

71. OCCURRENCE OF FITS OF AN EPILEPTIFORM NATURE IN RATS MAINTAINED FOR LONG PERIODS ON A DIET DEPRIVED OF VITAMIN B₆

BY HARRIETTE CHICK, M. M. EL SADR¹ AND ALASTAIR N. WORDEN²

*From the Division of Nutrition, Lister Institute, London, and
Roebuck House, Cambridge*

(Received 1 March 1940)

ABOUT 2 years ago Chick *et al.* [1938, 2] reported the occurrence of nervous symptoms in young pigs reared on a synthetic diet and deprived of certain members of the vitamin B₂ complex. The basal diet consisted of purified casein, purified maize starch, cotton seed oil, salt mixture, cod liver oil, with daily supplements of pure vitamin B₁, nicotinic acid and riboflavin. When daily doses of Edgar & Macrae's yeast eluate fraction which contains vitamin B₆ and of their purified yeast filtrate fraction were also given, the pigs developed satisfactorily, though not as well as a control group which received 4% of yeast in their diet as source of all the B-vitamins. When the filtrate factor was omitted the pigs did badly, became paralysed and died if the missing factor was not given. When the eluate factor was omitted the pigs also showed a subnormal rate of growth and in addition developed a tendency to epileptic fits. The following is the description given.

The fits resembled typical epilepsy as seen in the human being. Without warning the animal ran round the pen in excitement, screamed, then suddenly dropped as if shot. This was followed by a period of tonic spasm with extended legs, passing to a clonic stage with violent jerking of the limbs. A comatose stage succeeded with dry stertorous breathing gradually becoming shallow. A few minutes later the colour of the pig, which had become deathly white, improved, consciousness returned and the animal got up languidly and walked about as before the fit occurred. The duration of the fits was from a few minutes to a quarter of an hour. Between the fits the animals appeared normal. When the yeast eluate fraction was given to the pigs, no more fits occurred.

As far as we are aware, this is the first recorded instance of experimentally produced epilepsy of nutritional origin. Convulsive fits and other nervous symptoms have, however, been described in dogs, pigs, chicks or rats deprived of various members of the B-vitamin group.

Fouts *et al.* [1938] observed the occurrence of hypochromic microcytic anaemia, together with convulsive fits, in puppies about 4 months old reared on a synthetic basal diet of casein, sucrose, crisco, salt mixture, halibut liver oil, vitamin B₁ and riboflavin, to which was added a liver extract which contained the filtrate factor and nicotinic acid. If, in addition, an extract of rice polishings was given, to provide the rat antidermatitis factor, vitamin B₆, there were neither anaemia nor convulsions. Wintrobe *et al.* [1938] described ataxia with queer gait, and clonic, scratch-like, movements of the hind legs, in young pigs receiving a synthetic diet consisting of casein, sucrose, butter, lard, salt mixture, vitamin C and cod liver oil, to which whole yeast was added, at first adequate in amount

¹ Mission Member of the Egyptian University.

² Research Student in Animal Health, Ministry of Agriculture.

but gradually reduced to 0.1 g. per kg. body weight. Addition of vitamin B₁ and riboflavin did not prevent the nervous disorder. If the yeast was removed suddenly from the diet, there was prostration, and convulsions occurred [Wintrobe, 1939]. Oleson *et al.* [1939] mention paralysis among the different syndromes observed in rats receiving a synthetic basal diet supplemented with pure vitamin B₁, riboflavin, nicotinic acid, choline and the filtrate obtained after treatment of a liver extract with fuller's earth in acid solution. The rats also suffered from dermatitis; this was cured by pure vitamin B₆, but the paralysis only yielded to an impure preparation of the latter, viz. the eluate obtained from the fuller's earth adsorbate. Finally, Jukes [1939] has reported the occurrence of "spasmodic convulsions", sometimes leading to death, among chicks reared on a diet which included vitamin B₁, nicotinic acid, riboflavin and a preparation of pantothenic acid (filtrate factor); when pure vitamin B₆ was given in addition, there were no fits and growth was satisfactory.

In the course of investigations, extending over many years in this laboratory, of the effects on rats of deprivation of the B₂ group of vitamins and of its separate members, no nervous symptoms have ever been observed until recently. During the series of long-period observations described in the previous paper in this *Journal* [Chick *et al.* 1940], dealing with the skin lesions developed in deficiency of the different B₂-vitamins, fits of an epileptiform nature were observed in rats after long deprivation of the yeast eluate fraction (vitamin B₆).

