancer*, **37**, 364.
ZIMMERMAN, H. M. AND ARNOLD, H.—(1941) *Cancer Res.*, **1**, 919.—(1943) *Amer. J. Path.*, **19**, 939.—(1944) *Cancer Res.*, **4**, 98.