EXPERIMENTAL

The series of animals studied comprise the 34 rats deprived of the yeast eluate fraction (vitamin B₆) in experiments 1c, 2, 3 and 4a, of which fuller data are given in Table 2 of the previous paper (p. 587). To this reference should be made for all details, including composition of the basal diets used and the nature and amounts of the supplements given. Fits were seen without exception in all the rats in those groups which received basal diets containing carbohydrate in the form of sucrose or raw starch, and survived upwards of about 4 months. The fits usually occurred some weeks later than the dermatitis; the time of onset varied from 8 to 38 weeks after the animals received the experimental diet and did not normally occur sooner than about 20 weeks (see Table 1), though the rat often became restless and appeared to be hypersensitive some time earlier.

The fits showed the following stages.

i. A violent stage in which the rat would suddenly rush about wildly with protruding eyes, jumping to the floor of the room if not restrained, and leaping up into the air, sometimes uttering cries; this stage usually lasted less than 30 sec. In a few instances the eyes became suffused with blood which drained away through the naso-lachrymal duct. Occasionally the rats urinated during a fit and on one occasion vomiting of stomach contents was observed.

ii. A helpless condition in which there were muscular twitching of the limbs and tonic spasms while the rat lay helpless; sometimes the digits of one of the forepaws became clasped with those of the hind paw on the same side.

iii. A comatose condition when the rat sometimes became unconscious with a slowed and weakened heart beat and absence of corneal reflex.

iv. Gradual recovery, control being regained first of the fore part of the body, head and forepaws, and later of the hind limbs (see Plate 2, fig. 6).

The time taken for the whole fit was usually from 2 to 4 min. and all stages of severity were observed.

In the milder fits stage i might occur alone and the animal come to rest suddenly and remain quiet but conscious; in others stage i might be omitted and

Table 1.* *Incidence of dermatitis and fits in rats maintained for long periods on a synthetic diet supplemented with cod liver oil, pure vitamin B₁, riboflavin and purified yeast filtrate factor and deprived of the yeast eluate factor (vitamin B₆)*

Exp. no.	Litter	Carbohydrate in diet	Incidence of dermatitis			Incidence of fits			Notes
			No. of rats	No. of cases	Time of onset, weeks on diet	No. of cases	Time of onset, weeks on diet		
1c, 2	5, 6, 7	Sucrose	6	5	8-11	2	16-20	3 rats killed after 11, 12 and 18 weeks on exp. for histological study, † 1 rat cured with vitamin B ₆ at 10 weeks	
		Rice starch, raw	5	4	10-24	4	13-38	1 rat died at 16 weeks	
		Maize starch, raw	4	2	10-21	4	13-21		
3	8, 9	Sucrose	6	5	8-16	4	17-32	1 rat with severe dermatitis cured with vitamin B ₆ at 12 weeks, 1 rat died at 19 weeks	
		Rice starch, cooked	5	—	—	—	—	Observed for 30 weeks	
		Rice starch, raw	4	2	8	4	8-30		
4a	10	Rice starch, raw	4	3 1	8-9 30	3	21-28	1 rat died at 14 weeks	
4b†	10	Rice starch, raw	4	—	—	—	—	All healthy after 35 weeks	

* Reference should be made to Table 2 of the preceding paper which deals with the same series of animals.
 † By Dr El Sadr. ‡ Addition of pure vitamin B₆, 10-15 µg. daily.

the animal, without warning, suddenly become helpless with twitching paws. In others, again, recovery began immediately after stages i and ii, and there was no comatose stage.

When a fit was over, the rat usually remained very quiet for a long time, for an hour or more, with protruding eyeballs, and the hypersensitive condition persisted. A second fit could sometimes be induced if, for example, the tail were slightly pinched. Only on one occasion did an animal die in a fit.

The rats also showed minor nervous signs when fits did not occur. Such were occasional spasm of the ear muscles and eyelids and twitching of the shoulders when the daily doses were dropped into the mouth. Sometimes a rat would struggle and lunge to get free from one's hand when being dosed, which is quite unusual with rats which are accustomed to being handled every day and are quite tame.

Sometimes the fits were of daily occurrence and the slightest external stimulus seemed to precipitate them. Three such rats were sacrificed for bacteriological examination, in order to find out if the occurrence of the fits could be attributed to infection by a pleuro-pneumonia like organism, as was found to be the case with the "rolling disease" of mice [Findlay *et al.* 1938]. The examinations were carried out by Dr Emmy Klieneberger, to whom our thanks are due, but no evidence of a causal infective agent was found. Histological examination of the central nervous system by Dr J. G. Greenfield gave negative results (see Addendum).

In many instances, in a rat which had suffered from fits and had remained on the same diet, the fits ceased to occur but the animal remained nervous and

excited and showed protruding eyeballs. In such cases a fit could be induced sometimes by handling or stroking for 15–30 min. Sometimes attempts to obtain a drop of blood from the tail for haematological examination caused a fit to take place, and fits were also precipitated when rats were placed in an unaccustomed situation in order to take a photograph. It was very common, before a fit took place, to notice chattering of the teeth and also to observe the rat rubbing its nose and cheeks with its forepaws, as if these places were irritable.

The fits were somewhat similar in character to those observed by McCollum and his collaborators in rats deprived of magnesium [see Duckworth, 1939]. The description of the convulsions caused by Mg deficiency in the rat given by McCollum *et al.* [1939] shows a striking similarity to the fits described here. However, our basal diet contained 0.07% Mg and consequently our animals received an adequate supply.

Relation of fits to deprivation of vitamin B₆

The rats which developed fits were all deprived of the yeast eluate factor, that is of vitamin B₆ [see El Sadr *et al.* 1939, 2]. We have so far no evidence of the presence of any other dietary essential for the rat in the preparation of this factor which was used [Edgar *et al.* 1938]. It may have contained nicotinamide or nicotinic acid [Macrae & Edgar, 1937, p. 2229, n.], but there is no evidence that the rat requires either of these compounds [Macrae & Edgar, 1937; Chick *et al.* 1938, 1]. Of the 15 rats in Exp. 3, 7, distributed over the 3 groups receiving the 3 different types of carbohydrate, received 1 mg. nicotinic acid daily; these behaved in no way differently from the 8 which had no nicotinic acid.

The supposition that the fits were caused by lack of vitamin B₆ is also confirmed by the following facts. In Exp. 4 (*b*) the 4 rats which received 10 to 15 μ g. pure vitamin B₆ daily showed no signs of fits, in contrast to their litter-mates deprived of it. They also showed no skin affection and became healthy well-grown rats. After 33 weeks the males and females weighed respectively about 330 and 240 g., whereas their litter-mates deprived of vitamin B₆ weighed only about 110 g. irrespective of sex (see Fig. 3 of the previous paper, this *Journal*, p. 590).

The curative effect of pure vitamin B₆, 15 μ g. daily, was tried on 3 rats (Nos. 35, 36 and 51) suffering from fits after deprivation of eluate factor for periods of 15, 20 and 22 weeks, respectively. Of these one had no more fits; two continued to have them only during the first 2 weeks after the cure began; all three were observed for 2–3 months after the vitamin B₆ was given. Two of the above rats (Nos. 35 and 51) were suffering also from dermatitis when the cure with vitamin B₆ was given; these and others suffering from very severe dermatitis showed swift cure of the skin condition after administration of 15 μ g. vitamin B₆ daily.

Influence of carbohydrate in the diet

The results in Table 1 show that whereas fits occurred in all the rats receiving raw maize or rice starch, no fits occurred in the group in Exp. 3 which received the cooked rice starch, nor was there any dermatitis. The protection afforded by starch, as compared with sucrose, against the dermatitis caused by deficiency of vitamin B₆ is discussed in the previous paper (p. 591) and the suggestion is there made that it is due to traces of vitamin B₆ which adhere to the starch, and in some way become more available to the rat after cooking.

If this explanation be the true one it offers another argument for relating the occurrence of the fits to deprivation of vitamin B₆. It must be admitted, however,

that the diet given was deficient in other respects than in vitamin B₆. It is known that, in addition to riboflavin, yeast filtrate factor and vitamin B₆, there are present in whole yeast and in an extract of whole liver at least two other, at present unknown, water-soluble substances, which appear to be essential for the optimum nutrition of the rat (El Sadr *et al.* 1939, 1]. These unknown materials were probably absent from the diets given in this work, although traces may have been present as impurities in the starch and casein, and it must be admitted that the growth rates when vitamin B₆ was added to the diet fell somewhat short of the optimum (see Exp. 4*b*, Fig. 3 of the previous paper). Further, although successful pregnancies followed matings in this group, the litters were not reared. In any case, however, there is no evidence that the fits were caused by deprivation of either of the above unknown factors and there is much positive evidence that they were due to deprivation of vitamin B₆.

It will seem very strange that the epileptic fits described in this paper should not have been observed previously in any of the large numbers of rats deprived of vitamin B₆ studied in this laboratory. In the present case they occurred in every one of the 21 rats observed for 4 months or longer on the purified synthetic diet containing sucrose or raw purified starch as source of carbohydrate. While it is true that in the past a cooked basal diet containing starch as carbohydrate has usually been employed, recently sucrose has been used more frequently. The experiments of Series I in the previous paper are an example and here no fits were observed in rats deprived of yeast eluate fraction. None of these animals, however, was observed for a longer time than 14 weeks and in most cases the period was shorter.

The results of these experiments with rats fall into line with the previous ones on pigs by Chick *et al.* [1938, 2], alluded to above. The basal diets and the supplements given were similar. At the time when the experiments on pigs were carried out pure vitamin B₆ was not available, but preparations of the yeast eluate factor were found to be curative and it is reasonable to conclude that deprivation of vitamin B₆ was the immediate cause of the fits in the pigs as in the rats.

A connexion between deprivation of vitamin B₆ and the occurrence of the convulsive fits and other nervous symptoms in puppies, pigs, rats and chicks, described above on p. 595, is not so clear. In some of the observations, as for example those of Wintrobe and his colleagues on the pig, the diet was deficient in other known members of the vitamin B₂ complex; in others, as in those of Jukes on the chick, the basal diet was not composed of purified foodstuffs, although in this case pure vitamin B₆ was found to be preventive of the convulsions which occurred.

SUMMARY

1. Fits of an epileptiform nature were observed in rats maintained for long periods, 4 to 5 months and over, on a purified synthetic diet supplemented with cod liver oil, pure vitamin B₁, riboflavin and purified yeast filtrate factor.

2. The fits were prevented and cured by administration of pure vitamin B₆, 10–15 μ g. daily, and it is concluded that they were caused by deprivation of this vitamin.

3. No fits occurred when purified rice starch was the carbohydrate in the diet and the diet was cooked with water; it is suggested that this starch retains traces of vitamin B₆ which become more readily available to the animal after the starch has been cooked.

4. The nature of these fits in rats and the circumstances in which they were developed show the closest resemblance to the epileptic fits previously observed

in young pigs. The basal diets and the supplements given were similar in the two cases. It is reasonable to conclude that in the pigs they were also caused by deficiency of vitamin B₆.

Our thanks are due to Mrs C. E. Work and to Mr G. Flynn for assistance in the care of the experimental animals, to the former especially for her help during September when the rats made a temporary stay at the Lister Institute, Elstree, before they were finally settled at Roebuck House, Chesterton, Cambridge, through the hospitality of Sir Charles Martin, to whom we wish to express our gratitude.

We are also indebted to Dr S. Lepkovsky for a gift of pure vitamin B₆ and to Roche Products Ltd. for riboflavin and pure vitamin B₁ (aneurin).

REFERENCES

- Chick, Macrae, Martin & Martin (1938, 1). *Biochem. J.* **32**, 844.
 ——— (1938, 2). *Biochem. J.* **32**, 2207.
 ——— & Worden (1940). *Biochem. J.* **34**, 580.
 Duckworth (1939). *Nutrition Abstracts and Reviews*, **8**, 841.
 Edgar, El Sadr & Macrae (1938). *Biochem. J.* **32**, 2225.
 El Sadr, Hind, Macrae, Work, Lythgoe & Todd (1939, 1). *Nature, Lond.*, **144**, 73.
 ——— Macrae & Work (1939, 2). *Biochem. J.* **33**, 611.
 Findlay, Klieneberger, MacCallum & Mackenzie (1938). *Lancet*, **135**, 1511.
 Fouts, Helmer, Lepkovsky & Jukes (1938). *J. Nutrit.* **16**, 197.
 Jukes (1939). *Proc. Soc. exp. Biol., N.Y.*, **42**, 180.
 McCollum, Orent-Keiles & Day (1939). *Newer Knowledge of Nutrition*, 5th ed. New York.
 Macrae & Edgar (1937). *Biochem. J.* **31**, 2225.
 Oleson, Bird, Elvehjem & Hart (1939). *J. biol. Chem.* **127**, 23.
 Wintrobe (1939). *Amer. J. Physiol.* **126**, 375.
 ——— Mitchell & Kolb (1938). *J. exp. Med.* **68**, 207.

ADDENDUM

BY DR J. G. GREENFIELD

Results of histological examination of the tissues of rats suffering from fits

These rats, Nos. 40, 50 and 54, had been maintained on the diet deprived of vitamin B₆ for 31, 21 and 30 weeks, and had suffered from fits for 11, 5 and 13 weeks, respectively.

The brain and spinal cord and sciatic nerves were examined by the Marchi-Busch and Nissl methods.

The nerve cells throughout the cerebral nervous system were healthy, and there was no evidence of any falling out of nerve cells in the *Cornu Ammonis* or elsewhere. No Marchi degeneration was found in the medulla, spinal cord or sciatic nerves.

In the lungs of rat 40 there was considerable thickening of the alveolar septa in many places with fibro-cellular tissue. In some areas a few foamy cells were present in the alveoli. The bronchial walls were healthy. In the lungs of rat 54 the condition was similar but in places seemed to be more severe and acute, in that the alveoli were almost completely obliterated and more polymorphonuclear leucocytes were present in the alveolar septa. Such changes as the above are commonly present in rats maintained for long periods on artificial diets and would appear to bear no relation to the specific deficiency in this case.